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Original Article

Neuroprotective Role of Periplocin Against Aluminium Chloride-stimulated Alzheimer's Disease in a Rat Model by Modulation of Oxidative Stress and Inflammation

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ABSTRACT

Background: Alzheimer's disease is a prevalent neurodegenerative condition marked by the assembly of ß-amyloid deposits inside the brain parenchyma. Aluminium is a neurotoxin molecule that generates oxidative stress linked to various neurological disorders. **Objectives:** This study focused on the neuroprotective effects of periplocin, a natural compound, against aluminium chloride ($AlCl_3$)-stimulated diseased rat model. **Materials and Methods:** Four groups of twenty-four Sprague Dawley rats were established. For four weeks, the rats in the control group I was administered NaCl. $AlCl_3$ was provided to the second group of rats. An hour before $AlCl_3$ induction, rats in groups III and IV were given periplocin (25 and 50 mg/kg) orally. The rats were behaviourally examined before euthanization and analysis of the biochemical parameters was carried out after their sacrifice. **Results:** The learning ability and memory were impaired, lipid oxidation (MDA) was suppressed by antioxidant regulation (Reduced glutathione, Catalase, and Superoxide dismutase), and lactate dehydrogenase, $Na^+ K^+$ ATPase, acetylcholinesterase, and nitric oxide activity was inhibited upon periplocin administration. Additionally, the concentrations of inflammatory regulators such as Il-1β, TNF-α, and IL-6 were suppressed. **Conclusion:** Thus, periplocin might be a crucial neuroprotector against the advancement of Alzheimer's disease.

Keywords: Periplocin, Alzheimer's disease, Aluminium chloride, Neuroinflammation, Acetylcholinesterase.

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INTRODUCTION

Alzheimer's Disease (AD) is a widespread neurological disorder that is marked by gradual cognitive deterioration, dementia, and memory loss that affects predominantly older individuals across the world.[1] Loss of neurons, amyloid plaques, and neurofibrillary tangles are all characteristics of AD.[2] Around 50 million people are reported to have been diagnosed with Alzheimer's disease worldwide, with the number anticipated to rise to 152 million by 2050. The disease is currently the 5th highest reasonfor mortality in adults of 65 years of age, and the 6th among people of all age categories.[3]

In the progression of AD, parts of the brain linked with memory loss and cognitive dysfunction, such as the cortical and hippocampal regions, are most impacted.[4] Several behavioural facets of human existence including memory, critical thinking, navigation, learning capacity and speed, apprehension, calculation, and judgment are considered to get affected by AD.[5] Increases in glutamate concentration, neuroinflammation, and oxidative stresshave all been connected with AD pathogenesis.[6] Considering the several risk factors linked to this disease, it is difficult to understand its multifaceted pathophysiology and clinical aspects.[7]

The metal ion equilibrium in the brain is necessary for optimal cognitive functions, and its disruption has been recognized as one of the primary issues that contribute to neurodegenerative advancement.[4] Among the environmental factors contributing to the risk of AD, exposure to metals such as aluminium is one of the widely known factors. By stimulating the immune system and releasing different inflammatory mediators, aluminium exposure causes immuno-excitotoxicity, which leads to neurotoxicity.[8]

Al has been proven to penetrate the blood-brain barrier (BBB) via a particular transferrin receptor and cause severe loss of memory by disrupting a variety of typical neuronal functioning. Aluminium can serve as a cross-linker for amyloid protein, causing it to oligomerize and cause neurotoxicity.[9]

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Current treatment for Alzheimer's disease only gives relief from the symptoms with significant adverse effects that can worsen to stroke as well as even death.[10] As a result, new medications for treating or managing AD are urgently required. The focus of worldwide therapeutic research is now focusing on herbal medicine. Herbal plants have long been employed as traditional medicine since they are inexpensive and have few adverse effects. Numerous bioactive chemicals isolated from plants have been shown to minimize neuronal injuries in recent years, and they have become an important treatment alternative for related brain diseases.[4,11]

