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RESEARCH ARTICLE

Neuroprotective activity of alcoholic extract of *Operculina turpethum* roots in aluminum chloride-induced Alzheimer's disease in rats

Karthik Gangadhar, Prem Kumar N*

Abstract

Alzheimer's disease (AD) involves complex pathways leading to neuronal damage. Neurotoxicity, resulting from exposure to harmful agents such as chemotherapeutic drugs, heavy metals, dietary additives, illicit substances, and radiation, contributes to conditions like AD. This study explored the neuroprotective potential of an ethanolic extract of *Operculina turpethum* (EEOT) roots in rats subjected to AD induced by aluminum chloride. Wistar rats weighing between 230 to 250 g were used to investigate neuroprotective effects. Disease induction was achieved through $AlCl_3$ treatment, while therapeutic intervention was provided with EEOT root at low and high doses. The extract was administered orally once daily, mirroring the dosing schedule of the conventional AD medication, rivastigmine. Neuroprotective outcomes were evaluated through multiple parameters, including antioxidant enzyme levels (Catalase, SOD, LPO), total brain protein content, acetylcholinesterase activity, and behavioral assessments of motor learning, spatial learning, exploratory behavior, and cognitive function. The results demonstrated that EEOT root enhanced muscle strength (rotarod test) and improved learning ability (Morri's water maze test). Additionally, the EEOT root reduced anxiolytic-like exploration in the Hole board test. The results suggest that EEOT roots may help protect neurons, possibly mitigating the damage caused by aluminum chloride by lowering oxidative stress. This protective effect is likely linked to the preservation of normal acetylcholinesterase activity, which helps maintain cholinergic function. According to this study, EEOT roots may have applications as neuroprotective agents.

Keywords: Alzheimer's disease, *Operculina turpethum*, Acetylcholinesterase, Neuroprotective, Rivastigmine.

Introduction

Alzheimer's disease (AD), a neurodegenerative disorder, impacts approximately 35 million people worldwide. By 2030, estimates indicate that this figure could increase to 65.7 million. AD ranks as the fourth leading cause of death among the elderly population, potentially due to the accompanying cognitive decline and dementia, which heighten the risk of accidents for those afflicted. Jurcău *et al*., (2022) predict a sharp rise in the percentage of AD-affected individuals in the upcoming years. The condition's development has been attributed to a various factor, including neurofibrillary tangles due to aberrant tau protein phosphorylation and increased beta-amyloid plaque accumulation, both linked to certain risk genes (Kunkle *et al.,* 2019). AD is influenced by oxidative damage, aging, hormone deficits (including estrogen), and inflammation. The cholinergic theory suggests that the main causes of AD symptoms are the loss of acetylcholine receptors, the death of neurons that produce ACh, changes in synapses, and a decrease in cholinergic neurotransmission. Acetylcholinesterase (AChE), the enzyme that breaks down ACh, accumulates more as a result of these circumstances (Stanciu *et al*., 2020). Aluminum is expected to be heavily contaminated by food, deodorants, cooking utensils, and many industrial applications. Known to be extremely toxic to neurons, aluminum can penetrate the blood-brain barrier (BBB) in healthy individuals and saturate the entire brain. Aluminum damages the brain oxidatively, resulting in neuronal death, degeneration of cholinergic neurons, and accumulation of amyloid, all of which impair memory and learning (Singh and Goel, 2015). Protein misfolding in the cytoskeleton is

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caused by aluminum chloride, creating amyloid plaque and hyperphosphorylated tau protein. This has resulted in the broad approval of aluminum as an agent for inducing AD *in-vivo* models (Mirza *et al.,* 2017). Although cholinesterase inhibitors dominate the Alzheimer's drug market, they cannot completely halt disease progression, limiting their effectiveness. This could be related to the complexity of the disease's cause. Combining anticholinesterase activity with other neuroprotective properties might offer an effective AD treatment (Sharma *et al*., 2019). Over the last two decades, various medicinal plant preparations have demonstrated promise in reducing AD progression, highlighting their potential therapeutic benefits for memory impairment and age-related illnesses (Elmorsy *et al*., 2021). The study of flavonoids in mouse models of AD has been a significant advancement during the previous decade. Flavonoids, found in many plants, fruits, and vegetables, are powerful antioxidants with a variety of pharmacological effects. They scavenge free radicals chelate metals, and have anticholinesterase, anti-aging, neuroprotective, and anti-inflammatory activities. Flavonoids also promote learning and memory and have antidepressant and anti-amyloidogenic properties. Several flavonoids have been found to reduce beta-amyloid pathology (Teles *et al*., 2018). *Operculina turpethum* is a well-known medicinal herb with a wide therapeutic range. Secondary metabolites found in this herb include saponins, flavonoids, and glycosides. Studies have confirmed its pharmacological properties, such as laxative, ulcer-protective, anti-dyslipidemic, hypoglycemic, anti-inflammatory, and antimicrobial effects, attributed to its content of essential oils, glucose, and fructose (Gupta and Ved, 2017). The roots have also been used to treat obesity, haemorrhoids, cough, asthma, indigestion, intestinal gas, paralysis, gout, arthritis, sadness, and scorpion and snake stings (Singh *et al.,* 2023). According to several studies, this plant has laxative, neuropharmacological, immunomodulatory, nephroprotective, hepatoprotective, analgesic, antioxidant, antibacterial, antiulcer, antidiabetic, antiarthritic, and antidiarrheal properties. In the Charak Samhita, it is mentioned that dietary supplements for brain and neurological function, such as Trivrutta Kashya (herbal decoctions), are used as Rasayana (Sharma and Singh, 2012). The objective of this study was to determine if the ethanolic extract of *O. turpethum* (EEOT) roots could protect against AD in Wistar rats with the disease induced by aluminum chloride ($AlCl_3$).

