https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 28(2s) (February 2025); 1839-1848
Review Article

Neuroprotective Effects of Erythravine in Cerebral Ischemia-Reperfusion Injury

Chandarana Sanjana Rameshbhai[1*], Dr. Noopur Gandhi[2], Dr. Pragnesh Patani[3], Dr. Shweta Paroha[4], Dr. Nishkruti Mehta[5], Hetvi Shah[6]

[1*]Department of Pharmacology, Khyati College of Pharmacy, India.
[2]L. M. College of Pharmacy, Gujarat Technological University,India.
[3]Principal and Professor, Department of Pharmacology, Khyati College of Pharmacy, Palodia, Ahmedabad
[4] Professor, Department of Pharmaceutics, Khyati College of Pharmacy, Palodia, Ahmedabad
[5]Professor, Department of Pharmacology, Khyati College of Pharmacy, Palodia, Ahmedabad
[6]Department of Pharmacology, Khyati College of Pharmacy, India.

***Corresponding Author:** Chandarana Sanjana Rameshbhai
*Department of Pharmacology, Khyati College of Pharmacy, India, Email: sanjanachandarana48@gmail.com

Abstract
Background: Cerebral ischemia-reperfusion (I/R) injury remains a major cause of morbidity and mortality worldwide. It results from the temporary interruption of cerebral blood flow followed by the restoration of circulation, which paradoxically exacerbates neuronal injury through a cascade involving oxidative stress, inflammation, and excitotoxicity. The pathophysiological mechanisms include energy failure, ion imbalance, elevated intracellular calcium levels, generation of reactive oxygen species (ROS), and activation of inflammatory cytokines. These processes lead to cellular damage and death, primarily through necrosis and apoptosis.
Objective: To evaluate the neuroprotective potential of Erythravine, an alkaloid derived from *Erythrina mulungu*, in attenuating cerebral I/R injury in rats**,** including antioxidant and anti-inflammatory properties, which may contribute to neuroprotection.
Methodology: An experimental model of cerebral ischemia-reperfusion was induced in laboratory animals. The study involved the administration of Erythravine at therapeutic doses prior to and/or after the ischemic insult. Parameters such as infarct volume, neurological deficit scoring, oxidative stress markers (e.g., MDA, SOD, GSH), inflammatory cytokine levels (e.g., TNF-α, IL-1β), and histopathological changes in brain tissue were measured to assess the extent of neuronal damage and the protective effects of Erythravine.
Results: Erythravine-treated groups demonstrated a significant reduction in infarct size compared to controls. There was also marked improvement in neurological scores and a decrease in oxidative stress markers, indicating a reduction in lipid peroxidation and enhanced antioxidant defense. Furthermore, histological analysis revealed preserved neuronal architecture, and pro-inflammatory cytokine levels were notably reduced, suggesting an anti-inflammatory effect.
Conclusion: Erythravine exhibits a promising neuroprotective effect in cerebral ischemia-reperfusion injury by modulating oxidative stress and inflammatory responses. These findings support its potential as a therapeutic agent in the management of ischemic stroke and related neurological disorders. Further studies are warranted to elucidate its molecular mechanisms and evaluate its clinical applicability.

Keywords: Anti-oxidant, Anti-inflammatory, Cerebral ischemia reperfusion injury, Neuroprotective effects, oxidative stress, inflammation, and excitotoxicity.

***Author of correspondence: Email:** *pragatikamble7057@gmail.com*

Received – 02/01/2025 *Acceptance- 15/01/2025*

DOI: https://doi.org/10.53555/AJBR.v28i2S.7530

1. Introduction

1.1 Introduction of Disease: Cerebral Ischemia

Cerebral ischemia transpires when there is a substantial decrease or interruption of blood flow to the brain, leading to a deficiency of vital nutrients such as oxygen and glucose. This illness can be categorized as focal or global ischemia and may manifest as either transitory or persistent. The pathophysiological mechanisms triggered by ischemia encompass energy depletion, disruption of cellular ion homeostasis, acidosis, and elevated intracellular calcium levels ([Ca2+]), resulting in excitotoxicity and free radical-induced toxicity.[1]