Cortex periplocae is herbal medicine employed traditionally over the years that possesses immune system enrichment, anticancer, antiradiation, and anti-inflammatory properties.[12] Periplocin is a cardiac glycoside identified and isolated from the plant that is utilized to treat rheumatoid arthritis and bone and tendon strengthening.[13] It also has a powerful inhibitory impact on the propagation of various cancer cell lines like colon, lung, and gastric cancer.[14] Even though the effectiveness of periplocin as an anti-inflammatory drug in brain barrier tissues is yet to be determined, cardiac glycosides often possess a wide array of chemical variety as well as absorption, distribution, metabolism, elimination, and toxicity characteristics.[15]

With this background, the primary purpose of the current study is to assess the neuroprotective potential of periplocin against $AlCl_3$-stimulated oxidative damage, cognitive decline, transmembrane activity, and neuroinflammation in Sprague Dawley (SD)rats.

MATERIALS AND METHODS

Reagents and chemicals

For this study, periplocin from an analytical standard was bought. $AlCl_3$ was prepared in 0.9 percent saline, BCA protein assay kit, antioxidant ELISA kit Griess reagent, RNase, and Trizol for RNA isolation, Lactate dehydrogenase (LDH) detection kit, dinucleotide triphosphates, Taq polymerase, and reverse transcriptase enzyme were all purchased from Sigma-Aldrich. The other chemicals employed in the study were of analytical quality.

Animal model

Sprague Dawley rats of age 7–9 weeks and weight 170–190 g, were caged and provided tap water and pellets in a contained environment. Before the experiment, the animals were familiarized with the experimental environment for 7 days in the animal housing. The procedures were carried out to ensure that the experimental rats suffered as little as possible, and ethical approval for the same was obtained by the Institutional Animal Ethics Committee (IAEC).

Experimental design

The experimental animals were categorized into treatment groups before starting the experiment. As a result, the rats were separated into 4 groups, each with six rats. The rat's body weight was taken into account while administering the dose (B.W.). The animals in group I were given 0.1 percent NaCl for the duration of the trial (4 weeks) and were considered the control group. The rats in group II were administered $AlCl_3$ (100 mg/kg b.wt) dissolved in water. Periplocin (25 mg/kg and 50 mg/kg b.wt) was given orally to rats in groups III-IV, one hour before $AlCl_3$ stimulation. The animal groupings for various treatments are as follows: Group I –Control (0.1% NaCl) for 4 weeks; Group II – 500 ul of $AlCl_3$ (100 mg/kg b.wt) for 4 weeks; Group III – Periplocin (25 mg/kg b.wt) treatment 1h before $AlCl_3$ (100 mg/kg b.wt) exposure for 4 weeks; Group IV– Periplocin (50 mg/kg b.wt) treatment 1h before $AlCl_3$ (100 mg/kg b.wt) exposure for 4 weeks.

Food ingestion and weight fluctuations in the rodents were monitored daily. Before being sacrificed, all of the rats were put through behavioural testing. Pentobarbital sodium (40 mg/kg) was utilized to anesthetize the rats, and brain and blood samples were rapidly collected for biochemical investigation.

Estimation of behavioural indices

To study the memory and learning potential of rodent models, behavioural investigations employing the maze test were conducted. To perform the tasks in the rats, the RAM and MWM tests were employed.

Radial arm maze (RAM) test

As per the protocol of Foyet *et al.*[16] the RAM test was carried out with minor adjustments. Experimental rats were fed a rigorous diet to promote appetite during the study. In a fixed layout in the room, many visual extra-maze signals were supplied at a distance of 10–30 cm to the maze. At the termination of each arm, around feeding well (0.5 cm depth with a 1 cm diameter) was placed. The animals were stationed in the stage centre with feed pellets on the first day. The rats were then permitted to run to the baited arms for eating for the following five days. On the eighth day, the rats were subjected to the same training approach and were given reference and working memory exercises. The measurements to be recorded were the count of reference memory errors (RMEs) and the count of working memory errors (WMEs).

