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Barbaloin's Chemical Intervention in Aluminum Chloride Induced Cognitive Deficits and Changes in Rats through Modulation of Oxidative Stress, Cytokines, and BDNF Expression

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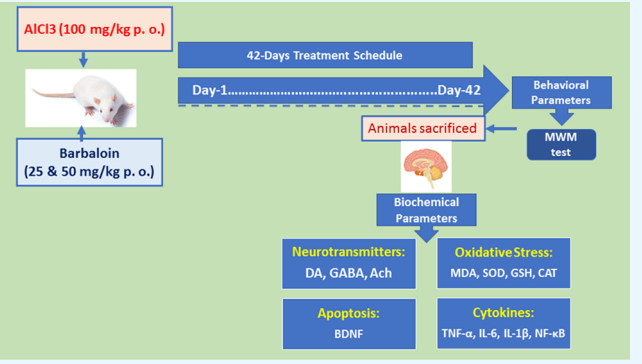
ABSTRACT: Alzheimer's disease (AD) is a long-term neurodegenerative condition characterized by impaired cognitive functions, particularly in the domains of learning and memory. Finding promising options for AD can be successful with a medication repurposing strategy. The goal of the research was to examine the neuroprotective characteristics of barbaloin in aluminum chloride ($AlCl_3$)-induced cognitive deficits and changes in rats through modulation of oxidative stress, cytokines, and brain-derived neurotrophic factor (BDNF) expression. Thirty male Wistar rats were subjected to $AlCl_3$ at a dosage of 100 mg/kg via the per oral route (p.o.), which induced cognitive decline. Morris water maze (MWM) is used to assess behavioral metrics. Assays for catalase (CAT), malondialdehyde (MDA), reduced glutathione (GSH), acetylcholinesterase (AChE), choline-acetyltransferase (ChAT), interleukins-1β (IL-1β), superoxide dismutase (SOD), tumor necrosis factor-α (TNF-α), nuclear factor kappa-B (NF-κB), interleukins-6 (IL-6), BDNF, and neurotransmitter levels [dopamine (DA), acetylcholine (Ach), and γ-aminobutyric acid (GABA)] were performed. Results: The transfer latency time was notably decreased, and substantial modifications in the concentrations of GSH, MDA, CAT, SOD, AChE, ChAT and observed modulations in the formation of interleukins-6 (IL-6), TNF-α, IL-1β, BDNF, and NF-κB were also evidenced after the treatment of rats with barbaloin in comparison to $AlCl_3$-induced control groups. Significant alterations in neurotransmitter levels (DA, Ach, and GABA) were also seen in barbaloin-treated groups in comparison to $AlCl_3$-induced groups. The current investigation has provided evidence that the administration of barbaloin yielded notable enhancements in cognitive function in rats through the inhibition of MDA, enhancing endogenous antioxidant enzymes, reduction of cytokine levels, and enhancement of neurotransmitter contents in the brain. These effects were observed in comparison to a control group treated with $AlCl_3$ and can be attributable to barbaloin's strong anti-inflammatory and antioxidant properties, and metal chelating properties may contribute to its neuroprotective effects. Barbaloin may also promote neuronal survival and enhance learning and memory by upregulating the expression of BDNF.



1. INTRODUCTION

Alzheimer's disease (AD) is a long-term neurodegenerative disorder characterized by impaired cognitive functions, particularly in the domains of learning and memory.[1,2] Additionally, it was noted that higher levels of reactive oxygen species (ROS) and their byproducts accelerate the onset and progression of AD.[3,4] AD is characterized by a broad spectrum of neurodegenerative abnormalities in addition to numerous cognitive difficulties. One of the pathogenic characteristics of AD is the disordered functioning of neurotransmitters, such as acetylcholine (Ach), γ-aminobutyric acid (GABA), serotonin (5-HT), and glutamate (Glu).[5] Excitatory and inhibitory neurotransmitters have been demonstrated to either exacerbate or diminish AD pathology, and their roles in learning, memory, and cognition have been well-documented.[6,7] Deficits in neurotransmitters 5-HT, Ach, and Glu have been observed during the early stages of AD, while disturbances in GABA and dopamine (DA) seem to manifest in later stages of the disease.[5,8,9]

Aluminum (Al) is a widely recognized neurotoxic substance implicated in the pathogenesis of AD.[10−12] Al has been observed to trigger the misfolding of cytoskeleton proteins

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within the brain, subsequently resulting in the production of amyloid β (Aβ) plaques.[13,14] Prolonged exposure to Al has been found to induce neurodegeneration and neuronal death through apoptotic mechanisms;[15] additionally, cognitive deficits and neuropathological alterations in the brain have been seen, leading to impaired learning abilities in rats.[16,17] Furthermore, research has also indicated that the presence of Al can lead to cognitive deficits in animal subjects.[18,19] It is widely postulated that the processes behind the induction of AD-like behaviors by aluminum mostly entail oxidative damage,[19] the process by which tau proteins become hyperphosphorylated,[20] accumulating the Aβ protein,[21] increasing the activity of acetylcholinesterase (AChE),[22] and apoptosis of neurons.[21] Furthermore, Al has the potential to induce brain dysfunction through glial changes, in addition to its effects on neuronal activity.[23] Neurotrophins, such as downregulation of the brain-derived neurotrophic factor (BDNF), produced and distributed throughout the brain,[24] have a significant impact on the growth, protection, and plasticity of neurons[25,26] and in the control of the neuro-immune axis and synaptic connections.[26] In neurodegenerative illnesses, BDNF and nerve growth factor (NGF) may be useful diagnostic and prognostic indicators.[27] The stimulation of gene expression driven by a cAMP response element-binding protein (CREB) is highly plausible in the context of memory formation.[28] Recent research has additionally demonstrated that activation of the PKA/CREB signaling pathway inside the hippocampus of rats is a crucial factor in the formation of long-term memory. However, it is worth noting that other pathways, such as PKC and CaMKII, also contribute to this process.[29] Based on the aforementioned findings, it is justifiable to propose that the signaling pathway including CREB and BDNF may also hold significance in the underlying mechanism of cognitive decline generated by aluminum chloride ($AlCl_3$) (Figure 1A).[30]

