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Evaluation of Neuroprotective Effects of Ethanolic Leaf Extract from *Causonis trifolia (L.)* in AlCl3 Induced Alzheimer's Disease in Wistar Rats

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ABSTRACT

This study examined the neuroprotective effects of ethanolic leaf extract from Causonis trifolia (L.) on Alzheimer's disease in Wistar rats produced by aluminum chloride (AlCl3). Bioactive substances like 9-octadecanoic acid were found by phytochemical and GC-MS studies, and molecular docking supported their interaction with important targets for Alzheimer's disease. In vivo tests revealed that, in comparison to untreated disease controls, treatment with the extract at doses of 150 and 300 mg/kg, along with donepezil, significantly increased locomotor activity, decreased anxiety, and improved memory retention in behavioral tests (IR Actimeter, Elevated Plus Maze, and Novel Object Recognition). Furthermore, the extract reduced neurodegenerative damage in striatal tissue and restored antioxidant enzyme activity (SOD and CAT). All things considered, the results show that Causonis trifolia has encouraging anti-Alzheimer's properties, most likely due to its antioxidant processes.

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CHAPTER ONE
INTRODUCTION

➢ *Introduction:*

The prevalence of age-related illnesses among the elderly has grown to be a significant worldwide health issue as the populace ages. Dementia results from the continuous and permanent loss of neurons caused by disorders connected to age. One such age-related illness is Alzheimer's disease (AD).

AD is a neurodegenerative disease. Approximately 36 million individuals worldwide struggle with AD, accounting for roughly 6% of the population over 65[1]. Multiple cognitive deficits, personality changes, and inappropriate behavior are characteristics of AD. Amyloid beta peptide and hyperphosphorylated tau protein are the main pathogenic markers seen in AD brain tissues, although the specific pathways causing these changes are yet unknown. Aggregating extracellular senile plaques (SP) cause amyloid B protein formation, whereas aberrant p-tau protein deposits generate neurofibrillary tangles (NFTs) [2]. It causes neuronal death, altered synaptic design and functioning, and eventually the clinical signs of AD.Both the cholinergic and glutamatergic neuronal signal transmission systems, which are important for cognition are impacted in Alzheimer patients, in particular, damaged cholinergic system is to blame for a reduction in memory and cognition in AD sufferer. Oxidative stress is recognized as both cause and treatment objective for AD. Excessive ROS production can ultimately harm neurons, lipids, and peptides. Additionally, AD is associated with mitochondrial malfunction, inflammatory reactions, impaired neurotransmissions, synaptic damage, hormonal changes, and irregularities in cell cycle. Acetylcholine levels rise in the brain, which lowers inflammatory cytokine production. In the case of elder people, a low level of ach can increase inflammation and exacerbate the disease. Long-term exposure to Some chemicals like streptozotocin, scopolamine, alcohol, heavy metals ($AlCl_3$, Cu, Zn, Pb), and reducing sugar(d-galactose) induces neuronal toxicity that leads to Alzheimer’s disease[3]. Various methods and models (open field, T-maze, Ymaze,Elevated plus maze,IR Actimeter) are developed to evaluate the AD. Therefore, the study aimed to identify the protective effect of Causonis trifolia (L.) extract on $AlCl_3$ neurotoxicity model for AD to compare the behavior changes and histopathology of hippocampus in Wistar rat.

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CHAPTER TWO
REVIEW OF LITERATURE

➢ *History of Alzheimer's Disease:*

Alzheimer's disease was first recorded in an autopsy on Auguste Deter, 55-year-old lady who passed away from a degenerative behavioural and cognitive disease, which was reported by Alois Alzheimer in 1906. Alzheimer noted the presence of two distinctive pathologies in Deter's brain: neurofibrillary tangles, which he correctly surmised were abnormal intracellular aggregates (and which were later shown to be composed of hyperphosphorylated and cleaved forms of the microtubule-associated protein tau), and neuritic plaques (which he called miliary foci), which were dystrophic neuronal processes surrounding a "special substance in the cortex" (Alzheimer et al. 1995). Glenner & Wong (1984) isolated and purified this "special substance," demonstrating that it was a 4.2-kDa peptide with a primary length of 40 or 42 amino acids, which they assumed was cleaved from a larger precursor. The peptide that Glenner and Wong identified today is called the Aβ peptide, which stands for amyloid-β peptide.

Brain tissue changes in Alzheimer's disease: Each neuron has long, branching extensions that allow them to connect. These connections, known as synapses, facilitate the flow of information through tiny bursts of chemicals that one neuron releases and another neuron absorbs. The brain is made up of trillions of synapses. These little connections help signals zip through the brain quickly, forming the foundation for our memories, thoughts, sensations, feelings, movements, and skills. Over time, researchers have discovered various changes in the brain that can disrupt these chemical signals, which may result in difficulties with thinking, learning, and daily activities, often seen in individuals with Alzheimer's disease. The accumulation of the protein fragment beta-amyloid into clumps (called beta- amyloid plaques) outside neurons and the accumulation of an abnormal form of the protein tau (called tau tangles) inside neurons are two of several brain changes associated with Alzheimer's disease. Beta-amyloid and tau have different roles in Alzheimer's. Plaques and smaller accumulations of beta-amyloid may damage neurons by interfering with neuron-to-neuron communication at synapses [8,25]. Inside neurons, tau tangles block the transportation of nutrients and other molecules essential for the normal function and survival of neurons while harming connections between neurons [9,10]. Beta-amyloid and tau accumulation is followed by damage to and destruction of neurons (called neurodegeneration) and other brain cells. Neurodegeneration, along with beta-amyloid and tau accumulation, is a key feature of Alzheimer's disease. The presence of toxic beta-amyloid and tau proteins is believed to activate immune system cells in the brain called microglia [11]. Microglia try to clear the toxic proteins and debris from dead and dying cells. Chronic inflammation may set in when the microglia can't keep up with all that needs to be cleared. Another brain change associated with Alzheimer's disease is atrophy (decreased brain volume) resulting from neurodegeneration and other factors [12]. While some degree of brain atrophy is common in older age, even in people who are cognitively healthy, atrophy is accelerated in people with Alzheimer's dementia [13]. Normal brain function is further compromised by decreases in the brain's ability to metabolize glucose, its main fuel.

➢ *Types of Alzheimer's Disease:*

In addition to biomarker evidence of Alzheimer's-related brain alterations, dementia caused by Alzheimer's disease is defined by observable memory, language, thinking, or behavioral symptoms that make it difficult for a person to function in everyday life[14].

➢ *Mild Alzheimer's Disease:*

While most people with mild Alzheimer's dementia may operate independently in many areas, they will probably need assistance with some tasks to maintain their safety and optimize their independence[15].

They could still be able to work, drive, and engage in their preferred pastimes. They can require extra time to finish everyday activities. It might be particularly difficult to make financial decisions and pay expenses.

➢ *Moderate Alzheimer's Disease:*

The intermediate stage of Alzheimer's dementia, which is frequently the longest stage, is characterized by increased memory and language difficulties, a higher risk of confusion, and difficulty doing multistep tasks like dressing and washing[16]. They could occasionally become incontinent, start having trouble identifying loved ones, and exhibit behavioral and impatient changes including anxiety and suspicion[17].

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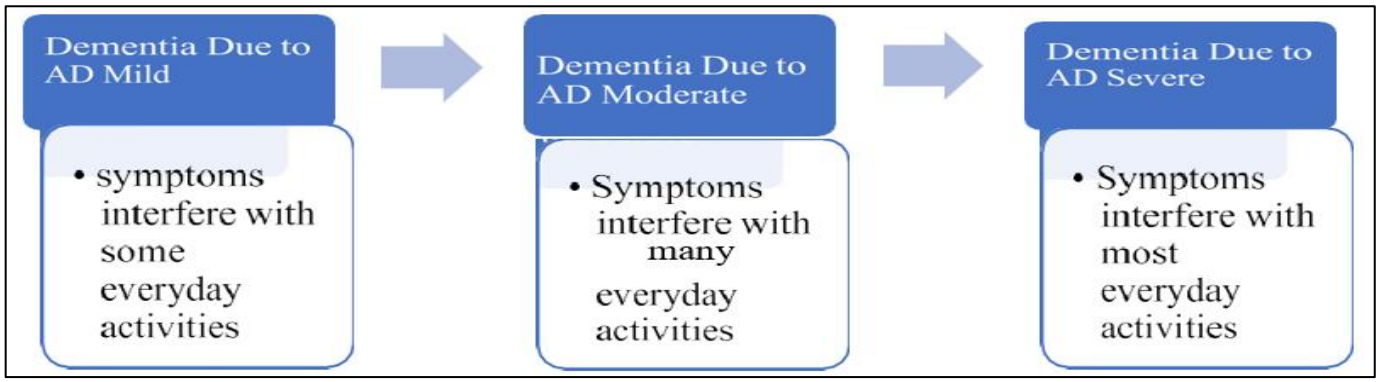

Fig 1 Flow Chart of Different Types of Alzheimer's Disease

➢ *Severe Alzheimer's Disease:*

People with Alzheimer's dementia who are in the severe stage have significantly reduced verbal communication skills and are likely to need 24-hour care. Walking may be impossible for certain people due to impairment to the parts of the brain involved in movement. They could thus spend the most of their time on a bed or in a wheelchair. Because they are less mobile, they are more susceptible to physical problems including blood clots, skin infections, and sepsis, which causes inflammation throughout the body and can lead to organ failure. It becomes challenging to eat and drink when the part of the brain that regulates swallowing is damaged. As a result, people may swallow food into their windpipe (trachea) rather than their esophagus (food pipe). Aspiration pneumonia is a kind of lung infection that can develop from food particles getting lodged in the lungs. One of the main causes of mortality for many people with Alzheimer's disease is aspiration pneumonia.[18]

➢ *Different Hypotheses Related to AD:*

- *Tau Hypothesis:*

Previously, Aß had been the focus of AD research. Researchers have now started to pay more attention to tau proteins since several investigations have indicated that they are among the essential components of NFTs. Tau protein is a functional monomeric, unfolded cell membrane protein and a member of microtubule-associated protein located within a neuron's cytosol. It Plays a critical role in tubulin stabilization. According to the tau hypothesis, normal tau is transformed into the paired helical filament (PHF-tau) and NFTS when the tau protein is hyperphosphorylated.[19,20,71] According to recent research, hyperphosphorylated tau that breaks down into oligomers (the toxic form of tau) is associated with neuronal damage, but still NFT itself does not seem to be linked to neurotoxicity that results in the development of neurodegeneration. [21,85]

- *Amyloid Cascade Hypothesis:*

According to the amyloid cascade theory, the pathophysiology of AD is significantly influenced by amyloid plaques, which are created by the buildup of Aß peptides and originate from the proteolytic dissociation of APP. Amyloid B precursor protein (ABPP), an intrinsic type I glycoprotein with a wide ectodomain, is the source of the Aß peptide.[49,50,51,52] Research has demonstrated that polypeptides (about 4 kDa) typically synthesised in soluble form make up the majority of amyloid plaques in AD.[21] There are many isoforms of the Aß protein, most of which have between 39 and 43 amino acids.[The ectodomain of the two APP isoforms, APP751 and APP770, is composed of 56 amino acids. The majority of the several Aß protein isoforms include between 39 and 43 amino acids. [22]

- *Oxidative Stress Hypothesis:*

The pathophysiology of AD is thought to be significantly influenced by oxidative stress. During mitochondrial respiration, the brain is known to use more energy and perform more tasks than any other organ, which also raises the risk of exposure to reactive oxygen species (ROS). Moreover, AD is intimately associated with lipid peroxidation, glycoxidation, protein oxidation, protein nitration, and molecular oxidative stress. Additionally, AD is intimately associated with Aß aggregation, which has been shown to result in oxidative stress.[21]

- *Cholinergic Hypothesis:*

The Cholinergic Hypothesis is the earliest of the AD causative ideas. According to this theory, the etiology of AD is caused by a reduction in neurotransmitters called acetylcholine in neurons. It has been proposed for over thirty years, implicated that the cognitive impairment observed in AD patients is caused by aberrant acetylcholine-containing neurons in the basal ganglia.[23]

- *Mitochondrial Cascade Hypothesis:*

Swerdlow was the first to suggest the mitochondrial cascade hypothesis in 2004. According to this idea, the primary cause of Aß accumulation, NFT development, and synaptic degradation in AD is mitochondrial abnormalities. It assumes that the aging brain

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and AD have comparable physiological pathways. Given that AD is characterized by systematic mitochondrial failure, it says that mitochondrial malfunction alone cannot adequately reflect the consequences of neuronal dysfunctions. [21,60,77]

- *Chemicals:*

Aluminium chloride (AlCl3) given by orally(175mg/kg) Aluminum chloride (AlCl3) can induce Alzheimer's disease (AD) by causing oxidative stress, inflammation, and apoptosis. [24]

Oxidative stress: AlCl3 can interact with plasma membrane lipids, causing lipid peroxidation and oxidative stress. Oxidative stress is linked to the progression of AD.

Inflammation: -AlCl3 can cross the blood-brain barrier and cause inflammation in the brain. This inflammation can lead to memory loss. [

Apoptosis: -AlCl3 can cause apoptosis by increasing the expression of the DAXX protein. DAXX is associated with the FAS protein, which is critical for apoptotic signaling.

- *Drugs:*

Donepezil (2.5mg/kg), orally administrated will use as a standard drug. Donepezil is commonly used in Alzheimer's disease because it is a cholinesterase inhibitor, which works by increasing the levels of the neurotransmitter acetylcholine in the brain, according to the National Institutes of Health (NIH).[25] Acetylcholine plays a crucial role in memory and cognitive function, and its levels are reduced in Alzheimer's disease. By inhibiting the breakdown of acetylcholine, donepezil can help improve or stabilize cognitive function and other symptoms associated with the disease.

- *Mechanism of Action:*

Donepezil works by binding to and inhibiting the enzyme acetylcholinesterase, which is responsible for breaking down acetylcholine in the brain. Donepezil hydrochloride, a piperidine derivative, is a fast-acting and reversible medication that works in the brain to block the enzyme acetylcholinesterase.[26] This enzyme is responsible for breaking down acetylcholine, a neurotransmitter. By stopping acetylcholinesterase from breaking down acetylcholine, donepezil increases the amount of acetylcholine available in the brain's synapses. This boosts cholinergic transmission, which is beneficial for treating Alzheimer's disease.[27]

Cholinergic Transmission by inhibiting acetylcholinesterase, donepezil increases the amount of acetylcholine available at the synapses, where nerve cells communicate. Cognitive Improvement: This enhanced cholinergic transmission can lead to improvements in memory, attention, and other.

➢ *Side-Effect of Donepezil Tablet:*

Diarrhoea, Feeling sick (nausua), Headache, Feeling sleepy in the daytime or feeling dizzy. [

➢ *Plant Description:*

Causonis trifolia commonly known as bush Grape, fox-grape, three-leaved wild vine or threleaf cayratia is a species of liana plant native to Australia and Asia. It has black-colored berries, and its leaves contain several flavonoids, such as cynadin and hydrocyanic acid is present in the stem, leaves and roots.[29]

- *Scientific Classification:*

Table 1 Scientific Classification:

Kingdom	**Plantae**
Clade	Tracheophytes,Angiosperms
Order	Vitales
Family	Vitaceae
Genus	Causonis
Spcies	C.trifoia
Biological Name	Causonis trifolia

The vernacular names for Causonis trifolia (L.) include: Bush grape, Fox-grape, Three-leaved wild vine, Three leaf cayratia, and Yugali.

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Fig 2 Pant Causonis Trifolia

- *Plant Morphology*

Kangaroo vine is a perennial climbing vine or shrub, popular when grown as a houseplant, partial Sun loving, bushy, Long height.[30]

- *Stems:*

Vine stem diameters were measured up to 7 cm.

- *Leaves:*

The underside of the pamphlet blades' utmost hairs are often hooked, especially on the midrib. With a lens, "oil painting blotches" are easily visible. The emulsion of tendrils splint-opposed has multiple branches, each of which ends in a distended structure that resembles a haustorium that grows in crevices and cracks. In the outgrowths, there is "oak grain." [31]

- *Flowers:*

Terminal or inflorescence leaf-opposed. flowers with a diameter of 4 mm. cup-shaped calyx, 0.2 mm long, without lobes. The apices are hooded, the outer surface is covered in hairs, and the petals are about 2.5 mm long. filaments that are about 1.5 mm long. Pale yellow, lobed disk, 0.8 mm high. pyramidal style. Each locule has two ovules. [31;32]

Fig 3 Leaf of Causonis Trifolia

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- *Fruit:*

globular, depressed fruits, 8-10 x 10-19 mm. About 5-7 x 2.5-5 mm seeds. The inner testa is brown and extremely hard, with a rugose surface, while the outer testa is soft and greenish brown. The cotyledons are slightly wider but shorter than the radicle, and the embryo is roughly 0.75–1 mm long.[31]

- *Seedlings*:

Elliptic to ovate cotyledons, measuring roughly 25–26 x 16–17 mm. The initial leaf is simple, toothed, or bifoliolate, with a tendency to trifoliate. The second leaf trifoliates. Hairs cover the lower surface, while the upper surface—aside from the midrib—is glabrous. At the tenth leaf stage, the stalk is roughly 0.8–1 cm long, and the middle leaflet blade is roughly 3.5–4 x 2.2–2.7 cm. Bases are oblique, stalks are between 0.2 and 0.6 cm long, and lateral leaflet blades measure roughly 2.5 to 3.5 x 2-2.5 cm. Seeds take 20 to 192 days to germinate.