Morris water maze (MWM) test

The MWM assessment was carried out by following the protocol of Ahmed and Gilani with minimal modifications.[17] For the investigation, a spherical stage with a puddle of water was established at the base. Before the treatment, the animals were permitted to swim from different places to the platform's end, and the duration it took them to get out of the water (Escape Latency-

ELT) was recorded. The rats' ELT to turn up at the platform after treatment was noted and plotted against the training day number.

Tissue homogenate

The brain sample was divided into 2 equal halves. One-half of the sample was preserved in saline (10%) for biochemical investigation, while the other portion was preserved in formaldehyde (4%) for histological investigation. The tissues were subjected to homogenization in PBS solution and centrifuged for 20 min at maximum speed (4°C) for biochemical investigation. The protein concentrations in the resultant supernatant were estimated with the BCA protein assay kit.

Estimation of oxidative-antioxidative indicators

Colorimetric and antioxidant ELISA test kits were utilized to analyse the homogenate oxidative stress marker, i.e., Malondialdehyde along with the antioxidant markers, i.e., glutathione, catalase, and superoxide dismutase (SOD), respectively. The complete protocols were carried out as per the manufacturer's guidelines.

Malondialdehyde (MDA)

The protocol was provided by Jain *et al.*[18] was utilized to estimate the tissue MDA concentration. PBS, BHT, and 30% TC were combined with the homogenate solution. The reaction mix was subjected to centrifugation (20 min, 3000 Xg) after a 1hr incubation at 37°C. After that, EDTA and 1% TBA were added to the supernatant. For at least 20 min, this mix was left for incubation at 80°C in a water bath. MDA was measured at 532 nm and represented as nmol/g of wet tissue.

Superoxide dismutase (SOD)

The SOD enzyme was calculated using the approach of Marklundand and Marklund.[19] The concentration of SOD was primarily assessed by its capacity to block superoxide-related decrease. The quantity of enzyme necessary to combat pyrogallol oxidation by 50% was considered to be one unit of SOD. SOD activity was measured in units/mg of protein.

Catalase (CAT)

The activity of CAT was estimated using Aebi's technique.[20] The quantity of enzyme required to breakdown 1mol of hydrogen peroxide (H_2O_2) per minute was defined as one CAT unit. The CAT enzyme activity was measured in units per milligram of protein.

Reduced glutathione (GSH)

The GSH level was determined using the Aykac *et al*. technique.[21] The reaction solution contained a combination of homogenate supernatant (2 ml), Millipore water (8 ml), and 1M NaCl (100 ml, pH 4.7). The activity was measured in nmol/mg protein and the absorbance was determined at 400 nm.

Estimation of lactate dehydrogenase (LDH)

An LDH toxicity detection kit was used to measure periplocin's tissue damage activity. In 0.1 M of K_3PO_4 buffer, the supernatant of homogenate was combined with Triton X-100 (6%, 40 µl) and 4.6 mM pyruvic acid (100 µl, pH 7.5). Using an ELISA microplate reader, the absorbance was determined at 340 nm. With the equation given below, the LDH release was calculated as: LDH leakage (%) = [A] sample / [A] control x 100; [A] Sample: Sample absorbance value. [A] Control: Control absorbance value.

Estimation of nitric oxide (NO) in $AlCl_3$triggered rats

The concentrations of NO were measured using the Griess reagent, as described by Tracey *et al.*[22] After dilution with PBS, the tissue homogenates were plated on a 96-well plate and left for incubation at 25°C for 15 min. A spectrophotometer was employed to measure the absorbance value against a blank at 540 nm.

