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Original Article

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

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ABSTRACT

Aim/Background: Alzheimer's Disease (AD) is a progressive neurodegenerative disorder with limited therapeutic options. Tephrosin (TP), a natural rotenoid, exhibits antioxidant and anti-inflammatory properties. This study investigated the neuroprotective effects of TP in an Aluminum Chloride ($AlCl_3$)-induced rat model of Alzheimer-like pathology. **Materials and Methods:** Twenty-four adult rats were divided into four groups (n=6): Control, $AlCl_3$, $AlCl_3$ + TP, and $AlCl_3$ + Donepezil. AD-like symptoms were induced by oral $AlCl_3$ (100 mg/kg) for six weeks. TP (10 mg/kg) or Donepezil (1 mg/kg) was administered orally during the last three weeks. Behavioral performance was evaluated using the wire hang and beam walking tests. Acetylcholinesterase (AChE) activity, oxidative stress markers [Malondialdehyde (MDA), Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD)], and pro-inflammatory cytokines [Interleukin-1β (IL-1β), Tumor Necrosis Factor-α (TNF-α)] were measured. Hippocampal histology was assessed using hematoxylin and eosin staining. **Results:** $AlCl_3$ exposure impaired motor function, increased MDA and AChE activity, elevated IL-1β and TNF-α levels, and caused hippocampal neuronal degeneration, while reducing GSH, CAT, and SOD activities. TP treatment significantly improved behavioral outcomes, restored antioxidant enzyme levels, reduced oxidative stress and inflammatory markers, and preserved hippocampal neuronal integrity. **Conclusion:** Tephrosin exerts neuroprotective effects against $AlCl_3$-induced Alzheimer-like pathology, likely via its antioxidant, anti-inflammatory, and anti-cholinesterase activities. These findings support TP as a promising candidate for AD therapy.

Keywords: $AlCl_3$, Alzheimer's Disease, Tephrosin, Oxidative Stress.

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INTRODUCTION

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia, accounting for 60-80% of cases globally.[1] It is estimated that by 2030, AD will affect more than 66 million people, and the number is projected to rise to 115 million by 2050.[2] AD is characterized by gradual memory loss, cognitive decline, and behavioral disturbances that eventually interfere with speech, motor function, and visuospatial orientation. Although dementia encompasses multiple forms, including Lewy body dementia, Parkinson's disease with dementia, frontotemporal lobar degeneration, vascular dementia, and normal-pressure hydrocephalus, AD remains the most prevalent, with many cases presenting mixed pathology.[3]

The pathogenesis of AD involves a complex interplay of genetic, environmental, and lifestyle factors. Age remains the strongest risk factor, with individuals over 65 years being at significantly higher risk. The presence of the APOE ε4 allele is another critical determinant of susceptibility. Women are disproportionately affected, particularly after age 80, partly due to hormonal differences and a higher tau protein burden despite similar amyloid β levels.[3] Cardiovascular comorbidities, metabolic syndromes, and sedentary lifestyles further increase the risk of developing AD.

Neuropathologically, AD is characterized by extracellular deposition of beta-amyloid (Aβ) plaques and intracellular Neurofibrillary Tangles (NFTs) consisting of hyperphosphorylated tau proteins.[4] These pathological aggregates disrupt neuronal

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signaling, leading to synaptic loss, neurotransmitter depletion, and selective neuronal death, particularly in hippocampal and cortical regions critical for memory. Alongside these hallmark lesions, there is evidence of widespread oxidative stress and neuroinflammation, both of which exacerbate neurodegeneration.[5]

Neuroinflammation is increasingly recognized as a central feature in AD pathology. Activated microglia and astrocytes release inflammatory mediators, including cytokines and chemokines, which sustain chronic inflammation and neuronal injury. Elevated levels of Tumor Necrosis Factor-Alpha (TNF-α) and Interleukin-1 beta (IL-1β) have been observed in AD brains, indicating that inflammatory processes contribute to disease progression.[5]

Environmental factors, particularly chronic exposure to aluminum, have also been implicated in AD. Aluminum is a common neurotoxin that readily crosses the blood-brain barrier, accumulates in brain tissues, and disrupts neuronal homeostasis.[6] Experimental evidence shows that Aluminum Chloride ($AlCl_3$) induces significant neurotoxicity by altering hippocampal structure, impairing neurogenesis, and triggering neuroinflammation.[7] Prolonged $AlCl_3$ exposure leads to excessive production of Reactive Oxygen Species (ROS), lipid peroxidation, and depletion of endogenous antioxidants such as Glutathione (GSH), Catalase (CAT), and Superoxide Dismutase (SOD), thereby promoting neuronal apoptosis.[7] Due to these pathological similarities, $AlCl_3$-induced AD-like models in rats are widely used to study disease mechanisms and evaluate the efficacy of therapeutic agents.[8]

Currently available treatments for AD, such as cholinesterase inhibitors and NMDA receptor antagonists, only offer symptomatic relief and do not halt disease progression. Therefore, there is an urgent need to explore new therapeutic agents, particularly from natural sources, that can target multiple pathogenic pathways, including oxidative stress and inflammation.[9] Phytochemicals are of particular interest due to their antioxidant, anti-inflammatory, and neuroprotective properties.

Tephrosin, a naturally occurring rotenoid compound isolated from plants of the Tephrosia, Lonchocarpus, and Derris species, has been traditionally utilized for its insecticidal properties.[10] Beyond this, recent studies have highlighted its pharmacological potential, demonstrating anticancer, antioxidant, and anti-inflammatory activities.[11] Unlike rotenone, a structurally related compound known for its mitochondrial toxicity, tephrosin exhibits lower cytotoxicity, making it a promising candidate for therapeutic use.[12] However, its potential neuroprotective effects in the context of AD remain largely unexplored.

