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Ailanthus altissima Extract Ameliorates Cognitive Decline and Neurodegeneration in an Aluminum Chloride–Induced Rat Model of Alzheimer's Disease

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ABSTRACT

Background: The extract of *Ailanthus altissima* possesses anti-inflammatory and neuroprotective properties. We aimed to investigate its effectiveness in mitigating the $AlCl_3$-induced Alzheimer's disease in a rat model.

Methods: A total of 32 adult male Wistar rats were divided into 4 groups each consisting of 8 rats. The control group received saline and the $AlCl_3$ group received $AlCl_3$ (0.2 cc 100 mg/kg, intraperitoneal) for 14 days. Two other groups received either 50 or 100 mg/kg of *Ailanthus altissima* after $AlCl_3$ treatment. Behavioral tests including open field test (OFT), elevated plus maze, and passive avoidance tests were performed. Nuclear factor kappa B (NF-κB), glial filamentous acidic protein (GFAP), and amyloid beta were stained using immunohistochemistry (IHC) in the hippocampus.

Results: $AlCl_3$ administration caused significant memory impairment, and treatment with *Ailanthus altissima* extract effectively mitigated these deficits in a dose-dependent manner. The effects were dose-dependent, and the group receiving 100 mg/kg extract presented the most significant results in the conducted behavioral tests. The IHC results for NF-κB showed that treatment with 50 mg/kg of extract significantly reduced NF-κB expression compared with the $AlCl_3$ group (6.64% vs. 13.3%, respectively, $p < 0.05$). A higher dose of 100 mg/kg resulted in an even greater reduction (4.45% vs. 13.3%, $p < 0.01$). Extract treatment reduced GFAP positivity in a dose-dependent manner, with the 50 mg/kg group showing a significant decrease to 11.8% ($p < 0.05$). The 100 mg/kg group demonstrated an even more substantial reduction to 7.7% ($p < 0.01$) compared with the $AlCl_3$ group (18.65%). Amyloid beta positivity was critically reduced in the 100 mg/kg-receiving group.

Conclusions: *Ailanthus altissima* extract shows potential as a neuroprotective extract. It counteracts $AlCl_3$-induced behavioral and neuropathological changes, suggesting promising therapeutic applications.

1 | Introduction

Alzheimer's disease (AD) is a progressive degenerative brain disease linked to amyloid beta (Aβ) plaque formation, neurofibrillary tangles of τ protein, and memory impairment [1, 2]. The underlying mechanisms of this diverse chronic neurodegenerative disease remain incompletely understood.

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Nonetheless, several variables, such as age, gender, heredity, lifestyle, and environmental circumstances, mediate the onset of AD. Several neuropathological features have been identified to be associated with AD, like extraneuronal senile plaques, intraneuronal neurofibrillary tangles (NFTs), cholinergic defects, mitochondrial impairment, calcium homeostasis imbalance, neurovascular damage, and overproduction of inflammatory mediators, which negatively affect the neuronal and glial cell functions [3, 4]. The formation of extracellular plaques containing Aβ is a pathologic feature of AD. It is now evident that Aβ activates microglia causing the release of proinflammatory cytokines which in turn induces the production of amyloid precursor protein (APP). This eventually results in higher production of Aβ [5, 6].

Oxidative stress has recently been drawing attention in AD research and the pathophysiology of other inflammatory diseases [7]. As the brain consumes relatively more energy, it is susceptible to the produced unstable reactive oxygen and nitrogen species. These chemicals interact with neurons (since they are composed of a large entity of polyunsaturated fatty acids) eventually leading to lipid oxidation, the alteration of the redox potential of β-amyloid metal ions, mitochondrial dysfunction, and finally neuron apoptosis [8].

Aluminum possesses neurotoxic properties for which several mechanisms have been proposed including impairing glucose metabolism, free radical damaging via increased lipid peroxidation, protein modification, impairing signal transduction, disturbing the axonal transport, and affecting phosphorylation of neurofilaments [9]. There is increasing evidence linking aluminum to the pathogenesis of AD. In animal models, aluminum contributes to the accumulation of tau protein and Aβ protein and induces neuronal apoptosis in vivo and in vitro, by endoplasmic stress from the unfolded protein response and mitochondrial dysfunction [9, 10]. Aluminum chloride ($AlCl_3$) is widely present in various commercially made items such as toothpaste, vaccines, pharmaceuticals, and bottled drinking water [11–14].

The tree-of-heaven *Ailanthus altissima* (Mill.) Swingle (family Simaroubaceae and genus Ailanthus) is native to China but can be found in many regions worldwide. The dried bark of the plant, widely designated as "Chunpi" in Chinese, has versatile usages in Chinese traditional medicine as a remedy for asthma, epilepsy, bleeding, and ophthalmic disorders [15]. Previous research on the composition of *Ailanthus altissima* found alkaloids, terpenoids, steroids, and flavonoids [16–18]. The presence of these phytochemicals may be linked to the therapeutic properties of *Ailanthus altissima*, especially on those with inflammation at the center of their pathophysiology.

In line with this, the incubation of Canthin-6-one (CO) alkaloids extracted from stem barks of *Ailanthus altissima* with lipopolysaccharide (LPS)-stimulated macrophages showed the following results: (a) inhibited the expression of inducible nitric oxide synthase, (b) suppressed the production of prostaglandin E2 (PGE2) and expression of cyclooxygenase-2, and (c) reduced monocyte chemotactic protein-1 (MCP1) and tumor necrosis factor (TNF) expression [19].

Additionally, the results of Jin et al. showed the exposure of BALB/c mice bone marrow-derived mast cells (mast cells are granulated leukocytes that mainly act as the diver cells of allergic reactions and release proinflammatory mediators once activated) to the extract of *Ailanthus altissima* showed that (a) COX-2-dependent PGD2 generation was inhibited dose-dependently, (b) leukotriene C4 production was inhibited, and (c) the extract inhibited c-kit ligand-dependent mast cells degranulation [20]. Moreover, *Ailanthus altissima* has been traditionally used in Chinese medicine as a remedy for neurological conditions such as epilepsy, anxiety, and memory weakness, indicating its potential for neuroprotection [15]. In addition, experimental studies have shown that chronic administration of its bark extract improves memory performance and enhances brain antioxidant levels in rats [21].

Previous phytochemical and pharmacological studies have identified several bioactive constituents that may underlie the observed therapeutic effects of *Ailanthus altissima* extract, including phenolic compounds and flavonoids [15], canthinone-type alkaloids [16, 19, 22, 23], monoterpenoids [17, 24], and steroidal constituents such as Chouchunsteride A–D [18, 25]. These bioactive compounds, through their antioxidant and anti-inflammatory biofunctions, are likely to underlie the behavioral improvements and histological protection observed in our treated groups, further supporting the neuroprotective potential of *Ailanthus altissima*.

2 | Materials and Methods

2.1 | Materials

Aluminum chloride ($AlCl_3$, $\geq$ 99% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Bark samples of *Ailanthus altissima* were obtained in late 2023 from Kermanshah, Iran, and taxonomically verified by the Herbarium, Faculty of Pharmacy, Kermanshah University of Medical Sciences (confirmed by Dr. Mohammad Bagher Majnooni). A voucher specimen (AA-2023–07) was deposited.

