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Original Research Article

Neuroprotective Effects of Santonin on Aluminum Chloride-induced Oxidative Stress Mediated Neuroinflammation in Rat Model of Alzheimer's Disease

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Abstract

Background: Alzheimer's disease (AD) is one of the neurodegenerative diseases that is marked by deterioration in cognitive functioning and behavioral capacities in a progressive manner. It is the most prevalent cause of dementia in the aging population and imposes a high social and economic cost on the global population.
Objectives: In this study, we have examined Santonin, which can prevent the brain damage caused by aluminum chloride ($AlCl_3$) in rats, a typical method used to simulate the effects of AD in the laboratory.
Materials and Methods: We administered $AlCl_3$ (100 mg/kg) to the rats to induce changes in them that resembled Alzheimer's and tested their behavior using the open field and elevated plus maze tests. Then, we analyzed their brains in terms of the presence of inflammation, oxidative stress, enzyme activities, and tissue damage.
Results: The rats receiving $AlCl_3$ had reduced motility and an increase in acetylcholinesterase, malondialdehyde, and inflammatory cytokines, including cyclooxygenase-2, tumor necrosis factor-alpha, and interleukin-6. Their antioxidant defense mechanisms, glutathione, superoxide dismutase, and catalase, also decreased. However, these effects were changed positively when we treated rats with Santonin. Their movement was better, and they responded quicker, and their brains became less inflamed and less oxidatively stressed. Santonin reduced levels of harmful proteins and enzymes and restored antioxidant activity in a dose-dependent manner.
Conclusion: Santonin helps reverse the brain changes and behavior problems caused by $AlCl_3$ in rats. It looks promising as a neuroprotective agent for AD.

Keywords

Aluminum chloride, Santonin, Alzheimer's disease, neuroinflammation

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Introduction

Dementia is a huge health problem around the world. By 2030, people expect around 78 million will be living with it. Alzheimer's disease (AD) is easily the most common type follows the same trend, and honestly, it is only becoming more of a problem (Alzheimer's Association Report, 2025; Zhang et al., 2024). AD is a growing global public health challenge, primarily driven by aging populations. In 2025, an estimated 7.2 million Americans aged 65 or older are living with Alzheimer's dementia. This represents about 11% of the US population in that age group. Globally, the burden of Alzheimer's and other dementias has been rising steadily: recent trend analysis shows a sharp increase in both incidence and prevalence over the past decades, projected to continue in the coming years. Frontiers+1 according to the OECD Health at a Glance 2025 report, across 19-member countries, 61 people per 1,000 aged 65 and over were estimated to be living with dementia (which includes Alzheimer's) in 2023—a figure expected to climb further.

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AD is a chronic and irreversible neurodegenerative condition that affects large portions of cerebral cortex and hippocampus. AD is connected with a collection of persistent forms of amyloidβ (Aβ) in plaques in extracellular spaces and blood vessel walls, and the microtubule protein tau in neurofibrillary tangles in neurons (Masters et al., 2015). Several risk factors, like as ageing, genetics, head trauma, vascular disease, infections, and environmental causes (like heavy metals and pollutants), are associated with AD, a complicated disorder (Breijyeh & Karaman, 2020). The individual's baseline risk is presumably established by inherited nuclear and mitochondrially-encoded genes; the pathogenic process of AD most likely starts 20–30 years before the first clinical indications appear. The initial susceptibility is probably altered by environmental variables such as metabolic syndrome, insulin resistance, dyslipidemia, inflammation, hypertension, and midlife obesity (Zvěřová, 2019). The neurodegeneration that is one of the primary causes is mediated by the neurotoxic oligomer Aβ peptide, which is the disease's neuropathological diagnostic criteria, and τ protein. Nevertheless, oxidative stress—a process that refers to disproportion favoring oxidants over antioxidants—is the primary cause and aggravating factor of these disorders (Huang et al., 2016).

The pathophysiology of AD, a debilitating condition of the elderly, is significantly influenced by oxidative stress. Most of the components of neurons (proteins, lipids, and nucleic acids) can be oxidized in AD owing to mitochondrial failure, augmented metal levels, Aβ peptides and inflammation. The brain is highly susceptible to oxidative stress than other organs. By encouraging Aβ deposition, tau hyperphosphorylation, and the eventual loss of synapses and neurons, oxidative stress pays to the development of AD. Given the association among oxidative stress and AD, antioxidants could potentially be useful in treating the condition as oxidative stress plays a key role in the development of AD (Chen & Zhong, 2014). When redox-sensitive signaling pathways are activated by high reactive oxygen species (ROS) levels in microglia and astrocytes, proinflammatory cytokines like interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α) are released. This sequence of events intensifies neuronal degeneration by generating a self-reinforcing loop in which oxidative stress and neuroinflammation reinforce each other (Tahir et al., 2024).

Heavy metals are closely linked to neurological diseases like AD, according to several studies. Because it heavily influences the many metabolic cascades in the nervous system, aluminum (Al) constitutes one of the primary heavy metals implicated in the development and initiation of neurodegenerative conditions (Chauhdary et al., 2019). Uptake of some metal toxicants, like Al, which were introduced into the body by drinking water contaminants, food pollutants, occupational exposure, and meals cooked in Al cookware, was directly associated to the commencement of AD. Al could ultimately accumulate in the brain and alter the blood–brain barrier (BBB). As a result, Al brain poisoning is known as a major risk of neurological diseases. Al may also impair the antioxidant mechanisms, alter brain chemistry and lead to injury to brain DNA (Chen et al., 2021). Therefore, it is proposed that the broadly adapted animal model that closely resembles human AD is $AlCl_3$-triggered AD in animals (Garcia et al., 2010).