Tephrosin, a naturally occurring rotenoid isoflavonoid found in Tephrosia, Lonchocarpus, and Derris species, has been found to have multiple pharmacological activities. It has shown potent anticancer effects, including cytotoxicity in various cancer cell lines, suppression of EGFR and ErbB2 internalization in colon cancer, and resensitization in paclitaxel-resistant ovarian cancer cells.[13] Tephrosin also has antioxidant properties, supporting free-radical scavenging and enhancement of endogenous antioxidant systems. It has also been shown to inhibit inflammatory mediators and interfere with NF-κB signaling pathways, suggesting potential anti-inflammatory effects in neurodegenerative and systemic inflammation.[14]

Considering these gaps, the present study aims to investigate the therapeutic efficacy of tephrosin in an $AlCl_3$-induced rat model of AD. Behavioral assessments, biochemical analyses of oxidative stress and inflammatory markers, and histopathological examinations of hippocampal tissues were performed to evaluate whether tephrosin can mitigate neuronal damage and restore normal brain function. This study provides insight into the possible use of tephrosin as a neuroprotective agent against AD-related neurodegeneration.

MATERIALS AND METHODS

Materials

Aluminium Chloride ($AlCl_3$), Tephrosin (TP), and Donepezil (DPZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents, including solvents, buffers, and assay kits, were of analytical grade and procured from Merck (Darmstadt, Germany). Fresh drug solutions were prepared daily in distilled water prior to administration to ensure chemical stability and accuracy of dosing.

Experimental animals and design

We acquired 24 Wistar male rats from an animal hospital that weighed between 150 and 180g. The animals were kept at a consistent temperature of 23±2.0°C with a 12-hr light and dark cycle, and they had unrestricted access to food and water. The studies were carried out in accordance with the Animal Ethics Committee's Guidelines for the Use of Experimental Animals. Then the experimental animals were randomly selected and divided into 4 groups, with 6 animals in each group 6 animals. Group I (Control): Received a standard laboratory diet and distilled water orally (p.o.) for 30 days. Group II (Disease Control-$AlCl_3$): Received aluminium chloride ($AlCl_3$) at a dose of 75 mg/kg body weight, administered orally (p.o.) once daily for 30 consecutive days to induce Alzheimer-like pathology. Group III ($AlCl_3$+Tephrosin): Received $AlCl_3$ (75 mg/kg p.o.) along with Tephrosin (TP) at a dose of 30 mg/kg body weight orally (p.o.) once daily for 30 consecutive days. Group IV ($AlCl_3$+Donepezil): Received $AlCl_3$ (75 mg/kg p.o.) along with the reference standard drug Donepezil (DPZ) at a dose of 5 mg/kg body weight orally (p.o.) once daily for 30 consecutive days. All treatments were administered via oral gavage, and dosing was adjusted daily based on the individual body weight of each animal.

Preparation of brain tissues

Diethyl ether was used to sedate the animals following their exploratory period. All of the experimental animals' separated brains were placed on an ice plate and sectioned, and then filled with ice-cold isotonic saline solution. Additionally, a sharp blade was used to separate the brains into the left and right hemispheres to remove the hippocampus. Four brains' hippocampus tissues were chosen at random and prepared for histological and ultrastructural confirmation, while the remaining brains' weighted hippocampuses were homogenised using a Teflon-glass homogeniser in ice-cold PBS of 0.1 mmol/L (pH 7.4). Following a 15-min centrifugation at 10,000 rpm, the homogenates (10%, w/v) were stored at -80ºC for further biochemical analyses.

Change in body weight measurement

On the first and last day of the experiment, the weight of the animals was recorded. The body weight was determined by comparing it to the initial weight on the first day of the trial.

Wire Hang Test

The forelimb strength (muscle strength) was evaluated using this test. On day 26, the wire hang test was conducted. A wire cage was utilised for this test. On top of the wire cage, the animal was put. Subsequently, the wire cage was inverted. The wire cage was kept between 0.5 and 8.6 meters above a cushioned surface, high enough to keep the rat from jumping down but not high enough to injure it if it did. It was determined how long it took for the delay to drop from the cable to the soft, flat pad. The rat was taken off the wire, and the duration was noted as 60 sec after the hanging period had exceeded that amount of time. Each rat underwent the trial three times.

Beam walking test

In particular, the hindlimb's motor coordination was evaluated using the beam walking test. The first step involved placing the animals in a corner of the thin beam, which measured 2.3 cm by 120 cm, and letting them cross it at least three times from end to end. The rats were allowed to move over the beam for 3 min throughout the test. In each trial, the number of foot slips and the amount of time it took to traverse the beam were

AChE Activity

To measure Acetylcholinesterase (AChE), 25 µL of the supernatant was combined with 10 µL of the AChE reagent, 20 µL of DTNB (5,5′-dithiol-bis [2-nitrobenzoic acid]), and 170 µL of Tris-HCl in a test tube. The reactive solution was mixed and then incubated at 37ºC for at least 10 min. After that, 10 µL of the substrate was mixed with AChE, and the absorbance at 412 nm was determined using spectrophotometry.

Estimation of MDA

The procedure outlined was implemented with minor adjustments. Pink chromophores that absorb at 530 nm are produced when carbonyl chemicals, including Malondialdehyde, combine with Thiobarbituric Acid (TBA). 250 µL of 20% Trichloroacetic Acid (TCA), and the tubes were then sealed. After 15 min of heating at 100ºC in a water bath, it was chilled for 30 min in a cold water bath. To allow the gases produced during the reaction to escape, the tubes were left open. Following a 5-min centrifugation at 1500 rpm, the absorbance of the supernatant was measured at 532 nm in comparison to the blank.[15] Estimation of antioxidant enzymes

The technique was founded on the observation that SOD in a sample prevents adrenaline from oxidising to adrenochrome. 2.5 mL of carbonate buffer (pH 10.2) was mixed with 0.2 mL of haemolysate to equilibrate the spectrophotometer. Then, 0.3 mL of newly made adrenaline was added to the mixture to initiate the reaction. The resulting mixture was homogenised by overturn, and the intensification in absorbance was monitored by reading it at 480 nm every 30 sec until 150.[16]