Materials and Methods

Collection and Verification of O. turpethum

O. turpethum roots were gathered from Bengaluru, Karnataka, India. The Central Ayurveda Research Institute's taxonomist, located in Thalaghattapura Post, Bengaluru, India-560109, identified the plant sample as *O. turpethum* Linn, a member of the Convolvulaceae family. The letter number SMPU/CARI/BNG/2023-24/2639, dated 12/02/2024, was used to verify the authenticity.

Plant Material Extraction

The EEOT roots were procured from Green Chem herbal extracts, Krishnasagara, Attibele industrial area Bengaluru, Karnataka, India.

Phytochemical Analysis

Chemical identification tests for steroids, glycosides, flavonoids, alkaloids, and triterpenoids were conducted according to standard procedures (Sharma & Singh, 2012).

Animals

Albino Wistar rats (200 ± 50 g) of either sex were housed at 25 ± 5°C with excellent ventilation in an animal facility on a 2-hour light/dark cycle, with the experimental protocol approved by the Institutional Animal Ethics Committee (Approval No. KCP/IAEC/PCOL/126/AUG-2023). The committee also accepted the animal housing conditions, which followed standard requirements (CPCSEA). The rats received a pellet diet with free access to water.

Experiment Design

$AlCl_3$ induced alzheimer's disease

Aluminum chloride (100 mg/kg) was given orally in saline for 30 consecutive days to induce to cause Alzheimer's. In addition, once daily low and high dosages of the EEOT roots were administered, there was a 60-minute gap between the two. The animals were evaluated behaviourally on day 31 following the administration of the test medication (EEOT) and the standard medication (Rivastigmine 1.5 mg/kg *p.o.*). They were then euthanized under light ether anesthesia. Brain samples were divided in half, with one half fixed in 4% formaldehyde for histological evaluation and the other half kept in 10% saline for biochemical investigation.

Five groups of six animals each were formed from the animals

Group1: Normal [Saline (1-mg/kg, *p.o.*)]
Group 2: Control [$AlCl_3$ (100 mg/kg, *p.o.*)] (Baburaj *et al.,* 2023)
Group 3: $AlCl_3$+ Low dose of *O. turpethum roots* (200 mg/kg, *p.o.*) (Kumar *et al.,* 2006)
Group 4: $AlCl_3$+ High dose of *O. turpethum roots* (400 mg/kg, *p.o.*)
Group 5: Standard [$AlCl_3$+Rivastigmine (1.5 mg/kg, *p.o.*)] (Kumar *et al.,* 2007)

Behavioural Assessment

Motor coordination test

During the test, the Rota rod device, which included a 7.0 cm-diameter drum, was set to rotate at a rate of 20 revolutions per minute. Motor coordination was examined by assessing the latency to fall within a 180-second test period, as described (Tabari *et al.,* 2019).

Elevated plus-maze (EPM) test

Rat behaviors on days 15 and 30 were investigated. Each experimental rat was placed centrally facing the open arm and observed for five minutes. A digital camera recorded their movements (Delgado *et al.* 2018).

The morris water maze

A circular pool containing opaque water, in which rats must learn to discover a submerged escape platform, was used. The hidden platform version tests spatial memory and is sensitive to hippocampal damage, as rats rely on spatial cues. In contrast, the visible platform version, which is influenced by lesions in the dorsal striatum, does not require hippocampal function. During both the acquisition and reversal training phases, swim latency (time to find the platform) and swim distance (distance traveled to locate the platform) are recorded (Nunez, 2008).

Hole board assessment

In the center of the board, rats were maintained gently, facing inward or, if needed, in a designated direction. The rat was free to roam about the board for a set period, typically between 5 and 10 minutes. During this time, record the rat's movements and interactions with the holes. Frequency head lowering in the hole served as a measure of exploratory behavior (File *et al.*, 1975).

Procedure to prepare homogenate of brain tissue

The rat brain was separated, weighed, and washed. After 15 minutes at 4000 rpm with 0.1M tris-phosphate buffer (pH 7.4), the brain tissue was homogenized into a 10% (w/v) homogenate. After centrifugation at high speed, the resulting supernatant was used for biochemical analysis (Ojha *et al*., 2022).

Oxidative Parameters

Catalase activity

To create a total volume of 3 mL, combine 1.95 mL of 0.05 M phosphate buffering solution (pH 7), 1-mL of 0.019 M hydrogen peroxide (H2O2), and 0.05 mL of a 10% (w/v) sample for the activity of catalase. A control cuvette containing the same components but without the sample is also prepared. At 240 nm, absorbance was measured, providing the basis for determining catalase activity (Sofic *et al.,* 2015).