Pharmaceutical treatments for AD are available; however, they only offer limited symptomatic relief and frequently do not stop or halt the illness's development. Some substances may potentially have unfavorable side effects and exacerbate cognitive impairment (Yiannopoulou & Papageorgiou, 2013). In addition to reducing secondary pathologies and preventing the initiation of the disease by seizing the primary targets, antioxidant treatment is one of the hopeful therapeutic methods for AD. It also slows or delays the progression of the disease, results in the halt or even restoration of neuronal injury after the start of the disease and subsequently avoids the onset of AD (Mathiyazahan et al., 2015). Santonin is a lactone chemical that is isolated from the flower buds of plants in the Compositae family, such as the crown daisy chrysanthemum or *Artemisia cina* Berg. Santonin has been used as a deworming medication for a very long period (Yang et al., 2018). Previous studies reveal that Santonin have anti-inflammatory, anti-pyretic, antioxidative, and analgesic effects (Al-Harbi et al., 1994; Rehman et al., 2025).

This study aimed to assess Santonin potential to guard against oxidative stress and inflammatory indicators driven on by the injection of $AlCl_3$. Rats were evaluated for lipid peroxidation, proinflammatory cytokines, antioxidant defense system activity, and behavioral state to correlate the biochemical and functional alterations in the brain and explore the potential mechanism of action. Additionally, histopathological analyses were included.

Materials and Methods

Materials

We purchased aluminum chloride ($AlCl_3$) and Santonin from Sigma-Aldrich. Prior to usage, each medication solution was made from scratch. Analytical grade reagents were all employed in this investigation.

Experimental Design

A total of 28 adult male Wistar rats (8–10 weeks old; 180–200 g) were obtained from the institutional animal facility. Upon arrival, the animals were housed in groups of two to four per polycarbonate cage, with free access to standard laboratory rat chow and pure drinking water. The standard diet (e.g., Purina Lab Diet 5001, USA or specify your facility's brand) consisted of approximately 21% crude protein, 5% fat, 4% fiber, 1.0% calcium, 0.8% phosphorus, and essential vitamins and minerals. Rats were maintained under controlled environmental conditions at 23°C ± 2°C, relative humidity of

50%–60%, and a 12-h light/dark cycle. We adhered to the Guidelines to the Use of Experimental Animals, and it was all approved by the Institutional Animal Ethics Committee. After 1 week of acclimatization, we randomly divided the rats into four groups of seven rats in each:

- Group I (Control): They got a standard diet and distilled water for 4 weeks.
- Group II ($AlCl_3$): These rats got daily intraperitoneal injections of $AlCl_3$ (100 mg/kg in 0.5 mL) for 4 weeks.
- Group III (Sant + $AlCl_3$): These received Santonin (20 mg/kg, by mouth) once a day, an hour before the $AlCl_3$ injection, and we kept this up for 4 weeks.
- Group IV (High-dose Sant + $AlCl_3$): These rats got a higher Santonin dose (40 mg/kg, by mouth) once a day for 4 weeks, then got the $AlCl_3$ injection each day.

All the rats were starved overnight (10–12 h) before being put under anesthesia and having their blood drawn; however, they were allowed to drink water. This maintained their metabolism in a steady state (Hamdan et al., 2022).

The dose of the $AlCl_3$ (100 mg/kg) is a typical dose that they always use since it consistently induces an oxidative and systemic toxicity in the rats. In the case of Santonin, previous research demonstrated that 20 and 40 mg/kg are safe and in fact, they prevent chemical damage. The low dosage (20 mg/kg) is the minimum effective dosage, and 40 mg/kg allows us to know whether there are greater levels of protection when higher dosage is used. These amounts gave us clear biological effects without causing extra suffering or death.

Brain Tissue Preparation

The animals were sedated with diethyl ether after their exploring phase. The separated brains of every experimental animal were put on an ice plate, sectioned, and filled with an iced isotonic saline solution. To remove the hippocampus, the brains were also separated into the left and right hemispheres using blade. The remaining brains' weighted hippocampi were homogenized using a Teflon-glass homogenizer in 0.1 mmol/L of iced saline (pH 7.4), while four randomly selected hippocampal tissues were prepared for histological and ultrastructural confirmation. For further biochemical studies, the homogenates (10%, w/v) were kept at −80°C after a 15-min centrifugation at 10,000 rpm (Hamdan et al., 2022).

Elevated Plus Maze Test

The elevated plus maze (EPM) test is utilized to evaluate test animals' spatial learning, memory, and anxiety levels (Pellow et al., 1985). The EPM equipment is made up of two open arms that are 50 cm by 10 cm and a plus-shaped maze. It is crossed by two identically sized closed walls that are 40 cm high. A 10 cm × 10 cm central square connected the open arms, giving the apparatus a plus form. The complete maze was kept in the room and raised 50 cm above the floor. After an inquiry, acquirement memory was assessed in Wistar rats. Rats were found on their own at the open arm in front of the surrounding arm. As a result, during the following 5 min, the rats' movements from open arm to closed one in maze were recorded, along with the number of times they returned to their home cages. When every paw was in an arm, the arm's entrance was considered complete. Following the augmented plus maze, it is said that rats with high anxiety would spend less time in the labyrinth's uncovered arms, whereas animals with lower levels of anxiety will freely explore the open arms (Pentkowski et al., 2021).

Open Field Test

Rats were in a brightly lit box that was noticeably larger than their home confinement as part of an open field test (OFT) to evaluate the animals' mental health (Walsh & Cummins, 1976). The device is a huge, square box with plywood sides that is 100 cm long, 100 cm wide, and 40 cm high. The box was separated into 25 rectangular parts, and its floor was treated with a black coating and sanitary resin cloth. To provide illumination, a 100-W lightbulb is positioned 50 cm above the OFT equipment center. As a measure of locomotor activity, the nurturing number and squares during a 5-min interval was recorded (Lopez et al., 2024).