The technique relied on measuring the concentration of yellow-colored TNB (5-thio-2-nitrobenzoic acid), which is produced when DTNB is used. Is reduced. The test and blank tubes were filled with 900 µL of Ellman's reagent and 100 µL of homogenate, respectively. The solution was homogenised and then placed at room temperature for half an hour. Optical densities were measured at 420 nm in comparison to a blank that contained 100 µL of 0.9% NaCl and 900 µL of reagent solution and was incubated in the same manner. Milli-moles per gramme of brain tissue (mmol/g tissue) was the unit of measurement for the concentration of decreased GSH in the brain tissues.[17]

The technique was predicated on the fact that Hydrogen Peroxide (H_2O_2) is reduced to Water (H_2O) and Oxygen (O_2) by the catalase found in the homogenate. A blue-green deposit of an unstable form of perchloric acid is created when H_2O_2 that has not been reduced by catalase binds to potassium dichromate. After that, heat broke this down to create a green complex that absorbs light at 570 nm. The calibration curve showed that the catalase activity was related to the optical density. The amount of H_2O_2 decomposed per milligram of protein was used to express the activity.[18]

Pro-inflammatory markers

Following the manufacturer's instructions, two pro-inflammatory cytokines, interleukin-1beta and tumour necrosis factor-alpha, were measured in the supernatant using readily available enzyme-linked immunosorbent assay kits (eBIOSCIENCE, San Diego, CA, USA). The results were shown in pg/mL.

Evaluation of histoarchitecture in brain tissues

The animals' brain tissues were quickly removed, and using sterile, clean scissors, they were then cleaned with ice-cold 1.15% potassium chloride. After that, tiny fragments of the hippocampal region were taken out, immediately immersed in 10% (V/V) formalin, embedded in melted paraffin wax, and stained with Haematoxylin and Eosin (H&E) staining. Next, a microtome was used to cut a little piece of tissue that contained paraffin from the wax block. A biological microscope was then used to observe the specimen slice (Chippenham, Wiltshire, UK).

Statistical analysis

Data from triplicate testing were provided as Mean±Standard Deviation (SD n=6). GraphPad Prism was used for the statistical analysis. Data are investigated using one-way ANOVA and Tukey's post hoc assay. p<0.01 and p<0.05, respectively, were considered statistically significant differences between the control group and the induced animal group.

RESULTS

Effect of TP on body weight in $AlCl_3$-intoxicated animals

Figure 1 depicts the effect of TP on body weight in $AlCl_3$-intoxicated animals. The weight of the rats in the $AlCl_3$-treated group was much lower than their starting weight (Figure 1), demonstrating the detrimental effects of $AlCl_3$ exposure on rat weight. As a result, the rats treated with $AlCl_3$ had a considerably lower final weight than the control and TP-administered groups.

Effect of TP on hanging time during the wire hang test in $AlCl_3$-intoxicated animals

Figure 2 shows the effect of TP on hanging time during the wire hang test. Based on the results, the hanging time was considerably reduced in the $AlCl_3$-treated group. The hanging time of the $AlCl_3$-treated group is lower than that of the control and TP-administered groups. Animals treated with TP demonstrated higher hanging time comparable to the animals treated with the control drug DPZ.

Effect of TP on handlimb motor coordination in $AlCl_3$ intoxicated animals

The effect of TP on the number of foot slips while walking (Figure 3a) and crossing time (Figure 3b) on the beam during the beam walking test for control and treatment groups. The number of foot slips and crossing time was high in the $AlCl_3$-administered group. Animals treated with TP demonstrated a low number of foot slips and crossing time, and the results were comparable with those of animals treated with DPZ.

Effect of TP on brain marker in $AlCl_3$-intoxicated animals

The effect of TP on AChE level in $AlCl_3$-intoxicated animals was depicted in Figure 4. Animals intoxicated with $AlCl_3$ exhibited significantly lower levels of AChE when compared to the control group. On treatment with TP, the level of AChE increased in animals intoxicated with $AlCl_3$. The level of AChE in animals administered with TP was comparable to that of animals treated with DPZ.

