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Bioevaluation of Ficus racemosa Linn. Latex for Memory and Learning Enhancing Activity in Animal Model

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Abstract: *Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive impairment, memory loss, oxidative stress, and neuronal degeneration. The present study was designed to evaluate the memory and learning enhancing activity of Ficus racemosa Linn. latex in intracerebroventricular streptozotocin (ICV-STZ) induced Alzheimer's disease in rats. Experimental Alzheimer's disease was induced in Sprague Dawley rats using ICV-STZ administration. Animals were divided into five groups including normal control, negative control, FR Latex 200 mg/kg, FR Latex 400 mg/kg, and donepezil treated standard group. Behavioral parameters such as transfer latency, retention time, and cognitive performance were evaluated using Elevated Plus Maze and Morris Water Maze models. Oxidative stress markers and antioxidant enzyme activities were also assessed. ICV-STZ induced rats showed significant cognitive impairment, oxidative stress, and memory deficits compared to normal control animals. Treatment with FR Latex significantly improved memory retention, learning behavior, and antioxidant enzyme levels in treated rats compared to negative control animals. The neuroprotective effect may be attributed to antioxidant and free radical scavenging properties of phytoconstituents present in the latex. The findings suggest that Ficus racemosa Linn. latex possesses significant neuroprotective and anti-Alzheimer activity.*

Keywords: Alzheimer's disease, Ficus racemosa latex, ICV-STZ, Neuroprotective activity, Memory enhancement, Donepezil

I. INTRODUCTION

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disorder characterized by gradual deterioration of memory, cognition, learning ability, and behavioral functions. It is the most common cause of dementia among elderly individuals worldwide and represents a major global health burden. The disease primarily affects the hippocampus and cerebral cortex, leading to impairment of memory processing, reasoning, language, and decision-making abilities. Histopathologically, Alzheimer's disease is characterized by extracellular deposition of amyloid-beta (Aβ) plaques, intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein, synaptic degeneration, mitochondrial dysfunction, neuroinflammation, oxidative stress, and neuronal loss. These pathological changes progressively impair neuronal communication and cognitive performance resulting in severe memory deficits and behavioral abnormalities (Ajit Kumar Thakur et al., 2018; Richard A. Armstrong et al., 2019).

The prevalence of Alzheimer's disease is increasing rapidly due to population aging and lifestyle-associated risk factors. According to global reports, dementia cases are expected to rise dramatically over the coming decades, with developing countries such as India showing the highest increase in disease burden. Bhagyashree S. R. et al. (2018) reported that the global number of dementia patients was approximately 47 million in 2015 and is expected to exceed

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135 million by 2050. Similarly, Suman Ray et al. (2023) highlighted that India is among the leading contributors to the global dementia burden due to increasing life expectancy and aging populations. Alzheimer's disease not only affects patients psychologically and physically but also imposes significant social and economic challenges on caregivers and healthcare systems.

Several pathological mechanisms are involved in the development and progression of Alzheimer's disease. Oxidative stress is considered one of the major contributors to neuronal degeneration in AD. Excessive production of reactive oxygen species (ROS) leads to lipid peroxidation, DNA damage, mitochondrial dysfunction, synaptic loss, and apoptosis of neuronal cells. Neuroinflammation and impaired cholinergic neurotransmission further aggravate cognitive dysfunction and neuronal injury. Ajit Kumar Thakur et al. (2018) reported that oxidative stress, mitochondrial dysfunction, amyloid toxicity, tau pathology, and cholinergic dysfunction are major mechanisms associated with Alzheimer's disease progression. Similarly, Hasan Ali et al. (2024) demonstrated that oxidative stress and neuroinflammation play central roles in Alzheimer's pathology leading to neuronal apoptosis and synaptic degeneration.

Currently available pharmacological therapies for Alzheimer's disease mainly include acetylcholinesterase inhibitors such as donepezil, rivastigmine, and galantamine, along with NMDA receptor antagonists like memantine. These drugs mainly provide symptomatic relief without completely preventing disease progression and are often associated with adverse effects such as nausea, vomiting, dizziness, hepatotoxicity, and gastrointestinal disturbances. Therefore, increasing attention has been directed toward medicinal plants possessing antioxidant, anti-inflammatory, and neuroprotective properties for the management of neurodegenerative disorders. Sofia Khanam et al. (2021) reported that herbal medicines and phytoconstituents may provide safer and promising therapeutic approaches for Alzheimer's disease management due to their low toxicity and multitarget pharmacological actions.

Medicinal plants contain numerous bioactive phytoconstituents such as flavonoids, alkaloids, tannins, terpenoids, glycosides, steroids, phenolics, and saponins which exhibit antioxidant and neuroprotective activities. Junaid R. Shaikh et al. (2020) reported that phytoconstituents present in medicinal plants are responsible for various pharmacological activities including antioxidant, anti-inflammatory, antidiabetic, antimicrobial, analgesic, and neuroprotective effects. Natural antioxidants can reduce oxidative stress, inhibit lipid peroxidation, improve cholinergic transmission, and protect neuronal cells against degeneration associated with Alzheimer's disease.

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II. PLANT PROFILE

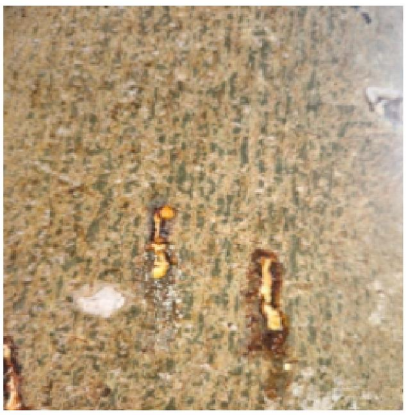

Fig. C Ficus racemosa fruit

Fig. D Ficus racemosa Leaves

Figure No. 3.1: Different parts of Ficus racemosa
[A] Stem[B] latex [C] Fruit [D] Leaves

2.1 Taxonomical classification is as follows: (Pahari N et al., 2022)

Sr No.	Taxonomic Rank	Classification
1	Domain	Eukaryota
2	Kingdom	Plantae
3	Phylum	Tracheophytes
4	Subphylum	Angiospermae
5	Class	Mangoliopsida
6	Order	Urticales
7	Family	Moraceae
8	Genus	Ficus
9	Species	F.racemosa