Measurement of acetylcholinesterase (AchE) enzyme activity

Ellman's approach[23] was adopted to determine the AchE activity. Tris–HCl (50 mM, 170 ml), supernatant (25 and 50 mg/kg, 250 ml), AchE (10 ml, 6.67 U ml^{-1}), and DTNB (5,5′-dithio-bis [2-nitrobenzoic acid]) (20 ml, 10 mM) in buffer were combined. After that, the reaction solution was incubated for 10 min at 37°C. The absorbance was determined at 412 nm after the addition of acetylthiocholine iodide (200 mM, 10 µl). The percentage of enzyme inhibition was estimated as follows: Inhibition percentage = 100 - Change of Absorbance of test/Change of Absorbance of blank × 100

Estimation of the transmembrane protein activity

The activity of the Na^+/K^+ ATPase was evaluated by following the protocol of Li *et al.*[24] The homogenate supernatant was left for incubation at 37°C in a mix of 0.2 ml each of buffer, distilled water, calcium chloride, and ATP. The mixture was then incubated for another 10 min at 37°C with 20% TCA (2 ml). Upon addition of ammonium molybdate (1 ml) and 4-aminonaphthol sulphonic acid (0.8 ml), a blue tint appeared. The resultant mixture's absorbance was analyzed at 650 nm.

Estimation of inflammatory cytokines in $AlCl_3$triggered rats

ELISA was utilized to study the levels of inflammation-associated cytokines, Il-1β, IL-6, and TNF-α. We performed ELISA tests on the diluted brain tissue extract to assess the concentrations of IL-1β (ab100768), IL-6 (ab100772), and TNF-α (ab100785) using abcam ELISA kits (abcam, USA) according to the manufacturer's instructions. The detection limits of each were 68.59 pg/ml -

50000 pg/ml for IL-1β, 40.96 pg/ml - 10000 pg/ml for IL-6, and 82.3 pg/ml - 20000 pg/ml for TNF-α.

Statistical analysis

The results were calculated with SPSS software (version 17.0) and displayed as the mean ±SD of triplicate trials. ANOVA was used to compare the experimental groups, followed by the Student-Newman-Keuls multiple comparison test. Statistical significance was assessed at $p<0.05$.

RESULTS

Impact of Periplocin treatment on behavioural parameters in $AlCl_3$ provoked animals

On the behavioral parameters of rats stimulated with $AlCl_3$ and treated with periplocin, the mean time taken to complete the maze was measured and compared between groups (Figure 1). The impact of Periplocin treatment on RMEs, WMEs, and TL time in the control and treated groups are depicted in Figure 2. In comparison with the group I animals, the RMEs, WMEs, and TL values of $AlCl_3$ induced rats were considerably higher to perform the tasks. When compared to $AlCl_3$-triggered rats, periplocin treatments at 25 mg/kg and 50 mg/kg resulted in a substantial decrease in all variables.

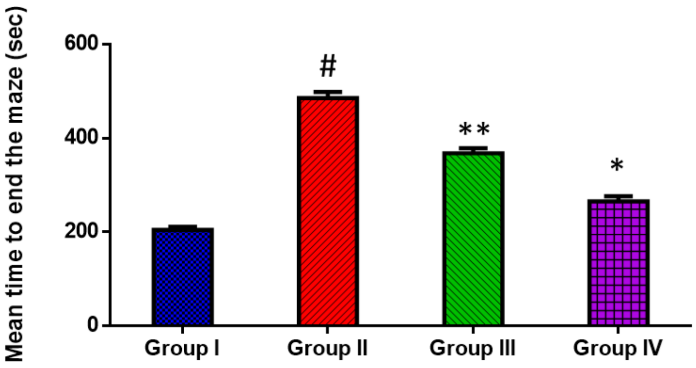

Figure 1: The mean total time to complete the maze in the periplocin treatment in $AlCl_3$-stimulated rats.
Periplocin was administered (25 and 50 mg/kg) to the rats in order to measure behavioural parameters and determine total time spent in the maze. The data are represented as Mean ± SD (*$p < 0.05$; ** $p< 0.005$, #$p < 0.001$).

