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**Research Article**

# Neuroprotective Effects of Hydroalcoholic Extract of Dracaena Reflexa Leaves in An Aluminum Chloride-Induced Alzheimer's Model in Mice

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## ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative illness marked by the deterioration and eventual death of neurons in various brain areas, leading to memory loss, cognitive decline, language difficulties, problems with daily functioning and no effective disease-modifying therapy for Alzheimer's disease. Dracaena reflexa, with in vitro anticholinesterase inhibition, its phytoconstituent tetracosane inhibiting beta amyloid aggregation, and anti-inflammatory and antioxidant properties, shows promise for Alzheimer's disease.This study is designed to evaluate the effect of hydroalcoholic extract of Dracaena reflexa in the prevention of Alzheimer's disease using an Aluminium chloride induced AD model in mice. In Swiss albino mice, 150 mg/kg of AlCl3 was administered to generate an Alzheimer's model, causing neurodegeneration and cognitive impairment, followed by two doses of hydroalcoholic extract of Dracaena reflexa alongside AlCl3 for 4 weeks. When evaluated through different memory, cognition and biochemical tests, it was found that hydroalcoholic extract of Dracaena reflexa could prevent or reverse the neurodegeneration caused by AlCl3 in Swiss albino mice. Improved histology appearance and increased neuroregeneration in the mice also substantiate the protective effect of hydroalcoholic extract of Dracaena reflexa leaves. Besides the elevated level of serotonin, GSH, catalase and reduced level of MDA, AChE level in the brain homogenate of the treated mice indicates its neuroprotective potential. Our experimental data suggest that the hydroalcoholic extract of Dracaena reflexa prevented the neurodegeneration in the brain and also produced memory and cognition improvement.

## INTRODUCTION

Neurodegenerative diseases such as Alzheimer's disease are presently the primary contributor to disability and the second most prevalent cause of death globally. Over the past three decades, there

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has been a notable increase in both fatalities and disabilities originating from neurological conditions, particularly in low- and middle-income nations.[1,2]Alzheimer's disease (AD) is a neurodegenerative illness marked by the deterioration and eventual death of neurons in various brain areas, leading to memory loss, cognitive decline, language difficulties, and problems with daily functioning. It affects 75% of individuals with dementia.[3]

Despite extensive efforts , no effective disease modifying therapy available for Alzheimer's disease.Over 20 compounds have undergone large phase 3, double-blind, randomized controlled trials with patients at various stages of AD, yet none has proven effective in slowing cognitive decline or improving overall functioning. These trial failures underscore the need for new approaches to clinical trial design for AD[4]. Existing treatments only partially alleviate symptoms and cognitive issues, making the development of an effective therapeutic agent for AD an urgent and critical challenge. Current medical research focuses on factors believed to contribute to AD, such as tau proteins and amyloid-beta deposits. Thus, the search for new, more effective drugs is very important and plants remains the paramount source of drugs. *Dracaena reflexa* (DR)is a tropical plant that is traditionally believed to have high medicinal value. Ancient medicinal practitioners have long held *Dracaena reflexa* for poisoning, dysentery, diarrhoea, and dysmenorrhea[5]. Tetracosane a phytoconstituent of *Dracaena reflexa*, which is identified from the GC-MS analysis also having potent inhibitory action on beta-amyloid aggregation. In vitro and in silico studies revealed that *Dracaena reflexa* inhibit acetylcholinesterase and butylcholinesterase enzyme that are known for hydrolysing acetylcholine in the synaptic cleft in the brain[6].Moreover, *Dracaena reflexa* possess antioxidant and anti-inflammatory properties, suggesting potential benefits for neuroinflammatory conditions tied to Alzheimer's disease [7,8]. So, This study is designed to evaluate the effect of hydroalcoholic extract of *Dracaena reflexa* in the management of Alzheimer's disease using Aluminium chloride induced AD model in mice.

## MATERIALS AND METHODS

### Plant Collection and Authentication

*Dracaena reflexa* leaves were collected from Medical College Campus, Thiruvananthapuram on December 2023 and was authenticated at Department of Botany, Karyavattom Campus, University of Kerala.

### Preparation of Plant Extract

The shade-dried powdered leaf was initially defatted with petroleum ether at 60–70°C. After drying, the remaining marc was extracted using the Soxhlet extraction method with an ethanol and water mixture in an 80:20 ratio, respectively, for up to 36 cycles. The hydroalcoholic extract was then concentrated under reduced pressure using a Rotary evaporator.

### Preliminary Phytochemical Screening

The defatted hydroalcoholic extract of *Dracaena reflexa* leaves was subjected to quantitative test to identify various phytochemicals such as alkaloids, flavonoids, saponins, steroids and tannins.