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10	Common name	Cluster Fig

Table No. 2.1 taxonomical classification Ficus racemosa Linn

2.2 Description: (Phari N et al., 2022)

Table No. 2.2: Description of Ficus racemosa Linn

Sr No	Plant Parts	Description
1	Appearance	F. racemosa is an evergreen, moderate to large, spreading, lactiferous, deciduous tree 15-18 m high, without prominent aerial roots (CitationVarier, 1995).
2	Fruits	The fruits are receptacles that are 3-6 cm in diameter, pyriform in large clusters from large branches or the main trunk
3	Roots	The roots of F. racemosa are long, brownish in colour, having characteristics odour and slightly bitter in taste.
4	Bark	Bark is reddish grey or greyish green, soft surface, uneven and often cracked, 0.5-1.8 cm thick
5	Seeds	Seeds are tiny, innumerable and grain-like. The outer surface of the bark consists of easily removable translucent flakes, greyish to rusty brown, uniformly hard and brittle
6	Latex	Milky white colour and viscous texture that readily adheres to surfaces

2.3 Cultivation Status:

Temperature: 20–35°C 68°F to 95°F)
Moisture: Moderate moisture, high watering
Light: Full sun light
Soil: Well-drained, fertile loamy soil (pH 6.0–7.5) (Ahmed and Urooj et al., 2010)

2.4 Chemical Constituent:

Table No.2.3 Chemical Constituent of Ficus racemosa Linn

Sr No.	Part of the Plant	Phytochemical constituents
1	Leaf	sterols, triterpenoids (Lanosterol), tetracyclic triterpene-glauanol acetate alkaloids, tannins, and flavonoids.
2	Stem-Bark	gluanol acetate, β-sitosterol, leucocyanidin-3-O-D-glucopyranoside, leucopelargonidin-3-O-D-glucopyranoside, leucopelargonidin-3-O-L-rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate, and α-amy. Lupenol, β-sistosterol, and stigmasterol.
3	Trunk-Bark	Upenol, β-sistosterol and stigmasterol.
4	Fruit	Glauanol, glauanol acetate, hentriacontane, sitosterol, glauanol acetate, glucose, tiglic acid, taraxasterol, lupeolacetate, friedelin, higher hydrocarbons.
5	Root	Cycloartenol, euphorbol and its hexacosanoate, taraxerone, and tinyatoxin.
6	Latex	a-amyrin, β-sitosterol, cycloartenol, cycloeuphordenol, 4-deoxyphorbol euphol, euphorbinol, isoeuphorbol, palmitic acid

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2.5 Total Phenolic, Flavonoid, & Flavonol Contents

Table No. 2.4: Total phenolic, flavonoid, and flavonol contents of Ficus Racemosa Latex.

	Total phenolic content (mg GAE/g DW)[a]	Total flavonoid content (mg QE/g DW)[b]	Total flavonol content (mg QE/g DW)[b]
F. Racsomsa Latex	40.68 ± 9.17	160± 48.51	119 ± 0.78

[a] Total phenolic content is expressed as mg gallic acid equivalents per g dry weight (mg GAE/g DW)
[b] Total flavonoid and flavonol content are is expressed as mg quercetin equivalents per g dry weight (mg QE/g DW)

2.6 Physicochemical Properties

Table No. 2.5: Physicochemical Parameters of Ficus racemosa Latex.

Sr. No.	Physicochemical parameters	Latex of Ficus racemosa
1	Loss on Drying (W/W) %	7.2 ± 0.18
2	Total Ash Value (W/W) %	3.8 ± 0.15
3	Acid Insoluble Ash Value (W/W) %	0.9 ± 0.04
4	Water Soluble Ash Value (W/W) %	1.7 ± 0.08

2.7 Medicinal Uses:

Anti-diabetic: Helps lower blood glucose because of flavonoids and tannins that enhance insulin secretion and glucose uptake.
Anti-inflammatory: Reduces inflammation due to β-sitosterol, which inhibits pro-inflammatory mediators.
Anti-ulcer: Protects gastric mucosa and reduces acid secretion because of leucocyanidin.
Antimicrobial: Shows activity against bacteria and fungi owing to phenolic compounds that disrupt microbial cell walls.
Antioxidant: Prevents oxidative stress–related damage because of polyphenols with free radical–scavenging action.
Hepatoprotective: Protects the liver against toxins due to triterpenoids that stabilize hepatocyte membranes.

III. MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and reagents

Table No. 3.1: List of Chemicals Used in Study

Sr. No.	Chemicals	Company
1	Petroleum ether	Thermosil Fine Chem Industries
2	Methanol	Thermosil Fine Chem Industries
3	Mayer's reagent	Prayogina Laboratories India
4	α – napthol	Burgoyne Burbidges & Co.
5	Conc. H_2So_4	Thermosil Fine Chem Industries
6	Glacial acetic acid	Samar Chemicals (India)

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7	Ferric chloride	Thermosil Fine Chem Industries
8	Ninhydrin solution	Thermosil Fine Chem Industries
9	Lead acetate	Thermosil Fine Chem Industries
10	Ammonia solution	Thermosil Fine Chem Industries
11	Streptozocin	Sovereign Pharma Pvt. Ltd.
12	Donepezil	Alkem Laboratories Ltd.
13	Anesthetic ether	S D Fine Chem Ltd.
14	Acetylcholine	Thermosil Fine Chem Industries
15	DTNB	BLD Pharmatech (India) Pvt. Ltd.
16	Ketamine Hydrochloride Injection IP	Themis Medicare
17	Xylazine Injection U.S.P.	Indian Immunological Limited
18	Dental Cement	D-tech
19	Povidone-Iodine	iNova Pharmaceuticals
20	Hair Removal Cream	CiplaHealth
21	Adrenaline Bitartrate	Vasocon

3.1.2 Apparatus and Instruments

Table No. 3.2: List of Instruments Used in Study

Sr. No.	Instruments	Company
1	Weighing balance	K- roy
2	Magnetic stirrer	Remi
3	Cooling centrifuge	Remi
4	Morris water maze apparatus	K- roy
5	Elevated plus maze apparatus	K- roy
6	Stereotaxic Apparatus	INCO
7	Stereotaxic driller	SAE SHIN
8	Sterile Surgical Blade	Niraj Industries PVT.LTD

3.2 Method

3.2.1 Collection & Authentication of Plant Materials

Ficus racemosa latex was collected from the local area of Yavatmal, Maharashtra. The plant material was identified and authenticated by Mrs A. M. Gaharwar, Assistant Professor of Vasantrao Naik College of Agricultural Biotechnology, Yavatmal. (Ref No. VNCABT/Ytl/Hort/13764/2026)

3.2.2 Collection and Preparation of Ficus racemosa Latex

Latex of Ficus racemosa was collected by making shallow incisions on the bark of healthy trees, and the exuding milky latex was collected in a clean container with the help of syringe. The latex was filtered through muslin cloth to remove debris and used fresh or stored at 4°C until further use. For pharmacological studies, the latex was suspended in Tween 80 and diluted with distilled water to obtain a uniform dosing preparation.