Powdered dried bark (100 g) underwent Soxhlet extraction with 70% ethanol, after which the solvent was evaporated under reduced pressure and the extract dissolved in normal saline.

Previous GC–MS profiling of *Ailanthus altissima* trunk bark identified α-pinene and β-pinene as the predominant volatile components [15, 21]. As phytochemical composition may vary with geographical origin and extraction conditions, these data are provided as reference information.

2.2 | Animals and Experimental Procedures

A total of 32 adult male Wistar rats, weighing 200–230 g, were provided by the Aftab Lorestan Institute (Iran). Animals were kept in a regulated laboratory environment (24 ± 2°C; 60 ± 5% humidity; and 12-h photoperiod) and allowed free access to food and drinking water [26]. All procedures were conducted in accordance with the guidelines of the Iranian National Institute of Health and approved by the Ethics Committee of Kermanshah University of Medical Sciences (IR.KUMS.AEC.1400.010).

Animals were randomly assigned to four groups ($n = 8$).

1. Control (saline, i.p., 14 days);
2. $AlCl_3$-treated (100 mg/kg, i.p., 14 days);

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3. $AlCl_3$ + *A. altissima* extract (50 mg/kg, oral, 14 days following $AlCl_3$);
4. $AlCl_3$ + *A. altissima* extract (100 mg/kg, oral, 14 days following $AlCl_3$).

The doses of *Ailanthus altissima* extract were selected based on previous in vivo studies demonstrating efficacy and safety within this range [26, 27]. This study followed a therapeutic protocol in which extract administration was initiated after aluminum-induced neurotoxicity.

2.3 | Behavioral Assessments

Behavioral tests were performed after treatment using standard protocols described previously, with minor modifications.

2.3.1 | Open Field Test (OFT)

Locomotor activity was assessed using the OFT as described elsewhere [28]. Rats were evaluated on Days 8 and 14. Total crossings, rearing frequency, and grooming behavior were recorded during a 10-min session.

2.3.2 | Elevated Plus Maze

Anxiety-like behavior was evaluated using the elevated plus maze according to established methods. Rats were placed in the open arm, and the latency to enter a closed arm was recorded on Days 8 and 14 [27].

2.3.3 | Passive Avoidance Test

Learning and memory were assessed using the passive avoidance paradigm as previously reported [29–31]. Initial transition latency (ITL) and step-through latency (STL) were recorded during acquisition and retention sessions conducted on Days 7-8 and 13-14, respectively.

2.4 | Histopathological and Immunohistochemical Analysis

Following behavioral testing, animals were euthanized with ketamine (80 mg/kg) and xylazine (10 mg/kg). Hippocampal tissues were isolated, fixed in 4% paraformaldehyde, and processed for hematoxylin and eosin staining [28, 29]. Histological evaluation was performed using a light microscope at 400 × magnification.

Immunohistochemical detection of nuclear factor kappa B (NF-κB), glial filamentous acidic protein (GFAP), and Aβ was conducted using standard affinity-based protocols with minor modifications [30]. Primary antibodies against NF-κB, p65, and GFAP were applied overnight, followed by incubation with appropriate secondary antibodies. Visualization was achieved using DAB, and sections were counterstained with hematoxylin.

Quantitative analysis was performed using ImageJ software. Three sections per animal and three random fields per section were analyzed by an investigator blinded to group allocation.

2.5 | Statistics

Statistical analyses were performed using GraphPad Prism v9.0. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Normality and homogeneity of variance were confirmed prior to analysis. Results are expressed as the mean ± SEM, with statistical significance set at $P < 0.05$.

3 | Results

3.1 | Elevated Plus Maze Test

According to Figure 1(a), the group exposed to $AlCl_3$, compared with the control, exhibited a notable increase in transfer latency, indicative of cognitive impairment (30.5 vs. 13.1 s, respectively, p value < 0.001). The group treated with 50 mg/kg of extract had a notable decrease in transfer latency on Day 8 when compared with the $AlCl_3$ group (17.41 vs. 30.5 s, respectively, $p < 0.01$). The group treated with 100 mg/kg of extract had a notable decrease in transfer latency on Day 8 when compared with the $AlCl_3$ group (11.8 vs. 30.5 s, respectively, $p < 0.001$), suggesting a notable enhancement in cognitive function.

The results on Day 14 also showed a similar trend in which the group exposed to $AlCl_3$, compared with the control, exhibited a notable increase in transfer latency (28.2 vs. 14.7 s, respectively, p value < 0.001). The group treated with 50 mg/kg of extract had a notable decrease in transfer latency on Day 14 when compared with the $AlCl_3$ group (18.4 vs. 28.2 s, respectively, $p < 0.05$). The group treated with 100 mg/kg of extract had a notable decrease in transfer latency on Day 14 when compared with the $AlCl_3$ group (13.4 vs. 28.2 s, respectively, $p < 0.01$).

3.2 | OFT

Figure 1(b) represents the results of the OFT. On Day 8, the $AlCl_3$-treated group showed the least number of observed rearing, grooming, licking, and crossing-line behaviors compared with the control group. In the case of rearing behavior, when compared with the control group, this group had the least mean number of observations (21.25 vs. 10.96, respectively, p value < 0.01). The $AlCl_3$-treated group, which received 50 mg/kg of extract, showed an improvement, but it was not statistically significant when compared with the $AlCl_3$ group (15.96 vs. 10.96, respectively, $p > 0.05$). However, the $AlCl_3$-treated group which received 100 mg/kg of the extract showed a notable improvement (20.57 vs. 10.96, respectively, $p < 0.01$).

A similar trend was seen in the results obtained on Day 14. The mean number of rearing behaviors dropped significantly in the $AlCl_3$ group when compared with the control group (3.42 vs. 12.98, respectively, $p < 0.01$). A 50 mg/kg dose of extract could not reverse the effect (8.20 vs. 3.42, respectively, $p > 0.05$). A 100 mg/kg extract dose could effectively reverse the effect (9.60 vs. 3.42, respectively, $p < 0.05$).

Importantly, no apparent motor impairment or abnormal locomotion was observed during habituation or testing sessions in any of the groups. The comparable general activity between extract-treated and control rats suggests that the differences observed in behavioral outcomes were primarily due to cognitive effects rather than motor dysfunction.