Fig 4 Flower of Causonis Trifolia

➢ *Pharmacological Activities: -*

- Antioxidant activities:

The primary source of the plant's remarkable antioxidant properties is its high phenolic content. These chemicals can reduce oxidative stress and perhaps minimize the chance of acquiring chronic diseases by neutralizing free radicals.[33]

- *Anti-Inflammatory Properties:*

Causonis trifolia has important characteristics like:

✓ BSA Inhibition Denaturation: The extract stops bovine serum albumin's (BSA)inflammatory process from denaturing.
✓ Protease Inhibition: This inhibits trypsin, a serine protease linked to inflammatory responses.
✓ Hemolysis Inhibition: By inhibiting heat-induced hemolysis, the extract demonstrates membrane-stabilizing qualities.[34]

- *Antidiabetic Effects:*

The plant exhibits encouraging antidiabetic effects by:

✓ α-Amylase Inhibition: The extract reduces the absorption of glucose by inhibiting α-amylase, the enzyme that breaks down starch.
✓ Enhancement of Glucose Uptake: It makes yeast cells more sensitive to insulin by increasing their uptake of glucose.

- *Antimicrobial Properties:*

Catechin, kaempferol, gallic acid, caffeic acid, and sinapic acid are among the phenolic and flavonoid compounds found in Causonis trifolia, which also has antioxidant properties.[35]

➢ *Geographical Distribution: -*

- Asia: - China, India, Bangladesh, Nepal, Pakistan, Philippines and Sri Lanka

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- Australia: - western Australia

➢ *Uses: -*

The plants material is used to treat splenopathy, neuralgia, tumors, and as a diuretic. Scurvy is treated with leaf decoctions, which are made from the juice of fresh leaves. The roots have anti-venom properties and are used as an astringent. While the powdered root tuber is taken orally with milk to promote the early healing of cracked bones, diabetic patients are given an aqueous extract of the root along with the infusion of seeds to regulate blood sugar levels. [36,37]

➢ *Aim & Objectives*

- *Aim:*

To evaluate the phytochemical screening and anti-Alzheimer's activity of the *Causonis trifolia (L.)*

- *Objectives:*

To study the phytochemical constituent of *Causonis trifolia (L.).* By the isolation, we may determine the active constituents which are responsible for Anti-Alzheimer activity in rodents

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CHAPTER THREE
MATERIALS & METHODS

➢ *Materials and Methods:*

- *Animals:*

Twenty-five adult Wistar rats were taken, age should be between four weeks to six weeks. Randomly divided them into five groups, each group containing five animals. They were kept in a well-maintained animal house where everything should be controlled. Placed in a stainless-steel cage, the Temperature should be in between 22º to 25º C. Humidity should be 55 to 70 percent.12/12 hours dark light cycle should be maintained with access to food and drinking water. The international standards for the care and use of laboratory animals were followed during the experimental procedures.

- *Chemicals:*

Aluminium chloride (AlCl3) given by orally(175mg/kg) Aluminum chloride (AlCl3) can induce Alzheimer's disease (AD) by causing oxidative stress, inflammation, and apoptosis.[39]

- *Drugs:*

Donepezil (2.5mg/kg), orally administrated will use as a standard drug.

- *Plant Extract:*

Ethanolic extract of *Causonis trifolia*

- *Preparation of Causonis Trifolia (L.) Extract and Authentication:*

*Causonis trifolia (*L.), also called "fox grape" (synonym: Cayratia trifolia (L.), we collected it from the local area of Uluberia and authenticated it from Shivpur Botanical Garden, Howrah. Authentication number: CIPT/2024/02.

- *Requirements:*

✓ Equipment's: Conical flask, filter paper, beaker, glass rod
✓ Chemicals: Petroleum ether, Ethanol Coarse powder of *Causonis trifolia*

- *Steps Involved in the Extraction Process:*

✓ Collect the plant leaves, wash them properly with fresh water, and dry them at room temperature for 7 to 10 days.
✓ Dry leaves of the *Causonis trifolia (L.)* were grinded properly to get a coarse powder. The total weight of coarse powder was 99.56 gm.
✓ The coarse powder was properly mixed in petroleum ether solvent at a 1:10 ratio and kept for 3 days.
✓ Petroleum ether and extract residue differentiated with filter paper and kept at room temperature to dry the residue.
✓ Dry residue went to the 3-day extraction method in an ethanolic medium. 6.Collected extract was stored in dry and cool places at 4ºC.

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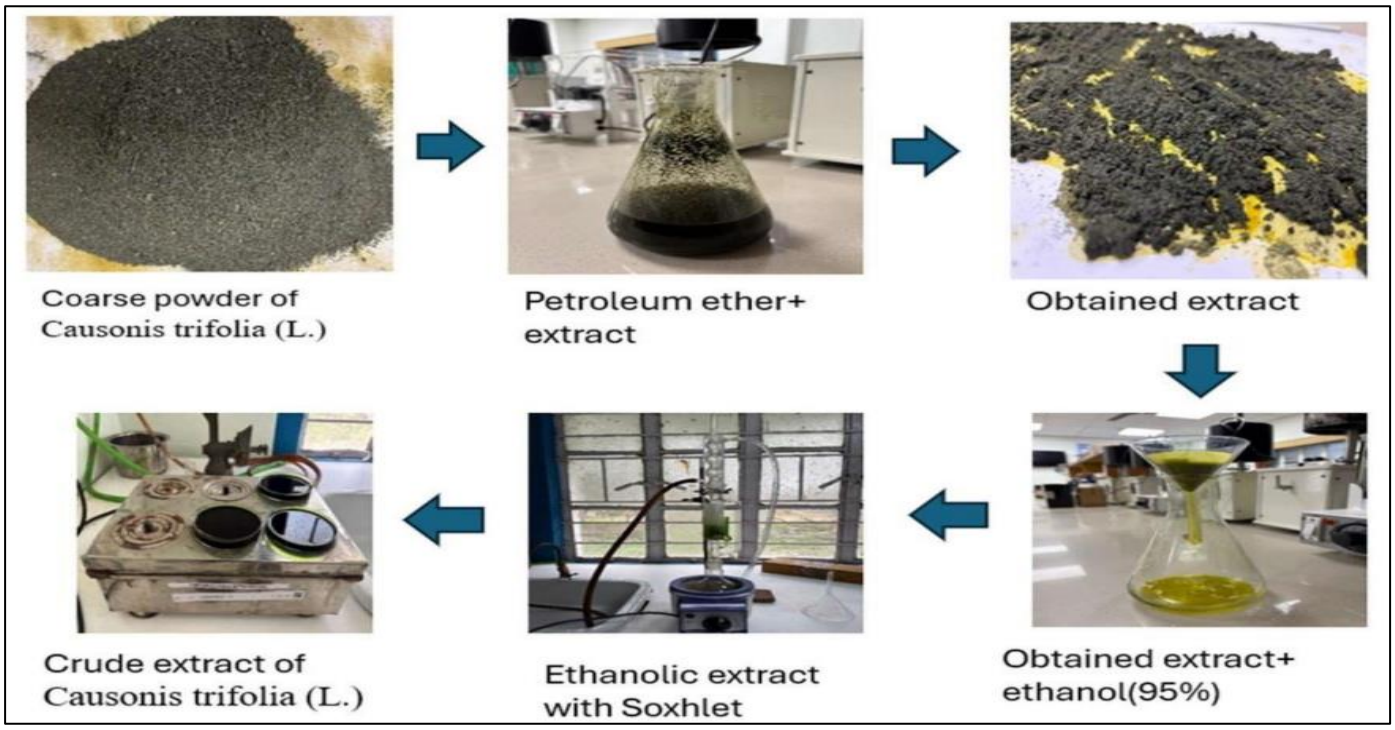

Fig 5 Extraction Procedure of Causonis Trifolia Leaf

➢ *Evaluation of Phytochemical Characteristics:*

Table 2 Test for Alkaloids:

Test	Procedure	Inference
Mayer's Test	Mix a few ml of extract solution of mercuric chloride and potassium iodide	Cream colored formed
Wagners's Test	1ml of extract solution few drops of iodine and potassium iodide were added.	Reddish brown
Dragendroff's test	1ml of extract solution + Potassium bismath nitrate	Reddish brown
Hager's test	1ml of extract solution few drops of saturated solution of picric acid	Yellow coloured

Table 3 Test for Flavonoids:

Test	Procedure	Inference
Shinoda test	Four pieces of magnesium filings are added to the ethanolic extract followed by a few drops of concentrated hydrochloric acid.	Pink colour formed
Sodium hydroxide test	About 5 mg of the compound is dissolved in water, warmed, and filtered. 10% aqueous sodium hydroxide is added to 2 ml of this solution.	This produces a yellow colouration.

Table 4 Test for Carbohydrate:

Test	Procedure	Inference
Molish's Test	Aqueous extract+ Alpha naphthol in alcohol+ con.H2SO4	Violet ring at the junction of two liquids
Barfaed's Test	Mix equal volumes of Barfaed's reagent and test sample then heat up and cool for a few minutes.	Red colour precipitated
Benedict's test	Mix equal volume of Barfaed's reagent and test sample then heat up.	Red colour appears

Table 5 Test for Protein:

Test	Procedure	Inference
Biuret Test	NaOH + 1% CuSO4	Violet or Pink colour
Millon's Test	Metalic mercury+ Nitric acid+ water	White precipitated which turns into red upon heating
Xanthoprotic test	Test sample+Conc.H2SO4+ Strong NH3 Solution test for sulpher-containing proteins NaOH+ Lead acetate	White PPT which turns into yellow upon heating, black or brownish due to pbs formation

➢ *Auto-Docking Process:* -

A complete procedure for molecular docking using Auto-Dock (AutoDock4 with Auto-Dock Tools, not Auto Dock Vina).

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This guide assumes you're working on a Linux or Windows system with Auto-Dock and Auto-Dock Tools installed. [41,42,43]

- *Auto Dock Docking Procedure: -*

✓ *Prepare Files*

▪ *Prepare Receptor (Protein)*

Download the protein structure from the RCSB PDB database (.pdb file).
Open in Auto Dock Tools (ADT):

- Load the PDB file.
- Remove water molecules (Edit > Delete Water).
- Add polar hydrogens: Edit > Hydrogens > Add > Polar Only.
- Add Kollman charges: Edit > Charges > Compute Gasteiger.
- Save as. pdbqt: File > Save > Write PDBQT.

✓ *Prepare Ligand (Small Molecule)*

- Draw or obtain ligand in .mol or .pdb format.
- Load into ADT.
- Add hydrogens: Edit > Hydrogens > Add > All Hydrogens.
- Compute Gasteiger charges: Edit > Charges > Compute Gasteiger.
- Detect torsions: Ligand > Torsion Tree > Detect Root, then define rotatable bonds.
- Save ligand as .pdbqt.

- *Set Up Grid Box*

✓ Open receptor in ADT.
✓ Set up grid box: Grid > Grid Box.

- Adjust center of the box (based on active site or known ligand position).
- Set dimensions (size in x, y, z).
- Recommended spacing: 0.375 Å.

✓ Save grid parameter file (.gpf): Grid > Output > Save GPF.

- *Set Docking Parameters*

✓ Docking > Macromolecule > Set Rigid Filename: Load your receptor. pdbqt.
✓ Docking > Ligand > Choose: Select your ligand. pdbqt.
✓ Set parameters under Docking > Search Parameters:

- Algorithm: Genetic Algorithm (GA).
- Number of runs: 10–100.

✓ Save docking parameter file (.dpf): Docking > Output > Save DPF.

- *Run AutoGrid and AutoDock*

Use command-line terminal (CMD/Terminal): bash

Copy Edit

Autogrid4 -p receptor.gpf -l receptor.glg

Autodock4 -p ligand.dpf -l ligand.dlg

✓ .glg = grid log file
✓ .dlg = docking log file (results)

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- *Analyze Results*

✓ Open. dlg in ADT.
✓ Analyze> Docking > Open and load. dlg.
✓ Analyze> Conformations > Play, Cluster to view binding poses.
✓ View binding energy, RMSD, and interactions.

- *Optional Tips*

✓ For batch docking, use scripting.
✓ Use AutoDockVina if you need faster performance.
✓ Use PyMOL or Chimera for better visualization of binding.

➢ *Catalase Activity Assay*

- *Sample Preparation:*

Our rat blood sample was taken by retroorbital puncture with anticoagulant (sodium citrate) and centrifuge at 1000×g for 10 minutes at 4ºC. Collect the supernatant and keep it on ice. [45,46]

- *Solutions Preparation:*

Use the following reagents in the kit to prepare the solutions accordingly-

Table 6 Different Reagents Provide in Estimation Kit.

Solution ID	Cat. No.	Solution Description	Quantity
Buffer-1	OPKA005-A	Sodium Phosphate Buffer	1100ml
Solution A	OPKA005-A	Peroxide Stock Solution	250ml
Solution B	OPKA005-A	Ammonium Molybdate Solution	110ml
Enzyme	OPKA005-A	Catalase (1000U/ml)	50ml

- *Enzyme Solution:*

Dilute the Catalase Enzyme by adding 10µl Enzyme to 90µl buffer solution to get enzyme solution of 100 U/ml. Keep in ice until use.[50,51,52]

✓ Substrate Solution: Dilute Peroxide solution by adding 25µl solution A to 10 ml of buffer 1(prepare just before use of the assay; stable for 1-2 days).
✓ Solution B: Ammonium Molybdate solution should be used undiluted.

- *Bioassay: 1st Set the Groups as:*

✓ Set-1: Buffer blank
✓ Set-2: Substrate blank
✓ Set-3: Positive control
✓ Set-4 (and other): Test sample
✓ Set-5 (and other): Test sample blank

- Now, follow the table as reference for reaction set up in micro-test plate:

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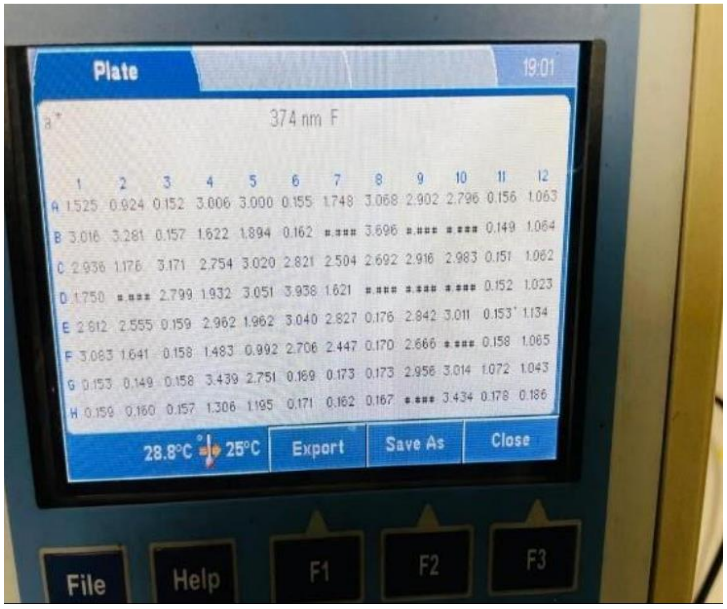

Fig 6 Different Lamda Value Showing in ELISA Reader

- *Experimental Procedure:*

✓ Add 90µl of substrate solution to the wells defined for set 2, 3 & 4 in the micro-titre plate.
✓ Add 100µl of buffer-1 to set-1, 10µl to set-2 and 90µl to set-5.
✓ Add 10µl of enzyme solution to set-3, serve as positive control.
✓ Add 10µl of sample to set-4 and set-5.
✓ Incubate at 37ºC for 5 minutes.
✓ Add 100µl of solution B to each wells of the groups Set 1,2,3,4,5….
✓ Incubate at 37ºC for 2 minutes.
✓ Measure the absorbance at 374nm with microplate reader (Microplate reader with 350nm or 405nm filter will be sufficient to measure the absorbance).
✓ Plate Setup:


Fig 7 ELISA Microplate Reader

- *Calculations of Activity:*

$$\textbf{Catalase activity (U/ml)} = \frac{2.303}{t} * [log \frac{S^0}{S-M}] * \frac{V_t}{V_s}$$

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- ✓ T: time
- ✓ Sº: Mean absorbance of set 2
- ✓ S: Mean absorbance of set 3
- ✓ M: Mean absorbance of set 1 (correction factor)
- ✓ Vt: total volume of reagents in test tube (200µl)
- ✓ Vs: volume of sample (10µl).

➢ *ELISA Procedure for SOD Test:*

- *Reagent Preparation:*

The 1X Wash Buffer was prepared by diluting 20 ml of the provided 25X Wash Buffer concentrate in 480 ml of deionized water. The Biotinylated Antibody and Streptavidin-HRP Conjugate for each assay (SOD, CAT, and GSH) were centrifuged briefly and diluted to the working concentration (100-fold) using the respective dilution buffers supplied in the kits.

✓ *Standard Preparation:*

The lyophilized standards were reconstituted with Standard Diluent as per kit instructions and gently mixed for 10–15 minutes to ensure complete dissolution. A series of two-fold serial dilutions was prepared to generate a standard curve for each assay, covering a broad concentration range. A blank (0 concentration) was included using only the Standard Diluent.

- *Assay Procedure*

All standards and test samples were run in duplicate. The procedure for SOD, CAT, and GSH assays followed the sandwich (for SOD and CAT) ELISA formats provided by the kit protocol:

- ✓ 100 µl of standards, blank, and diluted test samples were added to the respective wells of pre-coated 96-well microplates.
- ✓ The plates were sealed with adhesive covers and incubated at 37 °C for 1– 2 hours to allow antigen binding.
- ✓ Wells were then aspirated and washed four times with 1X Wash Buffer. After each wash, residual buffer was removed by firmly tapping the inverted plate on absorbent paper, and the exterior of the wells was wiped to eliminate interference during the reading step.
- ✓ 100 µl of the diluted Biotinylated Antibody was added to each well, followed by incubation at 37 °C for 1 hour.
- ✓ After washing as described, 100 µl of diluted Streptavidin-HRP Conjugate was added and the plates were incubated at 37 °C for 30 minutes.
- ✓ The washing step was repeated four times to remove unbound conjugates.
- ✓ 100 µl of TMB substrate was added to each well and incubated at 37 °C for 10–15 minutes in the dark. A blue color developed in positive wells.
- ✓ The reaction was terminated by adding 100 µl of Stop Solution, turning the color from blue to yellow.
- ✓ The absorbance was measured at 450 nm using a microplate reader within 10–15 minutes of adding the Stop Solution.