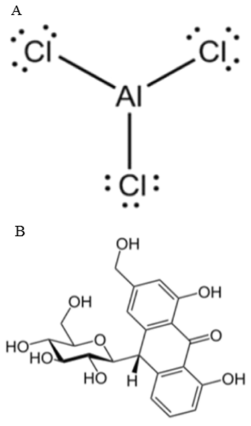

Figure 1. (A) Structure of aluminum chloride. (B) Structure of barbaloin.

Barbaloin (Figure 1B), the primary anthraquinone molecule derived from the exudates of aloe plant leaves, is commonly employed in the production of dietary supplements.[31,32] The plant in question is commonly referred to as the "Miracle Plant" or the "wound healer" due to its remarkable capacity to alleviate a wide range of human illnesses, hence promoting healing and providing solace to individuals.[33,34] Barbaloin (anthraquinone glycosides) has a complicated molecular structure. The compound consists of a chromone ring structure, accompanied by two polar side chains, namely, a glucose moiety and a monohydroxyanthraquinone moiety. The antioxidant and anti-inflammatory effects of barbaloin can be attributed to its chromone ring structure. The chromone ring possesses the ability to effectively scavenge free radicals and exert inhibitory effects on the synthesis of proinflammatory cytokines. The laxative and antibacterial actions of barbaloin can be attributed to its anthraquinone moiety.[34] Barbaloin exhibits several pharmacological characteristics, such as antioxidant, anticancer, antimicrobial, and anti-inflammatory activities[35−37] and are antineuroinflammatory.[38] Moreover, numerous articles have provided evidence that the use of Aloe vera gel induces hypoglycemia and hypolipidemic effects in animal models of noninsulin-dependent diabetes.[39−41] Previous research has revealed that barbaloin has been found to induce an excessive expression of interleukins-6 (IL-6), IL-1β, and tumor necrosis factor-α (TNF-α) through the activation of nuclear factor kappa-B (NF-κB). Furthermore, this effect was significantly diminished when the PI3K/AKT pathway was blocked. The aforementioned findings provide empirical evidence supporting the notion that barbaloin effectively reduces the production of ROS within cells by impeding the phosphorylation of PI3K and AKT. Consequently, this inhibition hinders the activation of NF-κB.[32,38] The anthraquinone moiety can also act as a metal chelator, which may contribute to barbaloin's neuroprotective effects against $AlCl_3$-induced neurotoxicity.

The current investigation has provided evidence that the administration of barbaloin has resulted in notable enhancements in cognitive function in rats through the inhibition of malondialdehyde (MDA), enhancement of endogenous antioxidant enzymes, reduction in cytokine levels, and improvement in neurotransmitter content within the brain. These effects were observed in comparison to a control group treated with $AlCl_3$ and can be attributable to barbaloin's strong anti-inflammatory and antioxidant, and metal-chelating properties may contribute to its neuroprotective effects. Barbaloin may also promote neuronal survival and enhance learning and memory by upregulating the expression of BDNF.

2. METHODS

2.1. Animals. A total of 30 male Wistar rats that weighed between 275 and 300 g were purchased from T. G. Lab and M.S., India, for the study. The subjects were accommodated in a controlled environment to maintain a room temperature of 22 ± 0.5 °C and exposed to 12 h cycles of light and dark. Their availability for food and drink was unrestricted. The experimental methodology received approval in accordance with the "CPCSEA Guidelines for Laboratory Animal Facilities" (IACUC/LNCP/2023/05).

2.2. Drugs and Solutions. $AlCl_3$ and barbaloin (Yucca Enterprises, MH, India) were used for measurement. All other chemicals utilized in this experiment were of superior quality. The quantifications of IL-6, IL-1β, NF-κB, and TNF-α were performed using a rat enzyme-linked immunosorbent assay (ELISA) kit obtained from MSW Pharma, India.

2.3. Research Design. The animals were chosen in a random manner and subsequently allocated into five groups with each group including six animals.

Group I was designated as the saline control and administered saline (10 mL/kg), Group II was designated as $AlCl_3$ control and administered $AlCl_3$ at a dosage of 100 mg/kg via the per oral route (p.o.), Groups III and IV administered

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barbaloin (25 mg/kg p.o.) and barbaloin (50 mg/kg, p.o.) and designated as $AlCl_3$ + barbaloin 25 and $AlCl_3$ + barbaloin 50, respectively, precisely 1 h before $AlCl_3$ is administered, Group V designated as per se group (barbaloin control) was administered barbaloin (50 mg/kg p.o.). The administration of all doses occurred on a daily basis for a consecutive period of 42 days. On the 42nd−47th days, behavioral test was performed. On the 47th day of the experiment, following a 12 h period of food deprivation, the animals were euthanized in accordance with ethical guidelines in order to obtain brain homogenates for subsequent biochemical analysis.[12] Animals were subjected to anesthesia using an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). Subsequently, euthanasia was performed via cervical dislocation. It is essential to note that the assessment of hallmarks of inflammation and oxidative stress and the estimation of NF-κB, BDNF protein expression, and neurotransmitter levels were performed using the brain homogenate.