Figure 2(a) provides information regarding line crossing behavior. $AlCl_3$ markedly reduced line-crossing activity compared with the control group on both Day 8 and Day 14. Although both extract-treated groups showed partial improvement, only the

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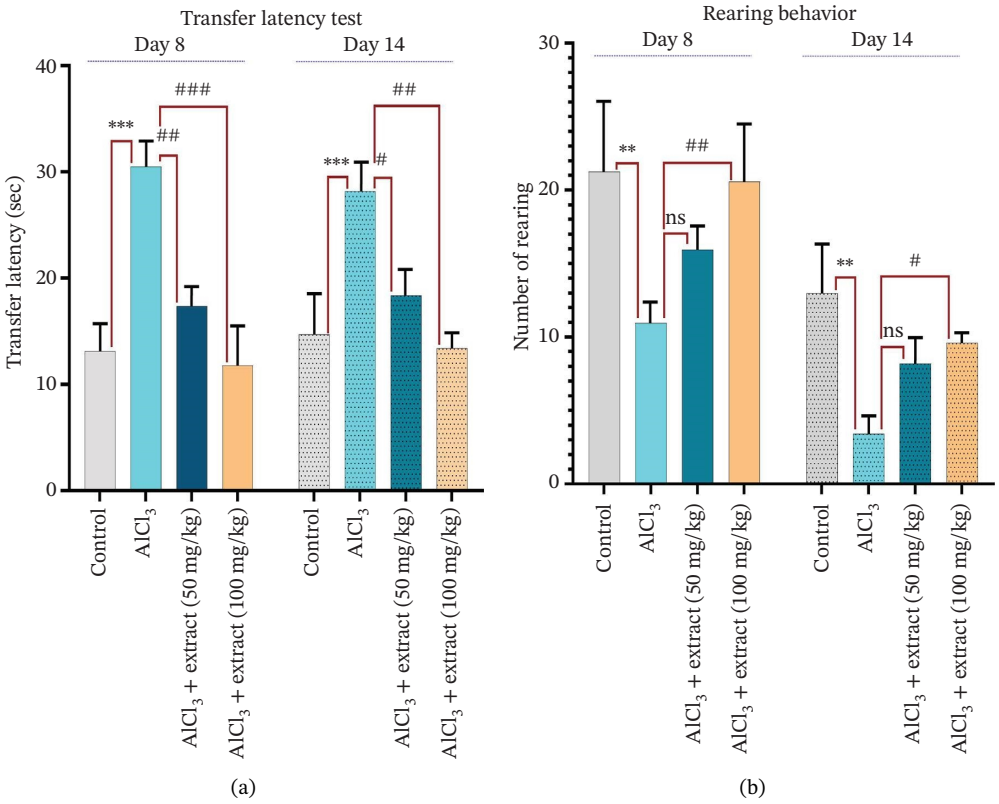


FIGURE 1 | (a) Transfer latency in rats across different treatment groups on Days 8 and 14. The graph illustrates the effects of $AlCl_3$ exposure and subsequent treatment with varying doses of extract on cognitive function in rats, as measured by transfer latency. $AlCl_3$-exposed rats showed significantly increased transfer latency compared with controls on both Day 8 (30.5 vs. 13.1 s, $p < 0.001$) and Day 14 (28.2 vs. 14.7 s, $p < 0.001$), indicating cognitive impairment. Treatment with 50 mg/kg and 100 mg/kg of extract significantly reduced transfer latency in $AlCl_3$-exposed rats on both days, with the higher dose showing more pronounced effects. On Day 8, the 100 mg/kg dose reduced latency to near-control levels (11.8 vs. 30.5 s in the $AlCl_3$-treated group, $p < 0.001$). Similar trends were observed on Day 14, suggesting that the extract may have a protective effect against $AlCl_3$-induced cognitive impairment. Figure 1(b) shows open-field test results. The $AlCl_3$-treated group exhibited significantly reduced behaviors compared with the control. 50 mg/kg extract treatment showed slight improvement, while 100 mg/kg extract significantly reversed $AlCl_3$ effects, particularly in rearing behavior. Similar trends were observed on Day 14, with the $AlCl_3$ group showing markedly decreased rearing. The 100 mg/kg extract dose effectively counteracted $AlCl_3$'s impact, while the 50 mg/kg dose had a limited effect.

100 mg/kg dose produced a significant increase in line-crossing behavior on Day 14.

Figure 2(b) shows grooming behavior, which was significantly decreased in the $AlCl_3$-treated group on both observation days. Treatment with 50 and 100 mg/kg extract significantly improved grooming behavior on Day 8, while on Day 14, only the 100 mg/kg dose produced a significant reversal of $AlCl_3$-induced deficits.

3.3 | Passive Avoidance Test

Figure 3 displays rats' memory and learning results as assessed by the passive avoidance test. As for STL, on the experiment days (Days 8 and 14), the procedure was repeated to assess the rats' cognitive abilities in recalling the previous day's electric shock experience and to determine whether the tendency to enter the dark room had diminished. If the rats remember the experience, they likely show a diminished tendency to enter the dark chamber. According to Figure 3(a), there were no significant changes observed in the ITL test results of the tested groups ($p > 0.05$). Figure 3(b) represents the data obtained from the STL test. In both observation days (8 and 14), $AlCl_3$-treatment reduced STL (117 and 126 s, respectively vs. 300 s, $p < 0.001$). In observations recorded on Day 8, the rat group that received 50 mg/kg of extract had a notably higher STL when compared with $AlCl_3$-treated rats (259 s vs. 117, respectively, $p < 0.001$). The rat group that received 100 mg/kg of the extract also demonstrated a notably higher STL when compared with $AlCl_3$-treated rats (261 s vs. 117, respectively, $p < 0.001$). When the test was performed on Day 14, the group that received 50 mg/kg of extract had a notably higher STL when compared with $AlCl_3$-treated rats (213 s vs. 126, respectively, $p < 0.05$). Treating with 100 mg/kg of the extract showed more profound results and could reverse the effects of $AlCl_3$ more effectively (251 s vs. 126, respectively, $p < 0.01$).

3.4 | Immunohistochemical

3.4.1 | NF-KB

The NF-κB protein family comprises NF-κB1 (p105/p50), NF-κB2 (p100/p52), RelA (p65), RelB, and c-Rel.

Once activated, NF-κB is translocated to the nucleus to promote the transcription of target genes, leading to the activation of oxidative stress and proinflammatory signals like NLRP3 inflammasome, which promotes increased inflammation and

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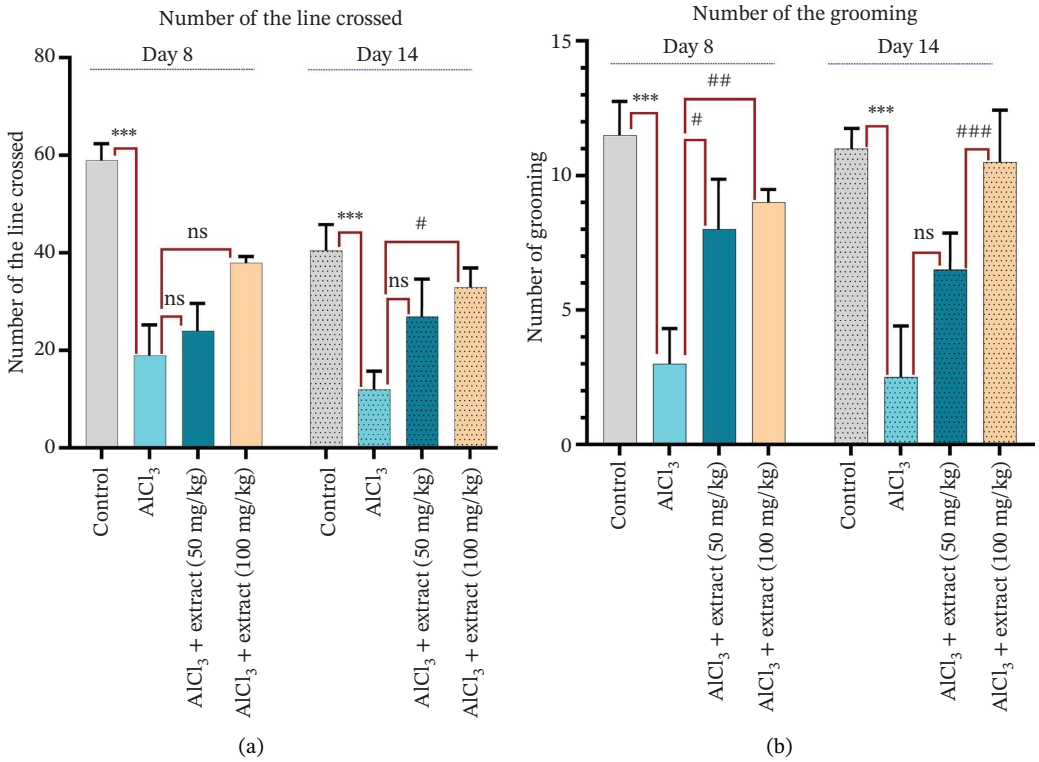