3.2.4 Phytochemical Screening

Methanolic extract was subjected to phytochemical screening for the detection of various active constituents.

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A. Test for Alkaloids

1. Mayer's test

1-2 drops of Mayer's reagent added to a few milliliters of filtrate (along the walls of the test tube). The precipitate was creamy/yellow in colour. This shows that alkaloids are present. (K. Sahira Banu et al., 2015)

B. Test for Carbohydrates

1. Molish's test

2 ml filtrate + 2 drops of alcoholic α – napthol + 1 ml conc. H_2So_4 (along the sides of test tube). A violet ring was obtained. This confirms the presence of Carbohydrates. (Vandana Singh et al., 2017)

C. Test for Cardiac Glycosides

1. Keller-Killani test

1 ml filtrate + 1.5 ml glacial acetic acid + 1 drop of 5 % ferric chloride solution + concentrated H_2So_4 (along the sides of test tube). A blue coloured solution (in the acetic acid layer). This confirms the presence of Cardiac glycosides. (Rama Swamy Nanna et al., 2013)

D. Test for Tannins

1. Braymer's test

1 ml filtrate + 3 ml distilled water + 3 drops of 10 % ferric chloride solution. Blue-green colour was obtained. This confirms the presence of tannins. (Uma KS et al., 2017)

E. Test for Proteins & Amino acids

1. Ninhydrin test

2 ml filtrate + 2 drops of Ninhydrin solution (10 mg Ninhydrin + 200 ml acetone). A purple coloured solution (Amino acids) was obtained. This confirms the presence of proteins & amino acids. (Gusthinnadura Oshadie De Silva et al., 2017)

F. Test for Phenolic Compounds

1. Lead acetate test

Plant extract dissolved in 5 ml distilled water + 3 ml of 10 % lead acetate solution. A white precipitate was formed. This confirms the presence of phenolic compounds. (Vishnu Balamurugan et al., 2019)

G. Test for Flavonoids

1. Ammonia test

Few ml filtrate + 5 ml diluted ammonia solution + concentrated H_2So_4. A yellow colour was obtained. This confirms the presence of flavonoids. (R. Suman Kumar et al., 2013)

H. Test for Anthraquinones

1. Borntrager's test

10 ml of 10 % ammonia solution + few ml filtrate (shaken vigorously for 30 seconds). A pink, violet, or red coloured solution was formed. This confirms the presence of Anthraquinones. (Mulugeta Alemu et al., 2024)

I. Test for Saponins

1. Foam test

Small quantity of the extract was shaken with adding 2 ml of water. Persistence of foam produced for 10 min indicates the presence of saponins. (Prashant Tiwari et al., 2011)

J. Terpenoids

1. Salkowski's test

Filtrate + few drops of concentrated H_2So_4 (shaken well & allowed to stand). Golden yellow layer (at the bottom). This confirms the presence of terpenoids. (Dipak Raj Pant et al., 2017)

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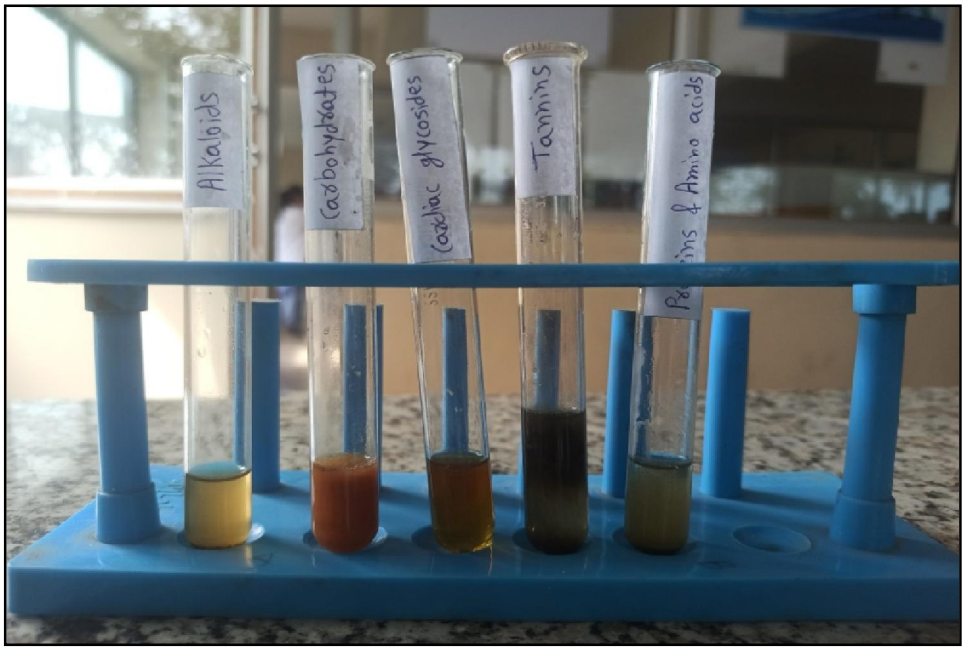

Figure No. 3.1: Phytochemicals screening of FR Latex for Alkaloids, Carbohydrates, Cardiac glycosides, Tannins, Proteins and Amino acids