The immediate result is that neurons may perish via necrosis or apoptosis. In the center of an infarct, where cerebral blood flow (CBF) is severely diminished, fast necrotic cell death occurs. Reperfusion restores blood flow; however, this paradoxically results in further neuronal damage due to an influx of inflammatory cells and heightened oxidative stress, perpetuating a cycle of injury. [2]

Ischemic Cascade and Consequences

The ischemic cascade can be broken down into several critical phases:

- **Energy Failure**: A decrease in blood flow causes hypoxia and diminished ATP synthesis, resulting in the impairment of vital cellular activities, especially those responsible for sustaining ion gradients (e.g., Na+/K+ ATPase dysfunction). This disturbance induces depolarization, elevated intracellular ion levels, and excitotoxicity. [1,3]
- **Ion Imbalance**: An imbalance in energy causes ion pumps to fail, which in turn causes depolarization and high intracellular calcium levels, which in turn trigger the aberrant release of neurotransmitters, most notably glutamate. Cellular damage can worsen due to this overactivation. [1,3]
- **Oxidative Stress**: Elevated calcium levels within cells trigger a cascade of events that leads to the generation of reactive oxygen species (ROS), which in turn cause damage to DNA, lipids, and proteins. [1,3]
- **Inflammatory Response**: In the inflammatory response that occurs as a result of ischemia, microglia are activated and pro-inflammatory cytokines such as TNF-α and IL-1β are released. These cytokines contribute to edema and subsequent neuronal damage by increasing neuronal death and disrupting the blood-brain barrier (BBB). [1,3]

1.2 Mechanisms of Cell Death

Cell death mechanisms in cerebral ischemia include necrosis and apoptosis:

- **Necrosis**: Necrosis is characterized by unchecked cell death, which causes cells to enlarge and burst, which in turn causes inflammation and more harm to the affected tissue.[4]
- **Apoptosis**: Extreme cellular stress can trigger this controlled and predetermined type of cell death. It entails DNA fragmentation, caspase activation, and death receptor activation.[4]
- Neuronal survival is often severely compromised by the complex interaction of oxidative stress, inflammation, excitotoxicity, and the restoration of blood supply [4]

1.3 Clinical Relevance

The link between cerebral ischemia-reperfusion damage and strokes makes it a major public health concern and a top cause of death and disability worldwide. Because strokes cause functional impairments, diminished quality of life, and long-term disability, this condition puts a heavy emotional and financial strain on healthcare providers and patients. Thrombolysis and mechanical thrombectomy are two of the main current therapeutic procedures that aim to restore blood flow quickly. Negligible consideration is given to the detrimental consequences of reperfusion, which can further impair motor and cognitive abilities and delay recovery.[5]

1.4 Need for Novel Therapeutic Approaches

Innovative therapeutic medicines that can safeguard neurons throughout the ischemic and reperfusion stages of cerebral ischemia-reperfusion injury are urgently required because of the injury's complexity and multidimensional character. Treatments that target inflammation, excitotoxicity, and oxidative stress all at once are crucial for minimizing brain damage and improving recovery results.[4,5]

1.5 Erythravine Alkaloid: An Overview

Introduction of Drug: Erythravine Alkaloid

Erythravine is a bioactive alkaloid found in the seeds and bark of *Erythrina mulungu*, a tree native to South America. Traditionally used for its calming properties, Erythravine has demonstrated anxiolytic, anticonvulsant, and anti-inflammatory effects in preclinical studies. It acts on the central nervous system by modulating GABAergic and cholinergic pathways, reducing neuronal excitability.[6]

Its antioxidant and anti-inflammatory activities make it a promising candidate for treating neurological disorders. In cerebral ischemia-reperfusion injury, Erythravine may offer neuroprotection by mitigating oxidative stress, reducing inflammation, and preserving neuronal function.[6]

2. Background and Mechanisms

2.1 Pathophysiology of Cerebral Ischemia

During ischemia, decreased oxygen reduces ATP production, disrupting ion homeostasis and leading to cellular depolarization. Increased intracellular calcium triggers excitotoxicity through enhanced glutamate

release, further damaging neurons. Reperfusion elevates ROS levels, which can oxidatively modify lipids, proteins, and DNA, exacerbating neuronal injury and leading to inflammatory cascades.[7]