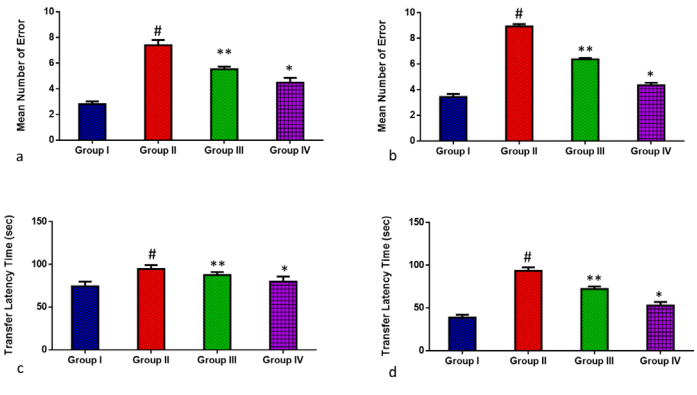

Figure 2: The impact of periplocin treatment on the behavioral parameters in $AlCl_3$-stimulated rats.
The change in reference memory errors (a), working memory errors (b), initial transfer latency time (c), and Retention transfer latencies time (d) by periplocin administration (25 and 50 mg/kg) are given. The data are represented as Mean ± SD (*$p < 0.05$; ** $p< 0.005$, #$p < 0.001$).

Impact of Periplocin administration on oxidative stress levels in $AlCl_3$ provoked animals

The impact of Periplocin treatment on oxidative stress levels was estimated by determining the MDA and antioxidant enzyme activities in brain samples, such as CAT, GSH, and SOD in the control and treated samples (Figure 3). The MDA levels of $AlCl_3$ administered to animals were substantially greater than in control rats, whereas Periplocin (25 and 50 mg/kg) administration significantly reduced the MDA level (Figure 3a). In contrast, the CAT, GSH, and SOD levels in the $AlCl_3$ administrated rats were considerably lesser than in the control samples (Figure 3b-3d). The findings may propose periplocin's remarkable antioxidant alleviation impact on $AlCl_3$-stimulated oxidative stress in rodents.

Impact of Periplocin administration on neuronal damage in $AlCl_3$ provoked animals

Figures 4a and 4b demonstrate the effect of Periplocin administration on neuronal damage as measured by LDH and NO activities in the tissue samples of the control and treated groups. When comparing the $AlCl_3$ induced group with the control rats, the data revealed that LDH and NO activity were considerably higher in the $AlCl_3$ induced animals. In comparison with the $AlCl_3$ induced animals, rodents treated with Periplocin at both concentrations demonstrated a reversed attenuated impact of LDH and NO activity with a notable difference.

Impact of Periplocin administration on cholinergic functioning in $AlCl_3$ provoked animals

The activity of Na^+ K^+ ATPase and AchE in the tissue samples of control and treated groups is shown in Figure 5 to evaluate the

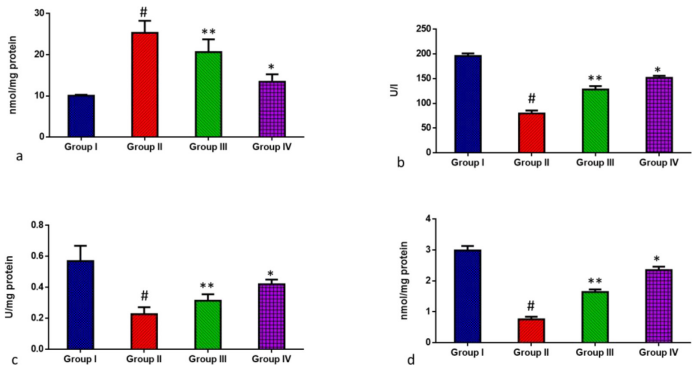

Figure 3: The oxidative stress level of periplocin in $AlCl_3$-stimulated rats.
The change in markers Malondialdehyde (a), Superoxide dismutase (b), Catalase (c), and Glutathione (d) by periplocin administration (25 and 50 mg/kg) are shown. The data are represented as (*$p < 0.05$; ** $p< 0.005$, #$p < 0.001$).