2.4. Behavioral Paradigm. *2.4.1. Morris Water Maze (MWM) Test.* MWM is utilized to evaluate spatial memory.[42] The MWM apparatus had a circular pool characterized by a dark color, containing water at a temperature of 22 ± 1 °C. The pool measured 150 cm in circumference and height (60 cm). The water tank, which had a circular shape, was divided into four quadrants of equal size. These quadrants were designated as the south, west, north, and east-beginning places. Additionally, a transparent platform with a diameter of 10 cm was positioned in the northern quadrant in the center. This platform was submerged 2 cm below the level of the water. The platform served as the sole means of escape from the water. The rats' spatial behavior was observed using a video camera that was affixed to a computer positioned directly above the central area of the MWM. This setup allowed for the measurement and recording of many parameters, including the distance covered (in centimeters) to reach the concealed platform, the rate of movement (in cm/s), and the duration of time (in s) spent in the target quadrant during each trial. The animals underwent a training regimen spanning 5 consecutive days with each training session occurring at around the same time. Throughout each day, the animals were subjected to four separate tests. On the sixth day, a memory assessment was conducted, wherein, in the probe testing, the platform was eliminated. During the given time frame, a duration of 60 s was allocated for their presence on the platform.[43]

2.5. Biochemical Parameters. *2.5.1. Brain Tissue Homogenization.* Following the completion of behavioral evaluations, the animals underwent biochemical assessments, including the examination of neuroinflammatory markers and estimations of neurotransmitters. Following euthanasia, the brains of the animals were isolated and subjected to a cleaning process using ice-cold isotonic saline solution. Subsequently, the brain tissue was centrifuged at a speed of 2000−3000 rpm for a duration of 15 min. The homogenate was separated and stored at 4 °C for enzymatic analysis.

2.6. Oxidative Stress Parameters. *2.6.1. MDA Determination.* In order to perform the experiment, the separated supernatant is subjected to the addition of trichloroacetic acid and thiobarbituric acid reactive substance (TBARS) solution. Subsequently, the mixture is subjected to boiling for a duration of 90 min, followed by immediate cooling in ice-cold water. Following centrifugation of the mixture at a force of 1500*g* for a minimum duration of 15 min, the resultant combination was subjected to spectrophotometric measurement at a wavelength of 532 nm. The MDA was quantified by measuring the amount of MDA in terms of μmol/g of brain tissue.[44]

2.6.2. Reduced Glutathione (GSH) Determination. To find out how much GSH is in the brain, 1 mL of trichloroacetic acid is added to a similar amount of brain homogenate to result in precipitation. The supernatant was supplemented with the DTNB reagent, also known as 5-5′-dithio-bis(2-nitro-benzoic acid) and a phosphate buffer solution (PBS). The absorbance at a wavelength of 412 nm was measured by using a UV spectrophotometer. The construction of a standard curve facilitated the quantification of the content of GSH. The findings were quantified in terms of GSH concentration, measured in mg/g of brain tissue.[45]

2.6.3. Superoxide Dismutase (SOD) Estimation. Xanthine oxidase and xanthine were added to the obtained supernatant and thereafter incubated for a duration of 30 min in a potassium phosphate buffer. The formation of a blue formazan compound was achieved by the addition of nitro blue tetrazolium to the mixture, followed by thorough mixing. The wavelength of this substance, specifically 550 nm, was subsequently determined by using spectrophotometry. The quantity of protein required to prevent 50% NBT reduction is utilized for the calculation of one unit of SOD activity.[46]

2.6.4. Catalase (CAT) Estimation. Tests include phosphate buffer and supernatant of brain homogenate, both present at a concentration of 50 nM. The combination was subjected to the addition of H_2O_2, and the measurement of absorbance was conducted utilizing spectrophotometry at a wavelength of 240 nm at regular intervals of 15 s. The measurement of the activity was quantified as μmoles/min/g of brain tissue.[47]

2.6.5. AChE and Choline-Acetyltransferase (ChAT) Estimation. A methodology akin to the one elucidated by Ellman was employed to quantify the degree of AChE activity, presented as μmol AcSCh/min/mg of protein.[48,49] The quantification of brain ChAT activity was conducted using commercial kits and hydroxylamine methods.

2.7. Neurotransmitter Levels. High-performance liquid chromatography (HPLC) was employed to ascertain the quantities of various neurotransmitters, including DA, GABA, and Ach.

2.8. Indicators of Biological Inflammation. The amount of cytokines, including IL-1β, IL-6, NF-κB, TNF-α, and BDNF, were estimated by the utilization of an ELISA kit. The amounts of IL-1β, TNF-α, BDNF, and IL-6 markers were quantified in picograms per milliliter (pg/mL), whereas the level of NF-κB was measured in nanograms per milliliter (ng/mL).[46]

2.9. Analysis of Statistics. A program called GraphPad Prism software, version 8.0 (GraphPad Software Inc.) was used to assess the data from the tests. The final results were then given as the mean value accompanied by the standard error of the mean (SEM). The data were assessed using a two-way one-way analysis of variance (ANOVA) for the MWM test, followed by Bonferroni's post hoc test. Furthermore, a one-way ANOVA was conducted using Tukey's test.