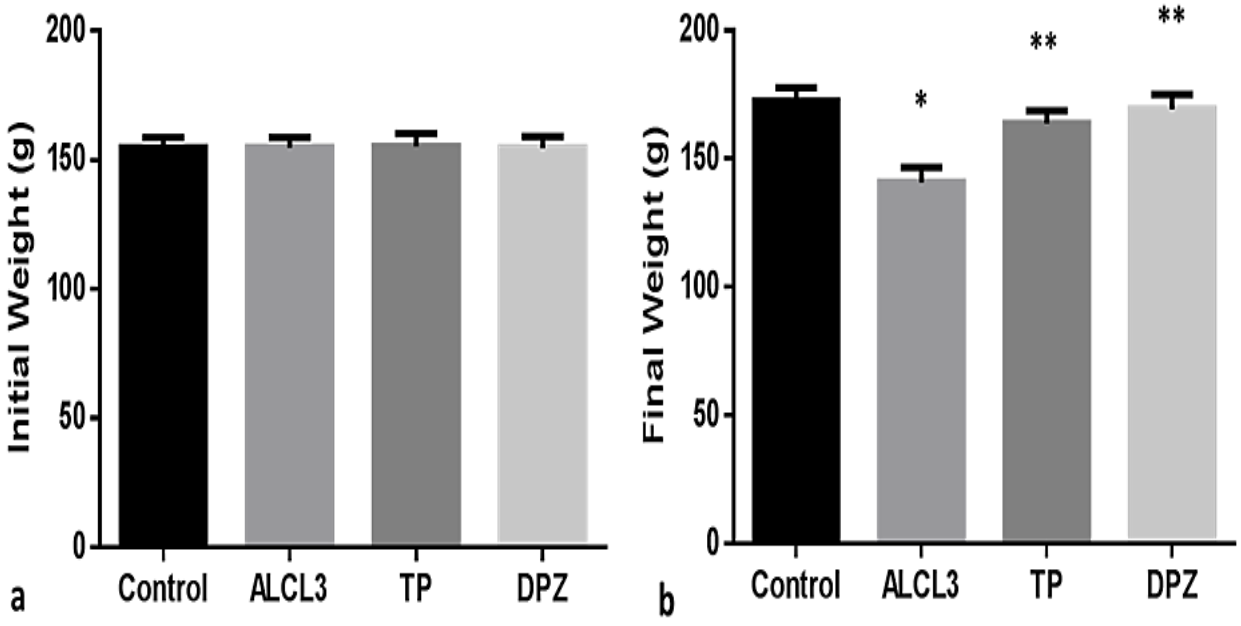

Figure 1: Effect of $AlCl_3$ on the Weight of Rats. a: Initial Weight (g) b: Final Weight (g). Results were given as mean±SD of three discrete experiments. Data are investigated using one-way ANOVA and Tukey's *post hoc* assay. '*' p<0.05 compared with control and '**' p<0.01 compared with $AlCl_3$-intoxicated group.

Effect of TP on antioxidant status in $AlCl_3$-intoxicated animals

Figures 5a-d depicts the effect of TP on antioxidant enzymes in $AlCl_3$-intoxicated animals. The level of MDA in $AlCl_3$-intoxicated animals was increased, and antioxidant enzymes, including glutathione, catalase, and SOD levels, were reduced in comparison to the control group. When the $AlCl_3$ intoxicated animals were treated with TP, the level of MDA was reduced, and antioxidant enzymes, including glutathione, catalase, and SOD levels, were significantly increased, indicating the antioxidant property of TP.

Effect of TP on pro-inflammatory cytokines in $AlCl_3$-intoxicated animals

The effect of TP on pro-inflammatory cytokines in $AlCl_3$-intoxicated animals was shown in Figures 6a and b. Animals treated with $AlCl_3$ exhibited high levels of pro-inflammatory cytokines, including TNF-α and IL-1β, whereas in the control group, the levels of TNF-α and IL-1β were low. Treatment of $AlCl_3$-intoxicated animals with TP showed a considerable drop in the level of IL-1β and TNF-α, indicating TP has anti-inflammatory action.

Effect of TP on histological changes in the hippocampus

Figures 7a-d displays the findings from the examination of histological slices of the rat brain following exposure to aluminium chloride. The control (group 1) exhibited a regular structure with complete-looking neurones in this particular brain layer, displaying the hippocampus's microarchitecture. The number of neuronal cells in the same layer of the hippocampus decreased in the $AlCl_3$-intoxicated animals (group 2), indicating a pathological alteration in comparison to the normal control. In addition to the treated TP and reference medications donepezil (groups 3 and 4), the extract improved the hippocampus structure to a level that was comparable to that of the normal control.

DISCUSSION

AD, a major healthcare concern of the twenty-first century, is getting more and more common around the world. Alzheimer's disease is a neurological condition that progressively impairs cognitive function. Aluminium has been identified as one of the possible risk variables for AD cause.[19] One of the most prevalent elements in the crust of the Earth is aluminium. Because of its frequent usage, which has led to extensive human exposure, it has garnered a lot of attention lately because of its possible neurotoxic consequences. Aluminium is widely present in the body, yet it serves no essential biological purpose (Figure 1). Alzheimer's disease is linked to its neurotoxic symptoms, which include oxidative stress and neuroinflammatory consequences. The aluminum-induced neurotoxic events, including calcium dyshomeostasis, oxidative stress, apoptosis, inflammation, Aβ deposition, and the development of neurofibrillary tangles in the brain.[20]

Thus, using biochemical and histological studies, the current work examined the possible neuroprotective function of TP against $AlCl_3$-induced Alzheimer-like ailments in early adult rats. The results of this investigation showed that body weight is negatively impacted by $AlCl_3$ intoxication. Our results, which

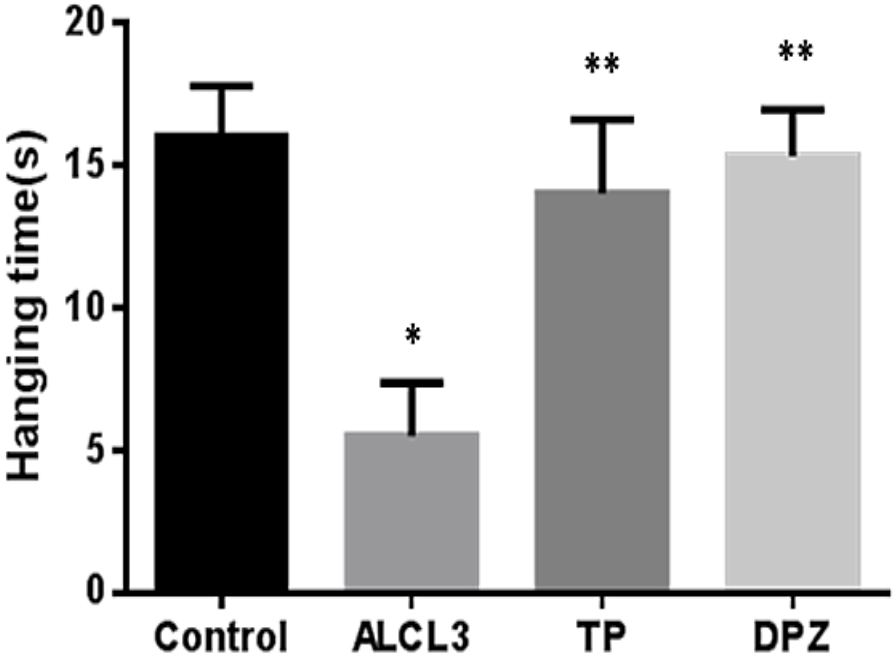

Figure 2: Effect of Tephrosin (TP) on hanging time during the wire hang test. Results were given as mean±SD of three discrete experiments. Data are investigated using one-way ANOVA and Tukey's *post hoc* assay. '*' $p<0.05$ compared with control and '**' $p<0.01$ compared with $AlCl_3$-intoxicated group.