➢ *Grouping of Animals:*

- *Animals were Divided into Five Groups, Each of Five Animals.*

- ✓ Group 1: The control group was administered by saline water.
- ✓ Group II: Disease control group orally administrated by aluminium chloride (175 mg/kg). (Negative control)
- ✓ Group III: Animal oral administered by donepezil tablet standard (2.5 mg/kg) and Alzheimer's induced with aluminium chloride(175mg/kg). (Standard control)
- ✓ Group IV: Animals were orally administered by extract(150mg/kg) which dissolved in CMC (0.3% w/w) solution and Alzheimer's induced with AlCl3. (Test control-1)
- ✓ Group V: Animals were orally administered by extract(300mg/kg) which is dissolved in CMC (0.3-0.5 w/w) solution and Alzheimer's induced with Aluminium chloride(175mg/kg). (Test control-2)

➢ *Pharmacological Screening of the Extract for the Following Activity by Using Various Animal Models to Check the Anti-Alzheimer's Activity:*

- *Ir-Acti-Meter:*

✓ *Principle:*

The Infra-red Acti-meter using infrared light beams, measures spontaneous activity. It is the perfect instrument for evaluating rodents' locomotor activity and exploration. The system is dependable for quick and simple drug testing in both day light and dark environments.

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- *The System Can be Used to Assess*

✓ Exploration of spontaneous locomotor activity.
✓ Curiosity (detection of nose-spokes in hole-board).

One square frame, a frame stand, and a hole board plate make up the IR Acti- meter system in its most basic form. There are a total of 32 infrared cells on the frame, 16 of which are on the X-axis and 16 on the Y-axis. The instrument control panel will show the total number of beam brakes in both acti-meter and hole board modes, as well as the number of brakes by an animal on each axis. When using in online mode, options for automatic cutoff, experiment name, comments, sex, group, and reading averaging are available.

- *Handling Procedure:*

✓ *Switch ON the instrument*

The display will show ORCHID SCIENTIFICS for few seconds and then will the XYZ Counts, T-counts (total of X, Y & Z) and timer. (Please note that Z axis is optional).

- X: represents number of beam breaks on X Axis (FRONT SIDE)
- Y: represents number of beam breaks on Y Axis (LEFT SIDE)
- Z: represents number of beam breaks on Z Axis
- T: represents total of number of beam breaks on X, Y & Z Axis.

The timer indicates the time from the start of the experiment.

T HB: represents hole board mode

Description of Keys:

ACT: represents acti-meter mode

PAUSE: To pause the experiment Timer & beam counts

RESET: To reset the counts and timer to zero position

Fig 8 IR-Actimeter

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- *Elevated Plus Maze (EPM):*

The elevated plus maze (EPM) is a test measuring anxiety in laboratory animals that usually uses rodents as a screening test for putative anxiolytic or anxiogenic compounds and as a general research tool in neurobiological anxiety research such as PTSD and TBI. The model is based on the test animal's aversion to open spaces and tendency to be thigmotaxic. In the EPM, this anxiety is expressed by the animal spending more time in the enclosed arms.

The validity of the model has been criticized as non-classical clinical anxiolytics produce mixed results in the EPM test. Despite this, the model is still commonly used for screening putative anxiolytics and for general research into the brain mechanisms of anxiety.

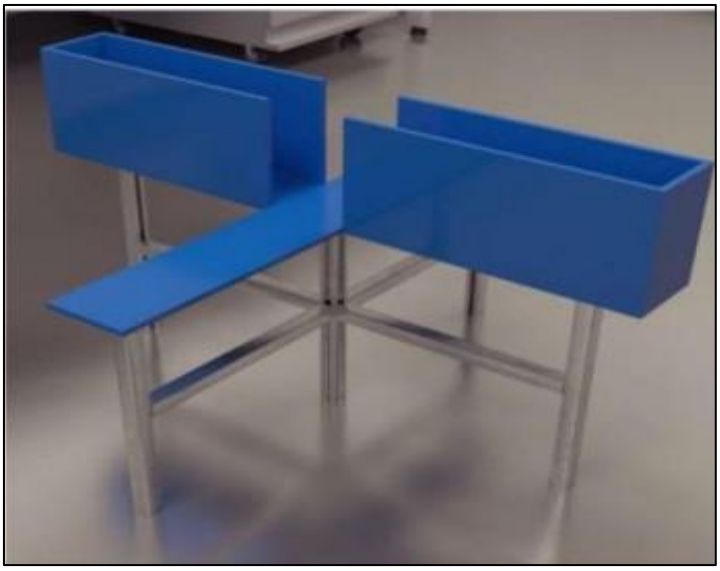

Fig 9 Elevated Plus Maze

✓ *Test Procedure:*

The EPM is a standard test of anxiety and spontaneous exploration, where the animals choose between exploring the closed arms, perceived as safer, or the more exposed open arms of the apparatus.

Three 5-minute testing sessions will be performed, with the initial exposure to the apparatus repeated after 1 day and again after 7 days, to assess possible differences in habituation.

A web camera placed above the apparatus will record the animals' behaviour. The digital tracking software will analyse the animals' locomotor activity (total distance) offline.

- *Novel Object Recognition (NOR) Task:*

✓ *Principle*

The novel object recognition (NOR) test (also called the novel object discrimination test, one-trial discrimination test, and novel object detection test) relies on the tendency of a fish to discriminate between two objects. This test requires no conditioning, external rewards, or aversive stimuli. On the alzheimeric condition animal behavour is non exploratory.

✓ *Test Procedure:*

On the first day, an animal was put into the black square arena (70×70cm, the same as used for OF testing) for 5min to allow habituation to the environment. The following day, two identical objects (glass jars full of pebbles) were added into the arena to opposite corners, 15cm from the walls and the rat spent there 5min exploring the objects

After a 1h retention interval, one object was shifted by 20cm and the animals was allowed to explore the objects for 3min (NOL test phase). On day 3, the animals explored the same identical objects in the same location as the previous day for 5min (NOR training phase).

After the 1h retention interval, one object was changed to a novel one (glass cuboid container of a comparable size) and the rat explored both objects for 3min (NOR test phase).

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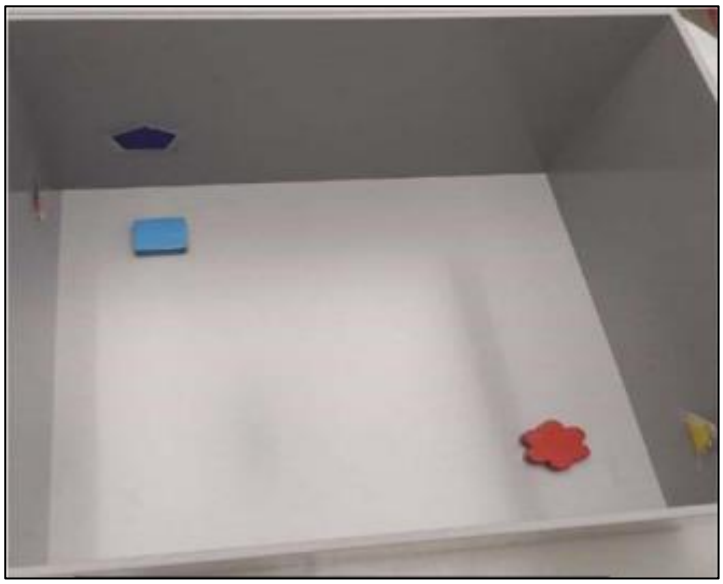

Fig 10 Novel Object

The behaviour of the animals were analyzed by a human observer using the Kenovea software, who noted object exploration and object climbing.

DISCRIMINATION INDEX = Tn – Tf

DISCRIMINATION RATIO = (Tn − Tf)/(Tn + Tf)

Where Tn= Time spent in novel object, Tf= Time spent in similar objevct

➢ *Statistical Analysis:*

The data obtained from animal experiments were analyzed with kinovea Software by GraphPad (version 3.10.4.2).

It will express as mean ± standard error of the mean.

Results was considered to be statistically significant at $p \leq 0.05$. Significance levels were as follows:

*Indicates $p \leq 0.5$ as significant

**indicates $p \leq 0.01$ as highly significant

***indicated $p \leq 0.001$ as very significant

……****indicated $p \leq 0.0001$ as very very significant

➢ *Brain Isolation Procedure:*

Brain isolation process in rats typically involves euthanasia, skull removal, and careful dissection to extract the brain, followed by further processing depending on the specific research goals. This may include separating specific brain regions, removing meninges, and isolating cellular or subcellular fractions.

- *Here's a More Detailed Breakdown:*

✓ *Euthanasia and Initial Steps:*

Rats are euthanized using methods like cervical dislocation (for mice) or decapitation (for both rats and mice).

The head and neck area are often sprayed with ethanol for sterilization. A midline incision is made on the scalp, and the skull is exposed.

✓ *Skull Removal:*

The skull is carefully removed using bone cutters or rongeurs, starting from the cerebellum and moving towards the olfactory bulb.

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The dura mater (outermost membrane covering the brain) is peeled back and removed with forceps.

Specific bones like the zygomatic arch, coronoid process, and angular process may be cut to facilitate access to the brain.

✓ *Brain Extraction:*

A spatula or forceps are used to gently lift the brain out of the cranial vault.

In some cases, the brainstem and cerebellum are carefully dissected away from the cerebrum.

✓ *Further Processing:*

- *Brain Region Dissection:*

Depending on the research, the brain may be further dissected into specific regions like the cortex, hippocampus, or cerebellum using fine scissors and forceps.

- *Meninge Removal:*

The remaining meninges (membranes covering the brain) are carefully removed using forceps.

- *Isolation of Cellular or Subcellular Fractions:*

The brain tissue may be homogenized and centrifuged to isolate specific cell types or subcellular components like synaptic membranes.

- *Tissue Preservation:*

The isolated brain tissue or fractions may be preserved in solutions like phosphate-buffered saline (PBS) or other buffers, often on ice.

- *Important Considerations:*

All steps are typically performed at 4°C to maintain tissue integrity and minimize degradation.

Protease inhibitors may be added to the buffer to prevent protein breakdown.

Aseptic techniques are crucial to prevent contamination during the isolation process, especially when working with cells or subcellular fractions.

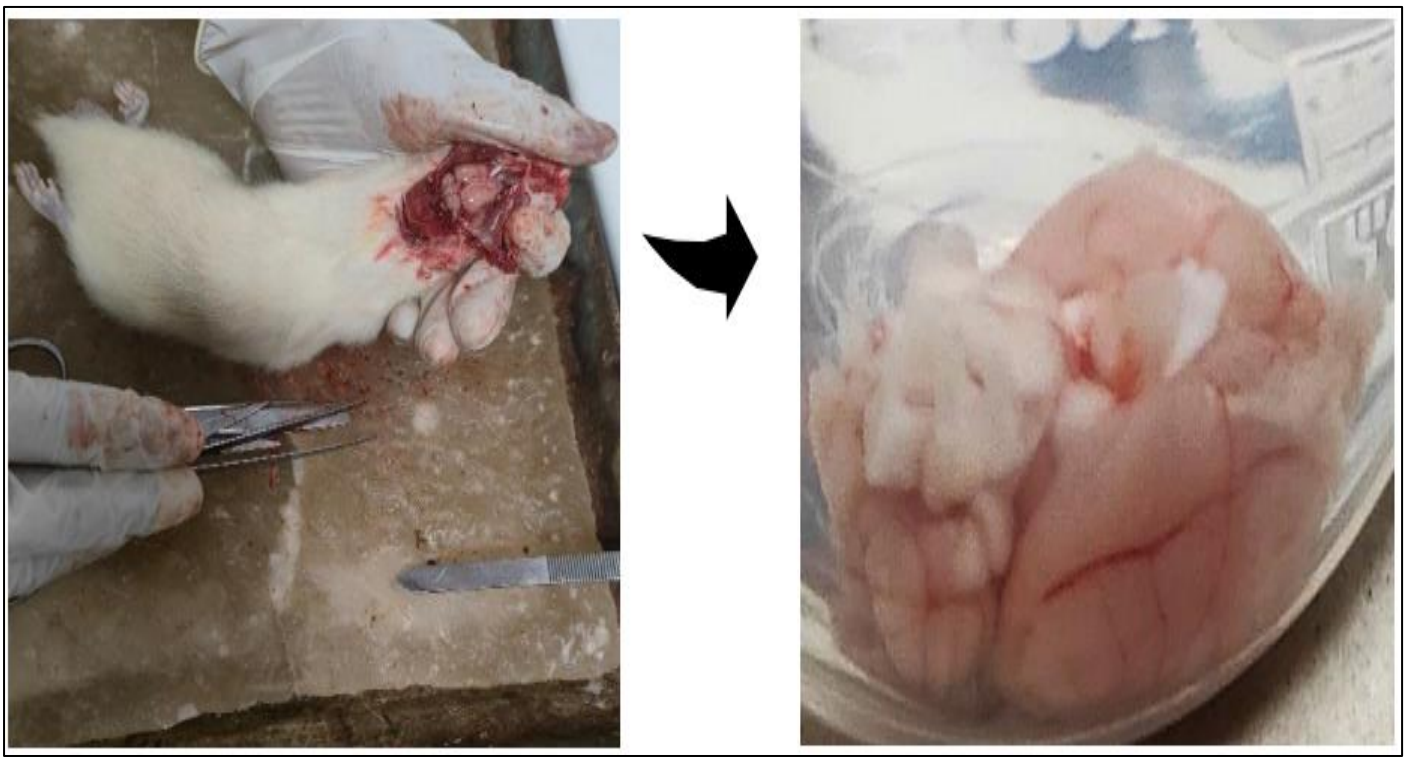

Fig 11 Brain Isolation Procedure of Wistar Rat

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CHAPTER FOUR
RESULTS AND DISCUSSIONS

A. Results:

➢ *Macroscopic Characters:*

- Size- Height- 12cm, Width- 7.5cm
- Shape- Oval
- Colour- Dark Green (upper surface), Pale Green (lower surface)
- Surface characteristics – Smooth & Slightly waxy
- Mergin- The leaf has teeth like structure

Fig 12 Causonis Trifolia Leaf

➢ *Phytochemical Analysis of the Ethanolic Leaf Extract of Causonis Trifolia (L.)*

Table 7 Name of Different Tests and Results

	Name of the Test	**Result**
Carbohydrate test	Molish's test	Positive
	Barfeed's test	Negative
	Benedict's test	Negative
Protein test	Biurat test	Negative
Alkaloids test	Mayer's test	Positive
	Dragendroff's test	Negative
Test for steroids	Salkowski's test	Negative
Test for glycosides	Liberman test	Positive
	Keller-killani test	Negative
	For anthraquinone glysosides	Positive
Test for flavanoids	Shinoda test	Positive
	Alkaline reagent test	Positive
Others test	Test for Phenol	Positive
	Test for Tannins	Positive
	Test for Saponin	Negative

➢ *Metabolic Profiling by GC-MS Analysis:*

GC-MS analysis of ethanolic leaf extract of *Causonis trifolia* revealed the presence of total 20 Phyto-constituents These are tabulated with there R.T, C.M,M.W, M.F, and structure The main compound of plant extract which is useful against Alzheimer's is 9-Octadecanoic acid.

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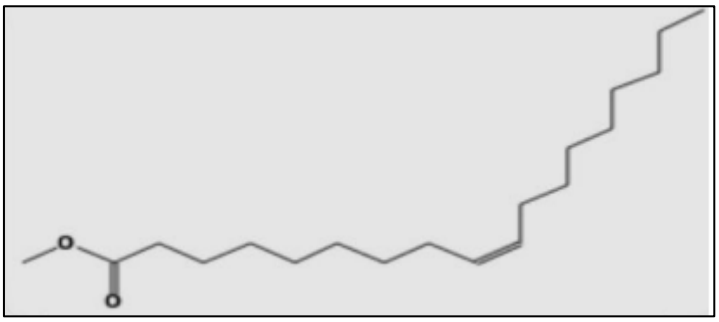

Fig 13 Molecular Structure of 9- Octadecanoic Acid

Table 8 Name of Different Compound Obtained from GC-MS Analysis

Peak.No.	Retention Time	Compound Name	M.wt	M.Formula	Structure
1	0.021	1-Nonadecanol	296.51	$C_{19}H_{40}O$	
2	0.056	1,1-Dodecanediol, diacetate	260.364	C14H28O4,	
3	0.225	2(3H)-Benzofuranone, hexahydro-4,4,7a-trimethyl	192.26	C12H16O2	
4	0.245	6,10-Dimethyl-4-undecanol	214.38	C14H30O	
5	1.124	Tetraborane(10)	53.32	B4H10	
6	1.153	2,4-Thiazolidinedione	117.13	C3H3NO2S	
7	1.282	1,3-Pentanediol, 2-methyl-	118.17	C6H14O2	
8	1.416	Ethyl Acetate	88.1	$C_4H_8O_2$	
9	1.483	1-Propanol, 2-methyl-	74.12	C4H10O	
10	1.895	Dodecanoic acid, 1,2,3-propanetriyl ester	354.57	C21H42O4	
11	2.057	Ethane, 1,1-diethoxy	118.18	C6H14O2	
12	3.740	3-Pentyl-pentanedioic acid	202.244	C10H18O4	
13	3.901	Cyclooctaneacetic acid, 2-oxo-	188.22	C9H16O3	
14	4.740	Decane, 1,10-diiodo-	394.09	C10H20I2	
15	5.255	4-Piperidinecarboxylic acid hydrazide	144.18	C6H12N2O2	
16	7.164	8-Heptadecanol, 8-methyl-	270.484	C18H38O	
17	7.305	Spiro[3,5-dioxatricyclo(6.3.0.0(2,7))undecan-6-one-4,2'cyclohexane], 1'-isopropyl-2,4'-dimethyl	235.35	C15H23NO2	
18	8.030	16-Hydroxyhexadecanoic acid	256.42	$CH_3(CH_2)_{14}CH(OH)COOH$	
19	11.253	9-Octadecynoic acid	280.45	C18H32O2	
20	14.290	trans-2,3-Epoxyoctane	126.19	C8H14O	

- *9-Octadecanoic Acid:*

It is an unsaturated fatty acid, which has a double bond at the ninth carbon atom. Also called as oleic acid. It prevents the breakdown of acetylcholine, a neurotransmitter crucial for memory and cognitive function, by the enzyme acetylcholinesterase (AChE). Also, oleic acid may possess anti-inflammatory and antioxidant qualities, which enhance its ability to fight against Alzheimer's disease.