FIGURE 2 | Effects of $AlCl_3$ and extract treatments on rat line crossing and grooming behaviors. (a) Line crossing behavior: $AlCl_3$ significantly reduced line crossings compared with control on both days. Extract treatments showed improvement, with 100 mg/kg dose significant on Day 14. (b) Grooming behavior: $AlCl_3$ decreased grooming compared with control. Both extract doses significantly improved grooming on Day 8, while only 100 mg/kg dose showed significant improvement on Day 14.

damage to neurons [31]. Investigation of NF-κB1 expression in the hippocampal nerve revealed that $AlCl_3$-induced AD increases the NF-κB1 expression as a sign of neuroinflammation when compared with the control (13.3% vs. 3.35%, respectively, $p < 0.01$). As expected, the rat group that received 50 mg/kg of extract showed a significant decrease when compared with the $AlCl_3$ group (6.64% vs. 13.3%, respectively, $p < 0.05$). The findings in 100 mg/kg of extract-receiving rats were much more profound (4.45 vs. 13.3%, respectively, $p < 0.01$) (Figure 4).

3.4.2 | GFAP

Reactive astrocytes are characterized by increased expression of GFAP, vimentin, and nestin [32]. The type III intermediate filament family, which includes vimentin, desmin, GFAP, and peripherin, preserves the structural integrity and architecture of different cell types [33].

GFAP is considered a biomarker of astroglial pathologies. It is the main intermediate protein of the mature astrocyte filaments, which is associated with their differentiation [34, 35].

The results of the specific immunohistochemistry (IHC) staining of GFAP are presented in Figure 5. According to this graph, the bar representing the GFAP in the $AlCl_3$ group towers over the control, indicating a substantial increase in GPAP positivity in this group (18.65% vs. 4.23%, respectively, $p < 0.001$). Interestingly, in both two groups of rats receiving extract, the rate of FPAP positivity was substantially reduced in which the rat group that received 50 mg/kg of extract showed a significant decrease when compared with the $AlCl_3$ group (11.8% vs. 18.65%, respectively, $p < 0.05$). The results of the group receiving 100 mg/kg dose of extract were much more profound (7.7% vs. 18.65%, respectively, $p < 0.01$).

3.4.3 | Aβ

Aβ peptide and hyperphosphorylated tau protein form protein aggregates in the cortex and hippocampus of the brains of individuals with AD. Through the amyloidogenic process, amyloid-β protein precursor (AβPP) is proteolyzed to produce Aβ peptide [36]. Figure 6 provides information regarding the IHC staining of Aβ. It depicts how effective the application of $AlCl_3$ could be in the induction of AD characterizations in the brain. A dramatic surge is evident in the percentage of Aβ positivity in the $AlCl_3$ group when compared with the control group (11.25% vs. 2.1%, respectively, $p < 0.001$). In both extract-receiving rat groups, the percentage of Aβ decreased; however, unlike the 100 mg/kg extract-receiving group, the other group did not show statistical significance when compared with the $AlCl_3$ group (7.1% in the 50 mg/kg-receiving group ($p > 0.05$) and 3.6% in the 100 mg/kg extract-receiving group ($p < 0.01$)).

4 | Discussion

AD is characterized by cognitive decline, memory impairment, and neuroinflammation, with Aβ accumulation and astrocytic activation contributing to disease progression. In this study, behavioral assessments revealed that $AlCl_3$ administration impaired memory and learning in rats, as evidenced by deficits in tasks evaluating cognitive performance. Treatment with

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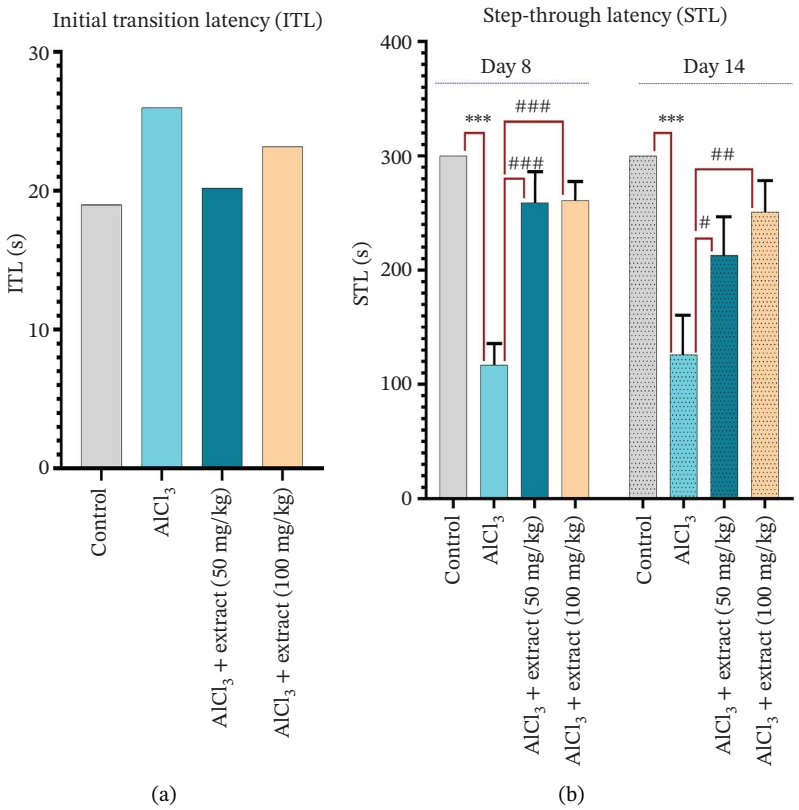


FIGURE 3 | Passive avoidance test in animals exposed to $AlCl_3$ and treated with extract. (a) There were no significant changes in the results of the ITL test observed in the tested groups ($p > 0.05$) and (b) step-through latency (STL) test results. $AlCl_3$ treatment significantly reduced STL on Days 8 and 14 compared with control ($p < 0.001$). Both extract doses (50 and 100 mg/kg) significantly increased STL compared with the $AlCl_3$ group on Day 8 ($p < 0.001$). On Day 14, 50 mg/kg extract showed improvement ($p < 0.05$), while 100 mg/kg extract demonstrated a more effective reversal of $AlCl_3$ effects ($p < 0.01$), indicating dose-dependent memory enhancement.