### Approval by IAEC

The study protocol was approved by the Institutional Animal Ethics Committee of Govt. Medical College, Thiruvananthapuram dated 30/01/2024 as per the approval No;01/01/IAEC/2024/GMCT.

### Animal Selection, Maintenance, and Care

28 healthy adult male Swiss albino mice weighing 25-40g were used for the study. Animals were procured from Sree Chitra Tirunal Institute for Medical Sciences and Technology , Poojapura. Animals were housed in polypropylene cages with stainless steel lids with provisions for keeping food and drinking water *ad libitum* as per

Committee for Control and Supervision of Experiments on Animals (CCSEA).

**Experimental Design**

Mice were divided into four groups of seven animals each (n =7).Alzheimer's model was induced in mice by administering 150mg/kg of $AlCl_3$ orally for 28 days. Hydroethanolic extract of *Dracaena reflexa* leaves was administered at doses of 200mg/kg(LDDR) and 400 mg/kg (HDDR) orally. The control group received 0.1% of CMC alone. The disease group received only 150mg/kg of $AlCl_3$ orally. The treatment groups received plant extract suspended in 0.1% CMC along with 150mg/kg of $AlCl_3$ orally. The drug treatment is considered as a preventive regimen and given concurrently along with the disease induction.

**Evaluation of Cognition**

**Novel Object Recognition Test**[9-13]

The novel object recognition test is a fast and efficient way to assess learning and memory in mice. It is non-invasive and spontaneous. This test is based on the fact that when animals are exposed simultaneously to familiar and novel objects, they approach frequently and spend more time exploring the novel object than the familiar one in order to satisfy their innate curiosity/exploratory instinct. The test assessment has three sessions: habituation, training, and test. Different sets were administered with corresponding drugs. The experiment started with habituation on the first day followed by training on day 2 and testing on the 3rd day. The result on day 3 was taken as response before the treatment and the procedure is again repeated on day 14 and day 28 of the treatment period.

**Open Field Test** [14-16]

Open field test consists of exposing an animal to an open arena, a new environment without any aversive or appetitive stimuli where the animal is left to explore freely for a fixed amount of time.

Day 1 Training: At any nook of the box the mice were placed and their behaviour was monitored for 5 min. After that, they were removed from the open field and returned to home cage.

Day 2 Testing: After 24 hrs, the mice were again set within the open field, the duration outlaid in the central area was recorded.

Day 7 test: The same procedure on day 2 is repeated. The result on day 7 was taken as the response before the treatment and the procedure was again repeated on day 14 and day 28 of the treatment period.

**Radial Arm Maze Test**[17 -18]

The Radial arm maze task is a spatial discrimination task that has been extensively used to investigate specific aspects of spatial working and reference memory. The radial arm maze consists of baited and unbaited arms. Food is the reward used to make the correct choice of entry into the arms. If the mice choose the baited arm, then it is recorded as a correct response, and the mice are considered to have retained memory.

Radial arm maze test consists of different sessions like food deprivation, handling and shaping, training, and testing sessions. Day 0 (Food deprivation): Food deprivation was carried out by restricting each animal with 2 grams of food pellets per day in the animal department. Day 1-3:Habituation The animals were habituated individually to the environment by placing it on the central platform and allowed to explore the RAM for 15 min/Day. Reinforcers or bait were placed in each arm. On the last day of habituation (day 3), the number of reinforcers was reduced to four arms, and the sessions ended when all eight arms had been visited. Day 4-11:Training The animals were trained individually in one session per day for 8 days. 1 piece of feed was positioned at the culmination of arms 1,3,5 and 7 and all the other four arms remain closed. The bait was placed in effectively conceal the food from view, while the animal was permitted to freely navigate the maze.

Day 12:Test session On the day of the test, the working memory error (WME), reference memory error (RME), and reference-working memory error (RWME) were analysed

**Shuttle Box Avoidance Test**[19,20]

Passive and active avoidance tests are behavioral tasks widely used to assess the different forms of learning and memory. Avoidance happens when a person or animal learns to predict an unpleasant event through conditioning and takes action to prevent it. Active avoidance requires performing a specific behaviour in order to escape or avoid the aversive stimulus. The procedure consists of habituation followed by a learning phase of five consecutive days. Retention trials are done on days seven and fourteen, following the learning phase.