➢ *Docking Results:*

Protein-ligand binding site give a good value, maximum score is -25.3. The modelled tau protein was phosphorylated and hyperphosphorylated from in silico perspective and later considered for active site identification based on the available tau crystal structure (PDB ID: 2MZ7) bound to microtubules.

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Table 9 Different protein-ligand binding site with value

CurPocket ID	Vina score	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)
C5	-25.3	167	-2, -19, -33	30, 30, 30
C3	-24.6	391	1, 5, -24	30, 30, 30
C2	-20.7	529	18, -4, -10	30, 30, 30
C4	-20.5	197	18, 12, -15	30, 30, 30
C1	-6.1	1173	23, 9, -24	30, 30, 30

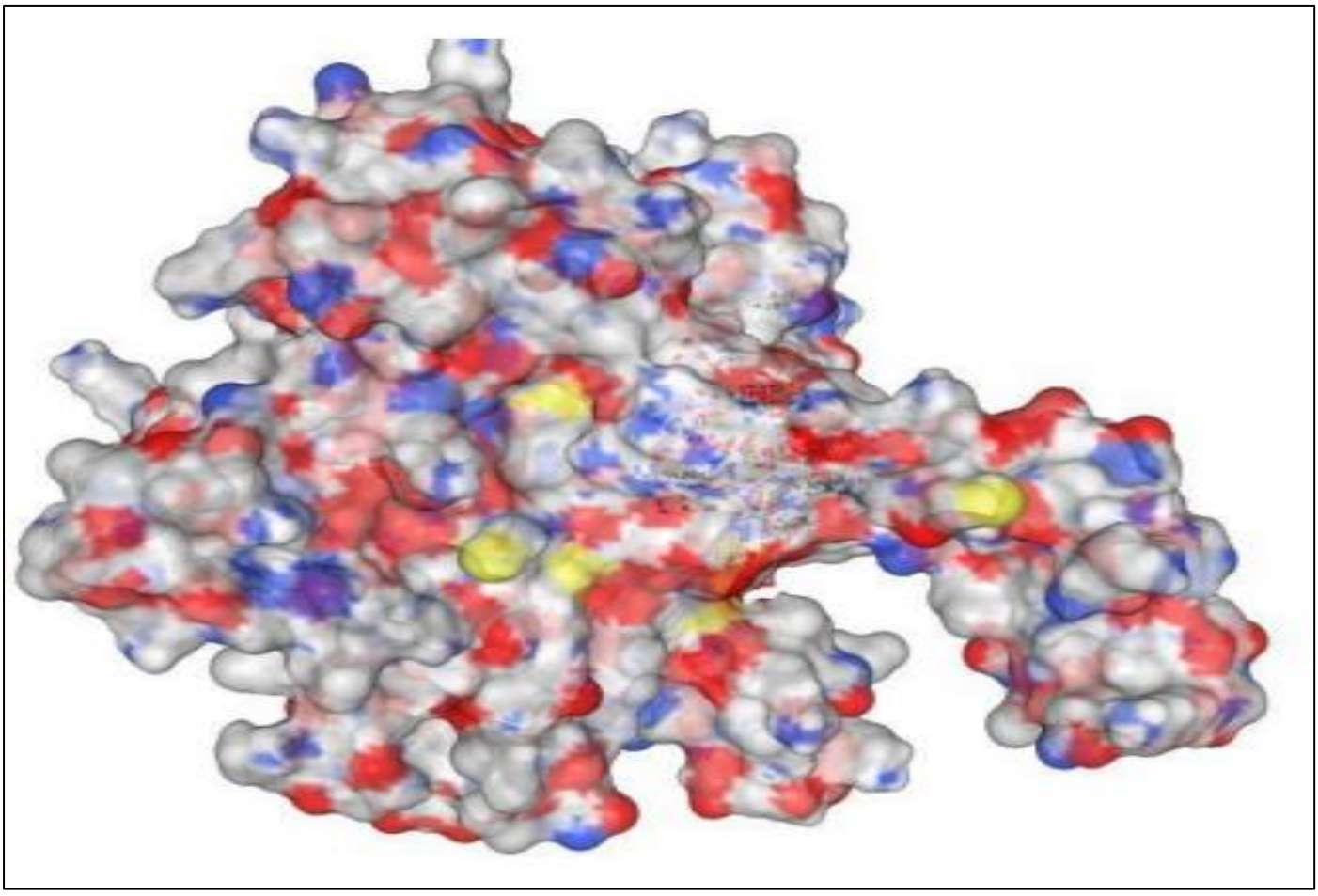

Fig 14 Docking Molecule

- *High-Performance Thin Layer Chromatography (HPTLC) Finger Printing Analysis: -*

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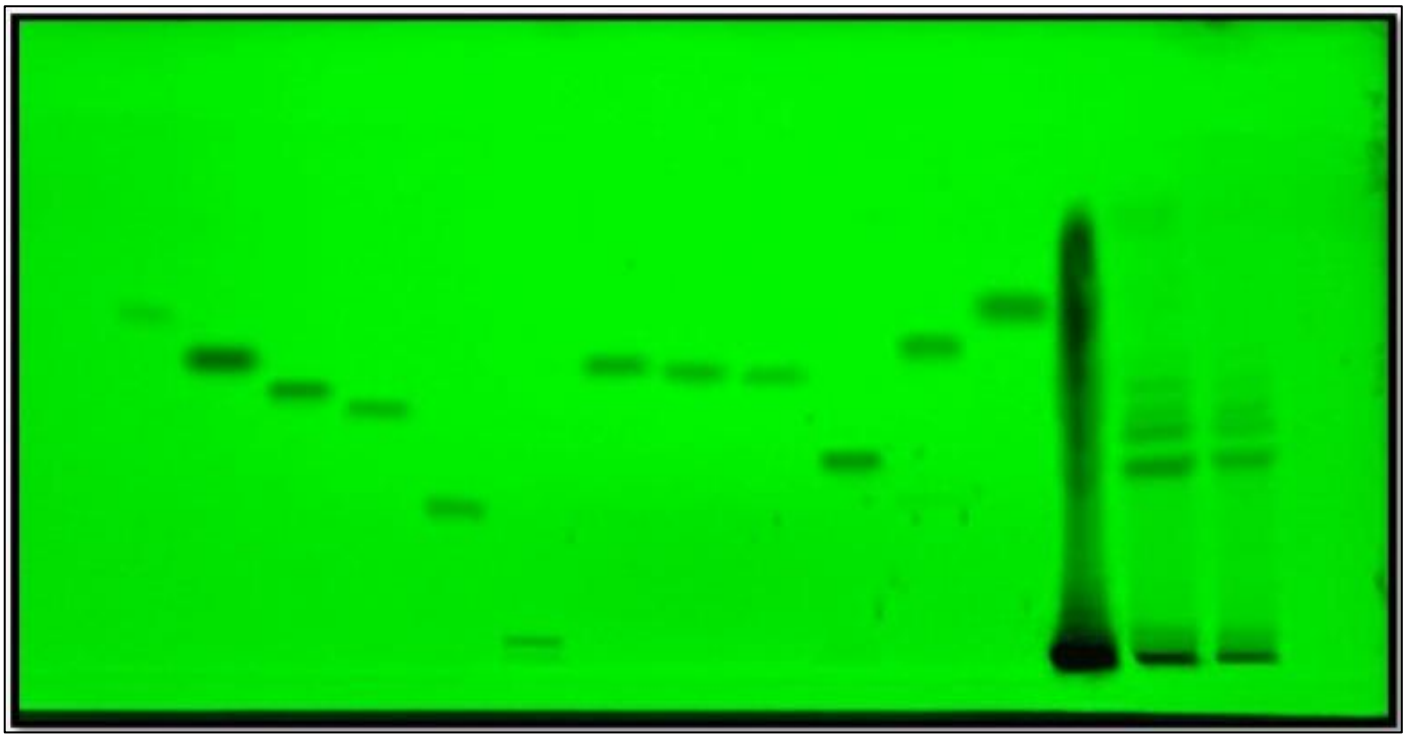

Fig 15 HPTLC Plate View at UV 254nm, of Standard and Samples Developed in a Mobile Phase of Chloroform, Ethyl Acetate, and Formic Acid (5:4:1v/v/v).

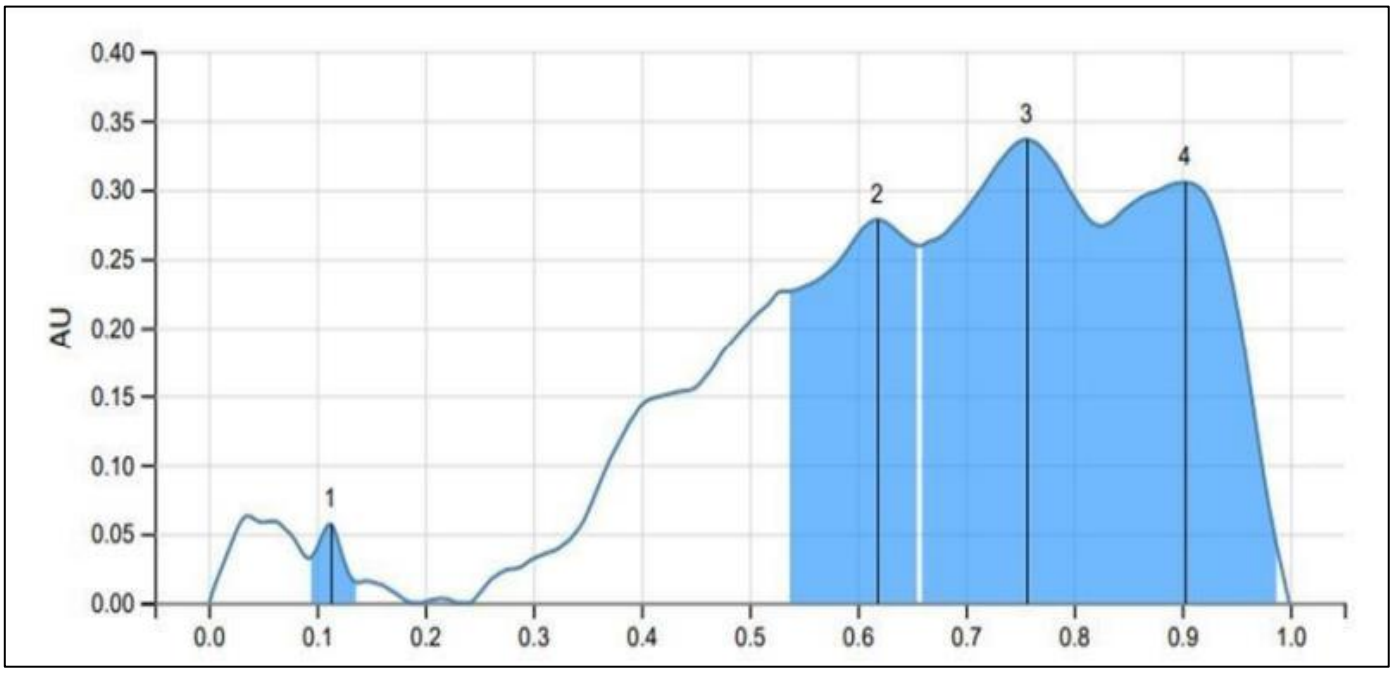

Fig 16 3 Peak of Naringenin and 2 Peak of Apigenin

Table 10 Isolation of Bio-Active Compounds by HPTLC Finger Printing of Ethanolic Extract of Causonis Trifolia (L).

Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.093	0.0322	0.112	0.0569	5.82	0.138	0.0148	0.00170	1.36
2	0.528	0.2263	0.618	0.2783	28.50	0.657	0.2593	0.03243	25.98
3	0.658	0.2593	0.756	0.3362	34.43	0.823	0.2738	0.04964	39.78
4	0.825	0.2737	0.902	0.3052	31.25	0.999	0.0000	0.04103	32.88

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HPTLC Finger printing confirm the presence of Naringenin (Rf 0.756) and Apigenin (Rf 0.618). Through HPTLC finger printing, presence of Naringenin and Apigenin were confirmed from the ethanolic extract which is responsible for curing Alzheimer's disease.

- *Naringenin:*

It is a natural flavanone, richly found in citrus and grape fruits, exhibits antioxidant potential, improves brain insulin signaling and cognitive functions and ameliorates AD-type neurodegeneration due to intracerebroventricular-streptozotocin (Khan et al., 2012, Yang et al., 2014)

➢ *IR Acti-Meter:*

Table 11 Mean Value of Different Groups, Measured, Locomotion Score by IR- Acti-Meter.

Group	Treatment	Locomotion score			
		Day 1	Day 7	Day 14	Day 21
I	Normal Control (Saline water)	444.8±33.24	465.67±19.31	460.8±29.38	478.56±26.82
II	Disease control (aluminium chloride treated(175mg/kg)	435.78±77.61	313.45±51.27	273.8±28.33	263.2±5.65
III	Standard control [Donepezil treated (2.5mg/kg)]	444.4±54.59	515.67±51.45**	575±26.57****	597.2±9.93****
IV	Test-1[Causonis Trifolia extract (150mg/kg)]	430.67±12.90	348.25±31	442.4±48.35**	484.25±56.54**
V	Test-2[Causonis Trifolia extract-2(300mg/kg)	378.67±22.39	445.45±25.75	461.4±24.56**	498±52.84***

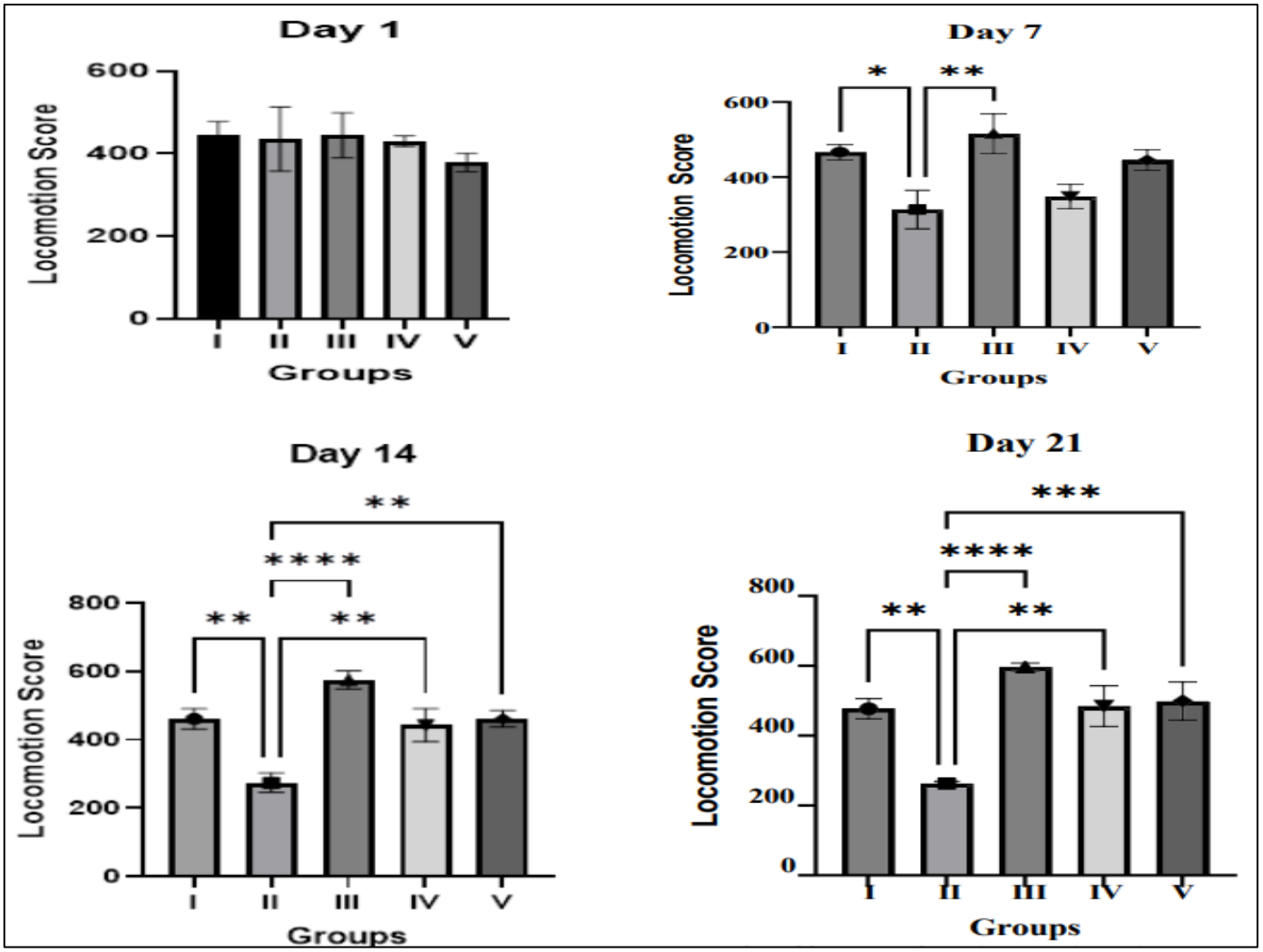

Fig 17 Graphical Representation of Effect of Ethanolic Leaves Extract (Doses 150 and 300 mg/kg) of Causonis Trifolia by IR Acti-Meter:

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Where,

Group-I: Normal control

Group-II: Disease control

Group III: Standard control

Group IV: Test control 1(Extract 150mg/kg)

Group V: Test control 2(Extract 300mg/kg)

Values are expressed as mean± SEM, n=5; *P<0.1 **P<0.01,***P<0.001, ****P<0.0001 considered statistically significant as compared to disease control group. Statistical analysis done by disease control group followed by Oneway

On day 14, Locomotor activity significantly increases, by our ethanolic extract. **p<0.01 compare to disease treated group and the standard drug Donepezil gives the more significant effect ***p<0.0001 Compare to disease treated group.