3. RESULTS

3.1. MWM Test. The MWM test assessed the cognitive abilities. In all groups, the trained rats' mean escape latency decreased over learning sessions. $AlCl_3$ control rats exhibited notably longer latency times on the 2, 3, and 4 days of training trials in comparison to the saline control rats ($P < 0.05$). Two-way ANOVA indicated that barbaloin administration substantially declines latency time in $AlCl_3$ control rats [F (4, 125)

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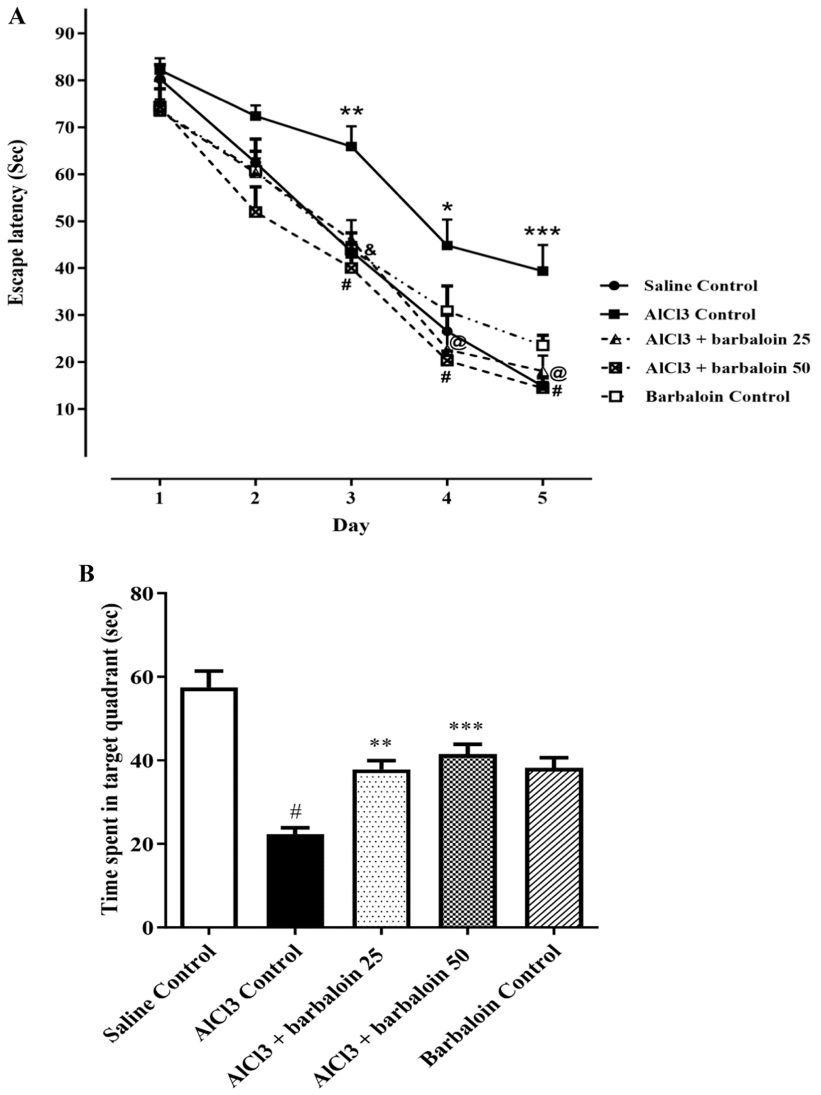

Figure 2. (A) Effect of barbaloin on MWM tests in the acquisition trail. Mean ± SEM (n = 6). *P < 0.05, **P < 0.01, and ***P < 0.001 vs saline control, &P < 0.05, @P < 0.01, and #P < 0.001 vs $AlCl_3$ control. Two-way ANOVA followed by Bonferroni post analytic test. (B) Effect of barbaloin on MWM tests in the probe trial. Mean ± SEM (n = 6). #P < 0.001 vs saline control, **P < 0.01 and ***P < 0.001 vs $AlCl_3$ control. One-way ANOVA was followed by Tukey's test.

= 177.7, P < 0.0001]. After conducting a post hoc test, it was discovered that rats administered with barbaloin (25 and 50 mg/kg) notably decline the latency time when compared to $AlCl_3$ control rats on 2, 3, and 4 days [F (4, 125) = 21.72, P < 0.0001] (Figure 2A).

During the 4 days of the trial, the rats' ability to remember and learn the location of the hidden platform is assessed during the probe trail. A one-way ANOVA revealed that $AlCl_3$ control rats spent considerably reduced the duration spent in the target quadrant when compared to saline control rats, and the influence of barbaloin treatment was found to have a considerable impact on the same (P < 0.05). Furthermore, a post hoc test reveals that the administration of barbaloin at doses of 25 and 50 mg/kg [F (4, 25) = 23.66, P < 0.0001] showed considerable increase in the duration of time spent in the target quadrant in comparison to $AlCl_3$ control rats (Figure 2B).

3.2. Biological Indicators. $AlCl_3$ control rats significantly decreased brain SOD, GSH, CAT, and enhanced brain MDA concentration when compared to the saline control rats (P < 0.0001). Barbaloin treatment enhanced brain SOD [F (4, 25) = 17.54, P < 0.0001], GSH [F (4, 25) = 9.149, P = 0.0001], CAT [F (4, 25) = 10.07, P < 0.0001], and lowered MDA [F (4, 25) = 8.833, P = 0.0001] level, respectively, compared to $AlCl_3$ control rats (Figure 3A−D).