Day 0: Habituation The mice were brought to the lab and made familiar with the apparatus by keeping them in the chamber one by one. Later they were returned to the home cage. Day 1-5 : Learning sessions The learning session consisted of 1 trial/day for five consecutive days. During the trial, mice exposed to conditioned stimuli (CS) after 30 seconds which is light and buzzer sound for 8 sec, followed by unconditioned stimuli (US) which is a foot shock of 0.4mA for 10 seconds. The mice kept in one compartment (right chamber) of the shuttle box and were exposed to buzzer stimuli followed by the foot shock through the grid floor. As the learning session progressed, the animal learned to escape to the unelectrified compartment (left chamber) as soon as the CS is delivered. This was recorded as an avoidance response. The stimuli were terminated when the mice escaped to the safe compartment or when 12 seconds after CS had elapsed, whichever occurred first. This learning session was continued till the end of five days. Day 7 and 14: Retention test Retention test was performed on days seven and fourteen soon after the end of the learning sessions. The retention trial consisted of the same experimental procedure repeated without giving a foot shock. Here the mice were individually placed in the right compartment and the CS of light and the buzzer sound were delivered. Jumping of the animal to the left safe compartment upon receiving the CS was recorded as an avoidance response. The learning and memory capacity of the animal was assessed based on the number of avoidance responses exhibited by the animal during 10 trial sessions on the test days.

**Sacrifice of the Animal**[21]

After the completion of the behavioral test, the animals were sacrificed by cervical dislocation. the skull was carefully cut open and the brain was exposed from its dorsal side. The whole brain was quickly excised and transferred to a petridish containing ice cold normal saline. The isolated brain was adopted for further works

**Biochemical investigation of Brain homogenate**

**Brain Homogenate Preparation**

The brain tissue was first washed with ice-cold isotonic saline. It was then homogenized in a 0.1M Phosphate Buffer (pH-7.4) at a ratio of 10 parts buffer to 1 part tissue by weight. After homogenization, the mixture was centrifuged at 10,000 rpm for 15 minutes. The resulting supernatant was separated and used in aliquots for further biochemical analysis.

**Estimation of Acetylcholine Esterase Activity**[22,23]

An aliquot of about 0.4ml of brain homogenate was added to a cuvette containing 2.6 ml phosphate buffer (0.1 M pH 8) and 100µl of 0.01M DTNB. This mixture was thoroughly mixed with bubbling air and absorbance is measured at 412 nm in the spectrometer. Absorbance is recorded as the basal reading when it attains a stable value. 20 µl of Acetylthiocholine (substate) was added and the ΔA was recorded. Changes in the absorbance per minute were then determined.

**Estimation of Malondialdehyde (MDA) Levels**[24-27]

MDA is commonly used as a marker for evaluating lipid peroxidation and is typically assessed using the Ohkawa method. To the tissue homogenate samples (less than 0.2 mL), add .2 mL of 8.1% SDS, 1.5 mL 20% Acetic acid solution (pH adjusted to 3.5 with NaOH), and 1.5 ml 0.8% aqueous TBA solution. Raise the total bulk to 4.0 mL with distilled water, then heat the mixture at 95°C for 60 minutes. After cooling with tap water, add 1.0 mL of distilled H2O & 5 of ml15:1 (v/v) mixture of n-butanol and pyridine, and shake vigorously. Following centrifugation at 4000 rpm for 10 minutes, the organic layer was collected and its absorbance at 532 nm was recorded against a blank. Tetra methoxy propane (TMP) served as an external standard, and the lipid-peroxide levels presented as nanomoles (nmol) of malondialdehyde (MDA).

**Estimation of Catalase (CAT) Levels** [28]

Catalase activity is assessed using UV spectroscopy. In the UV spectrum, hydrogen peroxide ($H_2O_2$) exhibits increasing absorption as the wavelength decreases. Catalase levels in the brain homogenate were measured as follows: 0.2 ml of tissue homogenate was mixed with 1.2 ml phos- buffer (0.05 M, pH 7.0), and the reaction was initiated by adding 1.0 ml of hydrogen peroxide (0.03 M). The absorbance decrease at 240 nm was recorded over 3 minutes, with an enzyme blank run simultaneously using 1.0 ml of distilled water instead of hydrogen peroxide. Catalase activity was calculated as micromoles of $H_2O_2$ degraded / min/milligram of protein.

**Estimation of Glutathione Level** [29-32]

The GSH level in the hippocampal and cortical regions of the brain was ascertained by Ellman method. The deproteination of brain homogenate was performed by mixing it with an equal volume of 10% TCA and centrifugated at 2000 rpm for 10 min. The supernatant was separated and used for GSH estimation. The supernatant was joined with 2 ml of phosphate buffer, 0.5 ml DNTB, and 0.4 ml of water. The mixture was shaken thoroughly and vortexed. The absorbance was taken at 412 nm within 15 min against the blank.