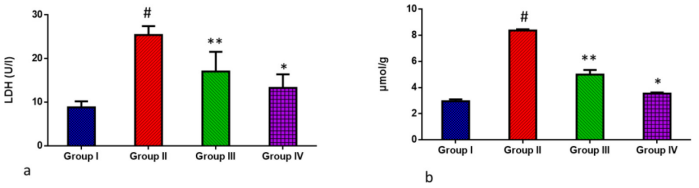

Figure 4: The neuronal damage effects of periplocin in $AlCl_3$-stimulated rats. The activities of lactate dehydrogenase (a) and Nitrite Oxide (b) by periplocin administration (25 and 50 mg/kg) are depicted. The data are represented as Mean ± SD (*$p < 0.05$; ** $p < 0.005$, #$p < 0.001$

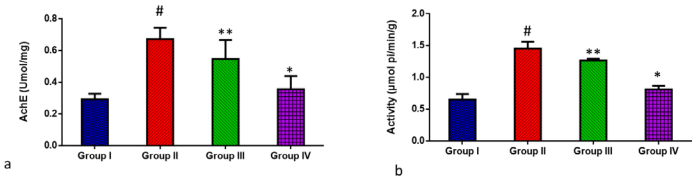

Figure 5: The impact of periplocin administration on cholinergic functioning in $AlCl_3$ provoked animals.
The activities of acetylcholinesterase (a) and $Na^+ K^+$ ATPase (b) by periplocin administration (25 and 50 mg/kg) are illustrated. The data are represented as Mean ± SD (*$p < 0.05$; ** $p < 0.005$, #$p < 0.001$).

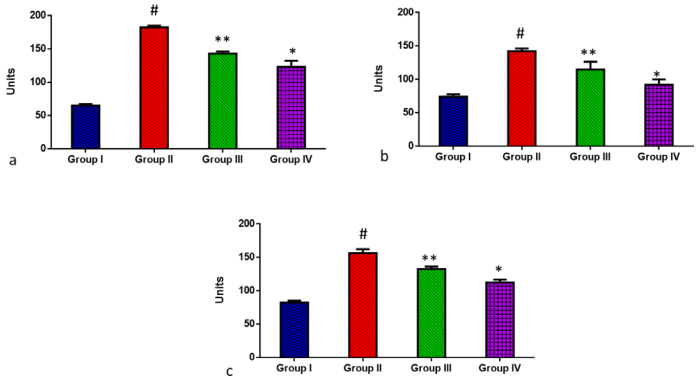

Figure 6: The impact of periplocin administration on inflammation-related mRNA gene expression in $AlCl_3$ provoked animals such as TNF-α (a), IL-6 (b) and IL-1β (c).
The gene estimation by periplocin administration (25 and 50 mg/kg) is shown. The data are represented as Mean ± SD (*$p < 0.05$; ** $p < 0.005$, #$p < 0.001$).

influence of Periplocin administration on cholinergic function. The levels of $Na^+ K^+$ ATPase as well as AchE were increased in tandem in animals from $AlCl_3$ provoked and control group, as shown in Figure 5a and 5b, with a notable difference between both the groups. Following that, the AchE and $Na^+ K^+$ ATPase levels in rats were considerably decreased by Periplocin treatment (25 and 50 mg/kg). This is due to the increase in cholinergic functioning by periplocin administration, which is crucial for memory, learning, and motor function in the brain.