Superoxide dismutase (SOD) activity

About 0.5 mL of the material and 0.5 mL of purified water were combined to create a tissue mix. After mixing for one minute with 0.25 mL of ethanol and 0.5 mL of cold chloroform, centrifuge the mixture for 20 minutes at 2000 rpm. Add 0.5 mL of EDTA (0.49 M) and 0.05 mL of carbonate solution (0.05 M, pH 10.2) to the precipitate for the test. 0.4 mL of epinephrine is used to initiate the reaction, and its optical density change at 480 nm is monitored every minute. Wet tissue SOD activity was expressed as U/mg (Nisticò *et al*., 1992).

Lipid peroxidation

Malondialdehyde (MDA) estimation entailed detecting thiobarbituric acid reactive compounds (TBARS). This method produces TBARS by reacting one MDA molecule with two thiobarbituric acid molecules under acidic conditions at 95°C for 20 minutes. The resulting pink chemical was then examined with a UV-visible spectrophotometer at 532 nm (Elhadidy *et al.,* 2018).

Tissue Parameters

Acetylcholinesterase activity

The Ellman method was employed to determine AchE levels in brain tissues, and the outcome was manifested as a percentage of the control group (Ellman *et al.*, 1961).

Assessment of total protein levels in neural tissue

The quantification of total brain protein was carried out using an autoanalyzer and an Erba kit.

Histopathological studies

The brains of both the EEOT-treated and control groups were dissected to assess cellular alterations. The brains were rinsed with water and then kept in a 10% formalin buffer for 24 and 48 hours. The samples were subsequently transported to the Koushik Laboratory and Clinic in Bengaluru for histopathological examination.

Statistical Analysis

The Dunnett test was used for post-hoc testing, whereas a one-way ANOVA was used for statistical analyses. A significance threshold of $p<0.05$ was applied, which were displayed as mean ± SEM.

Results

Preliminary Chemical Compound Screening and Root Constituents

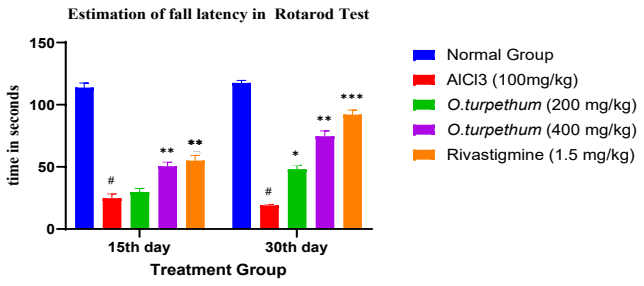
EEOT roots were found to contain glycosides, steroids, flavonoids, and alkaloids.

Behavioural Assessments

Rotarod test (Motor learning)

The group treated with both EEOT and $AlCl_3$ showed a statistically significant increase in muscle strength compared to the aluminum-treated group. $AlCl_3$ exposure significantly decreased muscle strength in the rotarod test compared to the untreated group. However, combining EEOT extract with $AlCl_3$ resulted in a marked improvement in muscle strength compared to the $AlCl_3$-only group as shown in Figure 1.

Evaluation of latency to fall in rats using rotarod—Day 15 and day 30



The statistical significance of rotarod test results (latency to fall) on Days 15 and 30 was analysed using Dunnett's post-hoc test performed after a one-way ANOVA. The data (n = 6) are displayed as mean ± SEM. *with $p < 0.05$, **with a $p < 0.01$, ***with a $p < 0.001$ vs. $AlCl_3$-induced group, #$p < 0.001$ vs. regular group.

Figure 1: Effect of treatment on fall latency in rats using rotarod test

Morris's water maze test

When compared to the control group, the aluminum chloride-treated group in the Morris water maze test exhibited a statistically significant decrease in escape latency. In contrast to the disease-induced group, EEOT administered at 200 and 400 mg/kg successfully mitigated the rise in escape latency caused by aluminum chloride. Comparable to the group treated with rivastigmine, the EEOT treatment group's results showed enhanced mental ability in the navigation of space task as illustrated in Figure 2.

Hole board apparatus

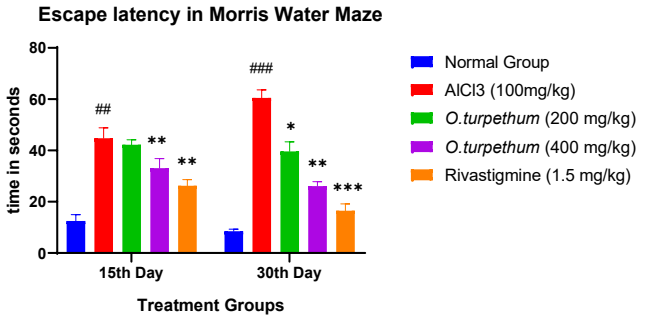
In the Hole board test, the aluminum chloride-treated group demonstrated more head dips than the control group. It was demonstrated that the administration of 200 to 400 mg/kg of EEOT significantly decreased the anxiety caused by the aluminum chloride therapy when comparing the treatment group to the disease-induced group. The therapy with EEOT decreased anxiety-related behaviours and yielded results similar to the group receiving standard medicine, rivastigmine as portrayed in Figure 3.

Elevated plus maze

In the elevated plus maze, the aluminum chloride-treated group demonstrated less amount of time spent in the open arms contrasted to the normal group. It was demonstrated that the administration of 200 to 400 mg/kg of EEOT significantly decreased the anxiety caused by the aluminum chloride therapy when comparing the treatment group to the disease-induced group. The therapy with EEOT decreased anxiety-related behaviours and yielded results similar to the group receiving standard medicine, rivastigmine as depicted in Figure 4 & 5.