**Estimation of Serotonin Level** [33-34]

The serotonin level was determined by Schulmpf method, The serotonin combined with o-phthaldiadehyde results in the development of fluorophore and is measured spectrofluorimetrically.The brain was isolated and the brain tissue was weighed and homogenized in 5ml HCI -Butanol for 1 min. The sample was centrifuged for 10 mins at 2000 rpm. The supernatant was collected and mixed with a centrifuge tube containing 2.5 ml of heptane and 0.31 ml of 0.1 M HCI. After 10 min of shaking and centrifuging under the same condition the two phases separated and the aqueous phase (0.2ml) was used for serotonin estimation.To the Aliquot(0.2ml) of aqueous extract, 0.25ml of OPT reagent was added. The mixture was heated for 10 min at 1000 c for the development of fluorophore. Then the sample was allowed to equilibrium with ambient temperature and absorbance was measured at 360-470 nm in the spectrofluorometer. A concentrated HCI of about 0.25 ml without OPT was taken as tissue blank. Serotonin (500µg/ml)was used as an Internal standard and was prepared by dissolving in distilled water and HCI-Butanol mixture in a ratio of 1:2.

**Histopathological Evaluation of Brain** [35-36]

Histopathological analysis was conducted on the brains of 2-3 mice randomly chosen from each group. The brains were promptly fixed in 10% phosphate-buffered formaldehyde, embedded in paraffin, and 5 µm longitudinal sections were made. These sections were stained with hematoxylin and eosin (H&E) and Congo Red stain examined under a microscope at 200 x magnification.

**Statistical analysis**

Results are expressed as Mean $\pm$ *SEM*.Statistical analysis were performed by ANOVA multiple comparison test using Graphpad prism software version 10.3.

**Results**

**Percentage yield**

% yield value of hydroethanolic extract of *Dracaena reflexa* leaves found to be 19%

**Preliminary phytochemical screening**

Investigation revealed the presence of Carbohydrate, Alkaloids, Phenols, Flavonoid, Tannins, Saponins and Terpenoids

**Evaluation of Cognition by Novel Object Test**

On day 0 all groups spent almost similar time near the novel object as shown in the **Fig.no.1**. All groups of animals except the disease group had spent more time with novel object on day 14 and day 28. The disease group showed comparatively lesser exploratory behaviour when compared to control group. Thus, the increased exploration time near novel object in drug treated groups shows the potential of the drug to prevent the cognitive impairment by $AlCl_3$.

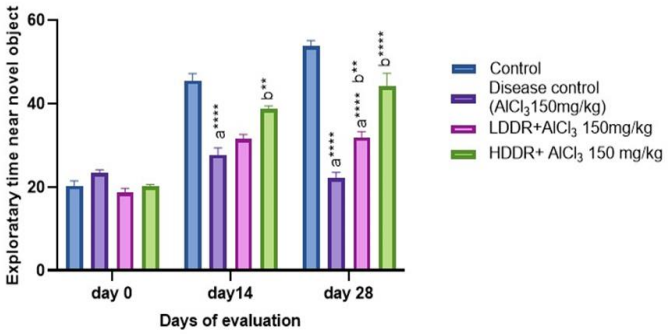



**Fig.no.1:Graphical representations of the effect of drugs on cognition by novel object recognition test**.

Results were expressed as Mean $\pm$ SEM for n=7 statistical analysis done by Two-way ANOVA along with Tukey's multiple comparison test. a**** indicates P <0.0001 compared to control group and b**** indicates P<0.0001,b** indicates P<0.01 compared to that of the disease group. LDDR-low dose of hydroalcoholic extract of Dracaena reflexa, HDDR-high dose of hydroalcoholic extract of Dracaena reflexa.

**Evaluation of Cognition by Open Field Habituation test**

On day 0 all groups spend almost similar time in the central area of the open field apparatus. As shown in **Fig.no.2**.All groups of animals except the disease group had spent more time in the central area of the open field apparatus on day 14 and day 28. The disease group showed comparatively lesser exploratory behaviour when compared to the control group. The group which received high dose and low dose of hydroalcoholic extract of *Dracaena reflexa* showed a significant increase in time spent in the central area.

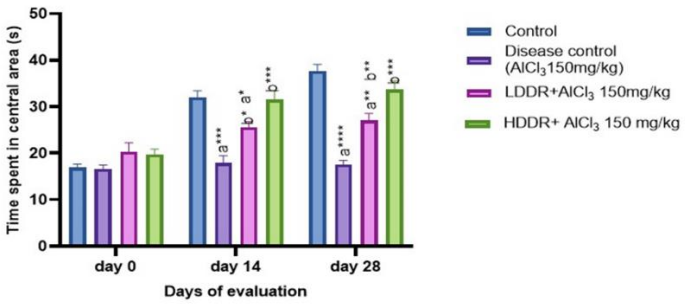



**Fig.no.2: Graphical representation of the effect of drugs on cognition by open field test.**

Results were expressed as Mean ±SEM for n=7 statistical analysis done by Two-way ANOVA along with Tukey's multiple comparison test. a**** indicates P <0.0001 compared to control group and b**** indicates P<0.0001,b**indicates P<0.01 compared to that of the disease group.LDDR-low dose of hydroalcoholic extract of Dracaena reflexa, HDDR-high dose of hydroalcoholic extract of Dracaena reflexa.