But on day 7, only the standard drug for Shows the significant effect **p<0.01 Compare to disease treated group.

On 21 days, our extract (300 mg/kg) showed more significant effect, ***p<0.001. and 150 mg/Kg extract shows significant effect (**p<0.01) Compare to the aluminium chloride group or disease treated group. On the same day the std drug (Donepezil) Shows much more significant ****p<0.0001 compare to disease treated group.

➢ *Elevated Plus Maze Test:*

- *Entry into Open Arm:*

Table 12 Mean Value of Different Groups Measured, Entry into Open Arm by Elevated Plus Maze

Group	**Treatment**	**Entry into open arm**			
		Day 1	**Day 7**	**Day 14**	**Day 21**
I	Normal Control (Saline water)	27.8±4.25	23.3±1.46	21.6±1.63	30±1.92
II	Disease control (aluminium chloride treated(175mg/kg)	25.4±3.90	29.8±1.98	14.4±2.13	9.3±0.8
III	Standard control [Donepezil treated (2.5mg/kg)]	10±0.70	14.4±2.13****	27.6±2.61**	29.8±4.25**
IV	Test-1[Causonis Trifolia extract (150mg/kg)]	9±0.70	14.4±2.13	24.8±1.98**	27.6±2.61**
V	Test-2[Causonis Trifolia extract- 2(300mg/kg)	9.4±1.02	22.8±1.98	27.6±2.61	28±2.4

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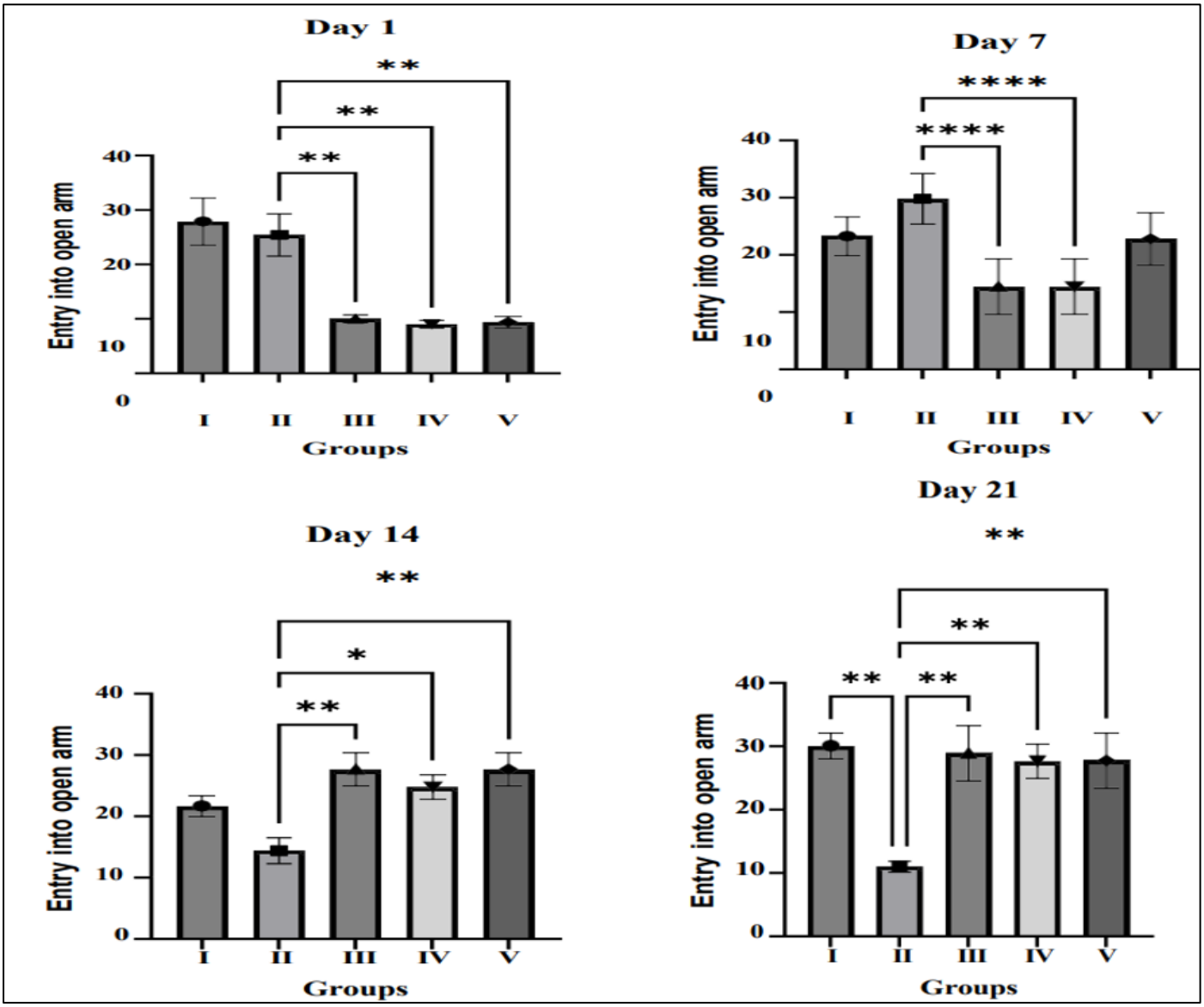

Fig 18 Graphical Representation of Effect of Ethanolic Leaves Extract (Doses 150 and 300 mg/kg) of Causonis Trifolia by Elevated Plus Maze (Entry into Open Arm)

Where,

Group-I: Normal control

Group-II: Disease control

Group III: Standard control

Group IV: Test control 1(Extract 150mg/kg)

Group V: Test control 2(Extract 300mg/kg)

On Day 14 and 21, animals were enter in the open arm significantly **p<0.01 which were treated with extract

Values are expressed as mean± SEM, n=5; *P<0.1 **P<0.01, ****P<0.0001 considered statistically significant as compared to disease control group. Statistical analysis done by disease control group followed by Onaway ANOVA Test.

- *Compare to Disease Treated Group but Our Low Dose Extract Showed Less*

significant effect, *p<0.5 on day 14 but insignificant in day 21. whereas on day 7, our extract (150mg/kg) showed Significant effect ** ** p<0.0001as like as standard drug (Donepezil) compared to the disease control group on the 7,14,21 our

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extract(150mg/kg) showed the more significant effect ****p<0.0001, *p<0.5, **p<0.01 compare to disease treated group.

On Day 14, 21 our extract(300mg/kg) showed significant effect by spending less time Compare to the disease control Group ** ** p< 0.0001, * ** p<0.001. Our lower dose extract also showed significant effect compare to disease treated group on 14, 21 days * ** p<0.001 but our standard drug showed more significant effect. On day 7 and 1 by reducing the time spent in close arm **p<0.01, *p<0.5 and **p<0.01 by our extract(150mg/kg) compare to disease control group

- *Time Spents in Close Arm:*

Table 13 Mean Value of Different Group Measured, Total Time Spend in Close Arm by Elevated Plus Maze

Group	Treatment	Time spent in close arm			
		Day 1	Day 7	Day 14	Day 21
I	Normal Control (Saline water)	115.4±5.78	122.4±7.02	120.3±7.28	119.4±6.79
II	Disease control (aluminium chloride treated(175mg/kg)	152.2±4.25	162.8±3	180.8±8.61	212.4±16.67
III	Standard control [Donepezil treated (2.5mg/kg)]	170±0.70**	140.6±2.13**	125.4±2.61****	122.4±9.24****
IV	Test-1[Causonis Trifolia extract (150mg/kg)]	171±0.70**	132.8±3.23***	140±1.98***	145.4±6.15***
V	Test-2[Causonis trifolia extract- 2(300mg/kg)	170.6±1.02**	146.2±1.98**	133.0±2.45****	1452.5±4.26***

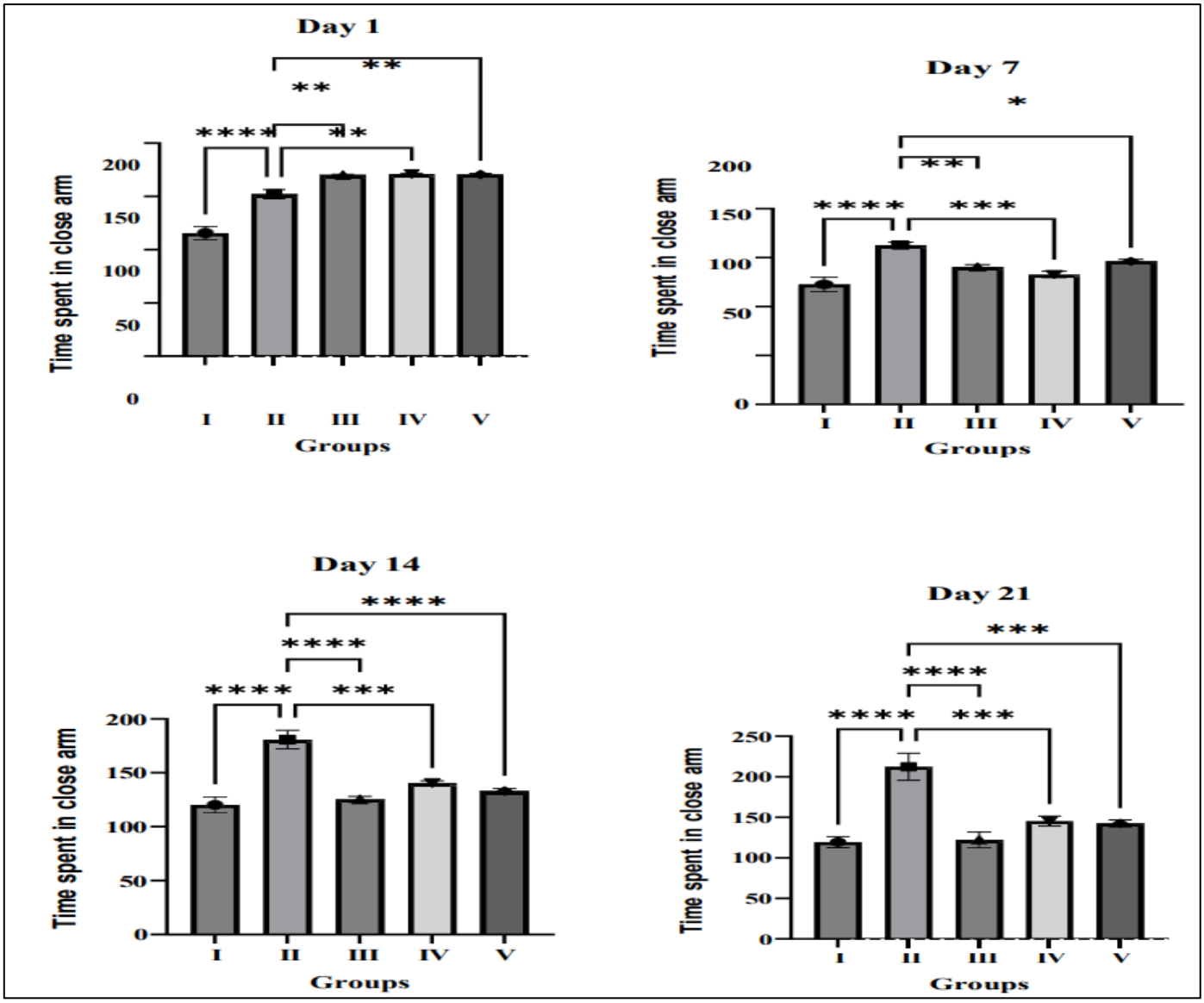

Fig 19 Graphical Representation of Effect of Ethanolic Leaves Extract (Doses 150 and 300 mg/kg) of Causonis Trifolia by Elevated Plus Maze (Time Spent in Close Arm)

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Where,

Group-I: Normal control

Group-II: Disease control

Group III: Standard control

Group IV: Test control 1(Extract 150mg/kg)

Group V: Test control 2(Extract 300mg/kg)

Values are expressed as mean± SEM, n=5;
*P<0.1 **P<0.01, ****P<0.0001
considered statistically significant as
compared to disease control group...

Statistical analysis done by disease control group followed by One-way ANOVA Test.

On day 14,21 our extract (300mg/kg) showed significant effect by spending less time compare to the disease control group ** **p<0.0001, * **p<0.001.

Our lower dose extract also showed significant effect compare to the disease treated group on day 14,21 days. * **p<0.001, but our std drug shows more significant effect, ** **p<0.0001.

On day 7 and 14 our extract (300mg/kg) also showed significant effect, **p<0.01, *p<0.5 by reducing the spending time at close arm and **p<0.01. by our extract (150mg/kg) compare to disease control group. Our standard drug also showed significant effect **p<0.01, compare to disease control group on day 7and day 14.

➢ *Novel Object Recognition Test:*

- *Total Distance Travelled:*

✓ *Similar Object:*

Table 14 Mean Value of Different Groups Measured, Total Distance for Similar Object by Novel Object Recognition Test

Group	Treatment	Total distance for similar object(cm.)			
		Day 1	Day 7	Day 14	Day 21
I	Normal Control (Saline water)	161.45±5.78	167.4±7.02	170.3±7.28	189.4±6.79
II	Disease control (aluminium chloride treated(175mg/kg)	152.2±4.25	132.8±3	117.8±11.61	102.4±16.67
III	Standard control [Donepezil treated (2.5mg/kg)]	158±0.70	170.6±2.13****	179.4±2.61****	192.4±9.24
IV	Test-1[Causonis Trifolia extract (150mg/kg)]	141±0.70	152.8±3.23**	160±1.98***	171.4±6.15
V	Test-2[Causonis Trifolia extract- 2(300mg/kg)	150.6±1.02	176.2±1.98****	186.0±2.45****	194.5±4.26

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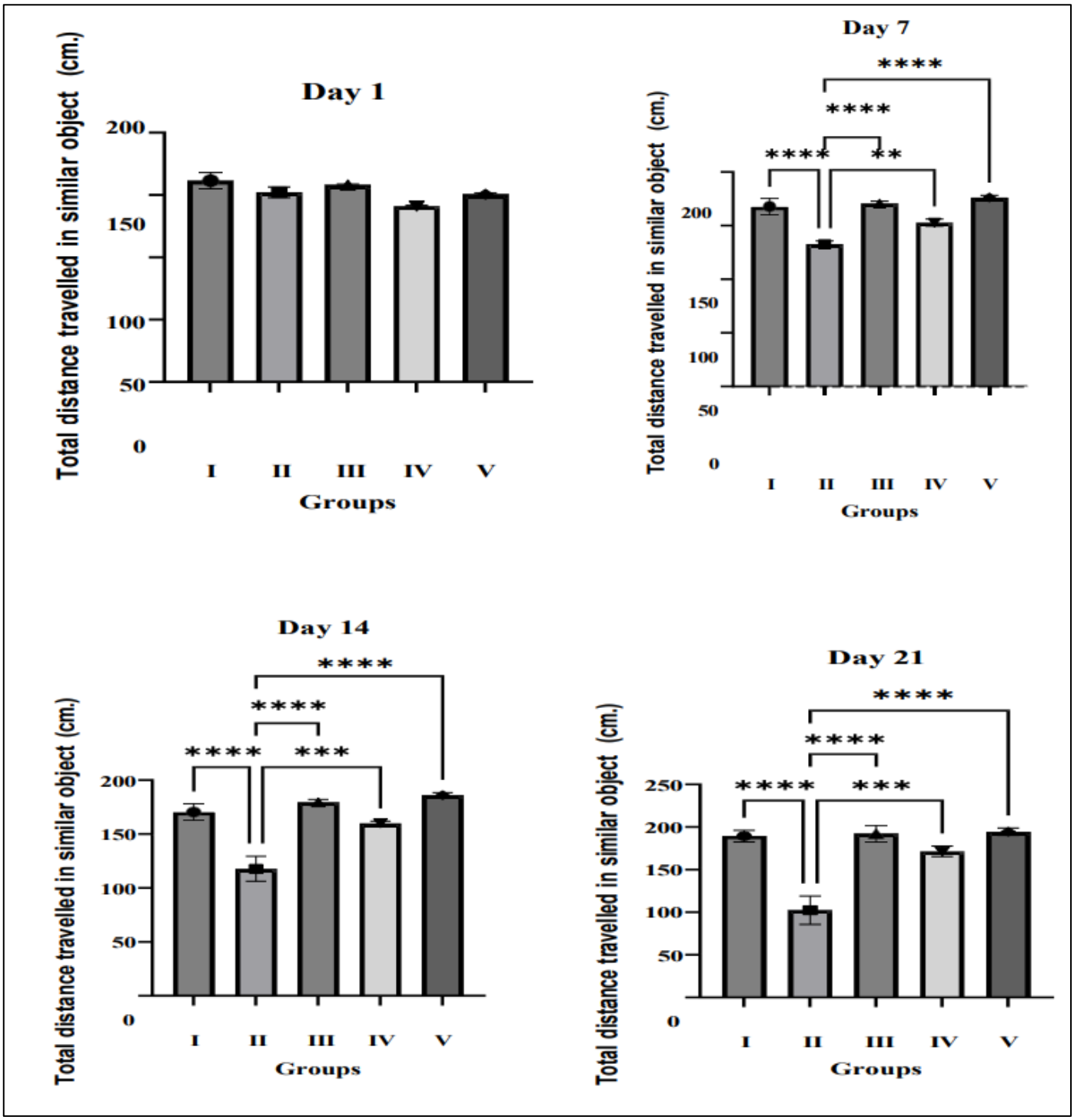

Fig 20 Graphical Representation of Effect of Ethanolic Leaves Extract (Doses 150 and 300 mg/kg) of Causonis Trifolia by Novel Object Recognition Test (Total Distance Travelled in Similar Object).