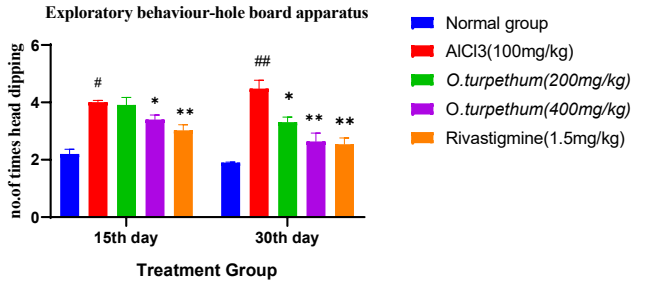
Acetylcholinesterase activity

Comparing the brains of animals in the normal group with those in the aluminum chloride-treated group, there



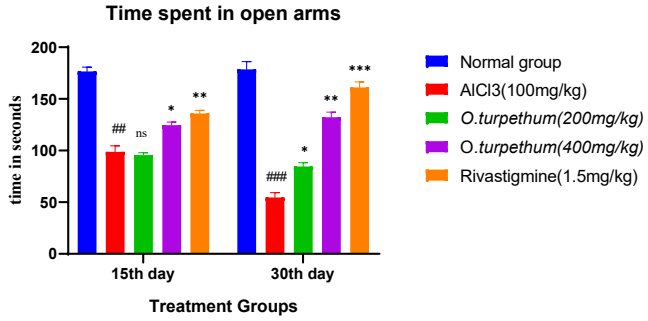
Rats' time to escape in the Morris water maze improved significantly on Days fifteen and thirty (mean ± SEM, n = 6), according to Dunnett's test after One-way ANOVA. Significant differences from the AlCl3 group are indicated by **with a $p < 0.01$, ***with $p < 0.001$, and ##$p < 0.01$, ###$p < 0.001$ when compared to normal rats.

Figure 2: Effect of treatment on escape latency in rats using Morri's water maze



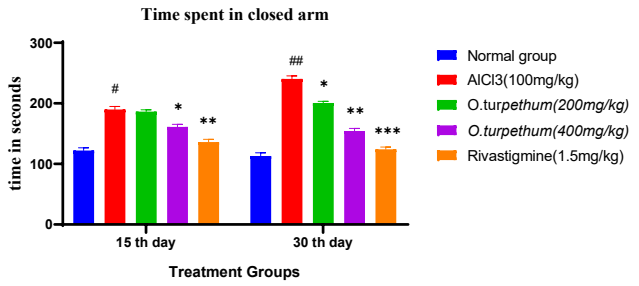
Results were shown as mean ± SEM (n = 6) in a one-way ANOVA study of head dip in rats that were given the Hole Board test on Days 15 and 30 and then Dunnett's test. Significant differences were observed between the AlCl3-induced group and normal rats, with *p less than 0.05, **p the following 0.01, which is ***$p < 0.001$, and #$p < 0.01$, ##$p < 0.001$

Figure 3: Effect of treatment on head dipping in rats using Hole bord apparatus



The data are shown as mean ± SEM (n = 6) in a one-way ANOVA evaluating the amount of time rats spent in open arms during the elevated plus maze test on Days 15 and 30, which was followed by Dunnett's test. *p less than 0.05, **p less than 0.01 and ***p less than 0.001 were significant variations from the AlCl3-induced group, while ##$p < 0.01$, ###$p < 0.001$ or greater was a significant difference from the normal group.

Figure 4: Effect of treatment on time spent (open arm) in rats using elevated plus maze apparatus



Dunnett's check was performed after a one-way ANOVA evaluated the rats' time spent in the closed chamber on Days 15 and 30 of the elevated plus maze. The mean ± SEM is used to present the data (n = 6). *p below 0.05, **p lower than 0.01 and ***p more than 0.001 were significant variances from the $AlCl_3$-induced group, while #$p < 0.01$, ##$p < 0.001$ was a significant difference from the normal group.

Figure 5: Effect of treatment on time spent (closed arm) in rats using elevated plus maze apparatus

was a substantial increase in AChE activity *(#p < 0.05)*. The administration of EEOT extract at a concentration of 400 mg/kg effectively inhibited the rise in AChE activity caused by aluminum chloride treatment as compared to animals in the illness control group (** *p < 0.01*). After EEOT extract therapy, similar outcomes to the group receiving rivastigmine treatment as illustrated in Figure 6.

Total protein

Compared to the normal group, the animals treated with aluminum chloride exhibited a considerable increase in protein levels. The administration of 200 and 400 mg/kg of EEOT extract significantly reduced the protein levels in comparison to the disease-induced group. Results from the EEOT extract treatment were similar to those of the rivastigmine-treated group, with an overall decrease in brain protein as portrayed in Figure 7.