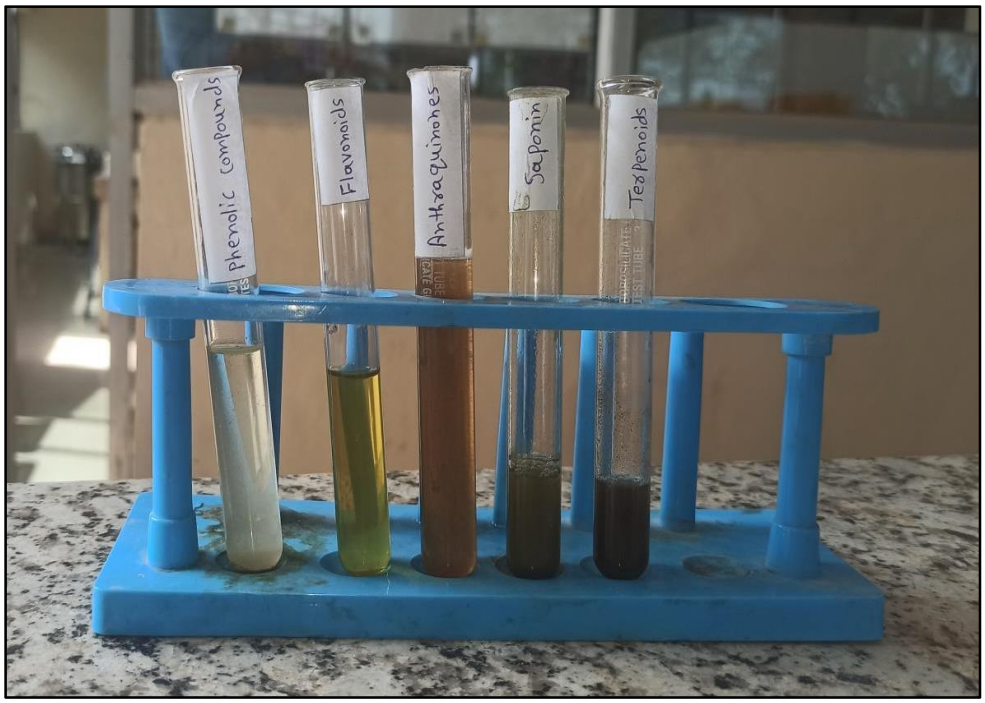

Figure No. 3.2: Phytochemical screening of FR Latex for Phenolic compounds, Flavonoids, Anthraquinones, Saponins and Terpenoids

3.3 Experimental Design

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3.3.1 Experimental Animals

8-week-old healthy female Sprague-Dawley rats (weighing 150-250 gm) were used for this study. Rats were housed in polypropylene cages with a wire mesh top and husk bedding and maintained under control condition of light (12h – light, 12h - dark), temperature (25 ± 2 ˚C), and humidity (60 ± 5 %) and fed with a standard pellet diet and water. The experiments were performed during day (8.00 – 16.00 hrs). The rats were housed and treated according to the rules and regulations of CCSEA and IAEC. The protocol for all the animal studies was approved by the Institutional Animal Ethics Committee (IAEC) with research project number 650/Po/Re/S-2002/2026/CCSEA/03

3.3.2 Animal Groups:

For this study, rats were divided into the following groups (n = 6)

Group 1 (Vehicle Control):- Rats received only normal saline solution for 21 days.

Group 2 (Negative Control):- Memory & learning impairment in rats were produced by using Streptozocin (2 mg/kg i.c.v.) (Moreira-Silva et al., 2019)

Group 3 (FR Latex 200 mg/kg):- Memory & learning impaired in rats were treated with Ficus racemosa Latex (200 mg/kg) orally for 21 days.

Group 4 (FR Latex 400 mg/kg):- Memory & learning impaired in rats were treated with Ficus racemosa Latex (400 mg/kg) orally for 21 days.

Group 5 (Standard):- Memory & learning impaired in rats were treated with Donepezil (5 mg/kg) orally for 21 days.

3.3.3 Learning & Memory Impairment State was Checked in all Animals by Different Animal Models Before & After Administration of Streptozocin

All animals in each group were assessed for the learning and memory impairment state by following the animal models.

1. Elevated plus maze apparatus
2. Morris water maze apparatus

The reading of all animals in each group was noted down. These readings were referred to as a 0 day before administration of Scopolamine. These readings were compared with the readings of the animal model after administration of Streptozocin i.e. after 21 days.

3.3.4 Induction of Alzheimer's Disease by Intracerebroventricular Streptozotocin (ICV-STZ)

Experimental Alzheimer's disease was induced in Sprague-Dawley rats weighing 150–250 g by intracerebroventricular (ICV) administration of streptozotocin (STZ) using a stereotaxic apparatus. The procedure was performed under sterile conditions according to the method described by Moreira-Silva D. et al. (2019) with slight modifications.

Animals were anaesthetised with ketamine (90 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). After confirmation of adequate anaesthesia, the rats were secured in a stereotaxic apparatus and placed on a heating pad to maintain body temperature at approximately 37 °C during the surgical procedure.

The dorsal region of the head was shaved and disinfected using povidone-iodine solution. A midline sagittal incision was made to expose the skull, and the bregma was identified as the reference point for stereotaxic positioning. Bilateral burr holes were drilled carefully at the predetermined coordinates corresponding to the lateral ventricles.

Streptozotocin was freshly prepared in 0.05 M citrate buffer (pH 4.5) immediately before administration and protected from light using aluminium foil. STZ was administered bilaterally into the lateral ventricles at a dose of 2 mg/kg body weight (2 µl/ventricle) using a Hamilton microsyringe connected to an infusion pump. The injection was delivered slowly at a rate of 1 µl/min. After administration, the needle was kept in position for a few minutes to prevent reflux and then withdrawn carefully.

Following the injection, the scalp was sutured with sterile surgical sutures and animals were allowed to recover under standard laboratory conditions. Post-operative care included administration of antibiotic and analgesic agents where

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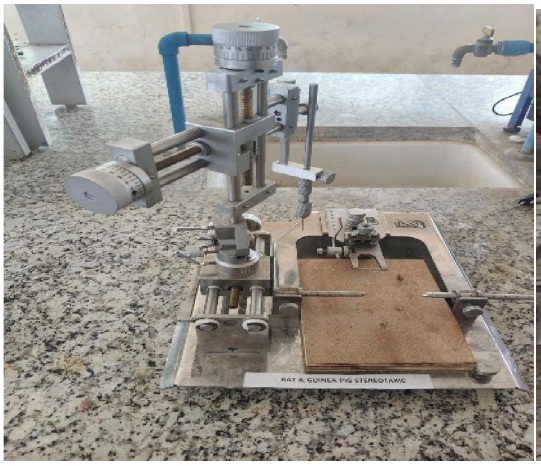
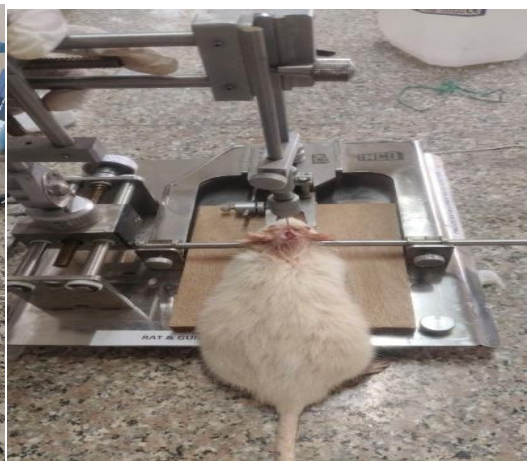
necessary. Behavioural and biochemical studies were performed after the recovery period to evaluate cognitive impairment and neurodegenerative alterations induced by ICV-STZ administration.