✓ *Novel Objects:*

Table 15 Mean Value of Different Groups Measured, Total Distance for Novel Object by Novel Object Recognition Test

Group	Treatment	Total distance in novel object(cm.)			
		Day 1	Day 7	Day 14	Day 21
I	Normal Control (Saline water)	121.45±5.78	122.4±7.02	130.3±7.28	109.4±6.79
II	Disease control (aluminium chloride treated(175mg/kg)	162.2±4.25	172.8±3	177.8±11.61	202.4±16.67
III	Standard control [Donepezil treated (2.5mg/kg)]	130±0.70	120.6±2.13	129.4±2.61	122.4±9.24
IV	Test-1[Causonis Trifolia extract (150mg/kg)]	141±0.70	132.8±3.23	127±1.98	111.4±6.15
V	Test-2[Causonis Trifolia extract- 2(300mg/kg)	120.6±1.02	136.2±1.98	126.0±2.45	114.5±4.26

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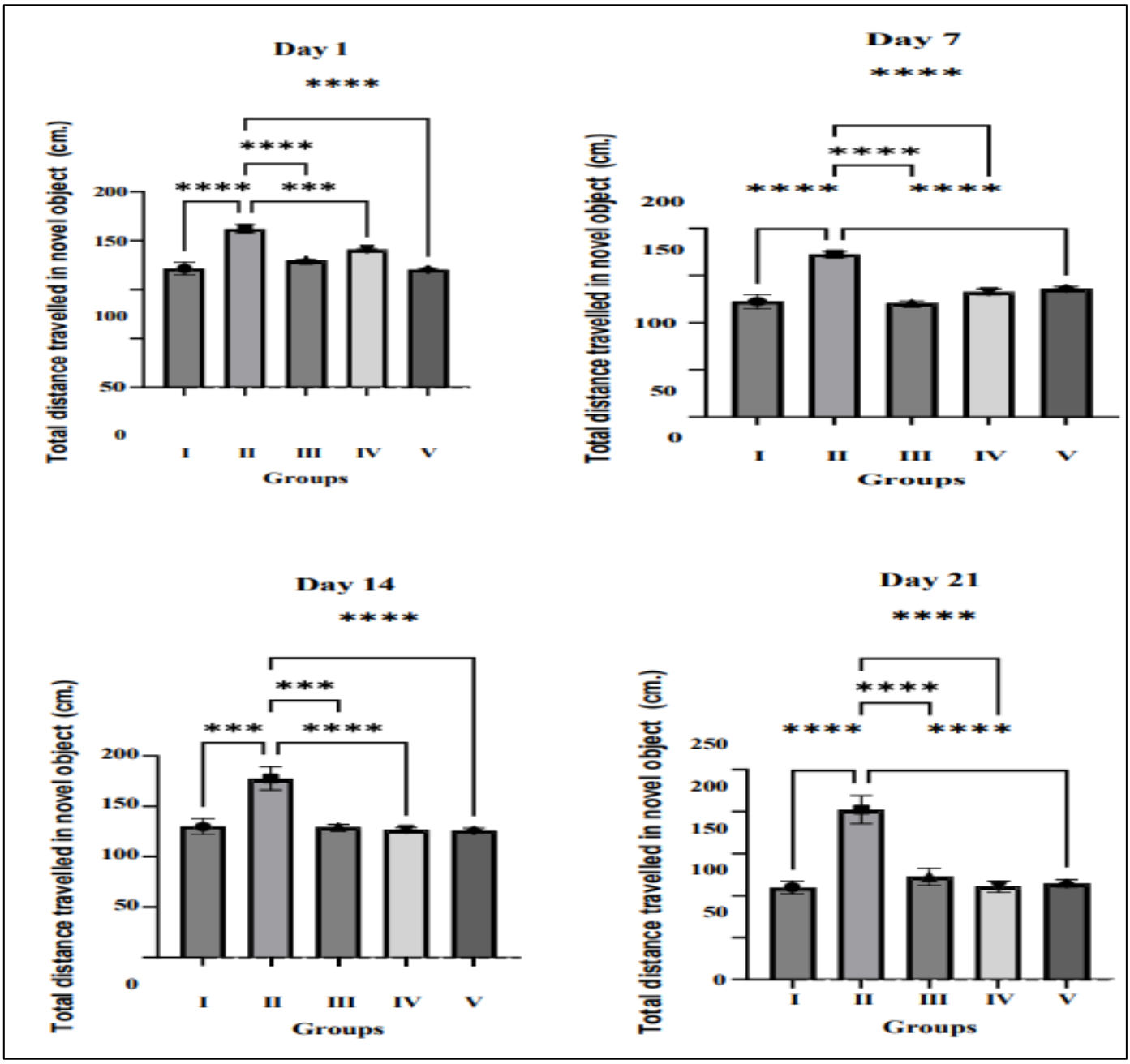

Fig 21 Graphical Representation of Effect of Ethanolic Leaves Extract (Doses 150 and 300 mg/kg) of Causonis trifolia by Novel Object Recognition Test (Total Distance Travelled in Novel Object).

Where,

Group-I: Normal control

Group-II: Disease control

Group III: Standard control

Group IV: Test control 1(Extract 150mg/kg)

Group V: Test control 2(Extract 300mg/kg

Values are expressed as mean± SEM, n=5; **P<0.01, ***P<0.001, ****P<0.0001 considered statistically significant as compared to disease control group.

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- *Time Spent:*

✓ *Similar Object:*

Table 16 Mean Value of Different Groups Measured, Time Spent for Similar Object by Novel Object Recognition Test

Group	Treatment	Time spent in similar object (sec.)			
		Day 1	Day 7	Day 14	Day 21
I	Normal Control (Saline water)	120.45±5.78	112.4±7.02	100.3±7.28	109.4±6.79
II	Disease control (aluminium chloride treated(175mg/kg)	162.2±4.25	152.8±4.2	167.8±11.61	172.4±16.67
III	Standard control [Donepezil treated (2.5mg/kg)]	130±0.70****	121.6±5.13**	109.4±2.61****	72.4±9.24****
IV	Test-1[Causonis Trifolia extract (150mg/kg)]	121±0.70****	112.8±3.23****	87±1.98****	71.4±6.15****
V	Test-2[Causonis Trifolia extract-2(300mg/kg)	100.6±1.02****	106.2±7.98****	98.0±2.45****	64.5±4.26****

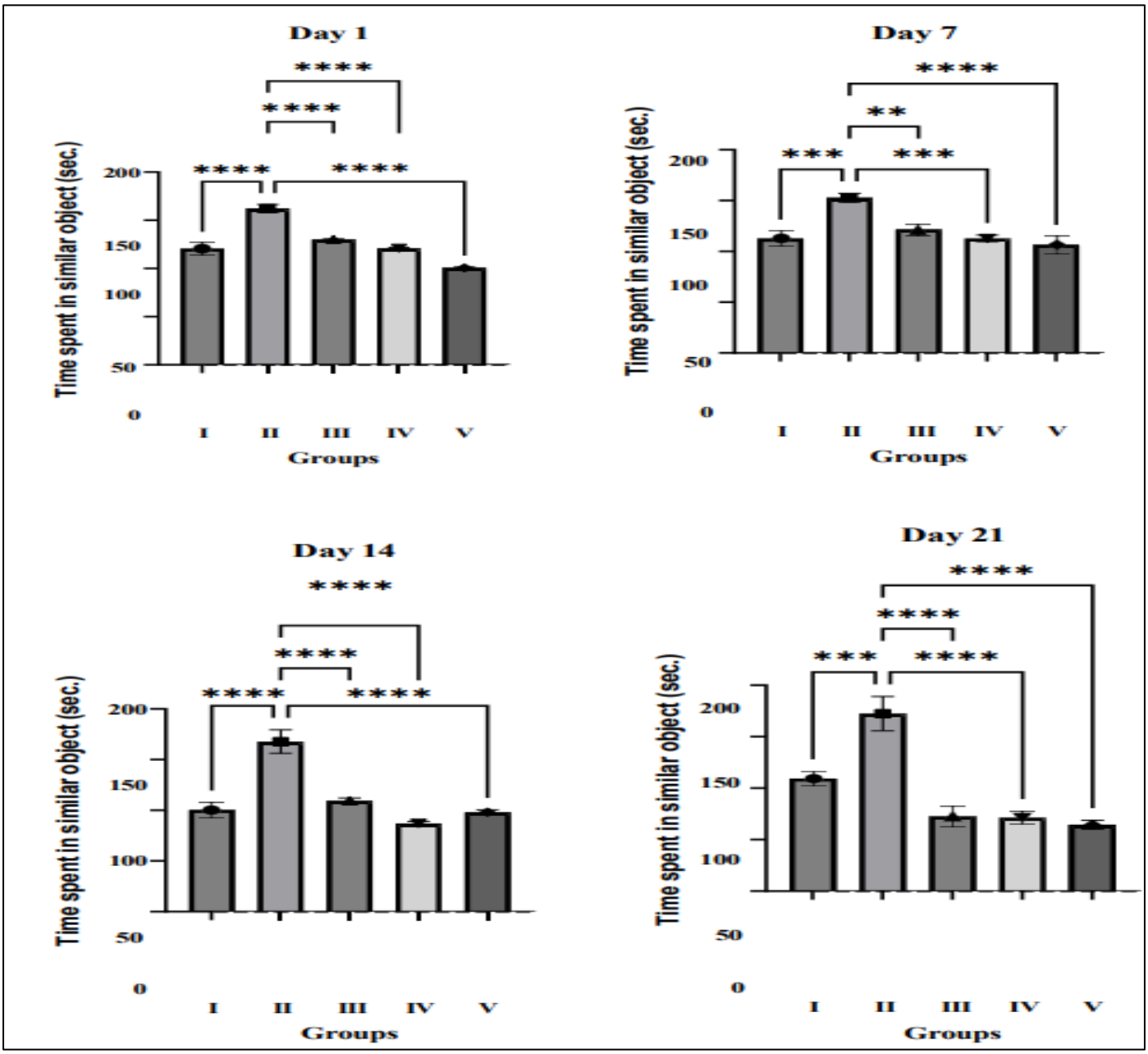

Fig 22 Graphical Representation of Effect of Ethanolic Leaves Extract (Doses 150 and 300 mg/kg) of Causonis Trifolia by Novel Object Recognition Test (Time Spent for Similar Object)

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Table 17 Mean Value of Different Groups Measured; Time Spent for Novel Object by Novel Object Recognition Test

Group	Treatment	Time spent in novel object (sec.)			
		Day 1	Day 7	Day 14	Day 21
I	Normal Control (Saline water)	151.45±5.78	162.4±7.02	170.3±7.28	179.4±6.79
II	Disease control (aluminium chloride treated(175mg/kg)	142.2±6.25	122.8±3.9	117.8±11.61	102.4±16.67
III	Standard control [Donepezil treated (2.5mg/kg)]	160±7.70	171.6±2.13****	179.4±2.61****	182.4±9.24****
IV	Test-1 [Causonis Trifolia extract (150mg/kg)]	161±8.70	182.8±3.23****	187±7.98****	191.4±6.15****
V	Test-2 [Causonis Trifolia extract-2(300mg/kg)	160.6±6.02	196.2±1.98****	198.0±2.45****	199.5±4.26****

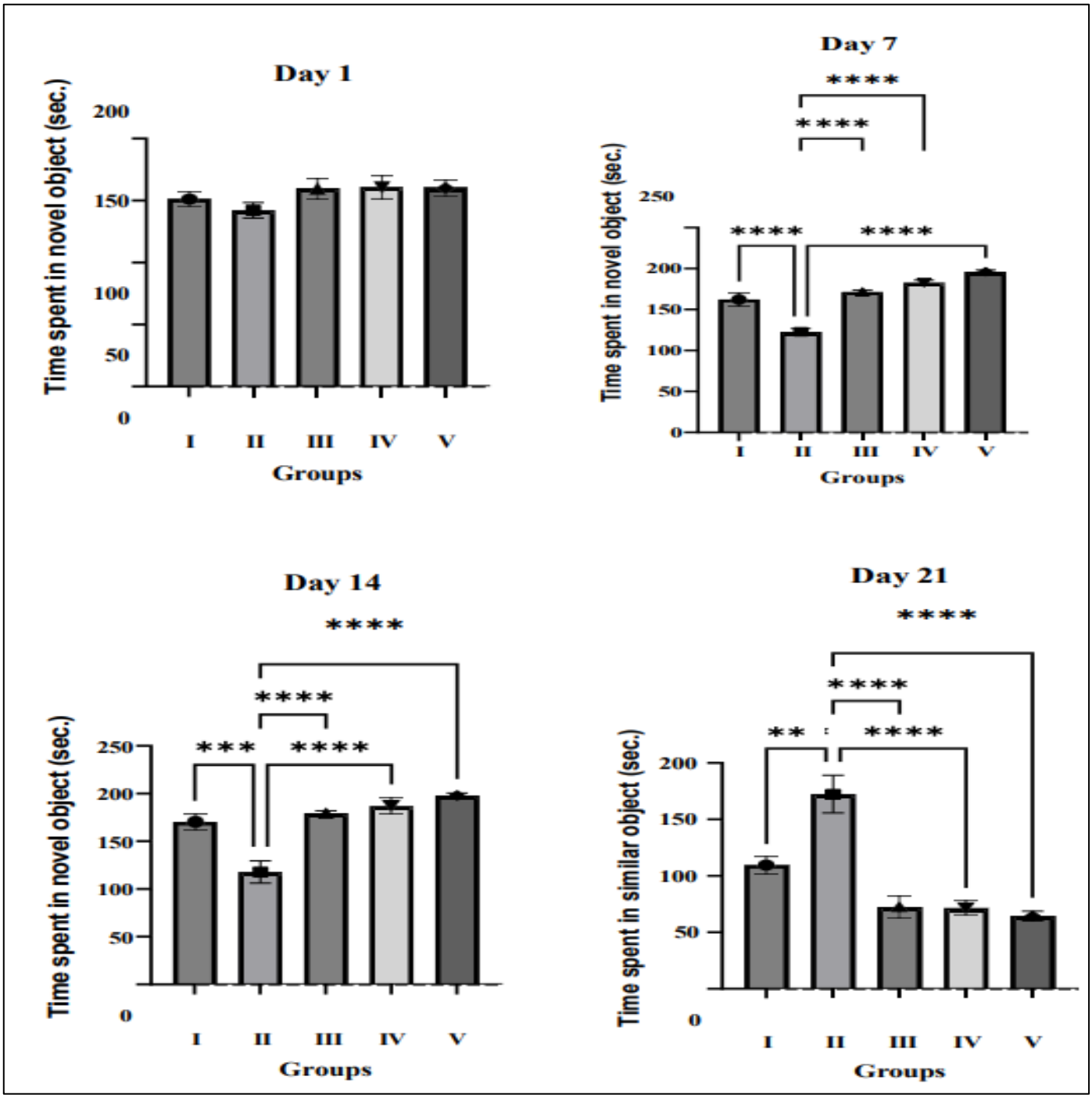

Fig 23 Graphical Representation of Effect of Ethanolic Leaves Extract (Doses 150 and 300 mg/kg) of Causonis Trifolia by Novel Object Recognition Test (Time Spent for Novel Object)

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- *Discrimination Index:*

Table 18 Mean Value of Different Groups Measured by Discrimination Index

Group	Treatment	Discrimination index			
		Day 1	Day 7	Day 14	Day 21
I	Normal Control (Saline water)	31±2.09	50±0.98	70±1.01	70±1.01
II	Disease control (aluminium chloride treated(175mg/kg)	20±5.31	30±2.28	50±5.98	70±3.02
III	Standard control [Donepezil treated (2.5mg/kg)]	30±2.28	50±4.90	70±4.57	110±4.95
IV	Test-1[Causonis Trifolia extract (150mg/kg)]	40±5.31	70±7.81	100±2.91	120±6.57
V	Test-2[Causonis Trifolia extract- 2(300mg/kg)	60±2.07	90±3.05	100±3.5	135±5.9