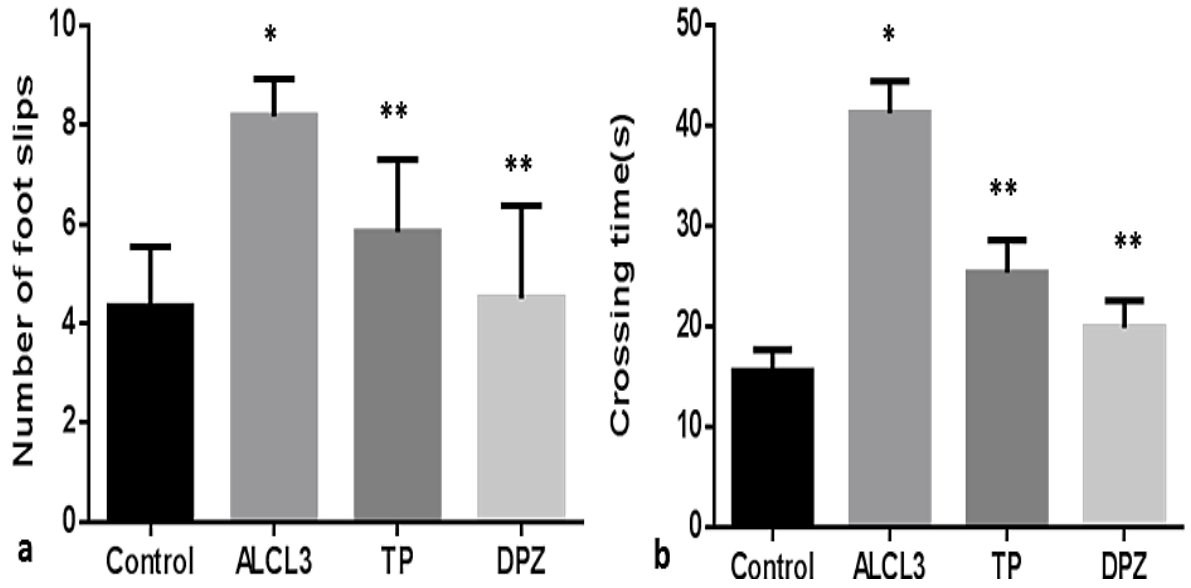

Figure 3: Effect of Tephrosin (TP) on the number of A: foot slips while walking and B: crossing time on the beam during the beam walking test for control and treatment groups. Results were given as mean±SD of three discrete experiments. Data are investigated using one-way ANOVA and Tukey's *post hoc* assay. '*' $p<0.05$ compared with control and '**' $p<0.01$ compared with $AlCl_3$-intoxicated group.

show significant variations in the weight of rats exposed to heavy metal poisoning, are consistent with previous research.

$AlCl_3$ may be harmful and disrupt the physiological and physical mechanisms that permit a decreased resting metabolic rate, food intake, and absorption when it interacts with apoplastic and symplastic targets on the plasma membrane.[21] Treatment with TP showed an insignificant difference in body weight, indicating the antioxidant property of TP, which inhibited the $AlCl_3$ activity. An effective method for assessing locomotor anomalies is the wire-hanging test. It is used to assess mice's stamina, balance, and grip strength.[22] The hanging time (muscle strength) in the $AlCl_3$-treated group was considerably reduced by $AlCl_3$. Neurodegeneration induced by aluminium chloride resulted in problems with coordination, motor dysfunction, and muscular weakness. The hanging duration of rats administered aluminium chloride was significantly reduced, suggesting that their motor function was affected.[23] TP was employed to assess the neuroprotective benefits. The hanging duration was considerably longer in the DPZ and TP group than in the $AlCl_3$ treatment group. The results indicate that neurodegeneration was reduced by TP.

The TP's capacity to enhance rats' motor coordination and balance was assessed using the beam walking test. The number of foot slips, which is used to assess motor coordination shortfall, may predict clinical sedation. The time required to finish the activity and the number of foot slips indicate a motor coordination deficit.[24] The study's findings showed that, in comparison to the control, donepezil, TP, the number of foot slips and crossing time of the $AlCl_3$ (neurotoxic control) group was considerably higher. The number of foot slips and crossing time in the $AlCl_3$-treated group was considerably increased by $AlCl_3$. Muscle damage caused by free radicals can also lead to changes in neuromuscular activity, muscle weakening, and ultimately, reduced muscle coordination (Figures 2 and 3). As a result, animals administered aluminium chloride alone performed poorly on the beam walking test.[25]

Acetylcholinesterase (AChE), a cholinergic enzyme, primarily occurs in postsynaptic neuromuscular junctions, especially in muscles and nerves. AChE's main function is to stop synaptic transmission and signalling between neurons in order to stop AChE from spreading and activating neighbouring receptors.[26] An increased activity of AChE contributes to the decline of neurotransmitter acetylcholine levels within the brain, which constitutes one of the leading causes of AD. In the current study, the findings support the description of substantial cholinesterase activation in the presence of $AlCl_3$ by demonstrating a considerable rise in AChE activity, contributing to a decline in AChE activity following $AlCl_3$ injection.[27] The administration of TP induced a significant increase in AChE activity, indicating that AChE activity had declined. Thus, TP has the ability to inhibit the enzymatic degradation (Figure 4).