Estimation of Acetylcholinesterase

The acetylcholinesterase (AChE) activity in rat brain tissues was quantified using a commercial AChE assay kit (Cell Signaling Technology, USA; Cat. No.: 703002). Brain tissues were homogenized in 9% (w/v) ice-cold buffered saline and centrifuged at 6,000 rpm for 10 min at 4°C. The resulting supernatant was collected for analysis. AChE activity was evaluated following the manufacturer's protocol, which is based on the classical Ellman principle. Briefly, the enzyme-mediated hydrolysis of acetylthiocholine releases thiocholine, which reacts with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) to form thionitrobenzoic acid (TNB), producing a yellow chromophore. Absorbance was recorded at 412 nm using a UV–vis spectrophotometer (Shimadzu UV-1800), and AChE activity was expressed as U/mg protein.

Analysis of Lipid Peroxidation and Antioxidant Levels

We measured brain antioxidants and markers of oxidative stress using standard biochemical tests. Brain tissue homogenates (10% w/v) were prepared in 0.9% saline and centrifuged at 6,000 rpm for 10 min at 4°C. The collected supernatant was used for the following assays.

Superoxide Dismutase

Superoxide dismutase (SOD) activity was quantified using the method of Kono and Fridovich (1982), based on the

inhibition of hydroxylamine oxidation. One unit of SOD activity was defined as the amount of enzyme required to inhibit the reaction by 50%, presented as U/mg protein.

Catalase

Catalase (CAT) activity was measured following Aebi (1984), based on the decomposition rate of 1 μM hydrogen peroxide (H_2O_2). Absorbance reduction at 240 nm was recorded over 3 min. Results were expressed as U/mg protein.

Reduced Glutathione

Glutathione (GSH) levels were quantified following Ellman et al. (1961) using DTNB reagent, which reacts with sulfhydryl groups to produce a measurable yellow chromogen at 412 nm, expressed as μmol/g tissue.

Glutathione Peroxidase

Glutathione peroxidase (GPx) activity was determined using the method of Flohé and Günzler (1984), which is based on the oxidation of GSH in the presence of cumene hydroperoxide. NADPH consumption was monitored at 340 nm and expressed as U/mg protein.

Lipid Peroxidation (Malondialdehyde)

Malondialdehyde (MDA), the end-product of lipid peroxidation, was estimated via the thiobarbituric acid-reactive substances (TBARS) assay. Brain homogenates were incubated with thiobarbituric acid (TBA) reagent, heated, and the pink chromogen was measured at 532 nm, expressed as nmol MDA/mg tissue.

Estimation of Inflammatory Markers by Enzyme-linked Immunosorbent Assay Kits

The levels of IL-6, cycyclooxygenase-2 (COX-2), and TNF-α were measured using the procedure outlined in the kit specifications that were acquired from RayBiotech, Canada (RayBiotech, Canada; IL-6: Cat. No. ELR-IL6-1, COX-2: Cat. No. EIA-COX2-1, TNF-α: Cat. No. ELR-TNFa-1). An enzyme-linked immunosorbent assay (ELISA) reader was utilized to test the absorbances at 450 nm right away against a blank.

Histopathological Analysis

After the anaesthetized experimental animals were killed, the brain tissues were collected. 10% formalin was used to fix the hippocampal tissues that were collected. Hematoxylin and eosin were used to stain the paraffin-entrenched hippocampal tissue. A magnification microscope was used to examine the $AlCl_3$-stimulated hippocampal lesions and if Santonin (Sant) lessened them (Elizabeth et al., 2020).

Statistical Analysis

The statistical analyses were done with Prism software V 10.2.2 (GraphPad Software, USA). All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism (version 10.2. 2). A one-way analysis of variance (ANOVA) was used to evaluate differences among multiple groups, followed by a Tukey's multiple comparison *post hoc* test to identify specific group-to-group differences. For comparisons involving only two groups, an unpaired two-tailed Student's *t*-test was applied. The assumptions of normality and homogeneity of variance were verified prior to performing parametric tests. Statistical significance was considered at $p < .05$, while values of $p < .01$ were regarded as highly significant.

Results

Effect of Santonin on Behavioural Status in Experimental Rats

The impact of Santonin on the behavioral state of experimental animals is depicted in Figure 1(A,B). In comparison to control, rats treated with $AlCl_3$ exhibited a much higher retention transfer delay. It is significant that the dose-based supplementation of Santonin demonstrated decreased retention transfer latency than the rats treated with $AlCl_3$ alone. The rat's mobility and other probing activities were observed using the OFT (Figure 1C). Compared to the control group, rats given $AlCl_3$ spent a lot more time not moving. Basically, their activity dropped off. When we gave Santonin to the $AlCl_3$-challenged rats, they started moving around more and spent less time sitting still. The control group stayed the same the whole time.

Effect of Santonin on Acetylcholinesterase Activity Level

Figure 2 represents the impact of Santonin on the AChE activity. The cholinergic dysfunction was evaluated based on AChE levels. The group that was treated with $AlCl_3$ showed that AChE activity significantly increased as compared to the control group ($p < .05$), which indicates impaired cholinergic transmission. Contrarily, rats that received Santonin at 20 and 40 mg/kg reported a significant decrease in AChE activity compared with the $AlCl_3$ group ($p < .05$). These results indicate that Santonin is capable of inhibiting the hyperactivity of the AChE caused by $AlCl_3$ thus the likelihood of Santonin relieving the cholinergic dysfunction.