**Evaluation of Cognition by Shuttle Box Avoidance Test**

On day 0, all groups show almost similar no of avoidance responses in the shuttle box. On day 14, the disease group showed a statistically significant decrease in avoidance response compared to the avoidance responses of the control group on the same day as shown in **Fig.no.3**.The group that received high dose and low dose of hydroalcoholic extract of *Dracaena reflexa* showed a significant increase in no of avoidance response compared to the disease group on the same day

On day 28, the avoidance responses of the control group were found to be increased However, the disease group had a decrease in avoidance responses when compared to the control group. Similarly, low and high doses hydroalcoholic extract of *Dracaena reflexa* treated groups showed a statistically significant increase in no of avoidance responses compared to the disease group.

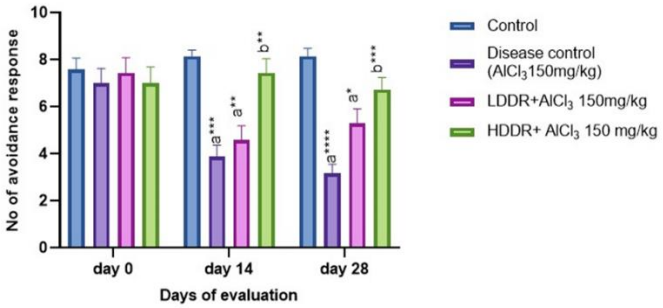



**Fig.no.3: Graphical representation of the effect of drugs on cognition by shuttle Box Avoidance test**.

Result were expressed as Mean ±SEM for n=7 statistical analysis done by Two-way ANOVA along with Tukey's multiple comparison test. a**** indicates P <0.0001 compared to control group and b**** indicates P<0.0001,b** indicates P<0.01 compared to that of the disease group.LDDR-low dose of hydroalcoholic extract of Dracaena reflexa, HDDR-high dose of hydroalcoholic extract of Dracaena reflexa.

**Evaluation of Cognition by Radial arm maze test**

From **Fig.no.4a**, On 14$^{th}$ day, working memory error of mice in the disease group is significantly increased compared with that of control group whereas the working memory error of the animals in the low dose and high dose group is significantly decreased compared with that of disease group.

Similarly, by 28$^{th}$ day, working memory error of mice in the disease group is significantly increased compared with that of control group whereas the working memory error of the animals in the low dose and high dose group is significantly decreased compared with that of disease group indicating retaining of memory.

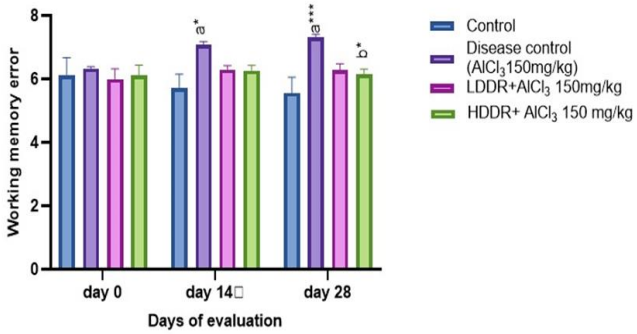



**Fig.no.4a: Graphical representation of the effect of drugs on cognition by radial arm maze test(WME).**

Results were expressed as Mean ±SEM for n=7 statistical analysis done by Two-way ANOVA along with Tukey's multiple comparison test. a*indicates a**** indicates P <0.0001 compared to control group and b**** indicates P<0.0001,b** indicates P<0.01 compared to that of the disease group. LDDR-low dose of hydroalcoholic extract of Dracaena reflexa, HDDR-high dose of hydroalcoholic extract of Dracaena reflexa.

From **Fig.no.4b** by 14th day, reference memory error of the animals in the low dose and high dose group is significantly decreased compared with that of disease group.

Similarly, by 28th day, reference memory error of mice in the disease group is significantly increased compared with that of control group whereas the reference memory error of the animals in the low dose and high dose group is significantly decreased compared with that of disease group indicating retaining of memory.