3.3. AChE and ChAT Estimation. $AlCl_3$ control rats significantly enhanced brain AchE and declined brain ChAT concentration when compared to the saline control rats (P < 0.0001). Barbaloin treatment declined brain AchE [F (4, 25) = 9.987, P < 0.0001] and enhanced ChAT [F (4, 25) = 15.43, P < 0.0001] level, respectively, in comparison to $AlCl_3$ control rats (Figure 4A,B).

3.4. Brain DA, GABA, and Ach Contents. In a similar situation, $AlCl_3$ control rats reduced DA, GABA, and Ach content in brain in the assessment of the normal control rats (P < 0.0001). Barbaloin treatment enhanced DA [F (4, 25) = 17.93, P < 0.0001], GABA [F (4, 25) = 11.55, P < 0.0001], and Ach [F (4, 25) = 8.279, P = 0.0002] contents in the assessment to $AlCl_3$ control rats (Figure 5A−C).

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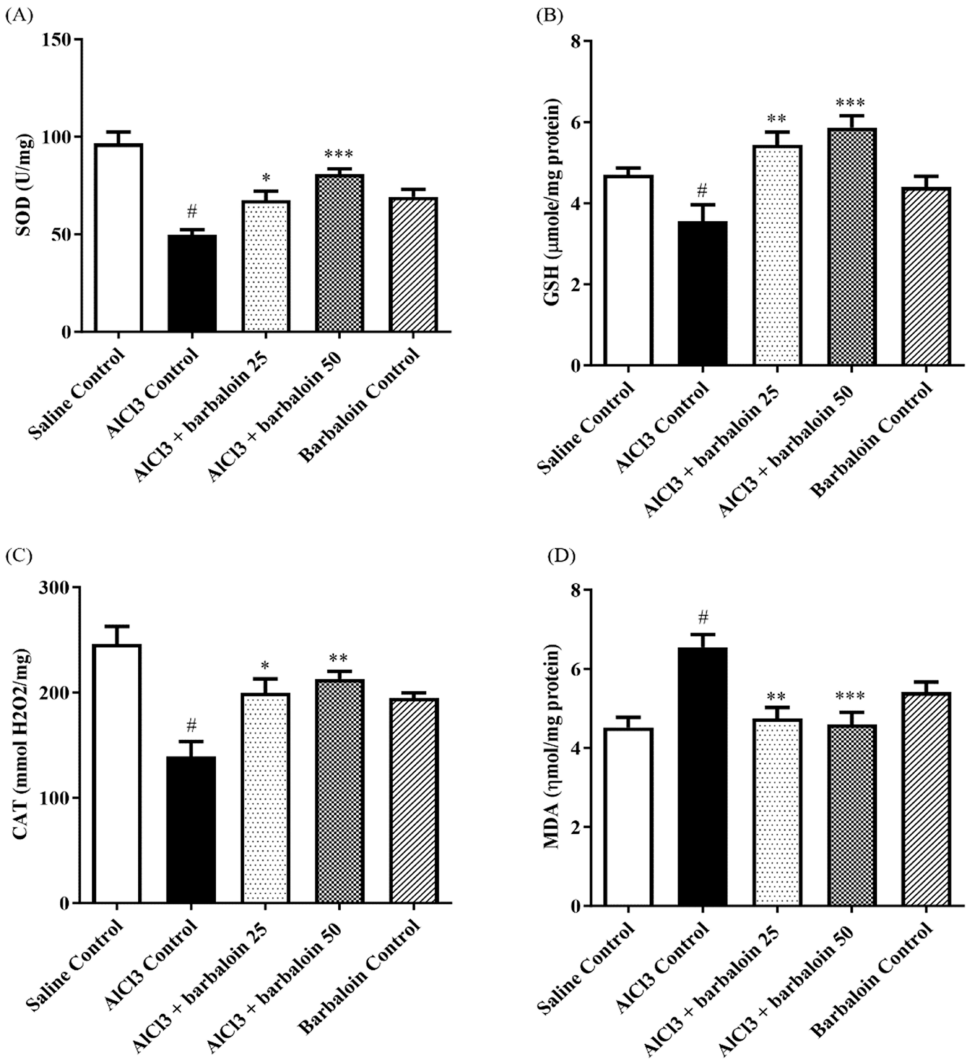

Figure 3. (A–D) Effect of barbaloin on SOD, GSH, CAT, and MDA levels. Mean ± SEM (n = 6). #P < 0.001 vs saline control, *P < 0.05, **P < 0.01, and ***P < 0.001 vs $AlCl_3$ control. One-way ANOVA was followed by Tukey's test.