Effect of Santonin on Lipid Peroxidation and Antioxidants in the Experimental Animals

Figure 3(A–D) shows the effect of Santonin on the antioxidant status and lipid peroxidation of the experimental animals. The level of SOD, GSH and CAT was significantly

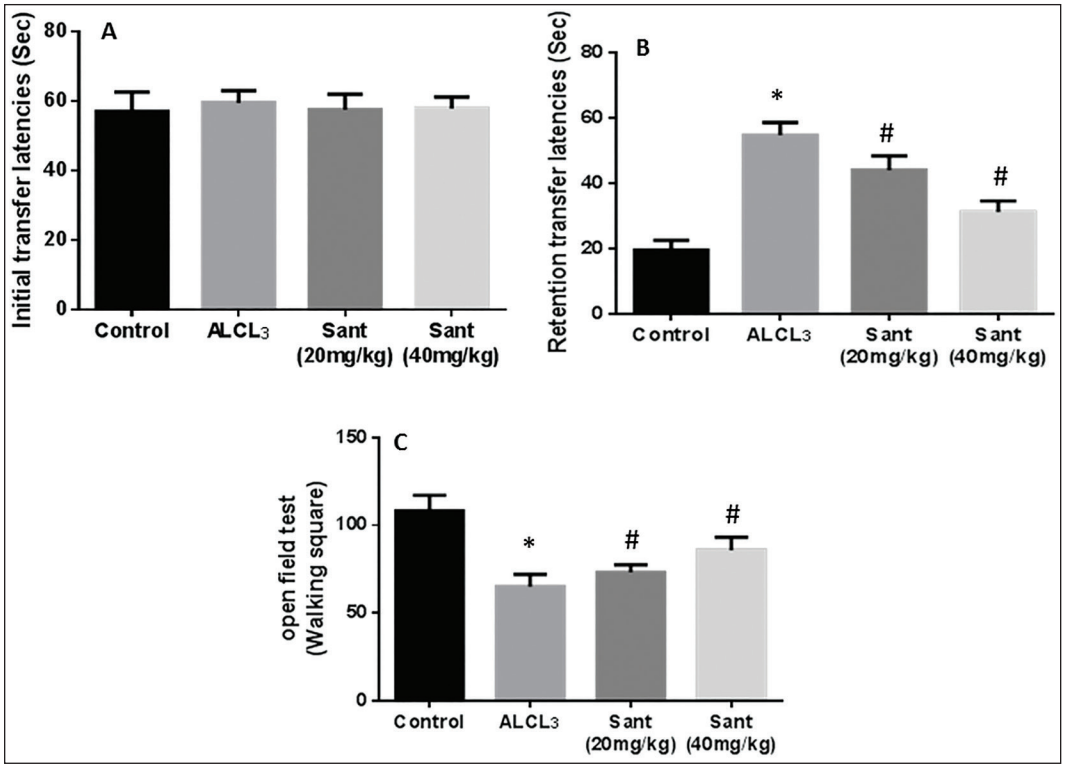


Figure 1. Effect of Santonin on Behavioral Activity of Experimental Rats.

Notes: The elevated plus maze (A) initial, (B) retention transfer latencies and (C) open field test in experimental rats were assessed in the experimental rats. Data are presented as mean ± SEM (n = 7 per group). * $p \leq .05$ compared with the control group and # $p \leq .01$ compared with the $AlCl_3$ group.

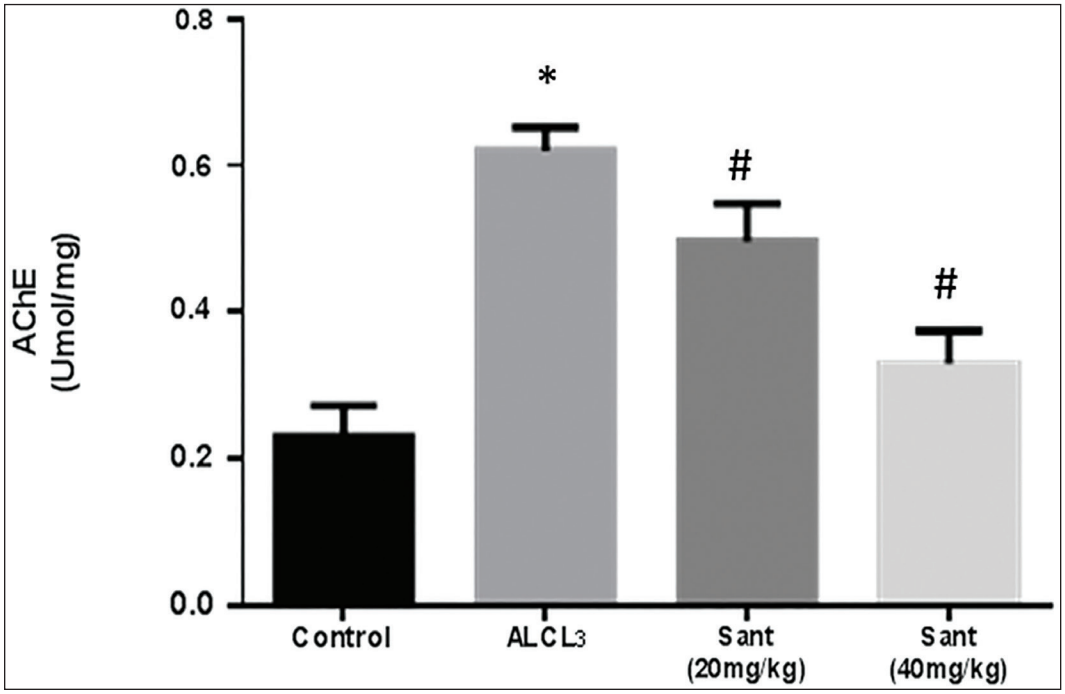


Figure 2. Effect of Santonin on Acetylcholinesterase (AChE) Activity in $AlCl_3$-treated Rats.

Notes: The AchE activity was evaluated in the experimental rats. Rats in the $AlCl_3$-treated group showed a significant increase in AChE activity compared with the control group, indicating aluminum-induced neurotoxicity. Treatment with Santonin (20 or 40 mg/kg) reduced AChE activity toward normal levels, demonstrating its protective effect. Data are presented as mean ± SEM (n = 7 per group). * $p \leq .05$ compared with the control group and # $p \leq .01$ compared with the $AlCl_3$ group.