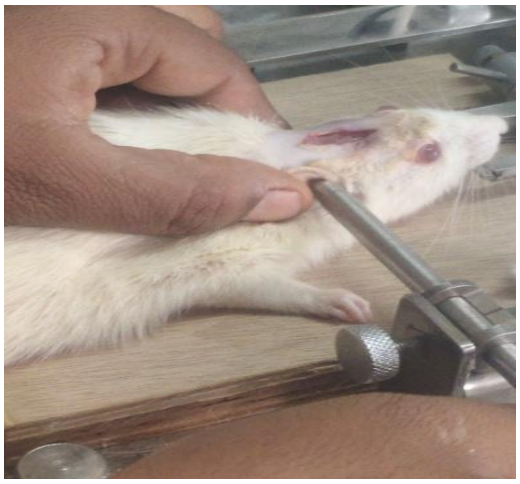


a) Stereotaxic apparatus



b) Rat secured in stereotaxic frame

C) Stereotaxic Drilling Machine



d) Close-up view of rat positioned in stereotaxic apparatus during surgery

Figure 3: Stereotaxic surgical procedure for intracerebroventricular (ICV) injection in a rat

Stereotaxic Coordinates

The stereotaxic coordinates used for ICV injection with reference to bregma were:

Anteroposterior (AP): −0.8 mm

Mediolateral (ML): ±1.5 mm

Dorsoventral (DV): −3.6 mm

Flow Chart of ICV-STZ-Induced Alzheimer's Disease Model

↓

Acclimatisation of Animals (7 days)

↓

Preparation of STZ in 0.05 M Citrate Buffer (pH 4.5)

↓

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Anaesthesia with Ketamine + Xylazine
↓
Fixing an Animal in a Stereotaxic Apparatus
↓
Shaving and Disinfection of the Scalp
↓
Midline Sagittal Incision on Skull
↓
Identification of Bregma
↓
Drilling Burr Holes at Stereotaxic Coordinates
↓
ICV Administration of STZ
(2 mg/kg, bilateral injection)
↓
Slow Injection Using Hamilton Syringe
↓
Needle Retained for a Few Minutes
↓
Withdrawal of Needle and Suturing
↓
Post-Operative Care and Recovery
↓
Behavioural and Biochemical Evaluation

Drugs

STZ (2 mg/kg i.c.v.) was used for memory impairment in rats. Donepezil (5 mg/kg p.o.) was used as the standard drug. Two different concentrations (200 mg/kg and 400 mg/kg) of the Ficus racemosa latex were prepared by dissolving the latex in distilled water with Tween 80 for suspension. All solutions were prepared freshly on test days and administered according to their standard routes.

3.3.5 Daily Dose of Ficus racemosa Latex Given to the Rats

A daily dose of Ficus racemosa Latex was given orally to group FR Latex 200 mg/kg and FR Latex 400 mg/kg for the duration of 21 days.

3.3.6 Study of Learning and Memory Impairment on 0 Day, On Day 7 & After 21 Days was conducted using

1. Elevated Plus Maze Apparatus
2. Morris Water Maze Apparatus

1. Elevated Plus Maze Apparatus

The initially constructed test apparatus was positioned 70 cm above the floor and featured two closed and two open arms, each measuring 45 cm in length by 10 cm in width. The open arms (50 x 10 cm) and closed arms (50 x 10 x 40 cm) of the elevated plus maze test were enlarged, and both featured tall surrounding walls with an open ceiling above the closed arms. The maze itself was also raised to a 50-centimeter height. Now arranged in the laboratory as a plus symbol, the elevated plus maze test apparatus has two open arms (25 x 5 cm, with a very small 0.5-cm wall) across from each other and perpendicular to two closed arms (25 x 5 x 16 cm), and it is raised 50 cm from the floor. The rat is placed in the middle of the 5 cm by 5 cm maze and is allowed to move freely. In rat models of CNS diseases, the transfer latency is evaluated to evaluate behaviour related to anxiety. While conducting the elevated plus maze test, an

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observer notes the outcomes. The test is recorded by a video camera that is connected to a computer within the lab. The Image EP application is used to gather and analyze the behavioural data. (Mani Vasudevan et al., 2007)

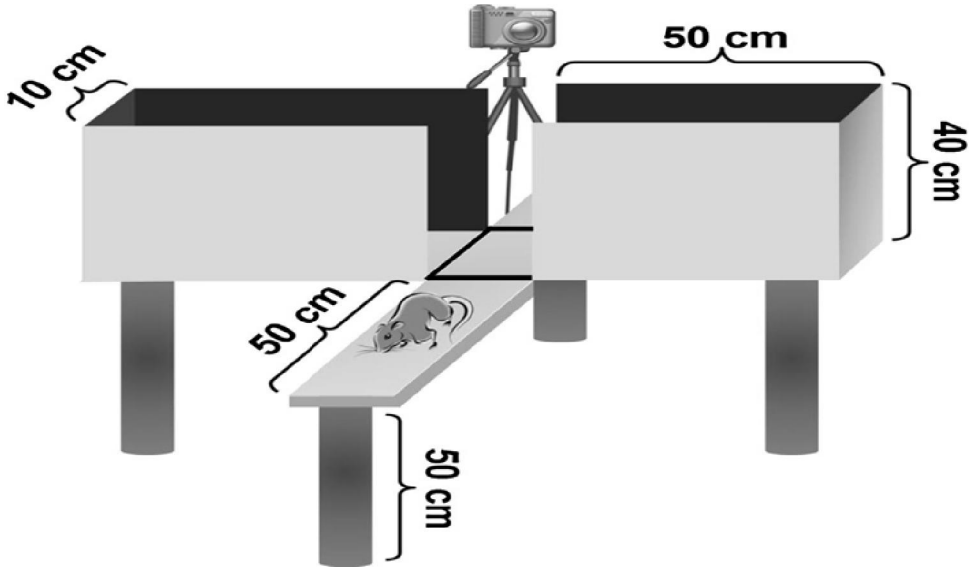

Figure No. 6.4: Elevated Plus Maze Apparatus

2. Morris Water Maze Apparatus

The process, methodology, and conclusion of memory testing. Briefly, a circular pool (170 cm in diameter and 45 cm in height) filled to a depth of 26 cm and kept at a temperature of 25°C served as the Morris Water Maze (MWM) for rats. White, non-toxic dye was used to turn the water opaque. Two threads that were fastened at a straight angle to one another on the pool rim helped split the tank into four equal quadrants. Within the pool's target quadrants (Q4 in the current study), a submerged platform with a white painted top surface was positioned below the water's surface. During the training, the platform's position remained constant. Every animal underwent four separate trials every day, separated by five minutes, for four days in a row (from the sixth day of drug administration to the ninth day), during which they were permitted to escape onto the concealed platform and stay there for twenty seconds. The rats were carefully lowered into the water between the training quadrants, facing the pool wall, with a different drop point for every trial. They were given 120 seconds to find the submerged platform. The rats were gently brought onto the platform and given 20 seconds to stay there if they couldn't find it in 120 seconds. The amount of time it takes an animal to go from the starting quadrant to the hidden platform in the target quadrant is known as escape latency (EL). (Md. Sahab Uddin et al., 2016)

For every animal, EL was recorded from the sixth to the ninth day. Each animal underwent four days of training trials in which the goal quadrant (Q4 in this study) remained constant and the beginning location was altered with each exposure as detailed below.