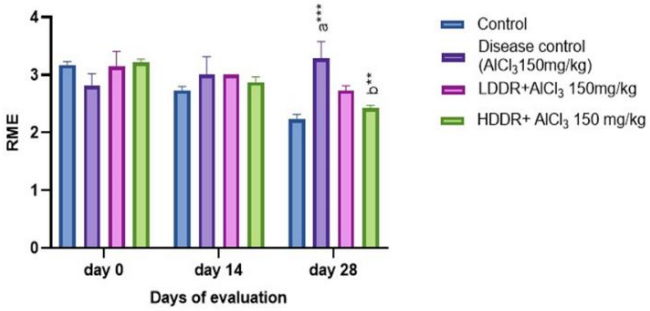



**Fig.no.4b:Graphical representation of the effect of drugs on cognition by Radial arm maze test(RME)**.

Result were considered as Mean ±SEM for n=7 statistical analysis done by Two-way ANOVA along with Tukey's multiple comparison test. a**** indicates P <0.0001 compared to control group and b**** indicates P<0.0001,b** indicates P<0.01 compared to that of the disease group. LDDR-low dose of hydroalcoholic extract of Dracaena reflexa, HDDR-high dose of hydroalcoholic extract of Dracaena reflexa.

**Biochemical Estimation of Brain Homogenate**

**Evaluation of brain Acetylcholinesterase in Albino mice**

The Aluminum chloride treatment showed an increased level of AChE that was significantly higher compared to the control . However the level is lowered for hydroalcoholic extract of *Dracaena reflexa* low dose and high dose treated groups compared to the control group.

From **Fig.no.5**, the brain AChE level was substantially elevated in the $AlCl_3$-treated group compared to the control group indicating $AlCl_3$ induced learning and memory impairment. The high dose and low dose treatments considerably

decreased AChE levels compared to the $AlCl_3$ treated group, which was statistically significant.

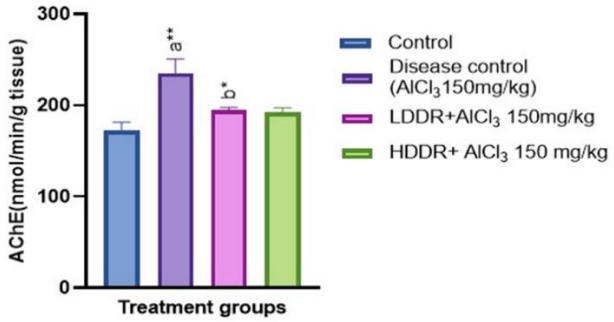



**Fig.no.5: Graphical representation of the effect of drugs on AChE level**..

LDDR-low dose of hydroalcoholic extract of Dracaena reflexa, HDDR-high dose of hydroalcoholic extract of Dracaena reflexa.

**Estimation of Serotonin**

From **Fig.no.6**, the brain serotonin level was significantly reduced in $AlCl_3$ treated group compared to the control group and indicating $AlCl_3$ induced learning and memory impairment. The high dose and low dose treatment considerably increased serotonin levels compared to the $AlCl_3$ treated group, which was statistically significant.

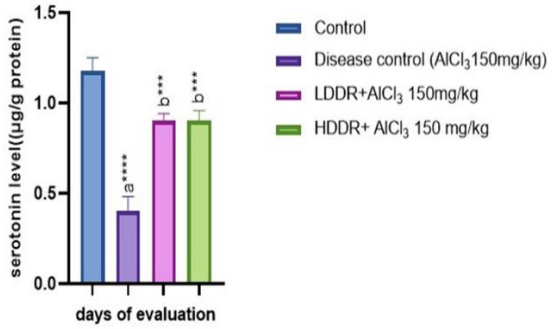



**Fig.no.6: Graphical representation of the effect of drugs on serotonin level**..

LDDR-low dose of hydroalcoholic extract of Dracaena reflexa, HDDR-high dose of hydroalcoholic extract of Dracaena reflexa.

**Estimation of Catalase Activity**

From **Fig.no.7,** the brain catalase level was significantly reduced in $AlCl_3$ treated group compared to the control group and indicating $AlCl_3$ induced learning and memory impairment. The high dose and low dose treatment considerably increased catalase levels compared to the $AlCl_3$ treated group, which was statistically significant.

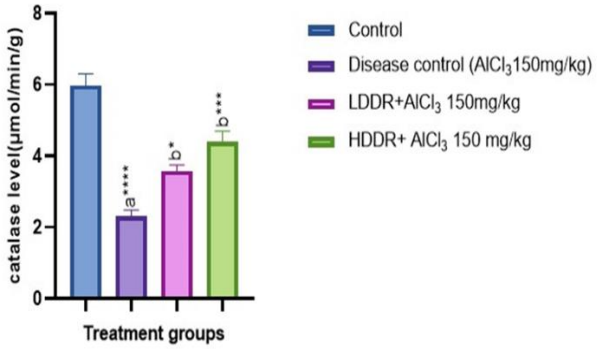



**Fig.no.7: Graphical representation of the effect of drugs on catalase level.**.

LDDR-low dose of hydroalcoholic extract of Dracaena reflexa, HDDR-high dose of hydroalcoholic extract of Dracaena reflexa.