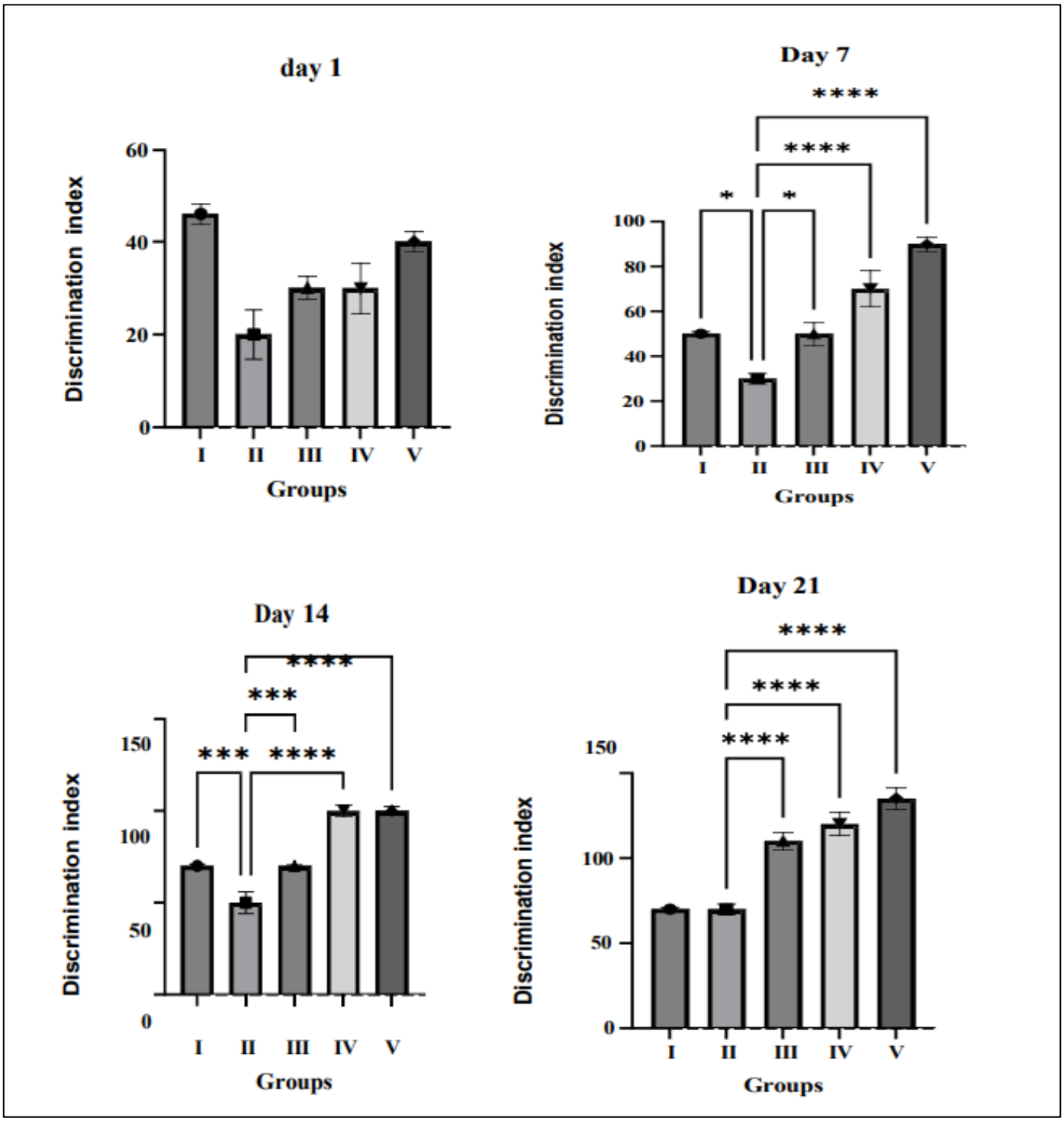

Fig 24 Graphical Representation of Effect of Ethanolic Leaves Extract of Causonis Trifolia by Novel Object Recognition Test. (D.I)

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Where,

Group-I: Normal control

Group-II: Disease control

Group III: Standard control

Group IV: Test control 1(Extract 150mg/kg)

Group V: Test control 2(Extract 300mg/kg)

Values are expressed as mean± SEM, n=5; *P<0.1 ,***P<0.001, ****P<0.0001 considered statistically significant as compared to disease control group. Statistical analysis done by disease control group followed by One way ANOVA test.

After treating with our extract (150mg/kg) and (300mg/kg) on day 7, day 14 & day 21 shows the significant effect on the novel objeet recognition test****p<.0001 compare to the disease Control gr where AlCl3 - was induced. But on day 7, the standard drug (Donepezil)" Showed Less significant effect *p<.0.5 than our. extract but on day-14 the standard drug showed the significant effect ***p<0.001 and day 21, the standard drug showed more significant effect****P<0.0001 like of our extract compare the to disease control groups.

The animals were spending more time. in the novel object than the familiar object

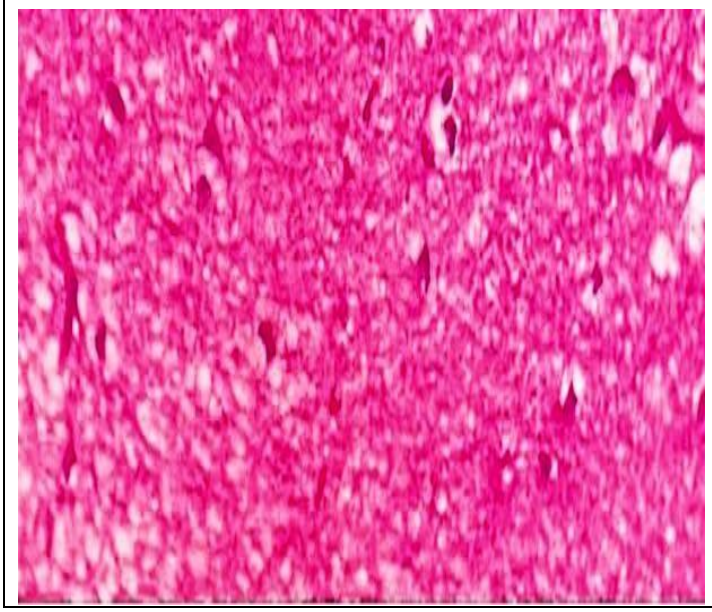
- *Data Analysis of SOD Test and Catalase Activity Test:*

The mean absorbance for each standard and sample was calculated. For SOD and CAT (sandwich ELISA), the absorbance values were directly proportional to the analyte concentration, and values were obtained by interpolating from the standard curve. For GSH (competitive ELISA), the absorbance values were inversely proportional to the analyte concentration, and a standard curve was used to determine unknown sample values. Results were expressed in appropriate units

Table 19 Mean Value of Anti-Oxidantal Enzymes (SOD and CAT) Activity

Group	Treatment	Antioxidant enzymes	
		SOD (µg/mg)	CAT (µg/mg)
I	Normal Control (Saline water)	26.30±0.61	25.85±0.09
II	Disease control (aluminium chloride treated(175mg/kg)	15.23±0.82****	9.87±0.66****
III	Standard control [Donepezil treated (2.5mg/kg)]	21.46±0.62***	29.18±0.50
IV	Test-1[Causonis trifolia extract (150mg/kg)]	29.05±0.85*	23.07±0.71
V	Test-2[Causonis trifolia extract-2(300mg/kg)	27.31±0.81**	22.01±1.05

A



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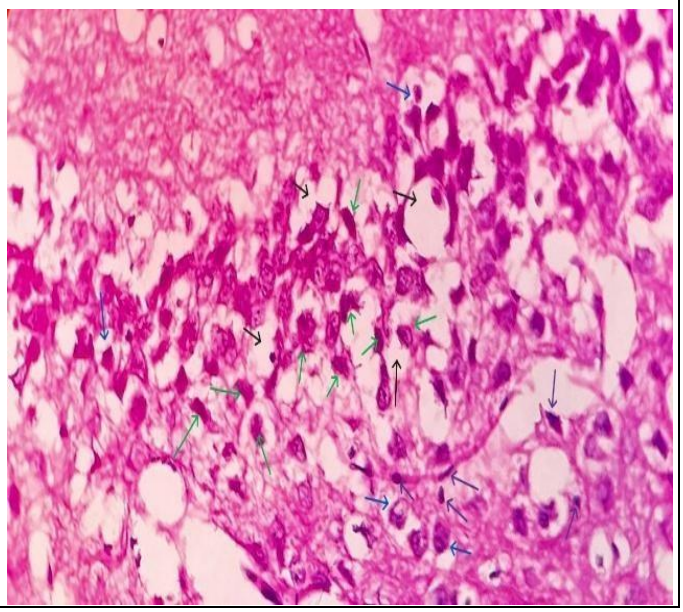

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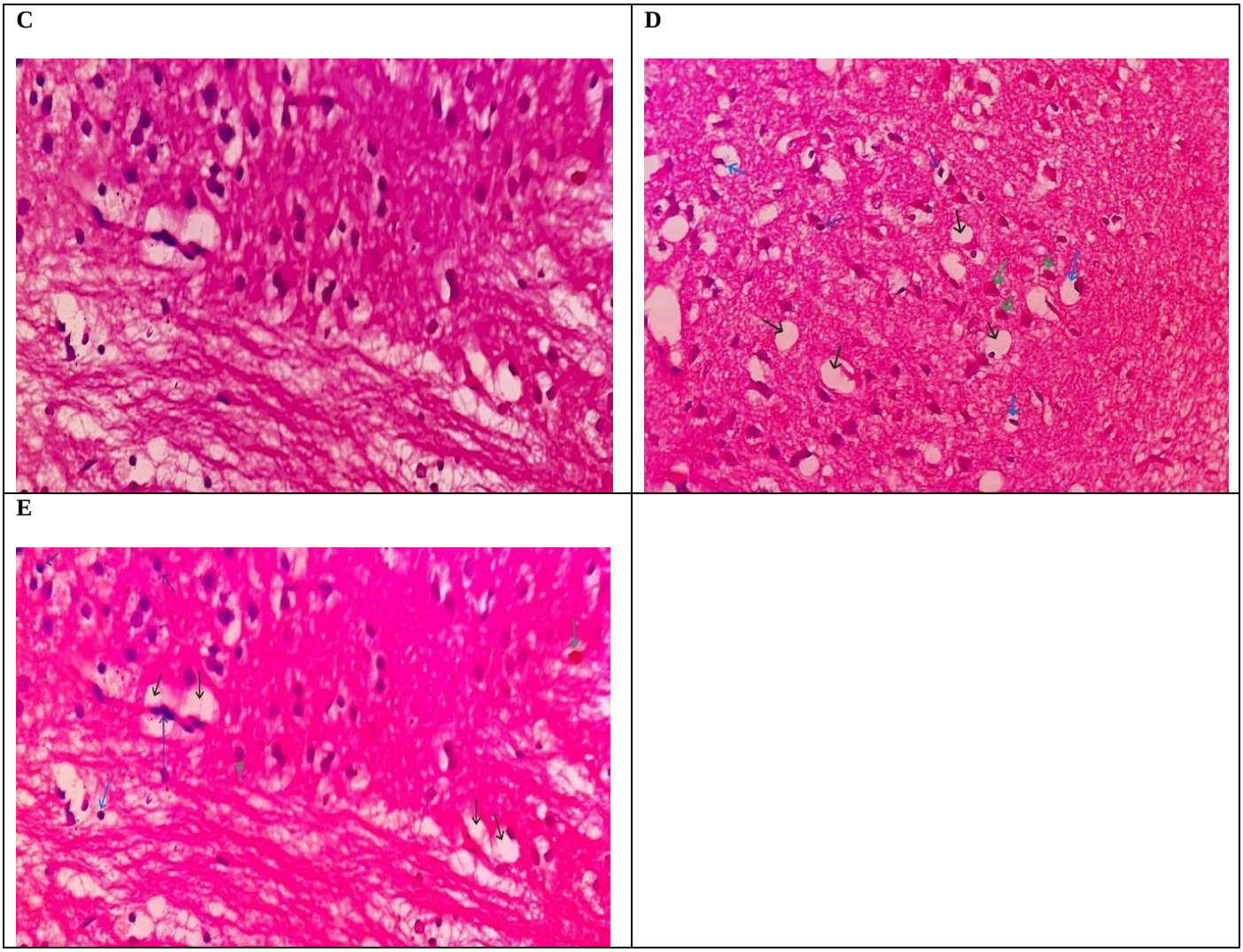

Fig 25 Haematoxylin & Eosin Staining of the Substantia Nigra Sections of the Wistar Rat.

(A) Normal Control: Cell layers are normal in size and uniformly arranged. (B)AlCl3 intoxicated: cellular infiltration (blue arrow), vesicular and enlarged nuclei (black arrow), hyperchromatic cells (green arrow), and some pyknotic cells (red arrows) in the striatum of the brain. (C) Donepezil treated: Shows a decrease in cellular infiltration (blue arrow), vesicular and enlarged nuclei (black arrow), hyperchromatic cells (green arrow), and some pyknotic cells (red arrows) in the striatum of the brain. (D) Extract lower dose: showed less expression of cellular infiltration (blue arrow), vesicular and enlarged nuclei (black arrow), hyperchromatic cells (green arrow), and some pyknotic cells (red arrows) in the striatum of the brain. (E) Extract with higher dose: Shows minimalistic expression of cellular infiltration (blue arrow), vesicular and enlarged nuclei (black arrow), hyperchromatic cells (green arrow), and some pyknotic cells (red arrows) in the striatum of the brain.

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CHAPTER FIVE
CONCLUSION

- *Conclusion:*

The study systematically evaluated the Anti-alzheimer's activity potential of *Causonis trifolia* (L.) leaf extract through a comprehensive series of phytochemical screenings, GC-MS profiling, HPTLC fingerprinting, in vivo pharmacological models, and molecular docking analysis. The ethanolic extract exhibited a diverse phytochemical composition, rich in flavonoids, phenols, and alkaloids—key constituents known for their therapeutic effects against Alzheimer's disease. GC-MS analysis confirmed the presence of active constituents like 9-octadecanoic acid, 2,4-thiazolidinedione, benzofuranone derivatives, and long-chain fatty alcohols that are capable of inhibiting pro-inflammatory mediators and oxidative stress. HPTLC analysis further verified the presence of Naringenin, flavonoids with well-established pharmacological benefits. Naringenin is responsible for anti-alzheimer's activity.Molecular docking provided mechanistic insight, showing stable interactions between active compounds and protein residues involved in inflammation and pain signaling pathways.9-Octadecanoic acid prevents the breakdown of acetylcholine, a neurotransmitter crucial for memory and cognitive function, by the enzyme acetylcholinesterase (AChE). Also oleic acid may possess anti-inflammatory and antioxidant qualities, which enhance its ability to fight against Alzheimer's disease. These in silico results supported the observed in vivo efficacy.Protein-ligand binding site give a good value , maximum score is -25.3. The modelled tau protein was phosphorylated and hyperphosphorylated from in silico perspective and later considered for active site identification based on the available tau crystal Animal studies demonstrated that the extract significantly reduced time spent in close arm in EPM tests, suggesting curing Alzheimer's disease. From EPM test, we obserbed that disease control animal have less activity and spent more time in close arm and less entry in open arm comparing to other groups.

From IR Acti-meter, spontaneous mortility increased when compared in standard control group, test control 1, Test control 2 and decreased in disease control group. Locomotor activity locomotor activity is the positive sign to recover from Alzheimer.

From novel object recognition test we got positive sign and more time spent in novel object comparing to disease control group.

In normal Control group: Cell layers are normal in size and uniformly arranged., In AlCl3 intoxicated: cellular infiltration, vesicular and enlarged nuclei , hyperchromatic cells , and some pyknotic cells in the striatum of the brain. In Donepezil treated group, it was seen that very less cellular dis arrangement observed, in test-1 gr represent less expression of cellular infiltration , vesicular and enlarged nuclei, hyperchromatic cells , and some pyknotic cells in the striatum of the brain in test -2 group, it was observed that minimalistic expression of cellular infiltration, vesicular and enlarged nuclei , hyperchromatic cells , and some pyknotic cells in the striatum of the brain.