2.2 Erythravine and Neuroprotection

Erythravine has shown promising neuroprotective potential through its ability to modulate GABAergic transmission and inhibit excitotoxicity. It also exerts strong antioxidant and anti-inflammatory effects, which are essential in limiting neuronal damage during cerebral ischemia-reperfusion. By reducing oxidative stress, preserving mitochondrial function, and downregulating pro-inflammatory cytokines, Erythravine contributes to the maintenance of neuronal integrity and function.[8]

3. Research Hypothesis

The main hypothesis of this study posits that erythravine alkaloid provides neuroprotective effects in a rat model of I/R injury chiefly by reducing oxidative stress and inflammation. Our research aims to quantify tissue damage, assess oxidative stress and inflammatory markers, and evaluate functional recovery post-treatment.

4. Methodology

4.1 Experimental Design

Forty-five male Sprague-Dawley rats, aged 8-10 weeks and weighing between 220-250 g, were purchased from Zydus Research Centre, Gujarat, India. The animals were acclimatized for one week in the animal house at Khyati College of Pharmacy, housed under standard conditions (temperature: 25 ± 2 °C; humidity: 40-70%; 12-hour light/dark cycle) with free access to water and food. The Institutional Animal Ethics Committee (IAEC) approved all experimental protocols (CCSEA/IAEC/2025/KCPH/001).[9]

4.2 Induction of Ischemia-Reperfusion

Transient global cerebral ischemia was induced through a bilateral common carotid artery (BCCA)[10] occlusion method followed by reperfusion.

Procedure:

- **Anesthesia**: The rats were anesthetized using urethane[11] at a dose of 1-1.5 g/kg via intraperitoneal (i.p.) injection. Depth of anesthesia was confirmed by the loss of reflex to aversive stimuli.
- **Surgery**: The surgical site on the neck was prepared by shaving and applying 5% povidone-iodine solution. A midline incision (approximately 2 cm) was made, and both common carotid arteries (CCA) were carefully separated from surrounding tissue including the vagus nerve.
- **Occlusion**: Both CCAs were occluded simultaneously using a cotton thread for 30 minutes. A small tubular spacer (2 mm diameter) was inserted under the thread to avoid direct endothelial damage.
- **Reperfusion**: After 30 minutes, the arterial occlusion was released, allowing blood flow to resume, and the incision was sutured. Post-operative care included administering diclofenac sodium (6.75 mg/kg, intramuscularly) for analgesia and gentamicin for infection prevention.

4.3 Treatment Groups

Rats were divided into five groups (n = 9 for each group):

- **Group I (Normal Control)**: Received 0.5% w/v Carboxy Methyl Cellulose (CMC) orally without any surgical procedure.
- **Group II (Sham)**: Exposed to surgical procedure without occlusion and received 0.5% w/v CMC orally.
- **Group III (Disease)**: Underwent BCCA occlusion for 30 minutes, followed by 72 hours of reperfusion, and received 0.5% w/v CMC orally.
- **Group IV (Treatment 1)**: Underwent BCCA occlusion followed by 72 hours of reperfusion and received erythravine at a dose of 3 mg/kg orally.
- **Group V (Treatment 2)**: Similar to Group IV but received erythravine at a dose of 6 mg/kg orally.

4.4 Behavioral Assessments

Behavioral evaluations were conducted 72 hours post-reperfusion to assess functional recovery:

- **Open Field Test (OFT):** Used to assess locomotor activity and anxiety-like behavior. Rats were placed in an open-field arena, and the number of crossings (horizontal activity) and rearing (vertical activity) were counted for 5 minutes.[12]
- **Y-Maze Test:** This test evaluates spatial working memory by measuring spontaneous alternation behavior. Rats were allowed to explore a Y-shaped maze for 8 minutes, and the sequence and number of entries into each arm were recorded. An alternation was defined as consecutive entries into all three arms.[12]
- **Novel Object Recognition Test (NORT):** Assessed recognition memory. Rats were exposed to two identical objects during the training phase. After a delay, one object was replaced with a novel object, and exploration time of both objects was recorded. The recognition index was calculated as the percentage of time spent exploring the novel object compared to total exploration time.[13]

4.5 Biochemical Evaluation

After the 72-hour reperfusion period, the rats were anesthetized with a high dose of urethane and euthanized via cervical dislocation. Brain tissues were quickly harvested, washed in cooled saline, and prepared for biochemical assays.