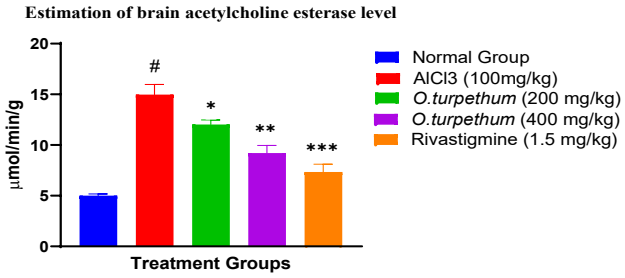
Estimation of Antioxidant Status of Brain

Catalase level

In the present study, rats induced with $AlCl_3$ showed a significant reduction in catalase levels compared to the normal group in brain tissue. The $AlCl_3$-induced alterations in brain antioxidant measures are considerably inhibited by pretreatment with EEOT at different dosages and treatment durations. Before receiving EEOT for 30 days at 200 and 400 mg/kg, the brain tissue of the other groups exhibited higher levels of catalase. The catalase levels in the rivastigmine-pretreated groups increased significantly as represented in Figure 8.

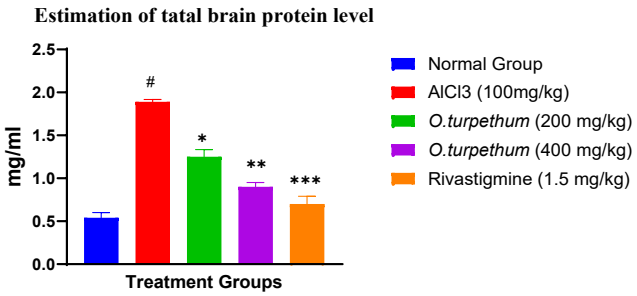
Estimation of SOD levels in the brain

In this study, rats exposed to $AlCl_3$ showed a significant reduction in superoxide dismutase (SOD) levels in brain tissue compared to the normal group. However, pretreatment with EEOT at varying doses and durations



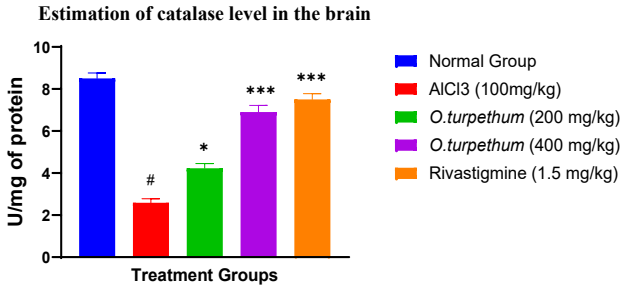
The significance level of brain acetylcholinesterase levels was evaluated using a one-way ANOVA, and post hoc comparisons were then performed using Dunnett's test. The results are shown as mean ± SEM (n = 6), with #with a $p < 0.001$ indicating differences from the normal group and*a $p < 0.05$, **with a $p < 0.01$ and ***a $p < 0.001$ indicating significance compared to the $AlCl_3$-induced group.

Figure 6: Effect of treatment on brain acetylcholinesterase (AChE) level



A one-way ANOVA assessed the significance of total brain protein levels, followed by Dunnett's test. The findings are presented as mean ± SEM (n = 6), with #with a $p < 0.001$ for the normal group and *with $p < 0.05$ **with p<0.01 and ***with $p < 0.001$ for the $AlCl_3$-only group.

Figure 7: Effect of treatment on brain protein level



One-way ANOVA assessed brain catalase levels, followed by Dunnett's test. The findings are presented as mean ± SEM (n = 6), with #with $p < 0.001$ for the normal rats and *with a $p < 0.05$, and ***with $p < 0.001$ for the $AlCl_3$-induced group.

Figure 8: Effect of EEOT roots treatment catalase level in the brain

significantly mitigated the $AlCl_3$-induced alterations in brain antioxidant parameters. Specifically, pretreatment with EEOT for 30 days at doses of 200 and 400 mg/kg resulted in higher SOD levels in brain tissue compared to other groups. Furthermore, the groups pretreated with rivastigmine

exhibited an even more substantial increase in SOD levels as depicted in Figure 9.

Estimation of lipid peroxidation in brains

In animals treated with aluminum chloride, there was a significant increase in lipid peroxidation levels compared to the normal group. However, treatment with the EEOT extract at doses of 200 and 400 mg/kg significantly reduced lipid peroxidation levels when compared to the disease-induced group. The EEOT extract effectively decreased overall lipid peroxidation, with results comparable to those seen in the rivastigmine-treated group as depicted in Figure 10.

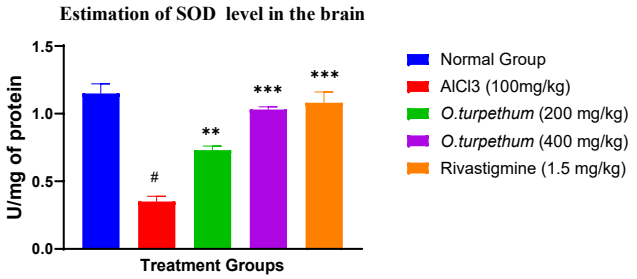
Histopathological Studies

Normal group, rat brain

Cortical region showed neuronal cells – normal – NAD + hippocampus region dentate gyrus showed normal neuronal morphology– NAD+. (X100) as shown in Figure 11a.