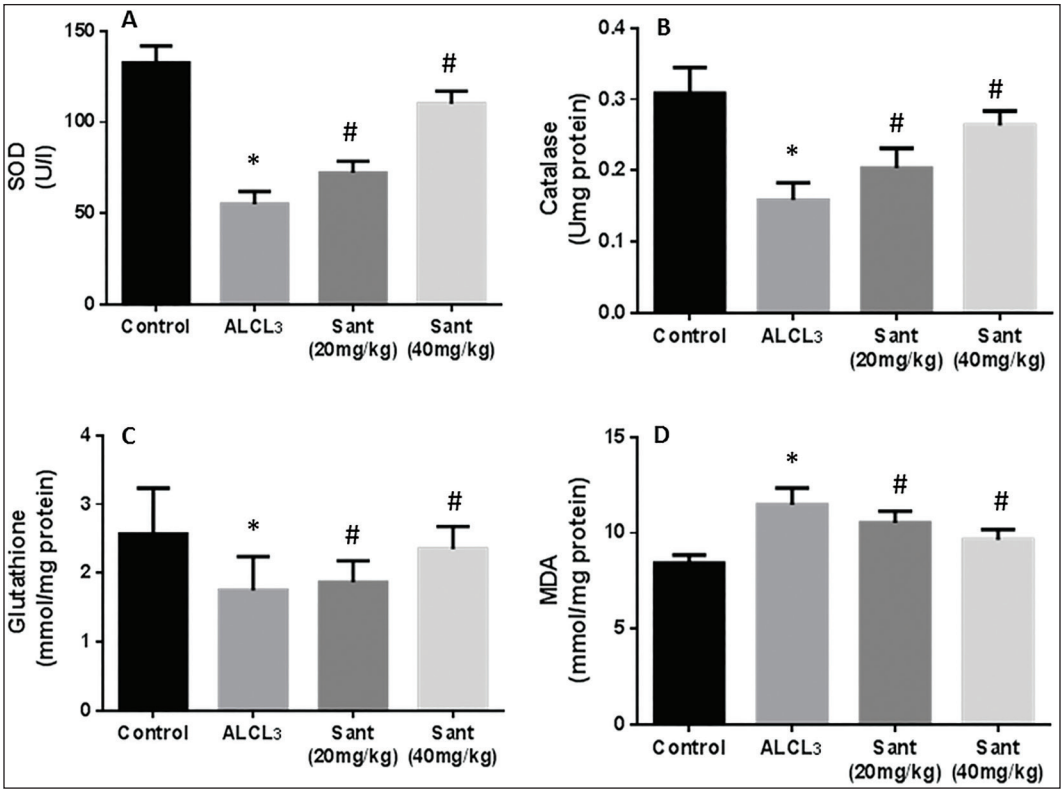


Figure 3. Effect of Santonin on Antioxidant Defense Markers and Lipid Peroxidation in the Brain Tissue of $AlCl_c$-treated Rats.

Notes: This figure shows the levels of major antioxidant enzymes and oxidative stress markers measured in brain tissue from all experimental groups. Aluminum chloride ($AlCl_3$) exposure is known to induce oxidative stress; therefore, evaluating these biomarkers helps determine the degree of neuroprotection provided by Santonin.The brain tissue on antioxidant levels was evaluated in the experimental rats. (A) Superoxide dismutase (SOD), (B) catalase, (C) reduced glutathione (GSH) and (D) malondialdehyde (MDA). Data are presented as mean ± SEM (n = 7 per group). * $p \leq .05$ compared with the control group and # $p \leq .01$ compared with the $AlCl_3$ group.

lowered in the brain tissues of the $AlCl_3$-treated rats with a significant rise in MDA content over the control group, and is a pointer of severe oxidative stress. Dose-dependent administration of Santonin substantially decreased MDA levels while restoring SOD, GSH, and CAT activities in the brain tissues of $AlCl_3$-exposed rats. These findings suggest that Santonin effectively mitigates oxidative damage and enhances antioxidant defense mechanisms in this AD model.

Effect of Santonin on Inflammatory Markers in the Experimental Animals

Figure 4(A–C) illustrates the impact of Santonin on inflammatory markers in the test animals. Anti-neuroinflammatory activity was tested utilizing neuro-inflammatory cytokines. Compared to control rats, animals treated with $AlCl_3$ exhibited noticeably greater levels of IL-6, TNF-α, and COX-2. Santonin (20 and 40 mg/kg) considerably diminished the COX-2, IL-6, and TNF-α in rats treated with $AlCl_3$. These results prove that Santonin may inhibit inflammatory cytokines that result in an inhibition of neuroinflammatory response in rats given $AlCl_3$.

Effect of Santonin on Histopathological Architecture of the Experimental Animals

Figure 5(A–D) shows the histological changes in the hippocampal regions of the control and experimental rats. Neuronal cells in the control rats had normal vesicular nuclei and were regular and healthy (Figure 5(A)). Neuron shrinkages, hyperchromatic nuclei, and vacuole spacing of neurons were seen in the hippocampal regions of AD rats that were triggered with Al (Figure 5(B)). Shrinkage of neurons was recovered, and the vacuoles surrounding the neurons were reduced when Santonin (20 mg/kg) was pre-supplemented with $AlCl_3$ (Figure 5(C)). When Santonin (40 mg/kg) was supplemented to AD rats, the animals showed large vesicular nuclei with distinct nucleoli and amphophilic cytoplasm.