Maintaining the cellular redox state is crucial for both the regular operation of the cell and, more crucially, its capacity to survive. It has been discovered that oxidant levels in disorders like AD are dysregulated, and this has been linked to the pathophysiology of the disease. Both an excess of oxidants and a depletion of antioxidants or the antioxidant defence system can be blamed for this loss of homeostasis.[28] Neurodegeneration brought on by elevated oxidative stress and disruption of intracellular signal transduction pathways may be linked to aluminium-induced neurotoxicity. $AlCl_3$ causes its harmful effects by fostering an oxidative environment inside cells, which is favourable to serious biological issues and illnesses.[29] The antioxidant defence system's

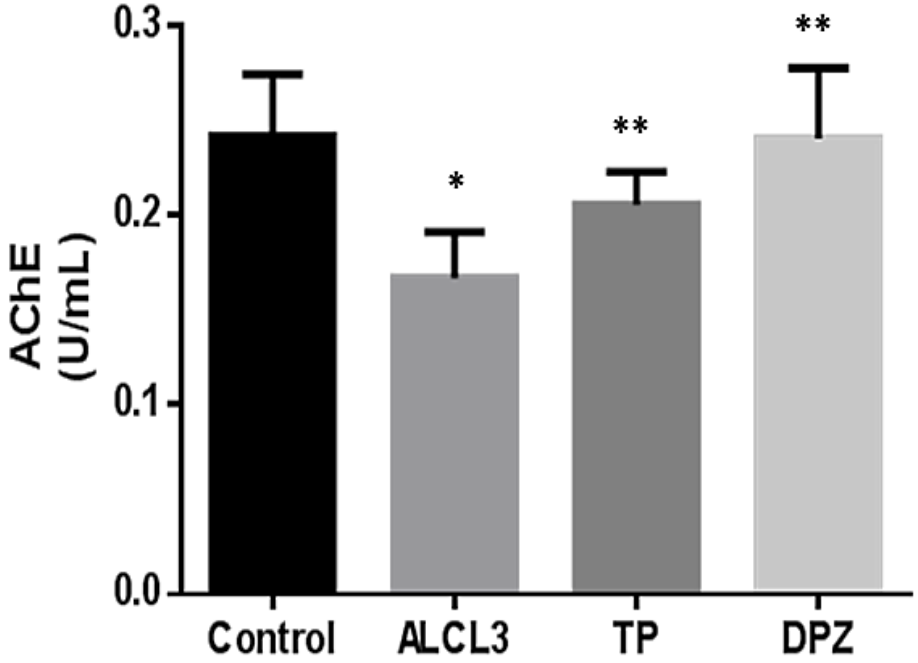

Figure 4: Effects of Tephrosin (TP) on Brain AChE Activity in $AlCl_3$-induced Rats: Results were given as Mean±SD of three discrete experiments. Data are investigated using one-way ANOVA and Tukey's *post hoc* assay. '*' $p<0.05$ compared with control and '**' $p<0.01$ compared with $AlCl_3$-intoxicated group.

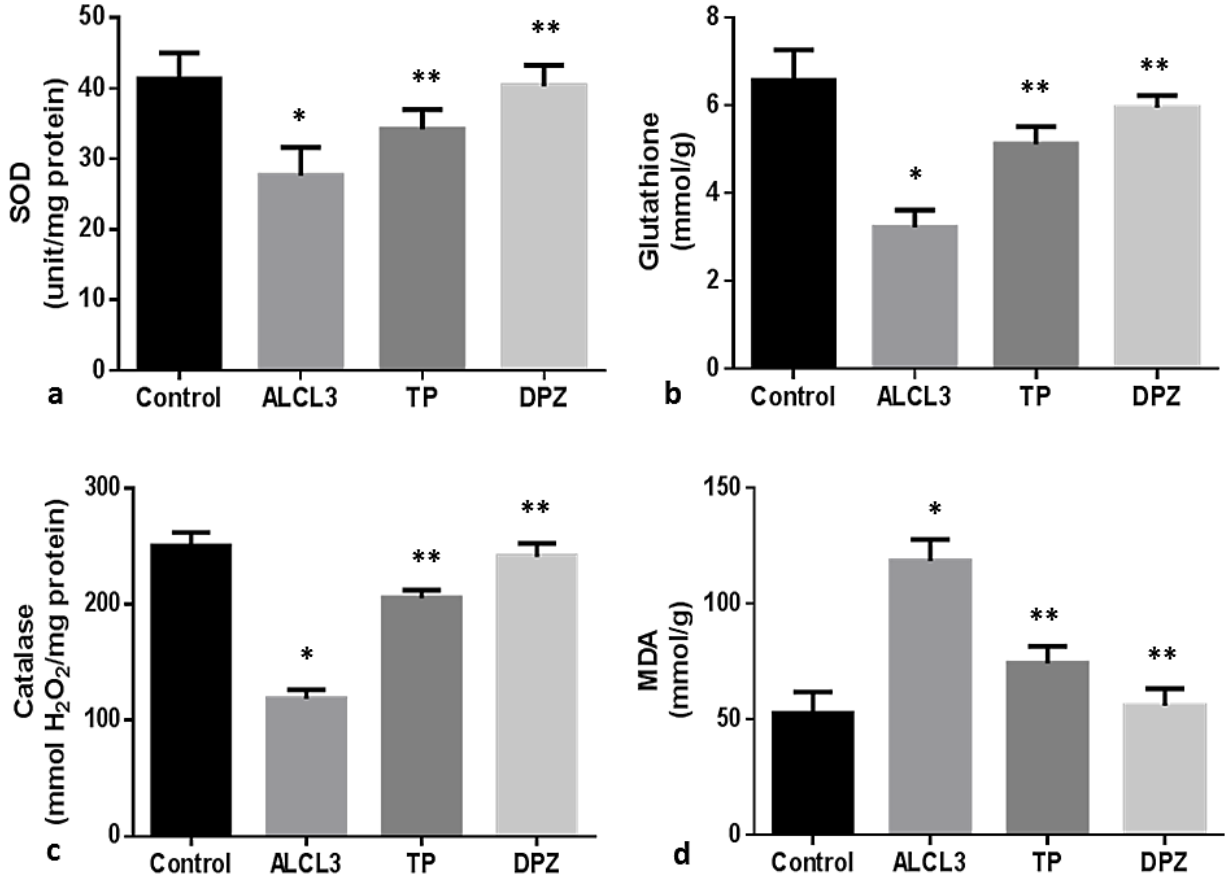

Figure 5: Effect of Tephrosin (TP) on rat brain antioxidant status in the rats. A: SOD (unit/mg protein) B: Glutathione (mmol/g) C: Catalase (mmol H_2O_2/mg protein) D: MDA (mmol/g). Results were given as Mean±SD of three discrete experiments. Data are investigated using one-way ANOVA and Tukey's *post hoc* assay. '*' $p<0.05$ compared with control and '**' $p<0.01$ compared with $AlCl_3$-intoxicated group.