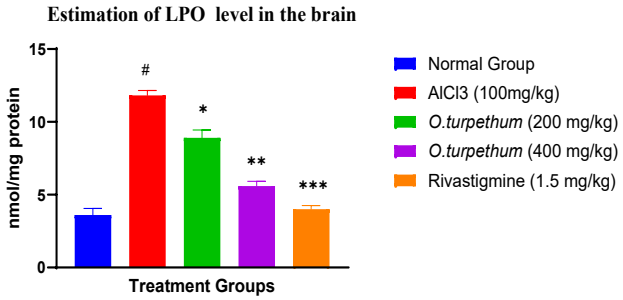
Rat brain, positive control

cortical region showed neuronal hyperplasia & gliosis – moderate 3+ (Changes brought on by chemical induction-



One-way ANOVA evaluated brain SOD levels, followed by Dunnett's test. The findings are shown as mean ± SEM (n = 6), with #$p < 0.001$ for the normal group and **with a $p < 0.01$ and ***with a $p < 0.001$ for the $AlCl_3$-induced group.

Figure 9: Effect of EEOT roots treatment on SOD level in the brain.



One-way ANOVA assessed brain LPO levels, followed by Dunnett's test. The findings are presented as mean ± SEM (n = 6), with #$p < 0.001$ in comparison to the normal group and *$p < 0.05$, **$p < 0.01$ and ***with $p < 0.001$ in comparison to the $AlCl_3$-induced group.

Figure 10: Effect of EEOT roots treatment on LPO level in the brain

Degenerative) area of the hippocampal brain displayed a dentate gyrus with moderately 3+ gliosis and pyknotic nuclei. SN area exhibiting moderate 3+ gliosis and inflammation (X100) as shown in Figure 11b.

Low dose of EEOT, rat brain

The cortical region of the rat brain displayed typical neuronal and glial cell morphology. Dentate gyrus in the hippocampal area displayed normal morphology. The area of the substantia nigra displayed normal, NAD+ neuronal cells (X100) Figure 11c.

High dose of EEOT, rat brain

the cortical region of the Rat Brain showed typical morphology of neuronal and glial cells. Dentate gyrus in the hippocampal area displayed normal morphology. area of the substantia nigra displaying normal, NAD+ neuronal cells (A favourable response caused by the test drug*) (X100) Figure 11d.

Standard group, rat brain

Neuronal cells in the cortical area were normal and NAD+. In the hippocampus's dentate gyrus, NAD+, which neuronal structure was normal (X100) Figure 11e.

Discussion

The current study evaluated the neuroprotective effects of an EEOT against $AlCl_3$-induced oxidative damage in Wistar rats.

Aluminum is acknowledged as a neurotoxin capable of breaching the blood-brain barrier after repeated exposure, which may influence various neurodegenerative disorders, including the neurological changes linked to AD (Nayak *et al.,* 2010).

According to Prakash *et al.,* (2013), $AlCl_3$ buildup is linked to increased oxidative damage in brain tissues, which causes lipid peroxidation and interferes with physiological and biochemical processes. In rats, it has been established that a daily oral dose of aluminum chloride ($AlCl_3$), ranging from 10 to 100 mg/kg body weight over a 30-day period, results in motor deficits, anxiety, and cognitive impairments (Zhao *et al*., 2020).

According to Auti *et al*. (2019), aluminum exposure causes changes in behaviour in the hippocampus and cortex. These changes are caused by oxidative stress, neuronal death, altered neurotransmission, and the buildup of amyloid plaque. Studying flavonoids in AD prone rats has been an important advance in the ten years since their discovery. Phytochemicals possess an extensive variety of pharmacological actions. Secondary metabolites are linked to enhanced antioxidant, metallic material chelators, elimination of free radicals, anticholinesterase, protection of neurons, anti-inflammatory, enhancing learning and memory, and having potent antidepressant and anti-amyloidogenic effects (Velmurugan *et al*., 2018).