Discussion

Around 2.4 million people globally already suffer from dementia, and that rate is predicted to elevate every 20 years. The common cause of dementia is AD, which is defined by the intraneuronal aggregation of tau protein (tau) and the

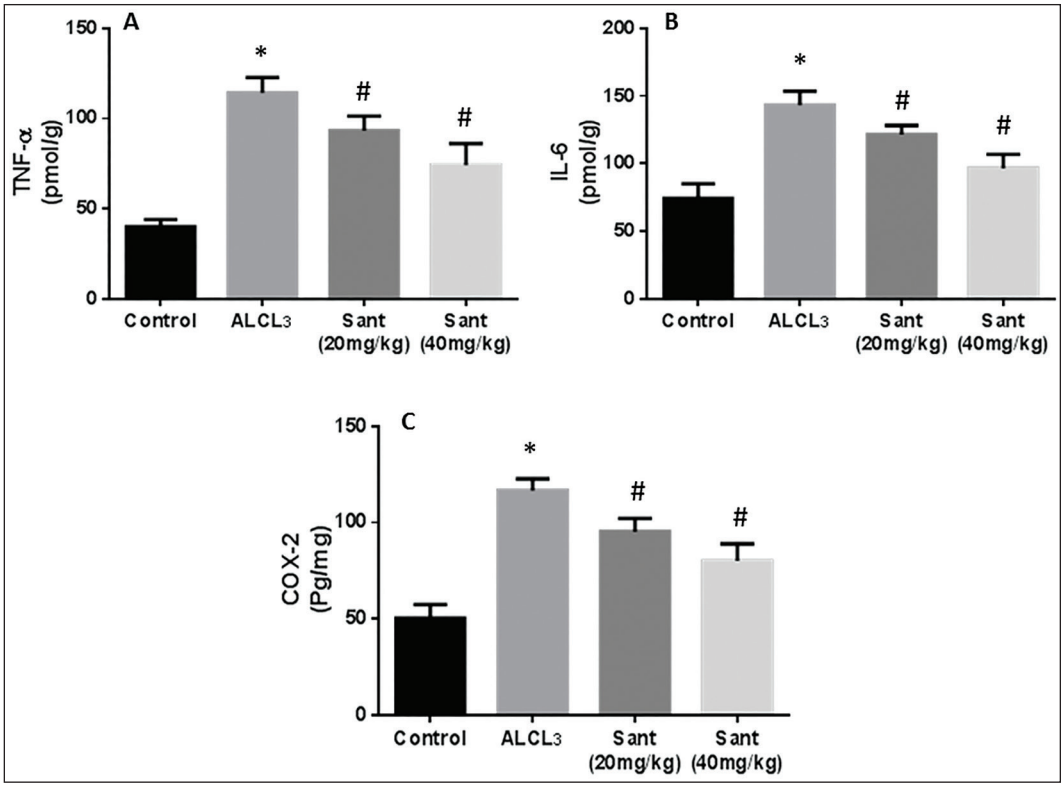


Figure 4. Estimation of Inflammatory Markers by Enzyme-linked Immunosorbent Assay (ELISA) Kits.

Notes: (A) Tumor necrosis factor-α (TNF-α), (B) interleukin-6 (IL-6), and (C) cyclooxygenase-2 (COX-2) levels were studied using ELISA kit specifications from Ray Biotech, Canada. The absorbance's were taken immediately at 450 nm against blank using an ELISA reader. Data are presented as mean ± SEM (n = 7 per group). * $p \leq .05$ compared with the control group and # $p \leq .01$ compared with the $AlCl_3$ group.

extracellular build-up of Aβ peptide (Aβ). Age, pollutants, heavy metals, smoking, traumatic brain traumas, obesity, high blood cholesterol, and diabetes are all contributing factors to the progression of AD (Calderon-Garcidueñas & Duyckaerts, 2018). One of the main factors known to contribute to the onset of AD is oxidative stress. In AD, neuronal cells are highly susceptible to severe oxidative damage, which contributes significantly to disease progression (Cassidy et al., 2020). Neuroinflammation is one of the major pathological characteristics that worsen tau pathology and amyloid-β(A) buildup. The inflammatory responses become persistent and the proinflammatory cytokines are released continuously into the brain making it a toxic environment to the neurons. Increased cytokine concentrations also cause mitochondrial dysfunction, which in most cases is facilitated by Aβ signaling pathways, and induce BBB permeability. All of these worsen the presence of oxidative stress, the disruption of neuronal integrity, and the promotion of the neurodegenerative mechanisms typical of AD (Calabrò et al., 2020).

In this work, we discussed the protective effects of Santonin against oxidative stress and inflammation in rats. $AlCl_3$ was used to cause oxidative stress and neuroinflammation and the effects of Santonin on lipid peroxidation, inflammatory mediators, antioxidant enzyme activity, and behavioral performance were assessed. In order to understand the neuroprotective mechanisms further, histopathological studies on brain tissue were also performed.

To measure the anxiety-like behavior, two established paradigms were used, namely the EPM and the OFT, which were described earlier by Knight et al. (2021). The EPM is a model of assessing anxiety-related reactions in rodents that is highly used and applicable in the case of conditions like PTSD (Kraeuter et al., 2018). The EPM also showed transfer latency (TL), which gave further implicit knowledge of learning and memory processes (Jadhav & Kulkarni, 2023).

We found that the baseline learning ability was similar, and that there was no difference in the initial TL in all groups under the first trial. Nonetheless, in the retention trial, there was a significant increase in TL in the rats treated with $AlCl_3$, indicating impaired memory retention. Conversely, rats that had been treated with Santonin had significantly shorter TLs, indicating that Santonin was effective in enhancing memory performance in the $AlCl_3$-exposed animals.