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REFERENCES

[1]. Villemagne VL, Burnham S, Bourgeat P, et al. Amyloid ß deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: A prospective cohort study. Lancet Neurol. 2013;12(4):357- 367.
[2]. Reiman EM, Quiroz YT, Fleisher AS, et al. Brain imaging and fluid biomarker analysis in young adults at genetic risk for autosomal dominant Alzheimer's disease in the presenilin 1 E280A kindred: A case-control study. Lancet Neurol. 2012;11(2):1048- 1056.
[3]. Jack CR, Lowe VJ, Weigand SD, et al. Serial PiB and MRI in normal, mild cognitive impairment and Alzheimer's disease: Implications for sequence of pathological events in Alzheimer's disease. Brain. 2009; 132:1355-1365.
[4]. Bateman RJ, Xiong C, Benzinger TL, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med. 2012;367(9):795-804.
[5]. Gordon BA, Blazey TM, Su Y, et al. Spatial patterns of neuroimaging biomarker change in individuals from families with autosomal dominant Alzheimer's disease: A longitudinal study. Lancet Neurol. 2018;17(3):241-250.
[6]. Braak H, Thal DR, Ghebremedhin E, Del Tredici K. Stages of the pathologic process in Alzheimer disease: Age categories from 1 to 100 years. J Neuropathol Exp Neurol. 2011;70(11):960-969.
[7]. Quiroz YT, Zetterberg H, Reiman EM, et al. Plasma neurofilament light chain in the presenilin 1 E280A autosomal dominant Alzheimer's disease kindred: A cross-sectional and longitudinal cohort study. Lancet Neuro. 2020;19(6):513-521.
[8]. Barthelemy N, Joseph-Mathurin N, Gordon BA, et al. A soluble phosphorylated tau signature links tau, amyloid and the evolution of stages of dominantly inherited Alzheimer's disease. Nat Med. 2020; 26:398-407.
[9]. Byard RW, Langlois NEI. Wandering dementia: A syndrome with forensic implications. J Forensic Sci. 2019;64(2):443-445.
[10]. Tom SE, Hubbard RA, Crane PK, et al. Characterization of dementia and Alzheimer's disease in an older population: Updated incidence and life expectancy with and without dementia. Am J Public Health. 2015;105(2):408-413.
[11]. Ganguli M, Dodge HH, Shen C, Pandav RS, DeKosky ST. Alzheimer disease and mortality: A 15-year epidemiological study. Arch Neurol. 2005;62(5):779-784.
[12]. Waring SC, Doody RS, Pavlik VN, Massman PJ, Chan W. Survival among patients with dementia from a large multi-ethnic population. Alzheimer Dis Assoc Disord. 2005;19(4):178183.
[13]. Brookmeyer R, Corrada MM, Curriero FC, Kawas C. Survival following a diagnosis of Alzheimer disease. Arch Neurol. 2002;59(11):1764-1767.
[14]. Larson EB, Shadlen MF, Wang L, et al. Survival after initial diagnosis of Alzheimer disease. Ann Intern Med. 2004;140(7):501-509.
[15]. Helzner EP, Scarmeas N, Cosentino S, Tang MX, Schupf N, Stern Y. Survival in Alzheimer disease: A multiethnic, populationbased study of incident cases. Neurology. 2008;71(19):1489- 1495.
[16]. Xie J, Brayne C, Matthews FE. Survival times in people with dementia: Analysis from a population-based cohort study with 14-year follow-up. BMJ. 2008;336(7638):258- 262.
[17]. Brodaty H, Seeher K, Gibson L. Dementia time to death: A systematic literature review on survival time and years of life lost in people with dementia. Int Psychogeriatr. 2012;24(7):1034-1045.
[18]. Todd S, Barr S, Roberts M, Passmore AP. Survival in dementia and predictors of mortality: A review. Int J Geriatr Psychiatry. 2013;28(11):1109-1124.
[19]. World Health Organization. Dementia: Key facts. Accessed October 10, 2023. Available at: https://www.who.int/news-room/factsheets/detail/dementia.
[20]. Smith AD, Smith SM, de Jager, CA, et al. Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: A randomized controlled trial.Plos One. 2010;5(9): e12244.
[21]. Kapasi A, DeCarli C, Schneider JA. Impact of multiple pathologies on the threshold for clinically overt dementia. Acta Neuropathol. 2017;134(2):171-186.
[22]. Brenowitz WD, Hubbard RA, Keene CD, et al. Mixed neuropathologies and estimated rates of clinical progression in a large autopsy sample. Alzheimers Dement. 2017;13(6):654-662.
[23]. National Institute on Aging. What are frontotemporal disorders? Accessed December 16, 2023. Available at: https://www.nia.nih. gov/health/what-are- frontotemporal-disorders.
[24]. Hogan DB, Jette N, Fiest KM, et al. The prevalence and incidence of frontotemporal dementia: A systematic review. Can J Neurol Sci. 2016;43(suppl): S96-109.
[25]. Amador-Ortiz C, Ahmed Z, Zehr C, Dickson DW. Hippocampal sclerosis dementia differs from hippocampal sclerosis in frontal lobe degeneration. Acta Neuropathol. 2007;113(3):245252.
[26]. Kane JPM, Surendranathan A, Bentley A, Thomas AJ, et al. Clinical prevalence of Lewy body dementia. Alzheimers Res Ther. 2018 Feb 15;10(1):19.
[27]. De Reuck J, Maurage CA, Deramecourt V, et al. Aging and cerebrovascular lesions in pure and in mixed neurodegenerative and vascular dementia brains: A neuropathological study. Folia Neuropathol. 2018;56(2):81-87.
[28]. James BD, Bennett DA, Boyle PA, Leurgans S, Schneider JA. Dementia from Alzheimer disease and mixed pathologies in the oldest old. JAMA. 2012;307(17):1798-1800.
[29]. Stojkovska I, Krainc D, Mazzulli JR. Molecular mechanisms of α-synuclein and GBA1 in Parkinson's disease. Cell Tissue Res. 2018;373(1):51-60.
[30]. Aarsland D, Zaccai J, Brayne C. A systematic review of prevalence studies of dementia in Parkinson's disease. Mov Disord.

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/26feb693

2005;20(10):1255.
[31]. Kawas CH, Kim RC, Sonnen JA, Bullain SS, Trieu T, Corrada MM. Multiple pathologies are common and related to dementia in the oldest-old: The 90+ Study. Neurology. 2015;85(6):535- 542.
[32]. Viswanathan A, Rocca WA, Tzourio C. Vascular risk factors and dementia: How to move forward? Neurology. 2009; 72:368-374.
[33]. Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in communitydwelling older persons. Neurology. 2007; 69:2197- 2204.
[34]. Schneider JA, Arvanitakis Z, Leurgans SE, Bennett DA. The neuropathology of probable Alzheimer disease and mild cognitive impairment. Ann Neurol. 2009;66(2):200-208.
[35]. Jellinger KA, Attems J. Neuropathological evaluation of mixed dementia. J Neurol Sci. 2007;257(1-2):80-87.
[36]. Jellinger KA. The enigma of mixed dementia. Alzheimers Dement. 2007;3(1):40-53.
[37]. Boyle PA, Lei Y, Wilson RS, Leurgans SE, Schneider JA, Bennett DA. Person-specific contribution of neuropathologies to cognitive loss in old age. Ann Neurol. 2018;83(1):74-83.
[38]. Boyle PA, Yu L, Leurgans SE, et al. Attributable risk of Alzheimer's dementia attributed to age-related neuropathologies. Ann Neurol. 2019;85(1):114-124.
[39]. Jellinger KA, Attems J. Prevalence of dementia disorders in the oldest-old: an autopsy study. Acta Neuropathol. 2010; 119:421- 433.
[40]. Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging- Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):280-292.
[41]. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on AgingAlzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):270-279.
[42]. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging- Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):263-269.
[43]. Jack CR, Albert MS, Knopman DS, et al. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):257-262.
[44]. Vermunt L, Sikkes SAM, van den Hout A, et al. Duration of preclinical, prodromal, and dementia stages of Alzheimer's disease in relation to age, sex, and APOE genotype.Alzheimers Dement. 2019; 15:888-898.
[45]. Sperling RA, Donohue MC, Raman R, et al. Association of factors with elevated amyloid burden in clinically normal older individuals. JAMA Neurol. 2020;77(6):735-745.
[46]. Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: A systematic review and meta-analysis. Lancet Neurol. 2016;15(7):673684.
[47]. Hunt A, Schonknecht P, Henze M, Seidl U, Haberkorn U, Schroder J. Reduced cerebral glucose metabolism in patients at risk for Alzheimer's disease. Psych Res: Neuroimaging. 2007; 155:147-154.
[48]. Bennett DA, Schneider JA, Arvanitakis Z, et al. Neuropathology of older persons without cognitive impairment from two communitybased studies. Neurology. 2006; 66:1837- 1844.
[49]. Knopman DS, Parisi JE, Salviati A, et al. Neuropathology of cognitively normal elderly. J Neuropathol Exp Neurol. 2003; 62:1087-1095.
[50]. Grontvedt GR, Schroder TN, Sando SB, White L, Brathen G, Doeller CF. Alzheimer's disease. Curr Bio. 2018;28:PR645-PR649.
[51]. Petersen RC, Lopez O, Armstrong MJ, et al. Practice guideline update summary: Mild cognitive impairment. Neurology. 2018;90(3):126-135.
[52]. Ward A, Tardiff S, Dye C, Arrighi HM. Rate of conversion from prodromal Alzheimer's disease to Alzheimer's dementia: A systematic review of the literature. Dement Geriatr Cogn Disord Extra. 2013;3(1):320-332.
[53]. Canevelli M, Grande G, Lacorte E, et al. Spontaneous reversion of mild cognitive impairment to normal cognition: A systematic review of literature and meta-analysis. J Am Med Dir Assoc. 2016;17(10):943-948.
[54]. Social Security Administration. Minimizing the risk of scams for people living with dementia. Accessed October 11, 2023. Available at: https://blog.ssa.gov/minimizing-the- riskof-scams-forpeople-living-with-dementia/#:~:text=People%20living%20with% 20dementia%20are, struggle%20to%20make%20financial% 20decisions
[55]. Memory and Aging Center, UCSF Weill Institute for Neurosciences. Executive functions. Accessed October 11, 2023. Available at: https://memory.ucsf.edu/symptoms/executivefunctions.
[56]. Heerema E. How executive functioning Is affected by dementia. Verywell Health. AccessedOctober 30, 2023. Available at: https://www.verywellhealth.com/executivefunctioningalzheimers-98596.
[57]. Beshir SA, Aadithsoorya AM, Parveen A, Goh SSL, Hussain N, Menon VB. Aducanumab therapy to treat Alzheimer's disease: A narrative review. Int J Alzheimers Dis. 2022; 2022:9343514.
[58]. Van Dyck CH, Swanson CJ, Aisen P, et al. Lecanemab in early Alzheimer's disease. N Engl J Med. 2023; 388:9-21.
[59]. Cummings J, Apostolova L, Rabinovici GD, et al. Lecanemab: Appropriate use recommendations. J Prev Alzheimers Dis. 2023;10(3):362- 377.

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/26feb693

[60]. Cummings J, Aisen P, Apostolova LG, Atri A, Salloway S, Weiner M. Aducanumab: Appropriate use recommendations. J Prev Alz Dis. 2021;4(8):398-410.
[61]. Cummings J, Rabinovici GD, Atri A. et al. Aducanumab: Appropriate Use Recommendations Update. J Prev Alzheimers Dis. 2022; 9:221- 230.
[62]. Cummings J, Zhou Y, Lee G, Zhong K, Fonseca J, Cheng F. Alzheimer's disease drug development timeline: 2023. Alzheimers Dement: TRCI 2023. doi:10.1002/trc2.12385.
[63]. Lim MM, Gerstner JR, Holtzman DM. The sleep-wake cycle and Alzheimer's disease: What do we know? Neurodegener Dis Manag. 2014;4(5):351-362.
[64]. Lloret M-A, Cervera-Ferri A, Nepomuceno M, Monllor P, Esteve D, Lloret A. Is sleep disruption a cause or consequence of Alzheimer's disease? Reviewing its possible role as a biomarker. Int J Mol Sci. 2020; 21:1168.
[65]. Rose KM, Fagin CM, Lorenz R. Sleep Disturbances in Dementia: What They Are and What To Do. J Gerontol Nurs. 2010;36(5):9-14.
[66]. Van der Mussele S, Le Bastard N, et al. Agitation-associated behavioral symptoms in mild cognitive impairment and Alzheimer's dementia. Aging Ment Health. 2015;19(3):247- 257.
[67]. Watt JA, Goodarzi Z, Veroniki AA, et al. Comparative efficacy of interventions for aggressive and agitated behaviors in dementia. Ann Internal Med. 2019;171(9):633-642.
[68]. Ralph SJ, Espinet AJ. Increased all-cause mortality by antipsychotic drugs: Updated review and meta-analysis in dementia and general mental health care. J Alzheimers Dis Rep. 2018; 2:1-26.
[69]. Maust DT, Kim HM, Seyfried LS, et al. Antipsychotics, other psychotropics, and the risk of death in patients with dementia: number needed to harm. JAMA Psychiatry. 2015; 72:438445.
[70]. Hebert LE, Bienias JL, Aggarwal NT, et al. Change in risk of Alzheimer disease over time.Neurology. 2010; 75:786-791.
[71]. National Institute on Aging. Accessed December 15, 2023. Available at: https://www.nia.nih.gov/health/what-causes-alzheimersdisease.
[72]. Saunders AM, Strittmatter WJ, Schmechel D, et al. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology. 1993; 43:14671472.
[73]. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: A meta-analysis. JAMA. 1997; 278:1349- 1356.
[74]. Green RC, Cupples LA, Go R, et al. Risk of dementia among white and African American relatives of patients with Alzheimer disease. JAMA. 2002;287(3):329-336.
[75]. Fratiglioni L, Ahlbom A, Viitanen M, Winblad B. Risk factors for lateonset Alzheimer's disease: A population-based, case-control study. Ann Neurol. 1993;33(3):258-266.
[76]. Mayeux R, Sano M, Chen J, Tatemichi T, Stern Y. Risk of dementia in first-degree relatives of patients with Alzheimer's disease and related disorders. Arch Neurol. 1991;48(3):269-273.
[77]. Lautenschlager NT, Cupples LA, Rao VS, et al. Risk of dementia among relatives of Alzheimer's disease patients in the MIRAGE Study: What is in store for the oldest old? Neurology. 1996;46(3):641-650.
[78]. Hebert LE, Weuve J, Scherr PA, Evans DA. Alzheimer disease in the United States (20102050) estimated using the 2010 Census. Neurology. 2013;80(19):1778-1783.
[79]. Nelson PT, Head E, Schmitt FA, et al. Alzheimer's disease is not "brain aging": Neuropathological, genetic, and epidemiological human studies. Acta Neuropathol. 2011; 121:571-587.
[80]. Bellenguez C, Küçükali F, Jansen IE, et al. New insights into the genetic etiology of Alzheimer's disease and related dementias. Nat Genet. 2022; 54:412-436.
[81]. Loy CT, Schofield PR, Turner AM, Kwok JBJ. Genetics of dementia. Lancet. 2014; 383:828-840.
[82]. Qian J, Wolters FJ, Beiser A, et al. APOE-related risk of mild cognitive impairment and dementia for prevention trials: An analysis of four cohorts. PLoS Med. 2017;14(3): e1002254.
[83]. Spinney L. Alzheimer's disease: The forgetting gene. Nature. 2014;510(7503):26-28.
[84]. Ward A, Crean S, Mercaldi CJ, et al. Prevalence of apolipoprotein e4 genotype and homozygotes (APOE e4/4) among patients diagnosed with Alzheimer's disease: A systematic review and meta-analysis. Neuroepidemiology. 2012; 38:1-17.
[85]. Mayeux R, Saunders AM, Shea S, et al. Utility of the apolipoprotein E genotype in the diagnosis of Alzheimer's disease. N Engl J Med. 1998; 338:506-511.
[86]. Evans DA, Bennett DA, Wilson RS, et al. Incidence of Alzheimer disease in a biracial urban community: Relation to apolipoprotein E allele status. Arch Neurol. 2003;60(2):185189.
[87]. Tang M, Stern Y, Marder K, et al. The APOE-e4 allele and the risk of Alzheimer disease among African Americans, whites, and Hispanics. JAMA. 1998; 279:751-755.
[88]. Weuve J, Barnes LL, Mendes de Leon CF, et al. Cognitive aging in black and white Americans: Cognition, cognitive decline, and incidence of Alzheimer disease dementia. Epidemiology. 2018;29(1):151-159.
[89]. Hendrie HC, Murrell J, Baiyewu O, et al. APOE ε4 and the risk for Alzheimer disease and cognitive decline in African Americans and Yoruba. Int Psychogeriatr. 2014;26(6):977- 985.
[90]. Reitz C, Jun G, Naj A, et al. Variants in the ATP-binding cassette transporter (ABCA7), apolipoprotein E epsilon 4, and the risk of late-onset Alzheimer disease in African Americans.JAMA. 2013;309(14):1483-1492.
[91]. Gottesman RF, Albert MS, Alonso A, et al. Associations between midlife vascular risk factors and 25-year incident dementia in the Atherosclerosis Risk in Communities (ARIC) cohort. JAMA Neurol. 2017;74(10):1246-1254.

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/26feb693

[92]. Bakulski KM, Vadari HS, Faul JD, et al. Cumulative genetic risk and APOE «4 are independently associated with dementia status in a multiethnic, population-based cohort. Neurol Genet. 2021;7: e576.
[93]. Rajan KB, Barnes LL, Wilson RS, et al. Racial differences in the association between apolipoprotein E risk alleles and overall and total cardiovascular mortality over 18 years. JAGS. 2017; 65:2425- 2430.
[94]. Kataoka S, Robbins DC, Cowan LD, et al. Apolipoprotein E polymorphism in American Indians and its relation to plasma lipoproteins and diabetes. The Strong Heart Study. Arterioscler Thromb Vasc Biol. 1996; 16:918-925.
[95]. Le Guen Y, Raulin AC, Logue MW, et al. Association of African ancestry-specific APOE missense variant R145C with risk of Alzheimer disease. JAMA. 2023;329(7):551- 560.
[96]. Granot-Hershkovitz E, Tarraf W, Kurniansyah N, et al. APOE alleles' associationn with cognitive function differs across Hispanic/Latino groups and genetic ancestry in the study of Latinosinvestigation of neurocognitive aging (HCHS/SOL). Alzheimer's Dement. 2021; 17:466-474.
[97]. Lott IT, Dierssen M. Cognitive deficits and associated neurological complications in individuals with Down's syndrome. Lancet Neurol. 2010;9(6):623-633.
[98]. National Down Syndrome Society. Alzheimer's Disease and Down Syndrome. Accessed December 15, 2023. Available at: https:// www.ndss.org/resources/alzheimers/.
[99]. Fortea J, Vilaplana E, Carmona-Iragui M, et al. Clinical and biomarker changes of Alzheimer's disease in adults with Down syndrome: A cross-sectional study. Lancet. 2020;395(10242):1988- 1997.
[100]. Fortea J, Zaman SH, Hartley S, Rafii MS, Head E, Carmona-Iragui M. Alzheimer's disease associated with Down syndrome: A genetic form of dementia. Lancet Neurol. 2021;20(11):930-942.
[101]. Hithersay R, Startin CM, Hamburg S, et al. Association of dementia with mortality among adults with Down syndrome older than 35 years. JAMA Neurol. 2019;76(2):152-160.
[102]. Bekris LM, Yu CE, Bird TD, Tsuang DW. Genetics of Alzheimer disease. J Geriatr Psychiatry Neurol. 2010;23(4):213-227.
[103]. Goldman JS, Hahn SE, Bird T. Genetic counseling and testing for Alzheimer disease: Joint practice guidelines of the American College of Medical Genetics and the National Society of Genetic Counselors. Genet Med. 2011; 13:597-605.
[104]. Lopera F, Marino C, Chandrahas AS, et al. Resilience to autosomal dominant Alzheimer's disease in a Reelin-COLBOS heterozygous man. Nat Med. 2023 May;29(5):12431252.
[105]. Arboleda-Velasquez JF, Lopera F, O'Hare M, et al. Resistance to autosomal dominant Alzheimer's disease in an APOE3 Christchurch homozygote: a case report. Nat Med. 2019;25(11):1680-1683.