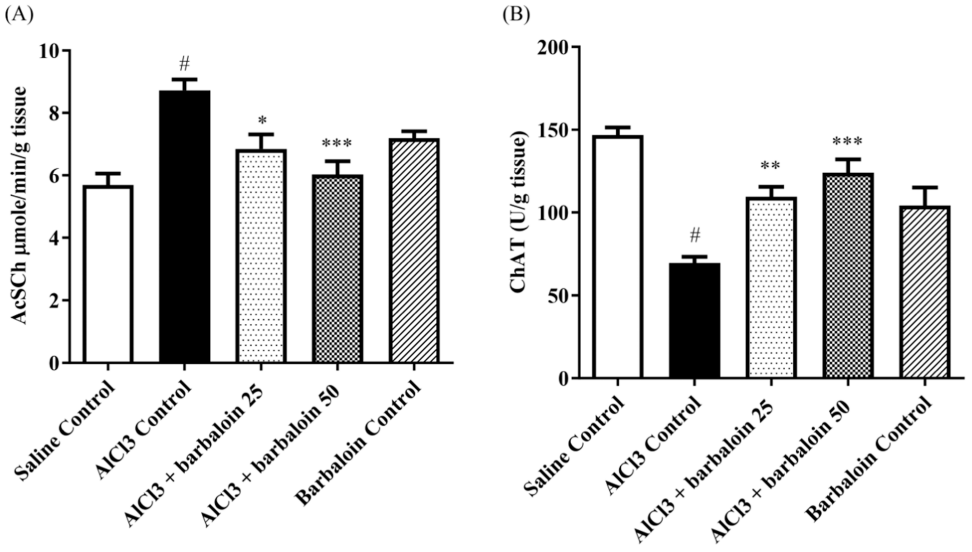

Figure 4. (A, B) Effect of barbaloin on AchE and ChAT content. Mean ± SEM (n = 6). #P < 0.001 vs saline control, *P < 0.05, **P < 0.01, and ***P < 0.001 vs $AlCl_3$ control. One-way ANOVA was followed by Tukey's test.

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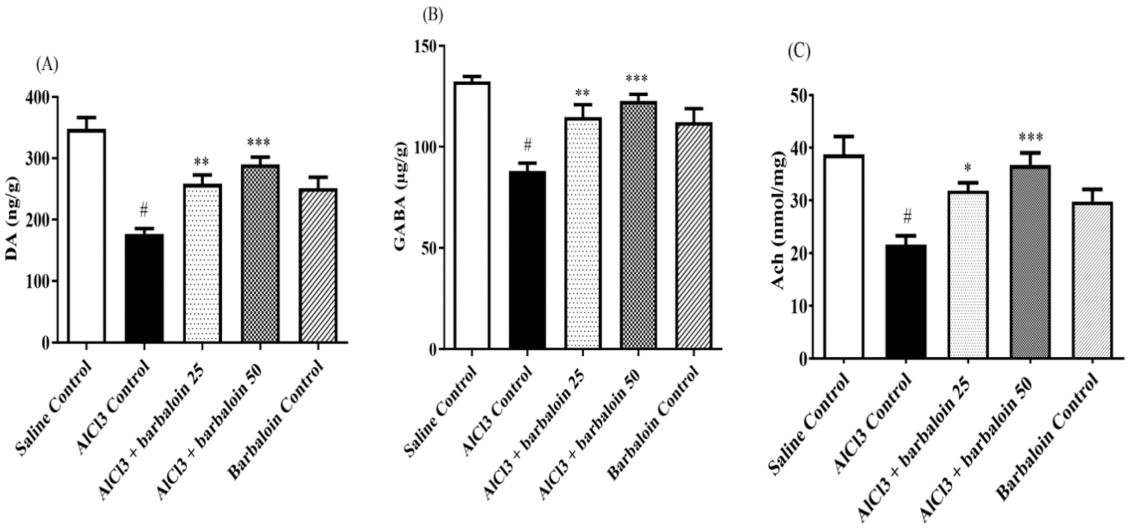

Figure 5. (A–C) Effect of barbaloin on DA, GABA, and Ach content. Mean ± SEM (n = 6). #P < 0.001 vs saline control, *P < 0.05, **P < 0.01, and ***P < 0.001 vs $AlCl_3$ control. One-way ANOVA was followed by Tukey's test.