Day 1 Q1 Q2 Q3 Q4.
Day 2 Q2 Q3 Q4 Q1.
Day 3 Q3 Q4 Q1 Q2.
Day 4 Q4 Q1 Q2 Q3.

Rats were placed in any of the three quadrants and given 300 seconds to explore the target quadrant on the fifth day, or the tenth day of the drug administration, after the platform was withdrawn. The average amount of time spent in each of the three quadrants—Q1, Q2, and Q3—was noted. The index of retrieval or memory was defined as the average amount of time spent looking for the missing platform in the target quadrant. The observer maintained the same posture

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at all times. The relative position of the water maze in relation to other laboratory objects was carefully monitored. On the 21st day, the last reading was taken. (Pankaj Phukan et al., 2019)

All data were collected for the Morris Water Maze test and compared with the normal control group, as well as the memory impairment induced rat and the Ficus racemosa latex extract treated memory impairment induced rat.

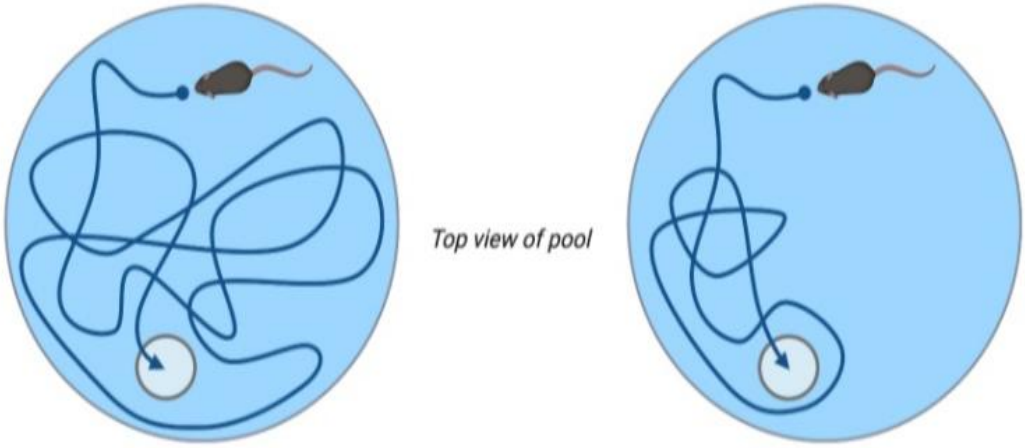

Figure No. 6.5: Comparison of the Activity of Alzheimer's Rat and Normal Rat in Morris Water Maze Apparatus

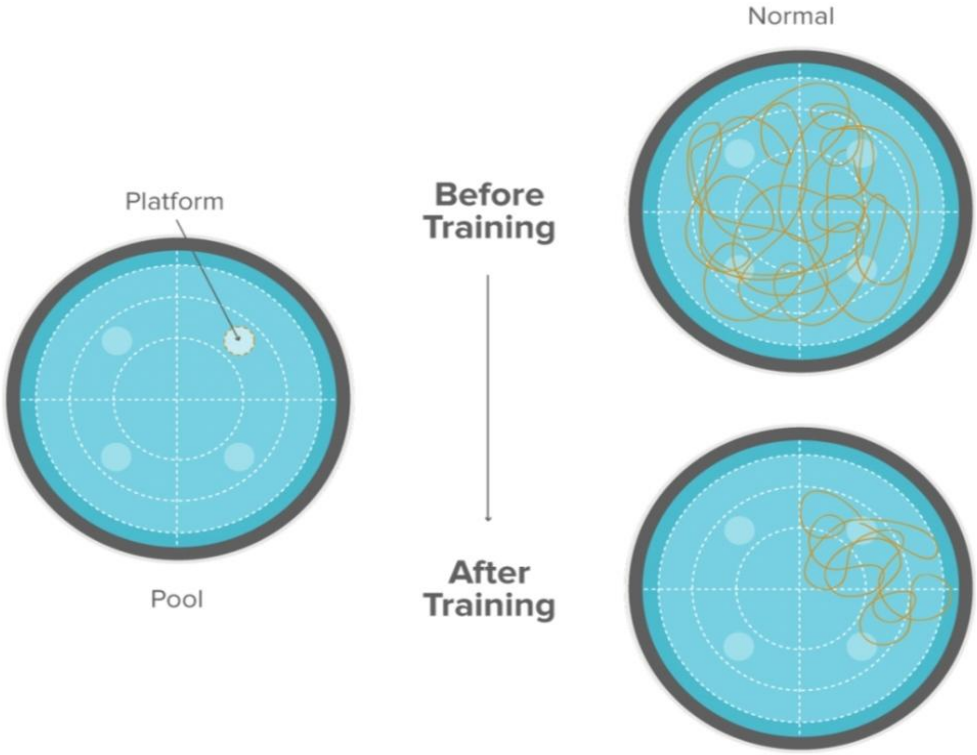

Figure No. 6.6: Representation of Before and After Training Activity of Rats in Morris Water Maze Apparatus

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3.3.7 Statistical Analysis

All data were expressed as the mean ± standard deviation. For statistical Analysis of the rats, group means were compared by one-way (ANOVA) followed by Dunnett's test, $p<0.01$ was considered a significant value.

IV. RESULTS

4.1 Effect of FR Latex on Memory and Learning Activity

ICV-STZ induced rats showed significant impairment in learning and memory compared to normal control rats. Treatment with FR Latex significantly improved cognitive behavior and memory retention.

4.2 Effect on Transfer Latency

Negative control animals showed increased transfer latency indicating memory impairment. FR Latex treated groups showed significant reduction in transfer latency compared to negative control animals.