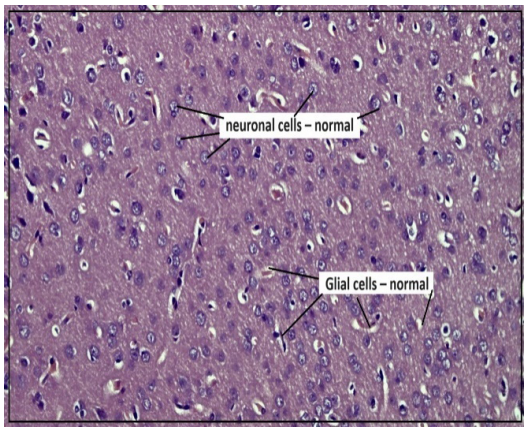


Figure 11a: - Normal group

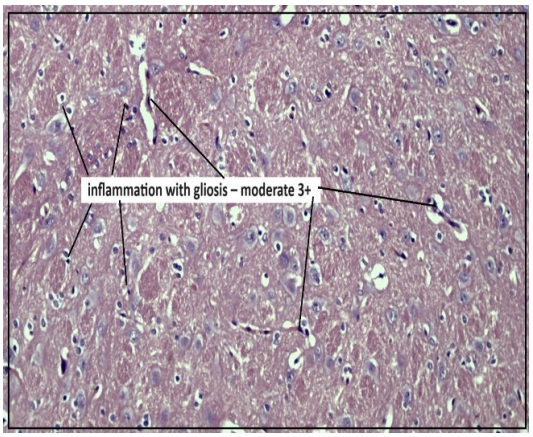


Figure 11b: - AlCl3 group

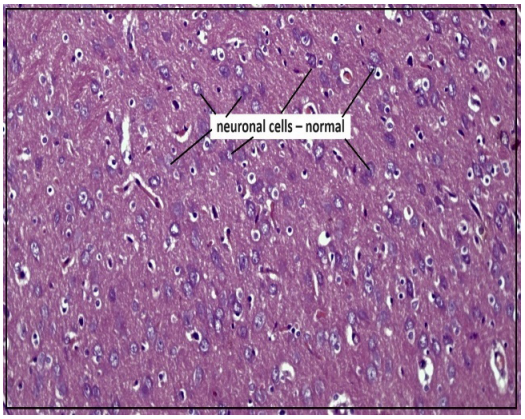


Figure 11c: $AlCl_3$+Low dose EEOT

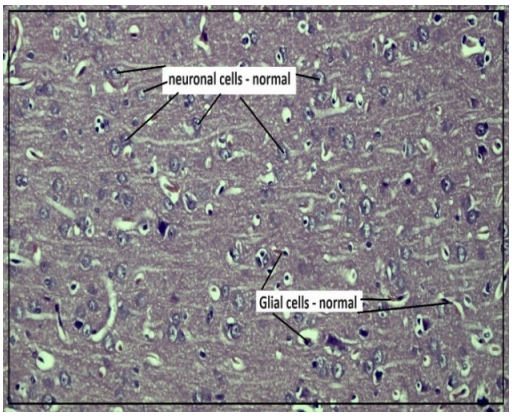


Figure 11d: $AlCl_3$+High dose EEOT

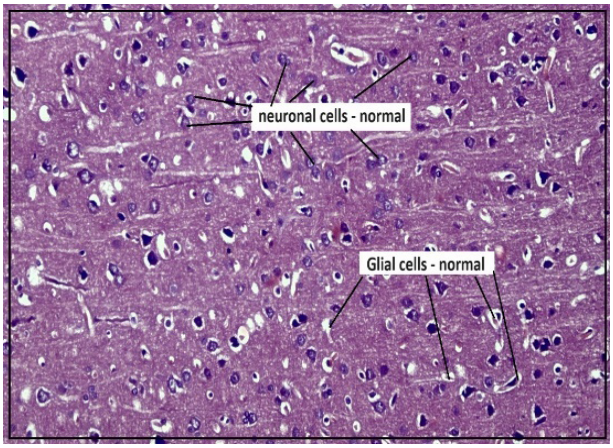


Figure 11e: $AlCl_3$+Rivastigmine

Figure 11: Histopathology in the rat brains

Furthermore, because flavonoids can pass across the blood-brain barrier after either acute or long-term administration, they may also directly impact the brain. Thus, these substances could be applied as a prophylactic to impede the advancement of illnesses such as AD (Teles *et al*., 2018). Plant antioxidants and the body's enzymes work together to shield the brain from oxidative damage (Maswada & Maswada, 2013). The plant *O. turpethum* is well-known for having a high flavonoid content and strong antioxidant activity (Sharma and Singh, 2012).

Behavioural assessments (Prema *et al*., 2016) revealed that rats administered $AlCl_3$ exhibited deterioration in their understanding and memory abilities. In the Morris water maze test, which measures cognitive processes of learning and memory, rats given $AlCl_3$ alone performed significantly worse than the normal group. All groups, memory and recall deficits were corrected by $AlCl_3$ therapy. An increase in memory and comprehension is reflected in a decrease in escape latency. In this inquiry, treatment with the ethanolic extract from the roots of *O. turpethum* significantly decreased the escape latency (EL) in rats compared to those that received only aluminum chloride, demonstrating clear improvements in memory and learning skills. The hole-board test is a standard procedure for assessing the behavioural effects of medicinal therapies, particularly in connection to anxiety, depression, or cognitive performance. Head-dipping behaviour is usually altered in terms of pattern and frequency by anxiolytic or anxiogenic drugs. Head dips were monitored for five minutes during the test. According to Brown & Nemes, (2008), rats exposed to aluminum chloride in their study exhibited elevated levels of anxiety as seen by an increased number of head dips per five minutes, a sign of reduced exploratory behaviour.

On the other hand, a large dose of treatment with the ethanolic extract from the roots of *O. turpethum* significantly decreased the escape latency (EL) in rats compared to those that received only aluminum chloride, demonstrating clear improvements in memory and learning skills. lowered anxiety levels, as seen by a drop in the frequency of head dips per five minutes. Kisipan *et al.,* (2022) reported that, to resolve ambiguities in the existing literature, a rotarod test study was conducted to determine the ideal aluminum chloride dose, duration, and form required to induce neurotoxicity. Exposure to aluminum chloride damages neurons' mitochondria, which reduces ATP synthesis, increases oxidative stress, and causes deficiencies in motor coordination and endurance, especially in the cerebellum and motor pathways. The application of the ethanolic extract of *O. turpethum* roots considerably ameliorated aluminum chloride-induced motor incoordination and memory deficits in the rats. Grundmann *et al.* (2007) reported that aluminum chloride treatment significantly increased closed-arm preference and reduced open-arm exploration in rats compared to disease controls in the elevated plus-maze test. This suggests that the sub-chronic exposure of aluminum chloride (100 mg/kg) causes anxiety in these animals. The EPM test revealed that rats administered 200 and 400 mg/kg of the ethanolic extract from the roots of *O. turpethum* spent considerably more time in the open arms and less time in the closed arms than rats treated with aluminum chloride.