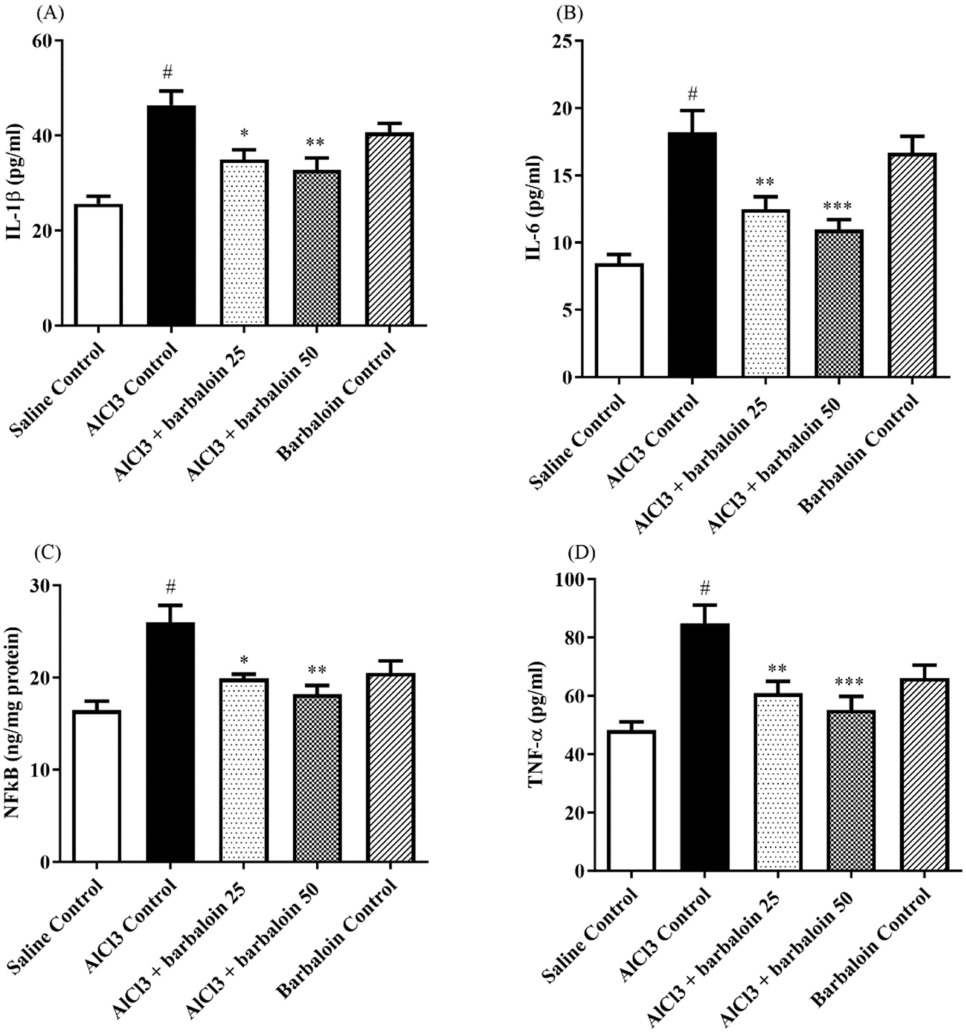

Figure 6. (A–D) Effect of barbaloin on cytokine IL-1β, IL-6, NF-κB, and TNF-α. Mean ± SEM (n = 6). #P < 0.001 vs saline control, *P < 0.05, **P < 0.01, and ***P < 0.001 vs $AlCl_3$ control. One-way ANOVA was followed by Tukey's test.

3.5. Neuromodulatory Cytokines. Cytokines IL-6, IL-1β, NF-κB, and TNF-α were prominently enhanced in $AlCl_3$ control rats when compared to saline control rats (P < 0.0001). Administration of barbaloin suggestively declined the IL-1β [F (4, 25) = 12.16, P < 0.0001], IL-6 [F (4, 25) = 13.48, P < 0.0001], NF-κB [F (4, 25) = 8.770, P = 0.0001], and TNF-α

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[F (4, 25) = 9.153, P = 0.0001] levels as compared to $AlCl_3$ control rats. Figure 6A–D depicts the results obtained from IL-1β, IL-6, NF-κB, and TNF-α tests.

3.6. BDNF Proteins. When compared to the saline control group, the rats' brains expressed levels of BDNF (P < 0.0001) that were significantly lowered in $AlCl_3$ control rats. Conversely, the doses of barbaloin considerably enhanced the protein expression/level of BDNF in $AlCl_3$-received animals [F (4, 25) = 9.158, P = 0.0001] in comparison to $AlCl_3$ control rats. Figure 7 expressed as BDNF proteins.

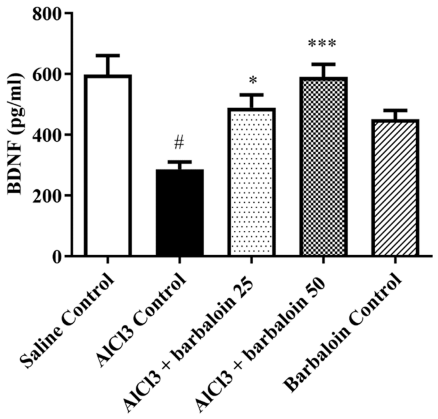

Figure 7. Effect of barbaloin on the BDNF level. Mean ± SEM (n = 6). #P < 0.001 vs saline control, *P < 0.05 and ***P < 0.001 vs $AlCl_3$ control. One-way ANOVA was followed by Tukey's test.

4. DISCUSSION

The primary aim of this research was to examine the potential neuroprotective impact of barbaloin in $AlCl_3$-induced AD rats on the cognitive deficits and changes in rats through modulation of oxidative stress, cytokines, and protein expression BDNF. According to the results, the administration of barbaloin resulted in notable enhancements in learning and memory performance in MWM tasks. Additionally, notable alterations were observed in the concentrations of GSH, MDA, CAT, and SOD, indicating alterations in the oxidative stress markers. Furthermore, barbaloin treatment led to improvements in IL-6, TNF-α, NF-κB, IL-1β, and BDNF, which are associated with various cellular processes. Moreover, barbaloin administration also resulted in considerable improvements in the levels of neurotransmitters such as DA, Ach, and GABA. These improvements were found to be disrupted by the consumption of $AlCl_3$.

Metals have been widely recognized as contributing factors to the development of neurodegeneration through oxidative stress. In recent studies, there has been a growing association between $AlCl_3$ and several neurological illnesses, including AD. $AlCl_3$ finds extensive application in the chemical industry for the synthesis of various products such as lubricants, paints, rubber, wood preservatives, antiperspirants, insecticides, and medicines. Moreover, it should be noted that $AlCl_3$ possesses harmful properties and has the potential to enter the food chain via consumption of contaminated drinking water and dietary intake.[50,51] Al is widely recognized as a neurotoxic substance capable of inducing neurodegeneration and symptoms resembling those of AD. Al induces these effects through many methods, including the promotion of oxidative stress,[52−54] increased Aβ deposition,[53,55] neuroinflammation,[17] and apoptosis.[15,52,56] When compared to the control group, $AlCl_3$-induced AD rats displayed a progressive increase in the escape latency time, indicating cognitive impairment (learning faults and a drop in memory function). The outcome aligns with earlier research on $AlCl_3$-induced AD rats.[52,53,56,57] In the current investigation, it was observed that the administration of barbaloin therapy resulted in a considerable reduction in escape latency during the learning phase. Additionally, the subjects allocated more time to the target quarter during the probe experiment.