Ailanthus altissima extract significantly improved these behavioral outcomes in a dose-dependent manner, suggesting that the extract can mitigate $AlCl_3$-induced cognitive deficits. These findings are consistent with prior studies demonstrating the extract's anxiolytic and memory-enhancing properties in rodent models [27], likely mediated by its antioxidant and anti-inflammatory activities that protect neuronal circuits and synaptic function.

At the molecular level, $AlCl_3$-induced neurotoxicity was associated with marked activation of NF-κB in the hippocampus, reflecting an inflammatory response. Elevated GFAP expression further indicated astrocytic activation, while increased Aβ deposition confirmed ongoing neurodegenerative processes. Treatment with *Ailanthus altissima* extract attenuated NF-κB activation, reduced GFAP expression, and decreased Aβ accumulation, highlighting its multifaceted neuroprotective effects. The modulation of these markers suggests that the extract may simultaneously suppress proinflammatory signaling, reduce astrocytosis, and promote mechanisms involved in amyloid clearance, which together contribute to improved cognitive function.

The observed neuroprotective effects are likely attributable to the diverse phytochemical constituents of Ailanthus altissima, including alkaloids, monoterpenoids, and steroidal compounds. Previous studies have shown that these compounds possess antioxidant properties, inhibit acetylcholinesterase and butyrylcholinesterase, and modulate inflammatory pathways [37–40]. It is plausible that the synergistic action of these bioactive compounds underlies the behavioral improvements and histological protection observed in our study.

Despite these promising findings, several limitations should be considered. Serum levels of proinflammatory cytokines (e.g., TNF-α and IL-6) were not measured, which could have provided further mechanistic insight into NF-κB modulation [41]. The extract was tested as a whole, without isolation or chemical standardization of active components via LC–MS or HPLC. Furthermore, the study lacked a standard treatment group (e.g., donepezil) and an extract-only group, which limits the ability to benchmark the extract's efficacy against established therapies. Nevertheless, the extraction method followed a validated hydromethanolic protocol previously applied in comparable AD models [42], ensuring consistency and reliability of the extract's bioactivity.

Future studies should focus on isolating and characterizing the active compounds responsible for the neuroprotective effects, evaluating serum biomarkers of inflammation, and including standard treatment and extract-only groups. Additionally, measuring early astrocytic markers such as GFAP and other emerging biomarkers like YKL-40 (CHI3L1) could provide deeper insights into the mechanisms of neuroinflammation and astrocytosis in AD [43, 44]. Collectively,

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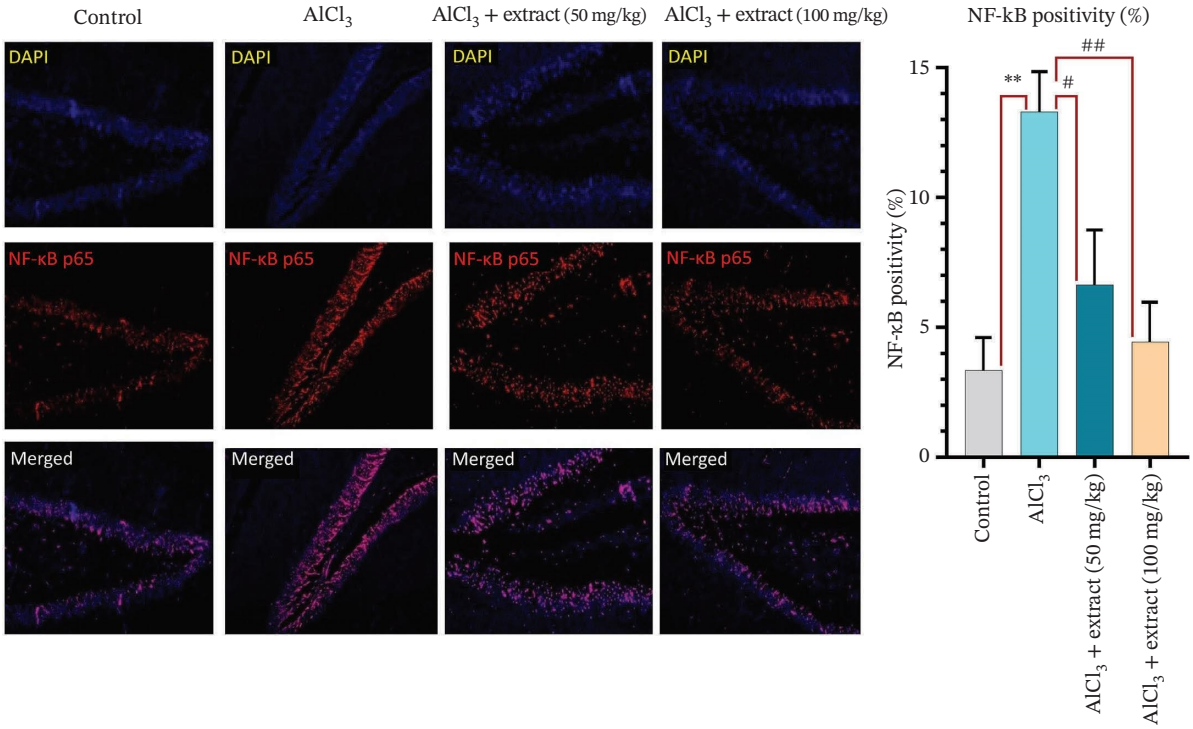


FIGURE 4 | The effect of $AlCl_3$-mediated AD on the expression of NF-κB. Immunohistochemical staining of NF-κB in the hippocampus. $AlCl_3$-induced AD significantly increased NF-κB1 expression compared with the control group (13.3% vs. 3.35%, $p < 0.01$). Treatment with 50 mg/kg of extract significantly reduced NF-κB expression compared with the AD group (6.64% vs. 13.3%, $p < 0.05$). A higher dose of 100 mg/kg extract showed an even more pronounced reduction in NF-κB1 expression (4.45% vs. 13.3%, $p < 0.01$).