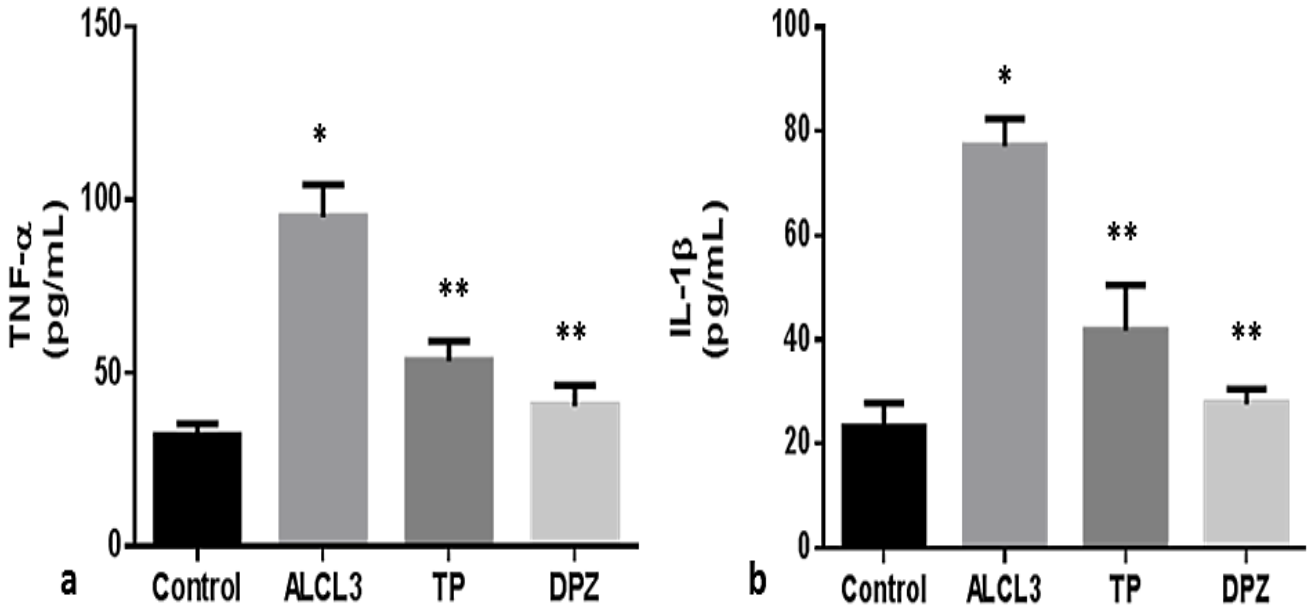

Figure 6: Levels of the proinflammatory cytokines (A) Tumor Necrosis Factor-Alpha (TNF-α) and (B) Interleukin-1beta (IL-1β) in hippocampal tissues of control and experimental groups of rats. Results were given as mean±SD of three discrete experiments. Data are investigated using one-way ANOVA and Tukey's *post hoc* assay. '*' $p<0.05$ compared with control and '**' $p<0.01$ compared with $AlCl_3$-intoxicated group.