Oxidative stress is a significant factor in the advancement and emergence of AD due to its exacerbation of Aβ and tau protein accumulation, impairment of mitochondrial activity and energy production, and antioxidant enzymes like CAT and SOD being depleted.[58] The addition of $AlCl_3$ results in a substantial elevation of oxidative stress,[59] hence triggering the synthesis of Aβ and subsequent accumulation within the hippocampus of transgenic mice, ultimately contributing to the development of AD.[60] The presence of oxidative stress was demonstrated by the increased concentrations of MDA in conjunction with a reduction in the levels of the antioxidants SOD and GSH. These findings are consistent with the research conducted by Auti and Kulkarni.[53] Justin Thenmozhi et al.[52] Based on the findings of our investigation, it is conceivable to suggest that the administration of $AlCl_3$ has the potential to modify the state of oxidative stress. The results of this investigation indicate that $AlCl_3$-induced AD rats can lead to reduction in the GSH, SOD, and CAT activities while increasing the amount of MDA, which serves as an indicator for lipid peroxidation. The administration of barbaloin resulted in a considerable increase in the SOD, GSH, and CAT activities while concurrently decreasing the MDA concentration.

Al has the potential to interfere with cellular and metabolic functions, such as neurotransmission,[61] and previous research conducted on rats has demonstrated that the administration of Al has been found to interfere with the functioning of the cholinergic system, resulting in the manifestation of neurobehavioral abnormalities.[62] There have also been documented cases of cholinotoxicity induced by heavy metal exposure.[63] In this work, the concentration of AChE was higher and lower ChAT in the $AlCl_3$-induced AD rats, whereas treatment with barbaloin rats showed significantly lower AChE levels and higher ChAT levels in comparison to $AlCl_3$-induced AD rats. AChE plays a pivotal role in the transmission of cholinergic neurotransmitters and is closely linked to regulatory functions and neurobehavioral processes.[64,65]

Several studies have suggested that many neurotransmitters play a critical role as neuroregulators and that pathological changes in these neurotransmitters lead to cognitive decline and behavioral deficits in AD.[66−68] The cognitive deficits and deterioration observed in AD are closely associated with the neuronal degradation and hypofunction of cholinergic neurons, making them the focal point of the "cholinergic hypothesis" among all neurotransmitters.[69] DA is generated within neurons located in the mesencephalon and subsequently diffuses to various regions of the brain including the hippocampus, cortex, and basal ganglia. The presence of dopaminergic disorders has been identified as a contributing factor in the pathogenesis of cognitive loss observed in individuals with signs of AD.[70] GABA holds significant prominence as a crucial inhibitory neurotransmitter inside the central nervous system. Severe cases of AD have been associated with notable decreases in

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GABA concentration, potentially contributing to the manifestation of behavioral and psychiatric symptoms observed in AD.[71] In the current investigation, the $AlCl_3$-induced AD rats were found to decrease the levels of DA, Ach, and GABA in the brain of rats. However, treatment with barbaloin was observed to restore the alterations in DA, Ach, and GABA levels.

The earliest event in AD that is responsible for its abnormal occurrence is inflammation. A transcription factor called NF-κB plays a key role in activating several genes related to inflammation. In neurodegenerative diseases such as AD, damage causes NF-κB to be activated, especially in a particular region of the brain. During the process of neuroinflammation, the nuclear localization of NF-kβ is achieved through the phosphorylation of IκBα. This phosphorylation event leads to the activation of transcription, namely, of proinflammatory cytokines such as TNF-α, IL-6, IL-1β, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2). Consequently, this activation initiates an inflammatory response.[51,72] In contrast, our findings indicate that barbaloin administration exerts a significant inhibitory effect on the $AlCl_3$-induced AD rats elevated levels of IL-6, IL-1β, TNF-α, and NF-κB, which are recognized as inflammatory indicators.

The administration of $AlCl_3$ resulted in a decrease in BDNF expression, consistent with a prior investigation that documented a lower level of BDNF expression in a rat model of AD produced by aluminum. The work elucidated the mechanism by which BDNF occurs, attributing it to synapse loss resulting from the toxic effects of Aβ.[53,73] The current study's findings demonstrated that barbaloin can strongly prevent $AlCl_3$-induced reductions in the BDNF protein level. Based on this evidence, barbaloin may inhibit $AlCl_3$-induced cognitive deficits and changes in rats through modulation of oxidative stress, cytokines, and protein expression BDNF.

5. CONCLUSIONS

In summary, the compound known as barbaloin has been found to mitigate cognitive deficits generated by $AlCl_3$. This effect is achieved by decreasing oxidative stress, which is activated by the NF-κβ pathway, cytokines, and the BDNF route. It is hypothesized that the high antioxidant properties of barbaloin contribute to this mechanism of action. The findings of this study indicate that barbaloin exhibits potential as a therapeutic agent for the management of neurodegenerative conditions. Additional research is required to substantiate the potential antialzheimer properties of barbaloin in relation to alternative models of AD prior to the commencement of clinical trials.

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Notes

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