Locomotor activity and exploratory behavior were assessed with the help of the OFT. Rats treated with $AlCl_3$ exhibited significant locomotion reduction that is in line with a heightened state of anxiety-like behavior (Firdaus et al., 2022). Notably, Santonin administration, especially at 40 mg/kg, restored exploratory activity and movement to near-normal levels. These behavioral outcomes indicate that

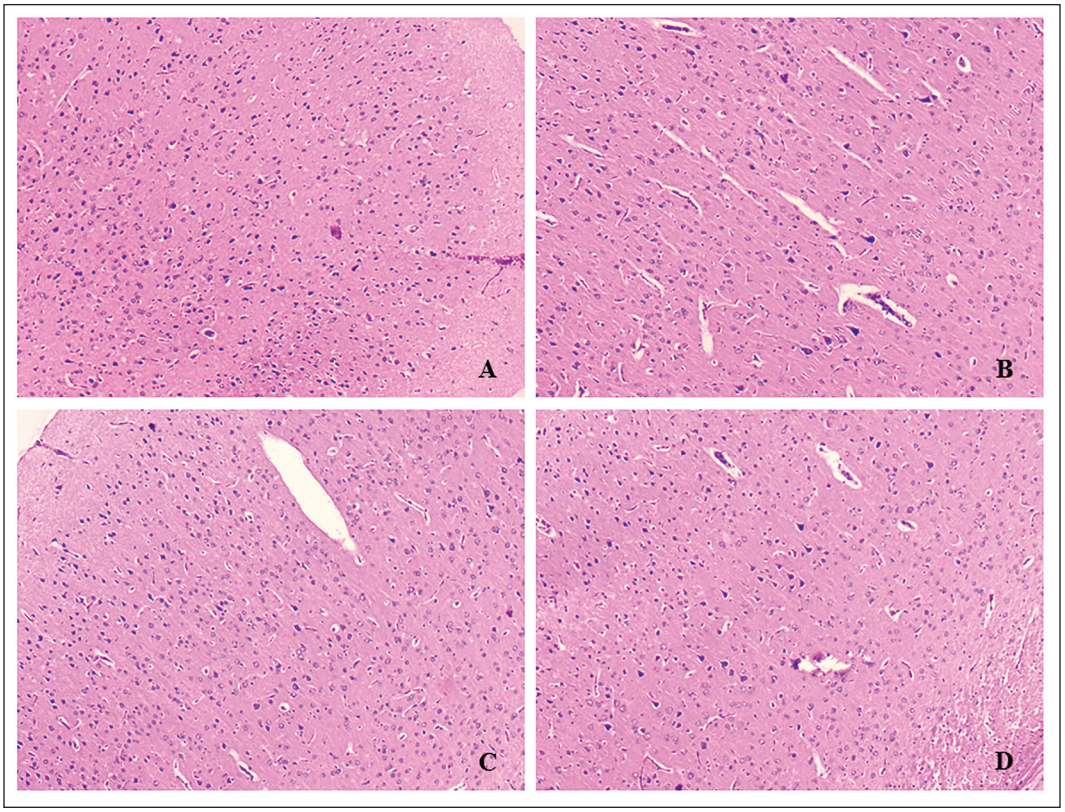


Figure 5. Representative Brain Histopathological Sections Showing the Effect of Santonin on $AlCl_3$-induced Neurotoxicity in Rats.

Notes: This figure presents microscopic images of brain tissue from all experimental groups, illustrating the structural changes caused by aluminum exposure and the protective effects of Santonin treatment. (A) Group I – Control: Brain tissue from rats fed a normal pellet diet, showing typical and intact neuronal architecture with no visible signs of degeneration or inflammation. (B) Group II – $AlCl_3$: Rats treated intraperitoneally with $AlCl_3$ (100 mg/kg; 0.5 mL) for 4 weeks. Sections show marked neuronal damage, including cellular shrinkage, vacuolation, disrupted neuronal layers, and indications of oxidative stress and neurodegeneration. (C) Group III – Santonin (20 mg/kg) + $AlCl_3$: Rats pretreated orally with Santonin (20 mg/kg) 1 h prior to the $AlCl_3$ injection for 4 weeks. Histology shows noticeable improvement compared with Group II, with reduced neuronal degeneration and partial restoration of normal tissue architecture. (D) Group IV – Santonin (40 mg/kg) + $AlCl_3$: Rats treated orally with Santonin (40 mg/kg) for 4 weeks and subsequently challenged with $AlCl_3$ (100 mg/kg). Sections show marked protection, including improved cellular integrity and reduced pathological alterations, indicating a stronger neuroprotective effect at the higher Santonin dose. All images were captured using light microscopy at the appropriate magnification (specify, e.g., 40× or 100×), allowing visualization of neuronal morphology and pathological changes.

Santonin mitigates $AlCl_3$-induced neurobehavioral deficits, reinforcing its potential neuroprotective properties. The cholinergic pathway is in charge of learning and memory. Acetylcholine (AChE) is broken down by an enzyme and is a cholinergic neurotransmitter. Through transmembrane protein (Na+K+ATPase) action, AChE is responsible for the integrity of the membrane of cholinergic neurons. Cholinergic transmission failure is connected to memory impairment in AD (Shunan et al., 2021). $AlCl_3$ has the potential to impede cholinergic transmission by influencing the production and elimination of neurotransmitters since it is a cholinotoxin (neurotoxin) that causes functional changes in cholinergic, dopaminergic, and noradrenergic neurotransmission (Borai et al., 2017). In this work, we noted that the $AlCl_3$-treated rats had a noticeably higher level of AChE. Other groups' levels of AChE were lower than those of the $AlCl_3$ group. The amount of AChE was diminished in experimental rats given Santonin, suggesting that AChE hydrolysis was inhibited and AD symptoms were avoided.