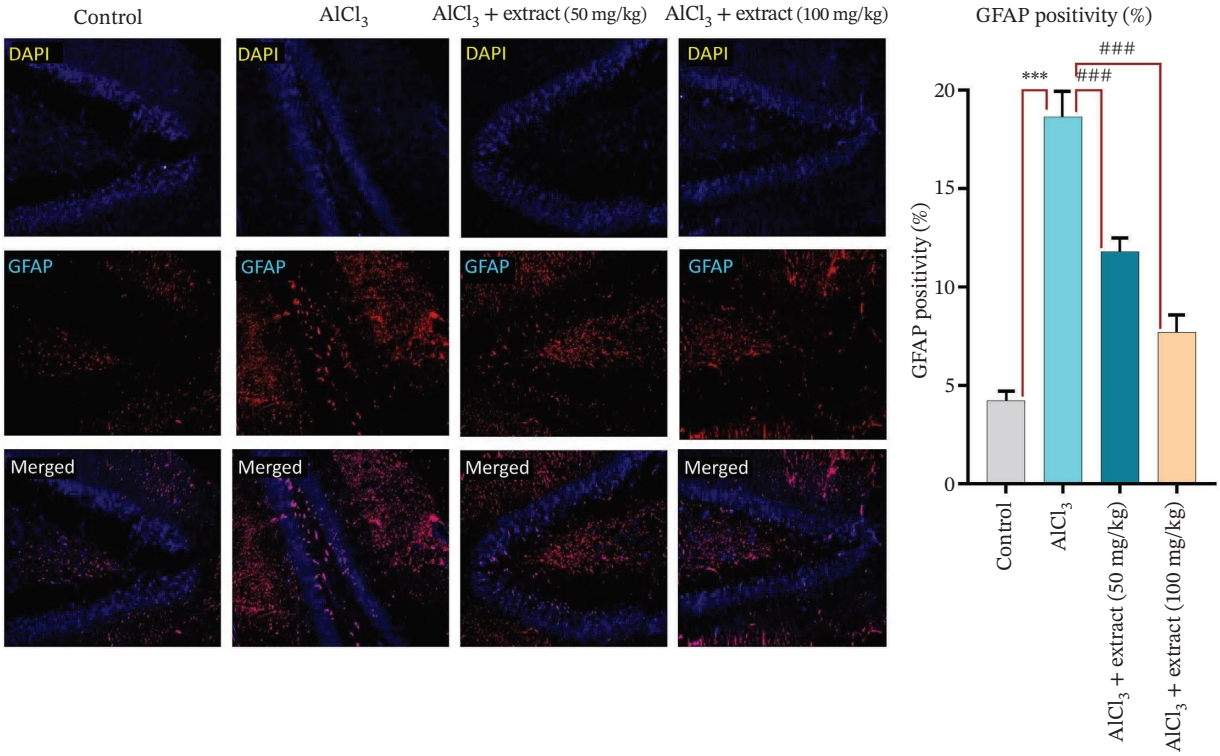


FIGURE 5 | GFAP immunohistochemistry staining in the hippocampus of rats. The $AlCl_3$ group shows a significant increase in GFAP positivity compared with the control (18.65% vs. 4.23%, $p < 0.001$). Extract-treated groups demonstrate reduced GFAP positivity, with the 50 mg/kg group showing a significant decrease (11.8%, $p < 0.05$) and the 100 mg/kg group exhibiting an even more profound reduction (7.7%, $p < 0.01$) compared with the $AlCl_3$ group.

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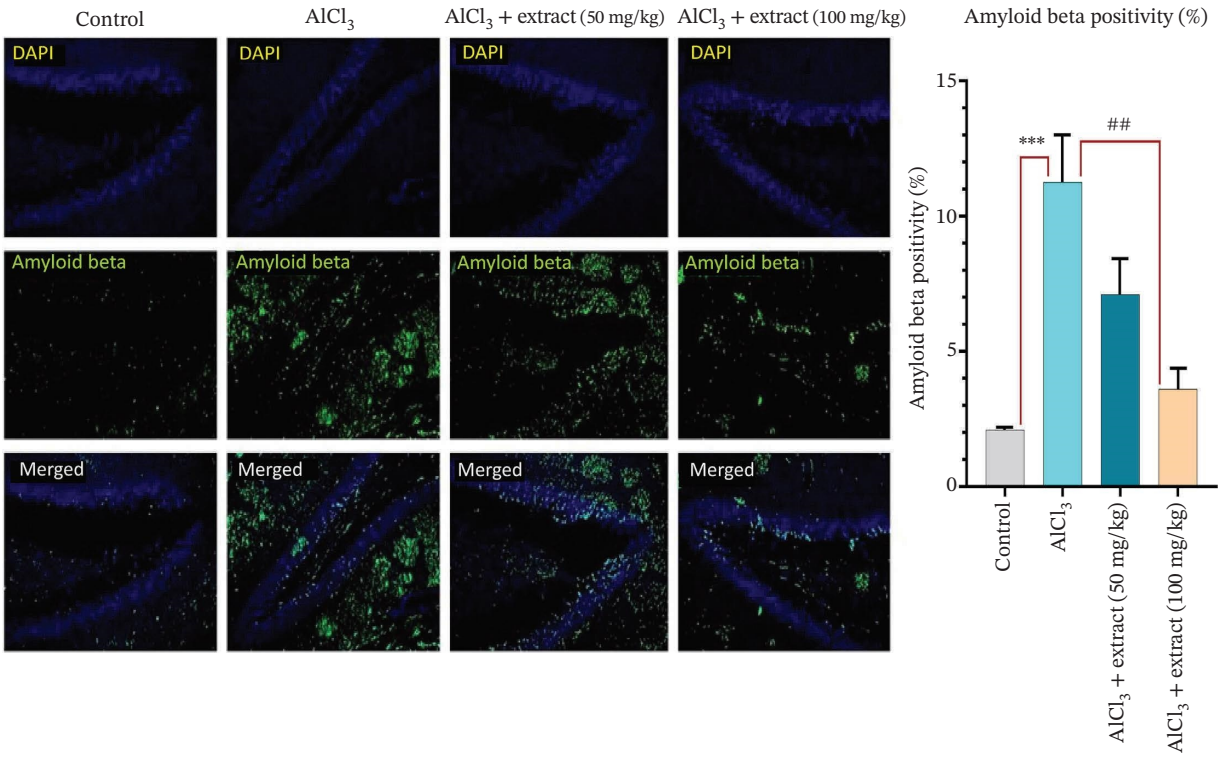


FIGURE 6 | Immunohistochemistry staining of amyloid beta. The $AlCl_3$ group shows significantly increased amyloid beta positivity compared with the control (11.25% vs. 2.1%, $p < 0.001$), demonstrating the effectiveness of $AlCl_3$ in inducing AD characteristics. Extract-treated groups show decreased amyloid beta levels, with the 100 mg/kg dose group exhibiting a statistically significant reduction (3.6%, $p < 0.01$) compared with the $AlCl_3$ group, while the 50 mg/kg dose group's decrease (7.1%, $p > 0.05$) was not statistically significant.

our findings suggest that *Ailanthus altissima* extract exhibits promising dose-dependent neuroprotective effects by modulating behavioral, histological, and molecular parameters in an experimental AD model.

5 | Conclusion

$AlCl_3$ could effectively develop AD-like cognitive and memory impairments and neurobiological impacts on the hippocampus including the increase of NF-κB, GFAP, and Aβ in our rat model, which could be reversed by taking the *Ailanthus altissima* extract. Both 50 and 100 mg/kg dose groups exhibited a statistically significant reduction. The observed reversing effects followed a dose-dependent pattern, showing the 100 mg/kg dose could be more effective in mitigating $AlCl_3$ effects. The outcomes of this study suggest that prolonged pretreatment with *Ailanthus altissima* extract may alleviate the effects of drugs, agents, and chemicals on the nervous system, and *Ailanthus altissima* extract could be used as a promising neuroprotective herbal product.

Nomenclature

AD	Alzheimer's disease
$AlCl_3$	Aluminum chloride
APP	Amyloid precursor protein
DAB	3,3′-diaminobenzidine
EDTA	Ethylenediaminetetraacetic acid
GFAP	Glial filamentous acidic protein
H&E	Hematoxylin and eosin
MCP1	Monocyte chemotactic protein-1
NF-κB	Nuclear factor kappa B
NHS	Normal horse serum
NFTs	Neurofibrillary tangles
OFT	Open field test
PBS	Phosphate-buffered saline
PGE2	Prostaglandin E2
TNF	Tumor necrosis factor

Author Contributions

M.M.G. performed lab work, was involved in project administration, and prepared the primary draft. D.E.A.K. performed lab work, revised the manuscript, and generated graphs. R.A., S.Z.H., and S.D.A. performed lab work. S.S. performed lab work and contributed to project administration. A.A. was involved in design and methodology of the study. M.H.F. contributed to conceptualization and supervision and provided input.