Several studies have shown that as AD worsens, the brain tissues of affected individuals undergo significant levels of oxidative stress. The brain eventually experiences oxidative damage, and this series of events eventually contributes to the development of Alzheimer's. Singh *et al*. (2016) Report that the intensification of the activities of the Fe2+ and Fe3+ ions cause aluminum chloride toxicity and oxidative damage. Aluminum chloride further activates a number of pathways in cells driven by redox-sensitive signals. Since free radicals often cause lipid peroxidation on long-chain polyunsaturated fats, well known for their occurrence in a range of dietary sources in extremely high amounts in brain cells, reactive oxygen species (ROS) are produced.

In a study on antioxidants, it was found that MDA levels were higher in rats with AD caused by $AlCl_3$, while SOD and CAT levels were lower in their brains. After four weeks of treatment with 400 mg/kg of EEOT roots, SOD and CAT levels greatly increased, whereas MDA levels significantly decreased. According to Singh *et al*. (2023), the plant extract exhibited *in-vivo* antioxidant properties, potentially reducing oxidative stress. Rats treated with aluminum chloride had higher total brain protein levels compared to those in the control group. Vivo antioxidant activity, reducing the damaging effects of ROS in the rat brain. Rats given aluminum chloride showed higher amounts of total brain protein than rats in the control group.

The ethanolic extract of *O. turpethum* roots at doses of 200 and 400 mg/kg markedly decreased protein levels in a dose-dependent manner, which was in contrast to the results in rats treated with aluminum chloride.

Giving $AlCl_3$ raises the activity of AChE, which lowers the levels of acetylcholine (ACh). This leads to cholinergic deficits that make it harder to remember things and learn new things. In contrast to the aluminum chloride ($AlCl_3$) intervention group, the *O. turpethum* root extract in this study dramatically decreased AChE activity in the rats' brains (Figure 3).

The plant extracts reduced $AlCl_3$-induced deficits and increased cholinergic neurotransmission by blocking AChE, hence preserving acetylcholine levels (Supriya *et al*., 2023). This implies protection against neuronal damage. and emphasizes the possibility of AChE inhibition as an AD treatment strategy. Mirza *et al.* reported that histological analysis showed typical neurons in the cerebral cortex and hippocampal regions in the negative control group, contrasting with the aluminum chloride-treated groups.

In rats treated with $AlCl_3$, neurons in the cerebral cortex appeared severely stained, atypically shaped, and loosely packed. Similarly, $AlCl_3$ exposure led to disarray and eccentric nuclei, resulting in an irregular morphological profile. Liaquat *et al*. 2019 reported localized gliosis and cerebral vascular blockage of cerebellar cortex cells. Many cognitive processes, including memory functions like storage, recall, representation of context, and the ability to navigate depend on the hippocampus. Impaired memory and learning in the hippocampus have been associated with aluminum chloride buildup in the prefrontal cortex and the hippocampus. In some circumstances, oxidative stress has been connected to a rise in apoptosis, especially when proapoptotic proteins are up-regulated.

Studies have demonstrated that rats chronically exposed to $AlCl_3$ exhibit fewer pyramidal cells and aberrant morphology in their disordered hippocampal cells (Al-Hazmi *et al*., 2021). The results highlight the need to protect the hippocampal structure from the harmful effects of aluminum chloride. An ethanolic extract of *O. turpethum* root extract increased the number and shape of hippocampal cells in rats that had been exposed to $AlCl_3$. This suggests that it may be able to lessen the negative effects of aluminum buildup on the hippocampus.

In this study, mixing $AlCl_3$ with an ethanolic extract of *O. turpethum* roots made more and better organized hippocampal cells in rats. This effect may result from the flavonoids' ability to protect hippocampal tissue from oxidative stress and apoptosis. While the disease control group exhibited signs of decreased vitality of brain cells, formation of vacuoles, necrosis, and scarring in the cerebral cortex region. indicating significant cell death.

The $AlCl_3$ group showed numerous intact neurons alongside glial cells (Ekundayo *et al*., 2022). These findings align with histological studies indicating significant neurodegeneration in the hippocampal region following four weeks of exposure to aluminum chloride. According to the current histology investigation, $AlCl_3$ also caused a sparse cell distribution and reduced the number of viable cells (Hussein *et al.,* 2020). Conversely, rats administered with both $AlCl_3$ and *O. turpethum* root extract demonstrated equivalent distribution and improved cell survival in the prefrontal cortex of the brain. Findings reveal that the ethanolic extract of *O. turpethum* root offers protection against $AlCl_3$-induced AD.

Conclusion

According to the current study, $AlCl_3$ has serious neurological effects on the etiology of AD, causing oxidative stress as well as impairments in learning and memory processes. On the other hand, the ethanolic extract of *O. turpethum* root, which includes flavonoids like luteolin and acacetin, may prevent or treat $AlCl_3$-induced hippocampal and cortical cognitive deficits. This is made possible by its anti-inflammatory and antioxidant properties, which raise neurotransmitter levels and strengthen CAT, SOD, and LPO's antioxidant capacities. Consequently, it might help to enhance memory, shield neural cells from oxidative stress, and help the brain resist the damaging influence of AD. Therapy with root extract from *O. turpethum* prevented the deleterious effects of aluminum chloride buildup in the brain in individuals, especially with heightened $AlCl_3$ sensitivity.

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