A burst of free radicals, including ROS and RNS, which are produced under pathological conditions, results in a disproportion between the production of free radicals and antioxidant mechanisms, an oxidative stress state (Peña-Bautista et al., 2019). SOD is believed to be the primary defense against oxidative stress because it produces less harmful H_2O_2 and O_2 from the hazardous superoxide anion. At the expense of GSH, catalase and GPx subsequently transform H_2O_2 into H_2O (Justin Thenmozhi et al., 2017). Al exacerbates lipid peroxidation and augments the release of free radicals, resulting in oxidative stress and subsequent neurotoxicity. Due to its impaired antioxidant status and elevated levels of free radicals, the brain is highly susceptible to oxidative stress and injury. Lipid peroxidation caused by ROS leads to the production of MDA, which is also a major biomarker of oxidative stress that causes

cell damage and membrane rupture. Although it reduces the activities of the antioxidant systems in the brain regions, repeated treatment with $AlCl_3$ significantly increases the MDA levels (Agrawal et al., 2024). As per the present investigation, the animals that were administered with $AlCl_3$ alone exhibited oxidative stress symptoms in the brain, as demonstrated by a significantly increased MDA level and a significantly reduced SOD, GSH, and CAT level. This was similar to the previous finding, which revealed that $AlCl_3$ increased MDA, leading to cell damage due to free radicals and a reduction in the antioxidant system (Qusti, 2017). Animals under the dose-dependent levels of Santonin treatment demonstrated a progressive decrease in MDA with a concomitant rise in SOD, GSH and CAT systems. The above enhancements are clear evidence of the antioxidant property of Santonin.

Activation of the microglia to secrete proinflammatory mediators, including interleukins, NF-kB, TNF-α and COX-2 triggers chronic neuroinflammation in Alzheimer disease (AD). TNF-α and COX-2 are not only increased in AD pathology, but they also play an active role in the development of Aβ plaques and hyperphosphorylation of tau proteins, which accelerates neuro-degradation (Sajad et al., 2025). The inflammatory cascade also enhances the synthesis of cytokines such as IL-6, TNF-α, IL-1β, iNOS and COX-2, which increases the inflammatory reaction. In line with the existing data, rats subjected to $AlCl_3$ show a remarkable rise in IL-6, IL-1β and TNF-α (Kazmi et al., 2024). Interestingly, Santonin treatment significantly decreased these high cytokine levels in $AlCl_3$ treated group. These findings suggest that Santonin has strong anti-inflammatory properties and could have neuroprotective effects against neurotoxicity caused by $AlCl_3$.

The results of our investigation have shown a very consistent and clear trend: decreased lipid peroxidation, increased neuronal integrity, and close correspondence to the existing literature on the topic, which indicates how plant-derived compounds offset neuroinflammation and oxidative stress. Such findings resemble the findings of Rai et al. (2025), who emphasized the therapeutic importance of phytochemicals in the regulation of the oxidative pathways. They also support the idea that proper mitochondrial activity and synapses are of the essence in healthy aging as put forward by Rai (2025). Besides, our findings align well with the viewpoints of Singh et al. (2024), who highlighted the role of synaptic plasticity in preventing the severity of the neurodegenerative disorder. Collectively, these links position our research in the wider context of Prakash et al. (2024) and Rai (2021) who propose that phytochemicals and targeted therapeutics will be incorporated into the treatment plans in the future.

Further understanding of the protective action of Santonin was obtained with the help of histopathological analysis. The brain tissues of the $AlCl_3$ treated rats showed characteristic features of neurodegeneration, such as neuronal shrinkage, compressed nuclei and pericellular vacuolations. Conversely, Santonin-treated rats had significantly better-preserved neuronal structure, and the morphology of tissues were much like the control group, which showed a strong level of neuroprotection.

Study Limitations

Although this study has relevant findings, there are various limitations. First, the comparatively brief treatment period can be inadequate to capture the chronic course of Alzheimer disease. Long-term research must be conducted to establish whether the protective properties of Santonin are more prolonged or cumulative. Second, male rats were the only participants, even though Alzheimer pathology and progression is different in both sexes. Both females and males should be included in future studies to increase the translational significance. Moreover, the current study was limited to biochemical markers and histopathology, but behavioral and cognitive testing, including Morris Water Maze or Y-maze were not conducted. The inclusion of such tests would assist in attaining the divide between the molecular transformations and the functional results.

Future Directions

Further research is required to study the pharmacokinetics of Santonin, such as the capacity to cross the BBB, using experiments that involve *in vivo* imaging and LC–MS/MS quantification of its pharmacokinetics. There is a need to test longer-term outcomes of behavior, neuroinflammatory events and any impact on amyloid and tau pathology. Prior to the idea of looking at clinical usage, it should be important to review safety profiles, optimize dosage schedules and examine potential drug–drug interactions. Since Alzheimer disease is a complex condition, extensive multidisciplinary research shall be essential in establishing the therapeutic potential of Santonin.

Conclusion

To conclude, Santonin exhibited significant neuroprotective properties in a rat model of $AlCl_3$-induced Alzheimer. It was effective in alleviating neuroinflammation, restoring redox equilibrium, reducing oxidative stress, and enhancing AChE activities. Santonin also reduced major inflammatory cytokines, such as TNF-6, IL-6 and COX-2 and enhanced behavioral improvement. Histologically, Santonin protected the cell structure and minimized tissue loss. Together, these results indicate that Santonin can be a promising agent in the future treatment of Alzheimer, but more mechanistic and longitudinal research is required to understand its full potential.

Abbreviations

Aβ: Amyloid-beta; AChE: Acetylcholinesterase; AD: Alzheimer's disease; Al: Aluminum; $AlCl_3$: Aluminum chloride; ANOVA: Analysis of variance; APOE: Apolipoprotein E; BBB: Blood–brain

barrier; CAT: Catalase; COX-2: Cyclooxygenase-2; DTNB: 5,5-Dithio-bis-2-nitrobenzoic acid; ELISA: Enzyme-linked immunosorbent assay; EPM: Elevated plus maze; IL: Interleukins; LOF: Large open field; MDA: Malondialdehyde; NDD: Neurodegenerative diseases; RNS: Reactive nitrogen species; ROS: Reactive oxygen species; Sant: Santonin; SOD: Superoxide dismutase; TNF-α: Tumor necrosis factor-α.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

This study is approved by Ethics Committee of Hunan University of Chinese Medicine (Approval Number: 2024-KY-68).

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Informed Consent

Informed consent was obtained from all participants (or from their legal guardians, where applicable) prior to their inclusion in the study.

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