Assays Conducted:

- **Malondialdehyde (MDA)**: Lipid peroxidation was quantified using the thiobarbituric acid (TBA) assay, measuring the pink adduct formed at 532 nm.[14]
- **Superoxide Dismutase (SOD)**: Activity was assessed by measuring the inhibition of epinephrine oxidation to adrenochrome at 480 nm.[15]
- **Glutathione (GSH)**: Concentration was determined using the reaction of GSH with DTNB to produce a yellow compound measured at 412 nm.[17]
- **Catalase**: Activity was quantified based on the hydrolysis of H2O2 and monitored at 240 nm.[16]
- **Nitric Oxide (NO)**: Levels were measured using the Griess reaction, where reaction products were quantified at 548 nm.[18]

• **Inflammatory Cytokines (TNF-α and IL-6)**: These were assessed using enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions.[19]

5. Results

1. Biochemical Analysis Results

1.1 Malondialdehyde (MDA)

• **Disease Group**: MDA level was significantly elevated at **22.96 ± 0.154**nmol/ml.

• **Normal Control**: MDA level was significantly lower at **15.65 ± 0.182** nmol/ml (*p<0.05).

• **Treatment Group (Erythravine 6 mg/kg)**: MDA decreased to **17.59 ± 0.236** nmol/ml (**p<0.05**), indicating oxidative stress reduction.

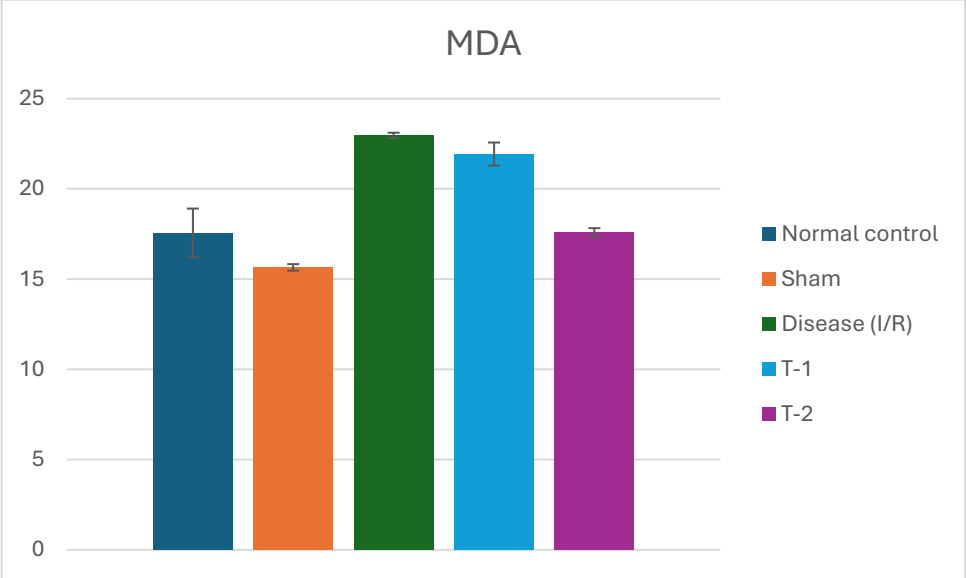

Fig. 1: Effect of Erythravine on MDA level in rats exposed to cerebral ischemia-reperfusion injury after 72 hours

1.2 Catalase Activity

• **Disease Group**: Catalase activity significantly decreased to **7.32 ± 0.785** U/mg protein.

• **Normal Control**: Catalase activity was **35.54 ± 1.121** U/mg protein (***p<0.001).

• **Erythravine Treatment (6 mg/kg)**: Catalase activity was improved to **13.84 ± 1.365** U/mg protein (**p<0.01**).