Impact of Periplocin administration on inflammatory cytokines expression in $AlCl_3$ provoked animals

The influence of Periplocin administration on the expression of inflammation-associated signalling pathways in control and treated animals is shown in Figure 6. In comparison with the control group, the levels of pro-inflammatory molecules such as TNF-α, IL-6, and IL-1β exhibited a tremendous increase in the $AlCl_3$ provoked rats. In animals from Groups III and IV, however, Periplocin administration (25 and 50 mg/kg) dramatically reversed the $AlCl_3$ impact.

DISCUSSION

The major clinical sign of Alzheimer's disease has been identified as cognitive deficits along with serious health issues caused by Al exposure. Al-stimulated neurochemical and biochemical aberrations have been linked to cognitive deficits, and comparable abnormalities have been observed in Alzheimer's disease.[25] Many age-related problems and neurodegenerative diseases are driven by enhanced oxidative stress, therefore the search for natural medicinal agents that boost cognitive function and enable neuroprotective effects via antioxidant action is of significant interest.[26,27] In the current investigation, periplocin was administered to $AlCl_3$-induced rats to determine whether it might alleviate behavioural, biochemical, and neurochemical problems, as well as to evaluate its potential as a neuroprotective drug against AD.

An association between aluminium-induced neurotoxicity and AD pathogenesis has been proposed in prior studies.[28] Long-term exposure to aluminium has been shown to produce disorientation, memory problems, and eventually dementia due to the brain's poor clearance of aluminium.[29,30] Increased AchE activity, oxidative stress, tau protein a reduction in BDNF, and aggregation of Aβ plaquesare among the additional variables that mediate aluminium contribution in AD pathogenesis.[31] This encouraged us to explore the effect of periplocin in an $AlCl_3$-stimulated rat model. Aluminium induction hampered the animal's capacity to learn as well as remember the platform's position in both maze examinations, most likely owing to a hippocampal deficiency. Interestingly, periplocin administration inverted this impact and increased the rats' learning capabilities and spatial memory. Black pepper has been demonstrated to improve memory in the $AlCl_3$ stimulated neurotoxicity animal model in previous investigations.[32]

The treatment of $AlCl_3$ resulted in a considerable increase in the duration necessary to complete the activity in the radial arm maze, indicating a reduction in working and reference memory.[4] In rats, increased Al intake causes Aβ build-up in the cortical and hippocampal brain areas, resulting in memory and learning impairments.[33] The Morris maze was employed to assess spatial memory, and animals administered with $AlCl_3$ alone had a lower likelihood to recall the position of the hidden platform, even after being instructed over several weeks. The memory deficit caused by $AlCl_3$ was reversed when periplocin was administered, demonstrating an elevated memorizing capacity of periplocin.

An aged brain is susceptible to oxidative stress because of its high propensity for oxygen uptake, elevated iron content, polyunsaturated fatty acid concentration in the membranes, low glutathione levels, and limited antioxidant defence capabilities.[31] Also, the brain is considered to be highly vulnerable to the negative impact of aluminium, and it is particularly susceptible to oxidative stress caused by excessive concentrations of free radicals along with inadequate levels of antioxidants.[34] As a result, the oxidant-antioxidant imbalance has been acknowledged as a crucial component in the progress of AD. Prolonged treatment of $AlCl_3$ led to elevated oxidative stress, as evidenced by a significant rise in lipid peroxidation as well as nitrite concentrations, along with a reduction in superoxide dismutase, reduced glutathione, and catalase levels in our study.