**Estimation of Glutathione level**

From **Fig.no.8**, the brain glutathione level was significantly reduced in $AlCl_3$ treated group compared to the control group and indicating $AlCl_3$ induced learning and memory impairment. The high dose and low dose treatment considerably increased glutathione levels compared to the $AlCl_3$ treated group, which was statistically significant.

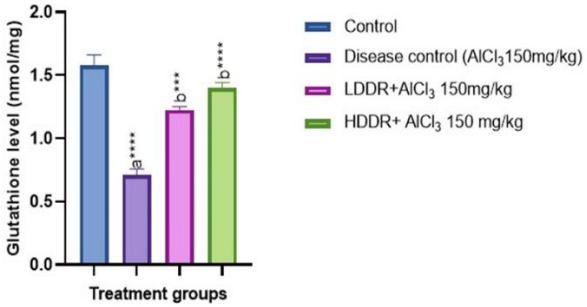



**Fig.no.8: Graphical representation of the effect of drugs on glutathione level**

.LDDR-low dose of hydroalcoholic extract of Dracaena reflexa, HDDR-high dose of hydroalcoholic extract of Dracaena reflexa.

**Estimation of Malondialdehyde level**.

**Fig.no.9**, the brain MDA level was significantly increased in $AlCl_3$ treated group compared to the control group and indicating $AlCl_3$ induced learning and memory impairment. The high dose and low dose treatment considerably decreased MDA levels compared to the $AlCl_3$ treated group, which was statistically significant.

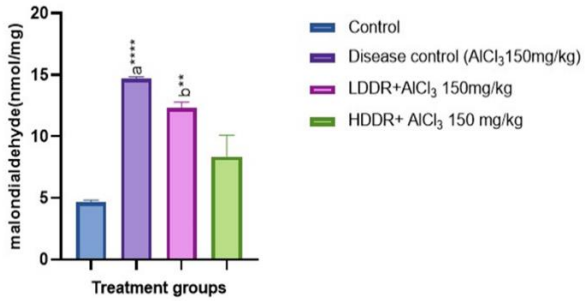



**Fig.no9:Graphical representation of the effect of drugs on MDA level.**

LDDR-low dose of hydroalcoholic extract of Dracaena reflexa, HDDR-high dose of hydroalcoholic extract of Dracaena reflexa.

**Histopathological Analysis of Brain**

**Histopathological Examination of Mice Brain using H and E Staining**

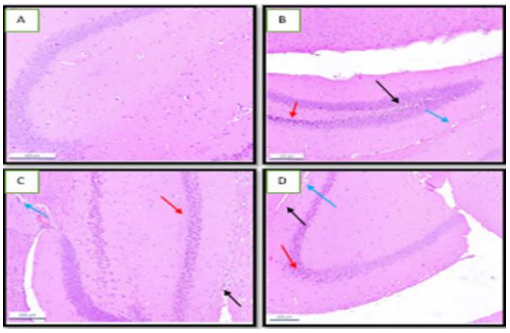


**Fig.no 5.10 : Photomicrograph of H &E -stained brain sections of different treated groups under 200X magnification.**

Black arrows represent the inflammatory cell infiltration, the Blue arrow represents neuron loss and Red arrow represents pyramidal cell degeneration.

A. Control
B. $AlCl_3$ 150mg/kg
C. $AlCl_3$ 150mg/kg + 200mg/kg of hydroalcoholic extract of *Dracaena reflexa*
D. $AlCl_3$ 150mg/kg +400mg/kg of hydroalcoholic extract of *Dracaena reflexa*

**Fig 5.10** (A,B,C,D) represents the brain sections of Swiss albino mice after different types of treatment for 4 weeks. Compared to the normal group, there is presence of amyloid plaques in disease group. At the same time, these plaques decreased in low dose and high dose treated groups, thus indicating the capacity of hydroalcoholic extract of *Dracaena reflexa* to inhibit the formation of amyloid plaque in the brain of albino mice.

**Histopathological Examination of Mice Brain using Congo Red Staining**

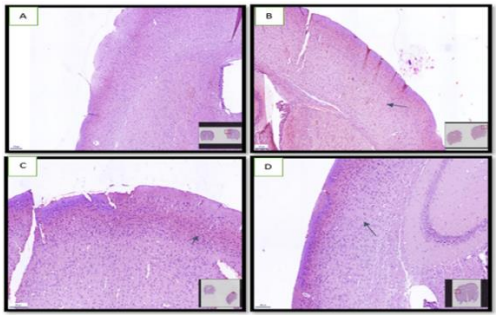


**Fig 5.11 : Photomicrograph of Congo red-stained brain sections of different treated groups under 200X magnification**.

Black arrow represents amyloid plaques.