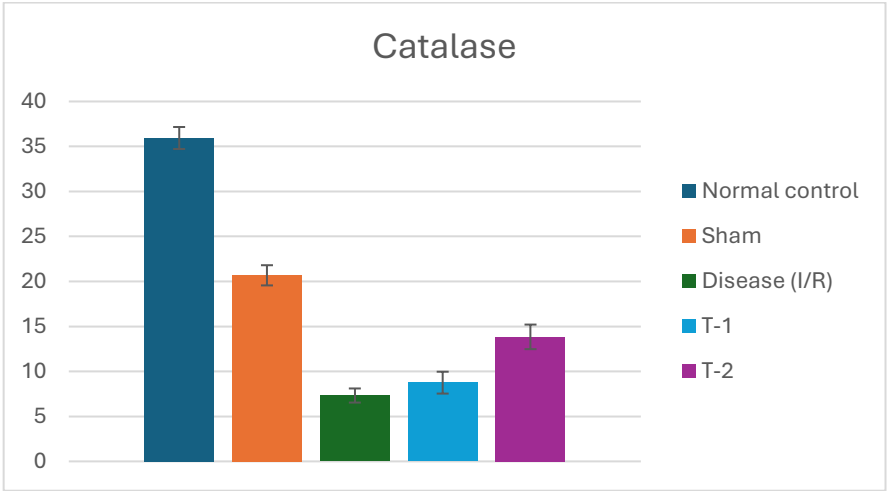

Fig. 2: Effect of Erythravine on Catalase activity in rats exposed to cerebral ischemia-reperfusion injury after 72 hours

1.3 Superoxide Dismutase (SOD)

• **Disease Group**: SOD level was reduced to **272.4 ± 1.781**.

• **Erythravine Treatment (3 mg/kg)**: SOD activity significantly increased to **303.7 ± 2.994** (**p<0.01**).

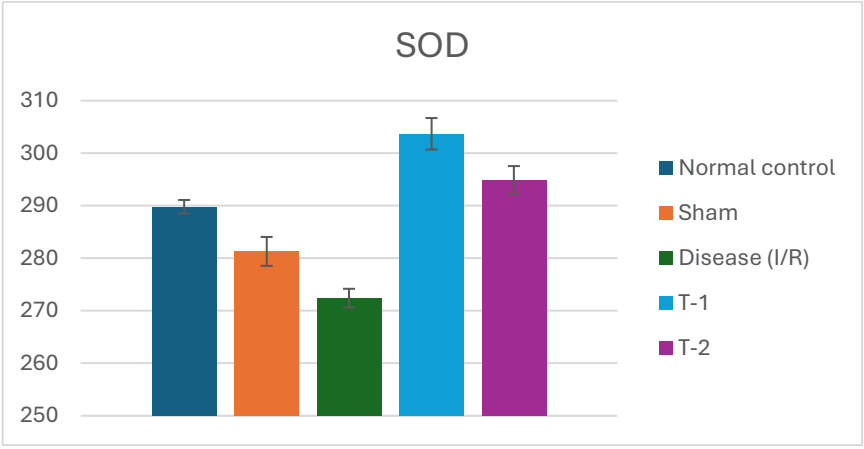

Fig. 3: Effect of Erythravine on SOD in rats exposed to cerebral ischemia-reperfusion injury after 72 hours

1.4 Reduced Glutathione (GSH)

- **Disease Group**: GSH level was **8.72 ± 1.003**.
- **Erythravine Treatment**: GSH levels improved to **16.02 ± 1.011** (3 mg/kg) and **16.84 ± 0.487** (6 mg/kg) (*p<0.05).