Elevated Plus Maze Apparatus

A. Transfer Latency

Table No. 4.2: Effect of FR Latex on transfer latency (TL) of rats in EPM apparatus

Sr. No.	Experimental Groups	Transfer latency (s) on Day 0	Transfer latency (s) on Day 7	Transfer latency (s) on Day 21
1.	Normal Control	16.2 ± 1.3	15.4 ± 1.1	14.2±1.3
2.	Negative Control	17 ± 1.6^{ns}	42.6 ± 3.2$^{@}$	68.4±4.3$^{@}$
3.	FR Latex (200 mg/kg)	16 ± 1^{ns}	34.8 ± 2.8*	31.5±2.4*
4.	FR Latex (400 mg/kg)	15.6 ± 1.1^{ns}	28.4 ± 2.3*	24.6±2.1**
5.	Donepezil (5 mg/kg)	16.1 ± 1.2^{ns}	22.8 ± 2**	18.4±1.8**

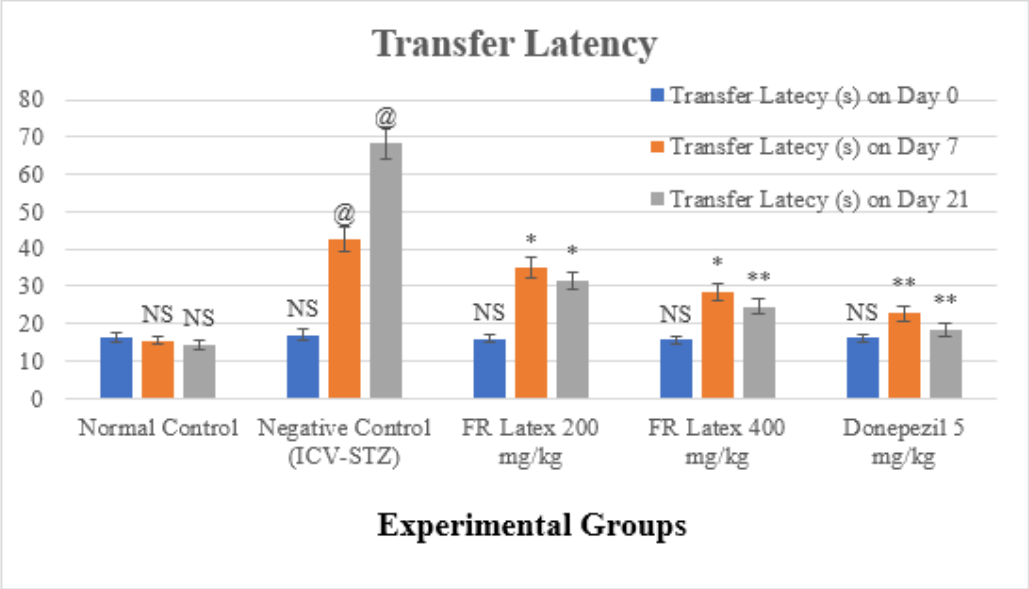

Figure No. 4.1: Effect of FR Latex on transfer latency of rats in EPM apparatus

All values are expressed as Mean ± SD $^{@}$ $p<0.01$ Significant increase in transfer latency was observed compared to the normal control group. ** $p<0.01$ Significant decrease in transfer latency was observed compared to the negative control group.

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Table No. 7.2 & Figure No. 7.3 show the effect of Ficus racemosa latex TL in EPM in memory-impaired rats. There was a significant ($p<0.01$) increase in TL in the negative control group as compared to the normal control group. There was a significant ($p<0.01$) decrease in TL in FR Latex 200 mg/kg, FR Latex 400 mg/kg, and Donepezil (5 mg/kg) treated group when compared to the negative control group.

4.3 Effect on Escape Latency Latency

Morris Water Maze Apparatus

Sr. No.	Groups	Escape latency (s) on Day 0	Escape latency (s) on Day 7	Escape Latency (s) on Day 21
1.	Normal Control	42.3± 3.2	16.8± 1.9	11.2±1.3
2.	Negative Control	43.1 ± 3.5^{ns}	$48.6 \pm 4.1^{@}$	$66.4 \pm 5.0^{@}$
3.	FR Latex (200 mg/kg)	42.8 ± 3.1^{ns}	$28.4 \pm 2.7^{*}$	$22.1 \pm 2.2^{*}$
4.	FR Latex (400 mg/kg)	41.9 ± 2.9^{ns}	$21.6 \pm 2.1^{*}$	$15.8 \pm 1.8^{**}$
5.	Donepezil (5 mg/kg)	42.0 ± 3.0^{ns}	$18.4 \pm 1.8^{**}$	$12.9 \pm 1.5^{**}$

A. Escape Latency: -

Table No. 4.3: Effect of FR Latex on escape latency (EL) of rats in MWM apparatus

Escape Latency

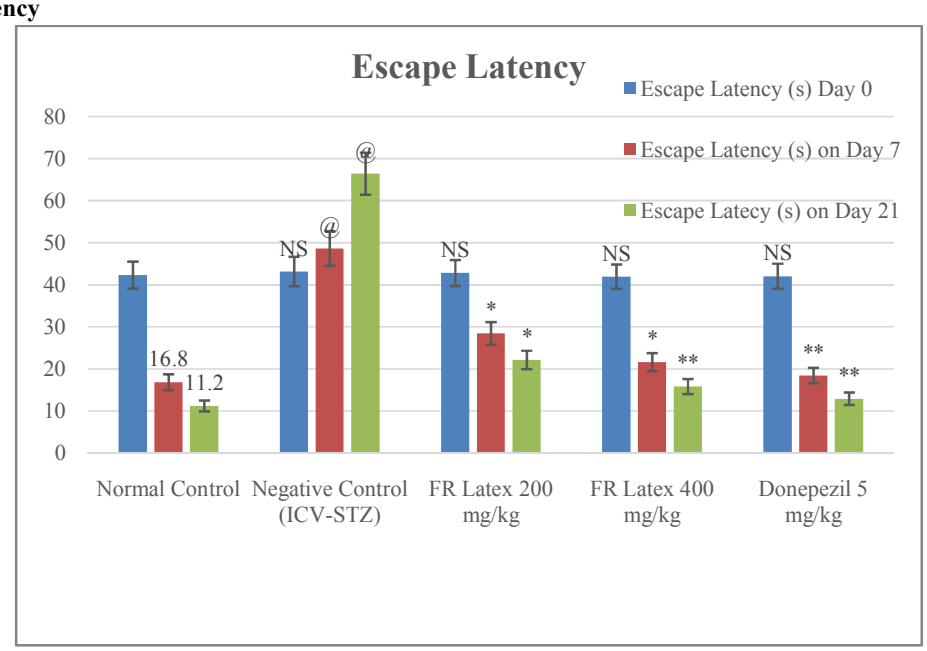

Figure No. 4.2: Effect of FR Latex on escape latency of rats in MWM apparatus

All values are expressed as Mean ± SD $^{@}$ $p<0.01$ Significant increase in escape latency was observed compared to the normal control group. $^{**}p<0.01$ Significant decrease in escape latency was observed compared to the negative control group

Table No. 7.3 & Figure No. 7.4 show the effect of Ficus racemosa latex on EL in MWM in memory-impaired rats. There was a significant ($p<0.01$) increase in EL in the negative control group as compared to the normal control group. There was a significant ($p<0.01$) decrease in EL in FR Latex 200 mg/kg, FR Latex 400 mg/kg, and Donepezil (5 mg/kg) treated group when compared to the negative control group.