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Use of AI. No artificial intelligence was employed at any stage of the research, manuscript production, or editing process.

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Ethics Statement

This study was approved by the Ethics Committee of Kermanshah University of Medical Sciences (IR.KUMS.AEC.1400.010).

Consent

No informed consent was required to prepare the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. A. R. Monteiro, D. J. Barbosa, F. Remião, and R. Silva, "Alzheimer's Disease: Insights and New Prospects in Disease Pathophysiology, Biomarkers and Disease-Modifying Drugs," *Biochemical Pharmacology* 211 (2023): 115522, https://doi.org/10.1016/j.bcp.2023.115522.

2. M. Kashif, P. Sivaprakasam, P. Vijendra, M. Waseem, and A. K. Pandurangan, "A Recent Update on Pathophysiology and Therapeutic Interventions of Alzheimer's disease," *Current Pharmaceutical Design* 29, no. 43 (2023): 3428–3441, https://doi.org/10.2174/0113816128264355231121064704.

3. Y. Shen, G. Zhang, C. Wei, et al., "Potential Role and Therapeutic Implications of Glutathione Peroxidase 4 in the Treatment of Alzheimer's Disease," *Neural Regeneration Research* 20, no. 3 (2025): 613–631, https://doi.org/10.4103/nrr.nrr-d-23-01343.

4. N. Gamez and R. Morales, "Role of Peripheral Amyloid-β Aggregates in Alzheimer's Disease: Mechanistic, Diagnostic, and Therapeutic Implications," *Neural Regeneration Research* 20, no. 4 (2025): 1087–1089, https://doi.org/10.4103/nrr.nrr-d-24-00066.

5. I. Blasko, R. Veerhuis, M. Stampfer-Kountchev, M. Saurwein-Teissl, P. Eikelenboom, and B. Grubeck-Loebenstein, "Costimulatory Effects of Interferon-Gamma and Interleukin-1 Beta or Tumor Necrosis Factor Alpha on the Synthesis of Abeta1-40 and Abeta1-42 by Human Astrocytes," *Neurobiology of Disease* 7, no. 6 (2000): 682–689, https://doi.org/10.1006/nbdi.2000.0321.

6. T. Ozben and S. Ozben, "Neuro-Inflammation and Anti-inflammatory Treatment Options for Alzheimer's disease," *Clinical Biochemistry* 72 (2019): 87–89, https://doi.org/10.1016/j.clinbiochem.2019.04.001.

7. M. M. Gravandi, Z. Pourmanouchehri, L. Behbood, et al., "Rutin-Loaded Chitosan Nanoparticles Alleviated Freund's Adjuvant Induced Rheumatoid Arthritis via Modulating Oxidative Stress and Inflammatory Parameters in Wistar Rats," *Naunyn-Schmiedeberg's Archives of Pharmacology* 397, no. 7 (2024): 4771–4790, https://doi.org/10.1007/s00210-023-02902-x.

8. B. Twarowski and M. Herbet, "Inflammatory Processes in Alzheimer's Disease-Pathomechanism, Diagnosis and Treatment: A Review," *International Journal of Molecular Sciences* 24, no. 7 (2023): 6518, https://doi.org/10.3390/ijms24076518.

9. R. Kandimalla, J. Vallamkondu, E. B. Corgiat, and K. D. Gill, "Understanding Aspects of Aluminum Exposure in Alzheimer's disease Development," *Brain Pathology* 26, no. 2 (2016): 139–154, https://doi.org/10.1111/bpa.12333.

10. A. Iranpanah, S. Fakhri, G. Bahrami, et al., "Protective Effect of a Hydromethanolic Extract from Fraxinus excelsior L. Bark Against a Rat Model of Aluminum Chloride-Induced Alzheimer's Disease: Relevance to Its Anti-Inflammatory and Antioxidant Effects," *Journal of Ethnopharmacology* 323 (2024): 117708, https://doi.org/10.1016/j.jep.2024.117708.

11. S. Majid Hosseini, E. Ghandali, H. Reza Moghimi, et al., "A Comparative Evaluation of Aluminum Chloride Hexahydrate Gel Iontophoresis Versus Tap Water Iontophoresis in People With Primary Palmar Hyperhidrosis: A Randomised Clinical Trial," *Indian Journal of Dermatology Venereology and Leprology* 90, no. 1 (2023): 52–58, https://doi.org/10.25259/ijdvl_975_2022.

12. Y. Irnidayanti, D. G. Maharani, M. H. Rizky, M. I. Noer, and V. Rizkawati, "Resveratrol-Tempeh Reduce Micronucleus Frequencies Bone Marrow Cells and Stimulate Osteocyte Proliferation in Aluminum Chloride-Induced Mice," *Brazilian Journal of Biology* 82 (2023): e266690, https://doi.org/10.1590/1519-6984.266690.

13. M. Bruze, E. Netterlid, and I. Siemund, "Aluminum-Allergen of the Year 2022," *Dermatitis* 33, no. 1 (2022): 10–15, https://doi.org/10.1097/der.0000000000000836.

14. E. Kocatürk, "How Infection and Vaccination Are Linked to Acute and Chronic Urticaria: A Special Focus on COVID–19," *Viruses* 15, no. 7 (2023).

15. X. Li, Y. Li, S. Ma, et al., "Traditional Uses, Phytochemistry, and Pharmacology of Ailanthus altissima (Mill.) Swingle Bark: A Comprehensive Review," *Journal of Ethnopharmacology* 275 (2021): 114121, https://doi.org/10.1016/j.jep.2021.114121.

16. Q. Shao, T. Y. Li, W. Quan, et al., "The Alkaloids With Neuroprotective Effect From the Root Bark of *Ailanthus altissima*," *Natural Product Research* 37, no. 22 (2023): 3751–3757, https://doi.org/10.1080/14786419.2022.2149518.

17. Q. Song, Z. K. Duan, Y. N. Tan, et al., "Isolation of Four New Monoterpenes From *Ailanthus altissima* (Mill.) Swingle and Their Enzyme Inhibitory Effects," *Fitoterapia* 176 (2024): 105984, https://doi.org/10.1016/j.fitote.2024.105984.

18. Z. H. Gao, Z. K. Duan, Z. T. Ma, et al., "Chouchunsteride A-D, Four New Steroids From the Leaves of *Ailanthus altissima* (Mill.) Swingle," *Steroids* 188 (2022): 109117, https://doi.org/10.1016/j.steroids.2022.109117.

19. S. K. Cho, M. Jeong, D. Jang, and J. H. Choi, "Anti-Inflammatory Effects of Canthin–6–One AlkaloidsFrom *Ailanthus altissima*," *Planta Medica* 84, no. 8 (2018): 527–535, https://doi.org/10.1055/s-0043-123349.

20. M. H. Jin, J. Yook, E. Lee, et al., "Anti-Inflammatory Activity of *Ailanthus altissima* in Ovalbumin-Induced Lung Inflammation," *Biological and Pharmaceutical Bulletin* 29, no. 5 (2006): 884–888, https://doi.org/10.1248/bpb.29.884.