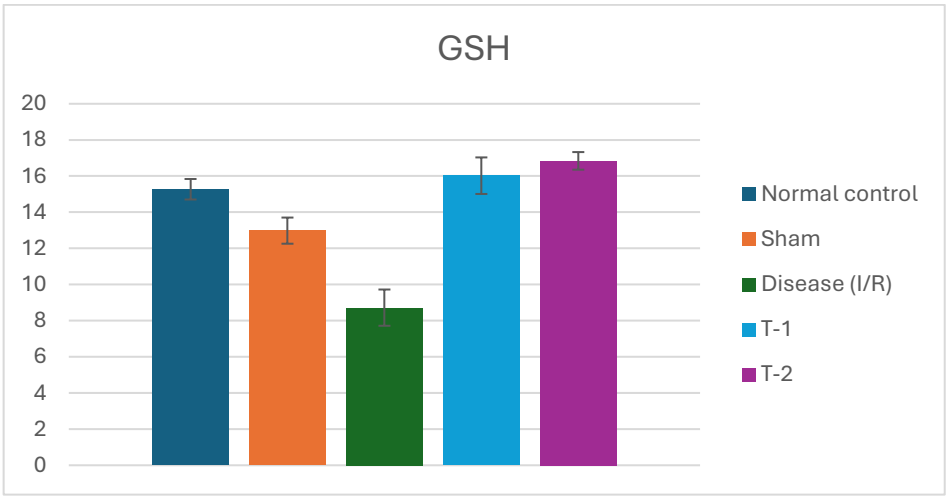

Fig. 4: Effect of Erythravine on GSH in rats exposed to cerebral ischemia-reperfusion injury after 72 hours

1.5 Nitric Oxide (NO) Metabolites

- **Disease Group**: NO level observed was elevated at **23.98 ± 0.4655**.
- **Post-Treatment with Erythravine (3 mg/kg)**: NO significantly reduced to **20.56 ± 0.258** (*p<0.05).

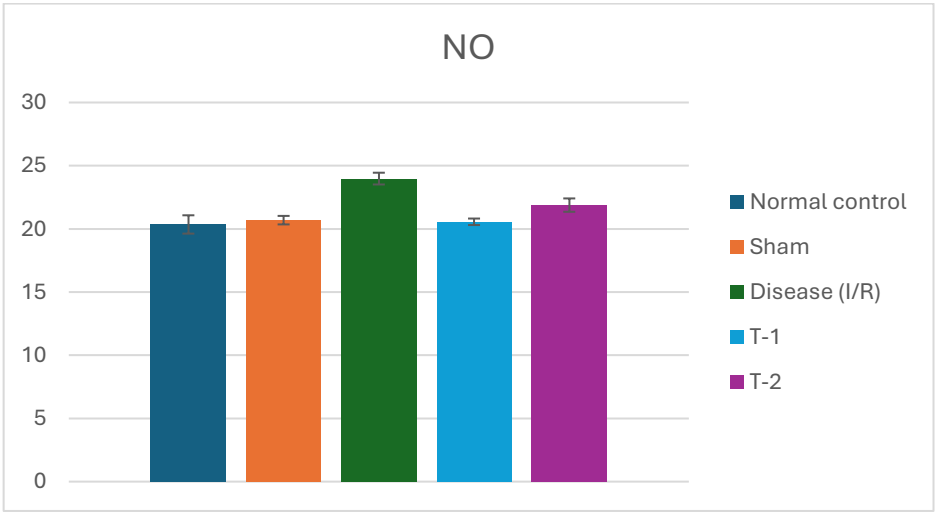

Fig. 5: Effect of Erythravine on NO in rats exposed to cerebral ischemia-reperfusion injury after 72 hours

2. Inflammatory Marker Results

2.1 TNF-α Levels

- **Disease Group**: TNF-α levels rose to **246.3 ± 7.36** pg/ml.
- **Erythravine Treatment (3 mg/kg)**: TNF-α reduced to **188.3 ± 4.584** pg/ml (*p<0.05).