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4.4 Effect on Retention Time

Retention time was significantly reduced in ICV-STZ induced rats. Treatment with FR Latex significantly increased retention time indicating improvement in learning and memory.

B. Retention Time: -

Table No. 4.4: Effect of FR LATEX on retention time (RT) of rats in MWM apparatus

Sr. No.	Experimental Groups	Retention time (s) on Day 0	Retention time (s) on Day 7	Retention time (s) on day 21
1.	Normal Control	26.8 ± 1.6	30.6 ± 1.9	34.4±2.1
2.	Negative Control	26.5 ± 1.5^{ns}	$17.2 \pm 1.4^{@}$	$10.4\pm1.2^{@}$
3.	FR Latex (200 mg/kg)	26.7 ± 1.4^{ns}	$21.8 \pm 2^{*}$	$23.5\pm1.5^{*}$
4.	FR Latex (400 mg/kg)	26.6 ± 1.5^{ns}	$25.4 \pm 1.6^{*}$	$18.5\pm1.9^{**}$
5.	Donepezil (5 mg/kg)	26.9 ± 1.5^{ns}	$28.1 \pm 1.8^{**}$	$32.2\pm2^{**}$

Retention Time

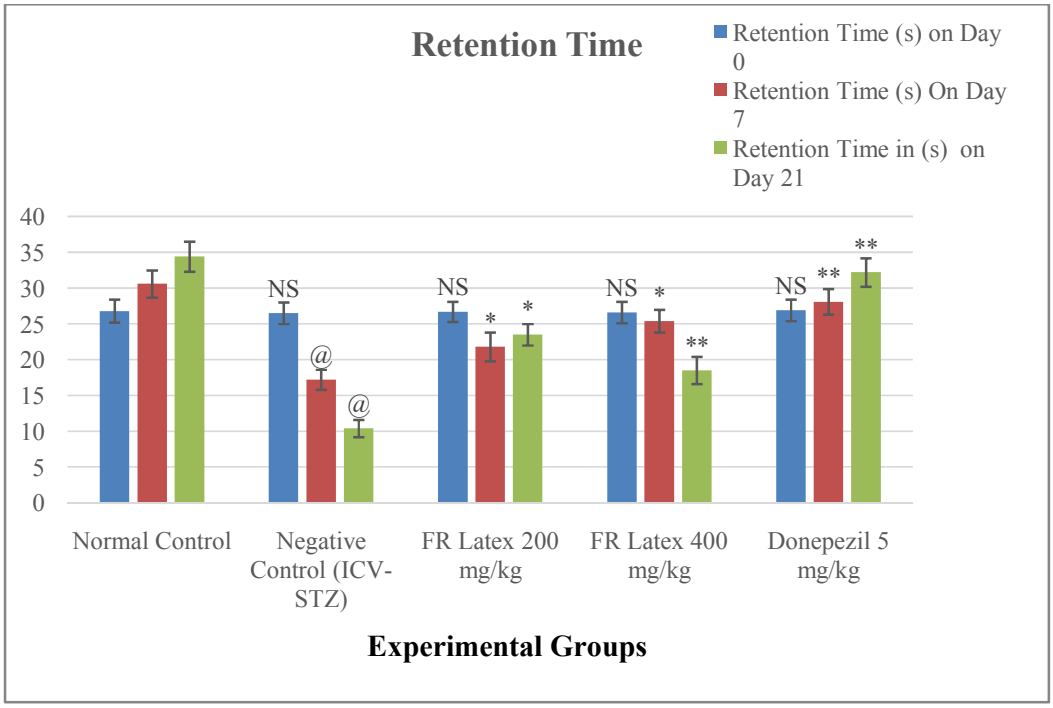

Figure No. 4.3: Effect of FR Latex on retention time of rats in MWM apparatus

All values are expressed as Mean ± SD $^{@}$ $p<0.01$ Significant decrease in retention time was observed compared to the normal control group. $^{**}p<0.01$ Significant increase in retention time was observed compared to the negative control group.

Table No. 7.4 & Figure No. 7.5 show the effect of Ficus racemosa Latex on RT in MWM in memory-impaired rats. There was a significant ($p<0.01$) decrease in RT in the negative control group as compared to the normal control group. There was a significant ($p<0.01$) increase in RT in the FR Latex 200 mg/kg, FR Latex 400 mg/kg, and Donepezil (5 mg/kg) treated group when compared to the negative control group.

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V. DISCUSSION

The present study demonstrated that intracerebroventricular administration of streptozotocin successfully induced Alzheimer-like cognitive dysfunction in rats. Negative control animals showed memory impairment, oxidative stress, and neuronal dysfunction compared to normal control animals.

Behavioral assessment using Elevated Plus Maze and Morris Water Maze revealed significant increase in transfer latency and reduction in retention time in negative control rats, indicating impairment in learning and memory behavior. Treatment with FR Latex significantly improved cognitive performance and memory retention in treated groups.

Oxidative stress is considered one of the major mechanisms involved in the pathogenesis of Alzheimer's disease. Excessive production of reactive oxygen species causes lipid peroxidation, mitochondrial dysfunction, inflammation, neuronal damage, and apoptosis. Treatment with FR Latex significantly improved antioxidant enzyme activities and reduced oxidative stress, suggesting potent antioxidant activity.

The neuroprotective activity of Ficus racemosa latex may be attributed to the presence of flavonoids, phenolic compounds, tannins, and terpenoids which possess antioxidant and free radical scavenging properties.

Donepezil treated animals also showed significant improvement in cognitive function confirming the validity of the experimental model. FR Latex treated groups demonstrated comparable neuroprotective activity suggesting its therapeutic potential in management of Alzheimer's disease.

VI. CONCLUSION

The present study concludes that Ficus racemosa Linn. latex possesses significant memory and learning enhancing activity against ICV-STZ induced Alzheimer's disease in rats. Treatment with FR Latex significantly improved cognitive behavior, memory retention, and antioxidant defense mechanisms.

The beneficial effects may be attributed to antioxidant and neuroprotective properties of phytoconstituents present in the latex. Therefore, Ficus racemosa Linn. latex may serve as a promising therapeutic candidate for management of Alzheimer's disease and related neurodegenerative disorders.

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First and foremost, I thank God Almighty for the strength and blessings that helped me complete this research successfully.

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