21. H. M. A. Rahman, S. Javaid, W. Ashraf, et al., "Effects of Long-Term *Ailanthus altissima* Extract Supplementation on Fear, Cognition and Brain Antioxidant Levels," *Saudi Pharmaceutical Journal* 31, no. 2 (2023): 191–206, https://doi.org/10.1016/j.jsps.2022.12.003.

22. H.M. Kim, et al., "A New Canthinone-Type Alkaloid Isolated From Ailanthus altissima Swingle," *Molecules* 21, no. 5 (2016).

23. Q. Shao, T.-Y. Li, W. Quan, et al., "The Alkaloids With Neuroprotective Effect From the Root Bark of Ailanthus altissima," *Natural Product Research* 37, no. 22 (2023): 3751–3757.

24. Q. Song, Z.-K. Duan, T.-N. Tan, et al., "Isolation of Four New Monoterpenes From Ailanthus altissima (mill.) Swingle and their Enzyme Inhibitory Effects," *Fitoterapia* 176 (2024): 105984.

25. Z.-H. Gao, Z.-K. Duan, Z.-T. Ma, et al., "Chouchunsteride A–D, Four New Steroids From the Leaves of Ailanthus altissima (Mill.) Swingle," *Steroids* 188 (2022): 109117.

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26. M. Mani and D. Rathore, "Pharmacological Evaluation of Anti-Fertility Effect of Stem Bark of *Ailanthus altissima* in Wistar Albino Rats," *Research Journal of Pharmacy and Technology* 9, no. 5 (2016): 497–500, https://doi.org/10.5958/0974-360x.2016.00092.5.

27. R. Frussa-Filho, J. R. Otoboni, F. T. Uema, and L. C. Sá-Rocha, "Evaluation of Memory and Anxiety in Rats Observed in the Elevated Plus-Maze: Effects of Age and Isolation," *Brazilian Journal of Medical and Biological Research* 24, no. 7 (1991): 725–728.

28. D. Wellington, I. Mikaelian, and L. Singer, "Comparison of Ketamine-Xylazine and Ketamine-Dexmedetomidine Anesthesia and Intraperitoneal Tolerance in Rats," *Journal of the American Association for Laboratory Animal Science* 52, no. 4 (2013): 481–487.

29. L. F. Van Pelt, "Ketamine and Xylazine for Surgical Anesthesia in Rats," *Journal of the American Veterinary Medical Association* 171, no. 9 (1977): 842–844, https://doi.org/10.2460/javma.1977.171.09.842.

30. J. Li, P. Y. Wen, W. W. Li, and J. Zhou, "Upregulation Effects of Tanshinone IIA on the Expressions of NeuN, Nissl Body, and IκB and Downregulation Effects on the Expressions of GFAP and NF-κB in the Brain Tissues of Rat Models of Alzheimer's disease," *NeuroReport* 26, no. 13 (2015): 758–766, https://doi.org/10.1097/wnr.0000000000000419.

31. D. Yang, J. Su, Y. Chen, and G. Chen, "The NF-κB Pathway: Key Players in Neurocognitive Functions and Related Disorders," *European Journal of Pharmacology* 984 (2024): 177038, https://doi.org/10.1016/j.ejphar.2024.177038.

32. N. A. Nor Nazli, S. Muthuraju, F. Ahmad, et al., "Characterisation of Primary Human Hippocampal Astrocyte Cell Culture Following Exposure to Hypoxia," *Malaysian Journal of Medical Sciences* 30, no. 1 (2023): 92–106, https://doi.org/10.21315/mjms2023.30.1.8.

33. B. Hwang and H. Ise, "Multimeric Conformation of Type III Intermediate Filaments but Not the Filamentous Conformation Exhibits High Affinity to Lipid Bilayers," *Genes to Cells* 25, no. 6 (2020): 413–426, https://doi.org/10.1111/gtc.12768.

34. N. V. Lyubimova, Y. S. Timofeiev, and N. E. Sushlinskii, "The Glio-Fibrillar Acid Protein in Blood Serum of Patients With Brain Tumors and Neurological Diseases of Non-Tumorous Etiology," *Klinicheskaia Laboratornaia Diagnostika* 61, no. 8 (2016): 466–469.

35. N. V. Lyubimova, Y. S. Timofeev, A. A. Mitrofanov, A. K. Bekyashev, Z. A. Goncharova, and N. E. Kushlinskii, "Glial Fibrillary Acidic Protein in the Diagnosis and Prognosis of Malignant Glial Tumors," *Bulletin of Experimental Biology and Medicine* 168, no. 4 (2020): 503–506, https://doi.org/10.1007/s10517-020-04741-9.

36. A. K. Torres, C. Jara, H. S. Park-Kang, et al., "Synaptic Mitochondria: An Early Target of Amyloid-β and Tau in Alzheimer's Disease," *Journal of Alzheimer's Disease* 84, no. 4 (2021): 1391–1414, https://doi.org/10.3233/jad-215139.

37. T. J. Huat, J. Camats-Perna, E. A. Newcombe, et al., "The Impact of Astrocytic NF-κB on Healthy and Alzheimer's Disease Brains," *Scientific Reports* 14, no. 1 (2024): 14305.

38. S. H. Alawdi, E. S. El-Denshary, M. M. Safar, et al., "Neuroprotective Effect of Nanodiamond in Alzheimer's Disease Rat Model: A Pivotal Role for Modulating NF-κB and STAT3 Signaling," *Molecular Neurobiology* 54, no. 3 (2017): 1906–1918.

39. A. Iranpanah, S. Fakhri, G. Bahrami, et al., "Protective Effect of a Hydromethanolic Extract From *Fraxinus excelsior* L. Bark Against a Rat Model of Aluminum Chloride-Induced Alzheimer's Disease: Relevance to its Anti-Inflammatory and Antioxidant Effects," *Journal of Ethnopharmacology* 323 (2024): 117708.

40. J.B. Pereira, S. Janelidze, R. Smith, et al., "Plasma GFAP is an Early Marker of Amyloid-β but not Tau Pathology in Alzheimer's Disease," *Brain* 144, no. 11 (2021): 3505–3516.

41. D. E. Komi, T. Kazemi, and A. P. Bussink, "New Insights Into the Relationship Between Chitinase-3-Like-1 and Asthma," *Current Allergy and Asthma Reports* 16, no. 8 (2016): 57.

42. D. Komi, "Investigation of Chitinase3like-1 (Chiti3L1) Gene Polymorphism (rs4950928) With Susceptibility to Allergic Asthma in Iranian Northwestern Azeri Population" (2020).

43. F. Baldacci, S. Lista, E. Cavedo, U. Bonuccelli, and H. Hampel, "Diagnostic Function of the Neuroinflammatory Biomarker YKL-40 in Alzheimer's Disease and Other Neurodegenerative Diseases," *Expert Review of Proteomics* 14, no. 4 (2017): 285–299.

44. L. Wang, T. Gao, T. Cai, et al., "Cerebrospinal Fluid Levels of YKL-40 in Prodromal Alzheimer's Disease," *Neuroscience Letters* 715 (2020): 134658.

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