Aluminium-stimulated reduction in both axonal mitochondrial turnover and synaptic vesicles trigger oxidative metabolite release in neurons, which might explain the apparent increase in MDA levels and reduction in GSH concentration caused by $AlCl_3$ administration.[35] MDA and NO levels in living organisms are key markers of oxidative stress. MDA is formed when ROS induces peroxidation of membrane lipids, resulting in membrane damage and degradation. NO is a neurotransmitter in the central nervous system, yet it is neurotoxic when it is produced in excess.[26,36] By clearing up the augmented ROS in the tissue samples, the combination therapy of $AlCl_3$ along with periplocin revived the oxidative stress indicators. Furthermore, elevated lipid peroxidation as a result of oxidative stress might explain the reduction in brain weight.[37]

The pathophysiology of Alzheimer's disease is strongly linked to an uncontrolled neuroinflammatory process, like the overexpression of inflammatory mediators like IL-1β, which in turn suppresses the formation of the brain-derived neurotrophic factors responsible for the conduct of physiological functions in the CNS.[38,39] Furthermore, patients with high levels of $AlCl_3$ demonstrated cognitive deterioration, which has been linked to impaired pro-inflammatory regulators including TNF-α.[31] Cardiac glycosides like periplocin may be beneficial in addressing inflammation at the brain's periphery, opening up new avenues for medication development for neuroinflammatory degeneration.[28] In the present investigation, the overproduction of TNF-α, IL-1β, and IL-6 in the tissue homogenate of aluminium-intoxicated rodents treated with periplocin was significantly suppressed, which indicates its potent anti-inflammatory effect.

AchE is a broadly distributed particularly potent enzyme in the brain that has several roles in cholinergic and neuromuscular synapses and is linked with building and preserving learning memory in the brain.[40] Owing to the action of transmembrane protein Na^+ K^+ ATPase, AchE is responsible for maintaining the cholinergic membrane stability. Memory loss in AD is connected to the malfunction of cholinergic transmission.[41] The treatment of $AlCl_3$ to rats led to a considerable elevation in AchE and transmembrane protein activity in our investigation. Periplocin administration in the $AlCl_3$-induced animals demonstrated a neuroprotective effect via inhibiting the AchE and transmembrane protein activity. Similarly, the suppressive action of a natural compound, fenugreek along with its active component on AchE has been discovered.[42] The amount of acetylcholine is elevated when the AchE potential is reduced, which ultimately has positive implications on cognitive performance.[2]

CONCLUSION

Prolonged exposure to aluminium chloride may promote lipid peroxidation due to ROS production, resulting in increased ACHE activity and lower acetylcholine levels, impairing memory as well as cognition. In the present investigation, Periplocin was found to attenuate $AlCl_3$-induced learning deficits, tissue damage, and cholinergic dysfunction via suppression of reactive free radicals and inflammatory mediators, owing to periplocin's powerful antioxidant and anti-inflammatory potential. These findings propose that Periplocin may be a possible therapeutic option for neurodegenerative diseases. However, before clinical trials, further research is required to confirm periplocin's neuroprotective activity in various Alzheimer's disease models.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

IAEC: Institutional Animal Ethics Committee; **RAM:** Radial arm maze; **MWM:** Morris water maze; **MDA:** Malondialdehyde; **SOD:** Superoxide dismutase; **GSH:** Reduced glutathione.

SUMMARY

- Periplocin is a cardiac glycoside identified and isolated from the *Cortex periplocae.*
- It exihibits Neuroprotective against aluminium chloride ($AlCl_3$)-stimulated diseased rat model.
- On treating the aluminium chloride ($AlCl_3$)-stimulated Alzheimer's disease with periplocin, the behavioral study and MDA level were significantly suppressed.
- Periplocin treatment suppressed lactate dehydrogenase, Na^+ K^+ ATPase, acetylcholinesterase, and nitric oxide activity as well as antioxidant regulation such as Reduced glutathione, Catalase, and Superoxide dismutase.

- The suppression of inflammatory regulators like Il-1β, TNF-α, and IL-6 were observed on treatment with periplocin.

Author Contribution

The authors contributed equally.

Ethics Approval

All work has been done under the guidelines of the Institutional Ethics Committee.

Consent to Participate

All authors have their consent to participate.

Consent for Publication

All authors have their consent to publish their work.

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