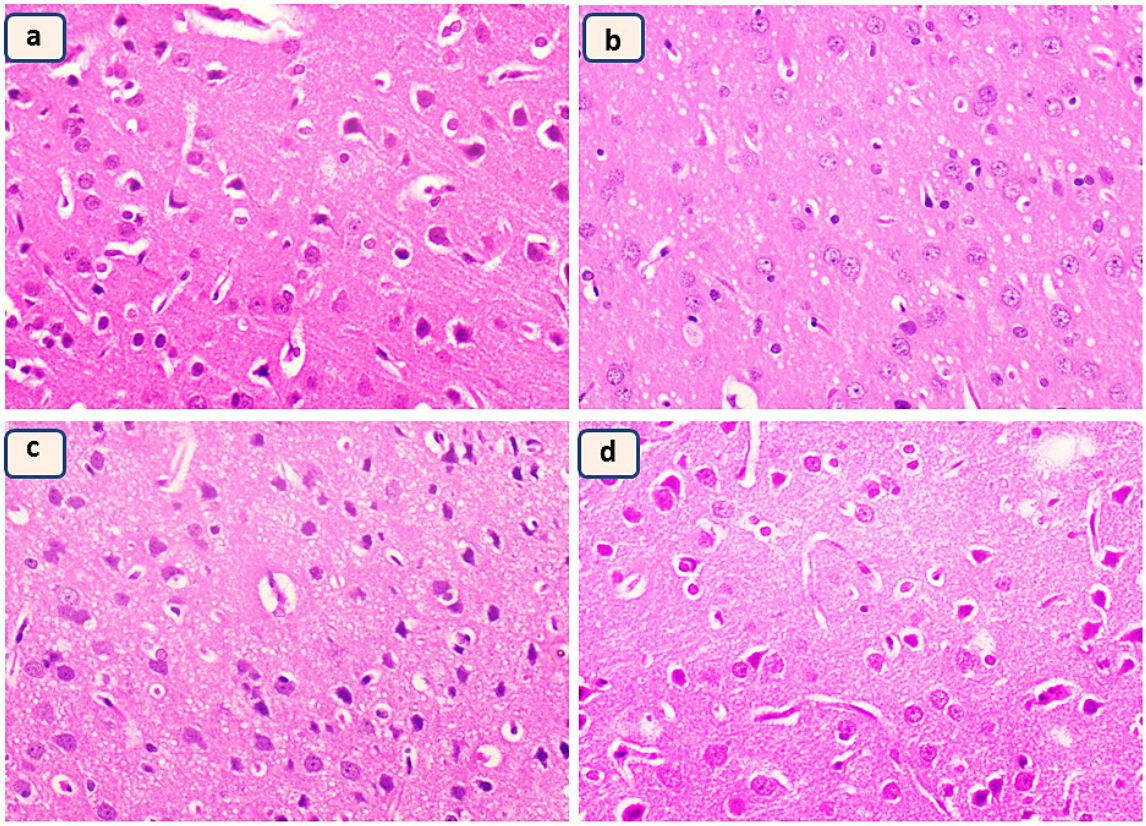

Figure 7: (a) Group I - control with basic diet; (b) Group II - Disease control Aluminium chloride ($AlCl_3$) (75 mg/kg/day p.o.) for 30 days induced rats; (c) Group III - Received $AlCl_3$ (75 mg/kg/day p.o.) along with Tephrosin (TP) (30 mg/kg) by weight; (d) Group IV - Received $AlCl_3$ (75 mg/kg/day p.o.) along with Donepezil (DPZ) (5 mg/kg p.o.) for 30 days.

first line, SOD, transforms superoxide anion into Hydrogen Peroxide (H_2O_2) and oxygen. Catalase then transforms H_2O_2 into H_2O. The primary non-enzymatic antioxidant, reduced Glutathione (GSH), either directly eliminates free radicals or acts as an enzyme's substrate that catalyses H_2O_2 detoxification.[30] According to the current study's findings, long-term exposure to aluminium dramatically reduced GSH, CAT, and SOD levels and activities while raising MDA. Elevated MDA levels point to the participation of free radical-induced cellular damage brought on by aluminium poisoning. On administration of TP, the levels of antioxidant enzymes, including GSH, CAT, and SOD, were significantly increased, and the level of MDA was considerably reduced. This indicates the antioxidant property of TP, which inhibited the oxidative stress in the brain (Figure 5).

$AlCl_3$ has been shown to alter antioxidant status, impact cellular homeostasis, and promote the synthesis of pro-inflammatory cytokines. The following processes underlie $AlCl_3$-induced neurodegeneration: disruption of intracellular calcium homeostasis, inflammatory signalling cascade activation, ROS formation, DNA damage, and epigenetic changes.[31] Cytokines, including IL-1β, IL-6, and TNF-α, have been shown to have a significant role in the pathophysiology of AD. A higher level of IL-1β indicates a significant risk of AD. TNF-α and IL-6 are two more AD-relevant cytokines that are further enhanced by IL-1β.[32] TNF-α and IL-1β were significantly increased in the hippocampus tissues of rats intoxicated with $AlCl_3$ in the current investigation, indicating that $AlCl_3$ primarily impacts macrophage activities and promotes the development of brain damage. When $AlCl_3$-intoxicated animals were treated with TP, the levels of TNF-α and IL-1β were considerably reduced. This indicates the anti-inflammatory property of TP, which was responsible for the decline in pro-inflammatory cytokine secretion (Figure 6).

Our histological results in rats treated with $AlCl_3$ revealed severe brain injury, which was consistent with the biochemical results and those from other investigations. In the $AlCl_3$-intoxicated animals, there were fewer neuronal cells in the same layer of the hippocampus, suggesting a pathogenic change (Figure 7). Together with the reference drugs TP and donepezil, the extract enhanced hippocampal structure to a degree similar to that of the normal control.

CONCLUSION

Ultimately, our research revealed that TP may offer protection against the inflammation and oxidative stress caused by $AlCl_3$ in Alzheimer's disease. Rats intoxicated with $AlCl_3$ showed reduced levels of lipid peroxidation products (MDA) and pro-inflammatory cytokines, fewer foot slips, and shorter crossing times after receiving TP. Furthermore, there was a significant increase in hanging time, AChE, and antioxidant enzyme levels. Additionally, TP treatment reduced the histological changes in the brain caused by $AlCl_3$. TP-treated rats are thereby protected from oxidative damage and inflammation caused by an excess of $AlCl_3$.

ABBREVIATIONS

TP: Tephrosin; **TNF-α:** Tumour Necrosis Factor-α; **$AlCl_3$:** Aluminium chloride; **SOD:** Superoxide dismutase; **AChE:** Acetylcholinesterase; **CAT:** Catalase; **IL-1β:** Interleukin-1β; **IR:** Insulin Resistance; **GSH:** Glutathione; **APOE:** Apolipoprotein E; **Aβ:** beta-amyloid; **ROS:** Reactive Oxygen Species; **H_2O_2:** Hydrogen peroxide; **Ach:** Acetylcholine; **MDA:** Malondialdehyde; **AD:** Alzheimer's disease; **ELISA:** Enzyme-Linked Immunosorbent Assay.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL STATEMENT

This study is approved by the Ethics Committee of Hunan University of Chinese Medicine (Approval Number: 2024-KY-66).

SUMMARY

In a rat model of Alzheimer's disease, this study thoroughly examined the preventive effects of tephrosin against inflammation and oxidative stress caused by Aluminium chloride. Throughout the trial, tephrosin reduces brain damage, AChE levels, and MDA activity. It raises SOD, GSH, and CAT levels, demonstrating tephrosin's anti-oxidative capabilities. Additionally, it decreased pro-inflammatory cytokine levels, demonstrating tephrosin's anti-inflammatory qualities. Additionally, tephrosin lengthened the duration of hanging and reduced the number of foot slips, suggesting that the animals' motor activity has improved. Tephrosin is therefore expected to be employed as a therapeutic medication to lessen the inflammation and oxidative stress brought on by Alzheimer's disease.

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