A. Control-0.1% CMC
B. $AlCl_3$ 150mg/kg
*C.* 150 mg/kg AlCl3 + 200 mg/kg Hydroalcoholic extract of *Dracaena reflexa*
*D.* 150 mg/kg AlCl3 + 400 mg/kg Hydroalcoholic extract of *Dracaena reflexa*

**Fig.no. 5.11** (A,B,C,D) represents the brain sections of Swiss albino mice after different types of treatment for 4 weeks. Compared to the normal group, there is presence of amyloid plaques in disease group. At the same time, these plaques decreased in low dose and high dose treated groups, thus indicating the capacity of hydroalcoholic extract of *Dracaena reflexa* to

inhibit the formation of amyloid plaque in the brain of albino mice

## DISCUSSION

The present study evaluated the neuroprotective activity of hydroalcoholic extract of *Dracaena reflexa* was using aluminium chloride-induced model and various memory and cognition parameters like central area exposure, exploratory behaviour, avoidance response, WME and RME were evaluated. 4 weeks of 150mg/kg $AlCl_3$ produced changes such as impaired memory, reduced cognition suggesting neurodegeneration associated behavioural changes This Aluminum chloride induced neurodegeneration associated behavioral changes were prevented by *Dracaena reflexa*. The extract significantly inhibited the cholinesterase enzyme. It was found that an increased level of serotonin level indicates its capacity to reduce the anxiety and memory impairment associated with neurodegeneration.It was found that extract treatment leads to an increase in the catalase and glutathione levels and a decrease in MDA levels, indicating a reduction in brain oxidative stress caused by $AlCl_3$.

From H& E staining results show that extract treated group showed decreased inflammatory cells compared to disease group suggest the extract able to reduce the neuro -inflammation related to $AlCl_3$ induction. The Congo-red staining showed lesser no of amyloid plaques in extract treated group compared to disease group revealing its capability to counteract the $AlCl_3$-induced neuronal damageevaluated. Various biochemical parameters, such as AChE, MDA, GSH, serotonin, and catalase levels were estimated in the mouse brains homogenate. The extract of *Dracaena reflexa* leaves showed extract are enriched with , flavonoids, tannins, phenols, alkaloids, saponins, lipids, glycosides, and steroids .The *Dracaena reflexa* genus was previously analyzed, and it was found to contain various phenols and flavonoids. The n-Butanol fraction had the highest phenolic content at 92.72 ± 0.79 mg GAE/g extract, while the n-hexane fraction had the lowest phenolic content at 44.72 ± 0.79 mg GAE/g extract.(Bilal *et al*., 2023) ROS are typically generated during metabolic processes. An excessive build-up of ROS can harm fatty acids, DNA, and proteins, resulting in tissue injury and inflammation (Magder, S *et al* 2006). To bolster the immune system, it's essential to eliminate these ROS using antioxidants. *Dracaena reflexa* possess antioxidant and anti-inflammatory properties, suggesting potential benefits for neuroinflammatory conditions associated with Alzheimer's disease.The administration of $AlCl_3$ into the mice is now an established way of generating Alzheimer's model.AD pathological features could be counteracted by potential anti amyloid bioactive natural components.(Yaghmaei *et al*.,2013) From the biochemical,memory and cognition parameters and histopathological examination indicates that the protective activity of hydroalcoholic extract of *Dracaena reflexa* that effectively reduced brain plaques and enhanced neurogenesis and cognition in animal model.

## CONCLUSION

The present study evaluated the neuroprotective activity of hydroalcoholic extract of *Dracaena reflexa* using aluminum chloride-induced Alzheimer'model and various memory and learning parameters like central area exposure, exploratory behavior, avoidance response, working memory error, and reference memory error were evaluated. Various biochemical parameters such as AChE, MDA, GSH, serotonin, and catalase levels were estimated in the mouse brain of homogenate. The various phytochemicals identified in the hydroalcoholic extract of leaves of *Dracaena reflexa* were alkaloids, flavonoids polyphenols, phenols, sterols, terpenoids, tannins, and saponins. $AlCl_3$ treatment produced significant changes such as impaired memory, and reduced cognition suggesting neurodegeneration-

associated behavioural changes. The concomitant administration of hydroalcoholic extract of *Dracaena reflexa* could prevent the $AlCl_3$ induced neurodegeneration in a dose dependent manner. The extract could reduce the cognition and memory impairment caused by simultaneous administration of $AlCl_3$ The extract could produce an AChE inhibitory action and found elevated serotonin and antioxidant enzymes. The neuroprotective effect may be due to the AChE inhibition elevation in antioxidant enzymes and associated inhibition of free radical-induced injury to neurons. The neuroprotective effect in memory and cognition may be also due to Acetylcholinesterase inhibition, and elevated antioxidant and serotonin levels. So, the hydroalcoholic extract of *Dracaena reflexa* may be a useful neuroprotective agent requiring further research.

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**Conflict of Interest**

All the authors have no conflict of interest

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