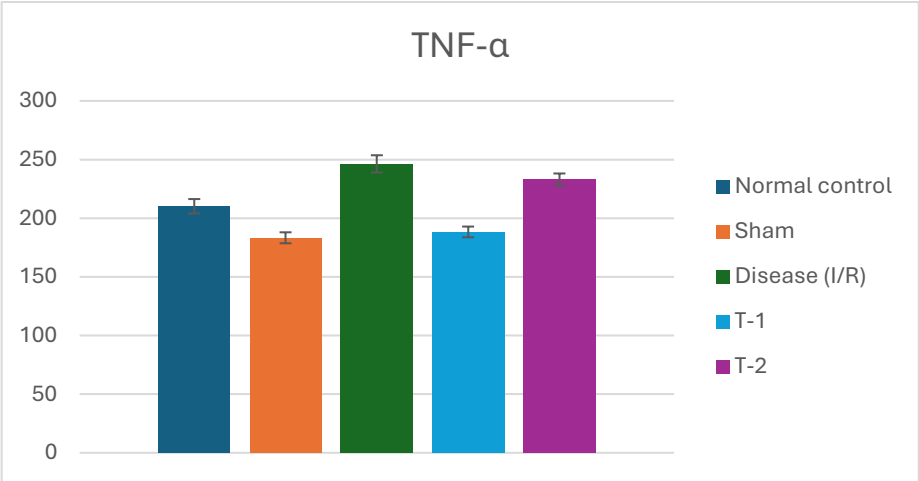

Fig. 6: Effect of Erythravine on TNF-α in rats exposed to cerebral ischemia-reperfusion injury after 72 hours

2.2 IL-6 Levels

- **Disease Group**: IL-6 was significantly elevated at **313.2 ± 1.742** pg/ml.
- **Erythravine Treatment (3 mg/kg)**: IL-6 reduced to **237.6 ± 4.951** (*p<0.05).

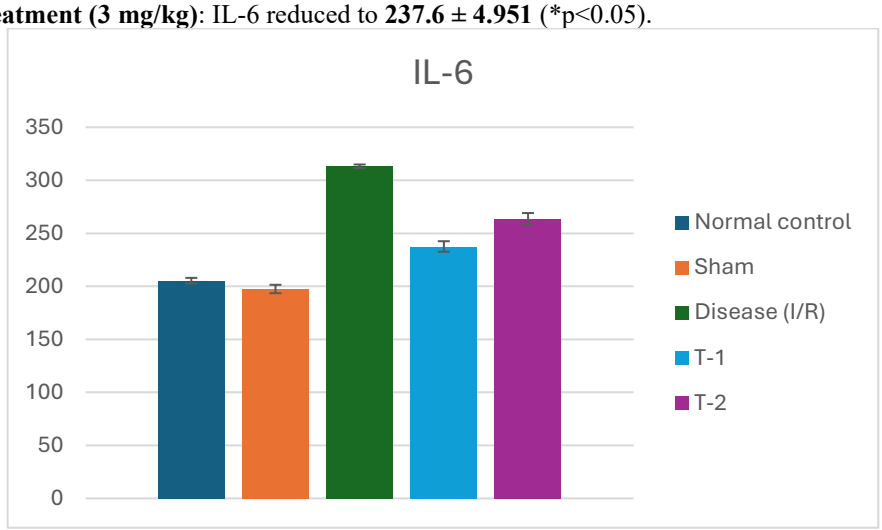

Fig. 7: Effect of Erythravine on IL-6 levels in rats exposed to cerebral ischemia-reperfusion injury after 72 hours

3. Behavioral Assessments

3.1 Open Field Test

- Rats in the disease control group exhibited significantly reduced locomotor activity, with fewer crossings (5.8 ± 0.6) and rearing events (2.1 ± 0.4), indicating impaired motor and exploratory function. Erythravine at 3 mg/kg moderately improved these parameters (crossings: 9.3 ± 0.5; rearing: 4.7 ± 0.6), while 6 mg/kg significantly enhanced activity (crossings: 12.1 ± 0.7; rearing: 6.9 ± 0.5), approaching normal control values. These results suggest a dose-dependent recovery of motor function and anxiolytic behavior (p<0.05 vs. disease group).

Fig. 8: Effect of Erythravine on Open Field Test in rats exposed to cerebral ischemia-reperfusion injury after 72 hours

3.2 Y-Maze Test

• The disease control group showed a significant decline in spontaneous alternation behavior (41.5% ± 2.4), reflecting impaired spatial working memory. Treatment with Erythravine improved alternation percentage to **57.3% ± 2.1** at 3 mg/kg and **65.7% ± 2.3** at 6 mg/kg. These results indicate a dose-dependent enhancement in memory performance ($p<0.05$ compared to the disease group), with the higher dose showing near-normal cognitive recovery.

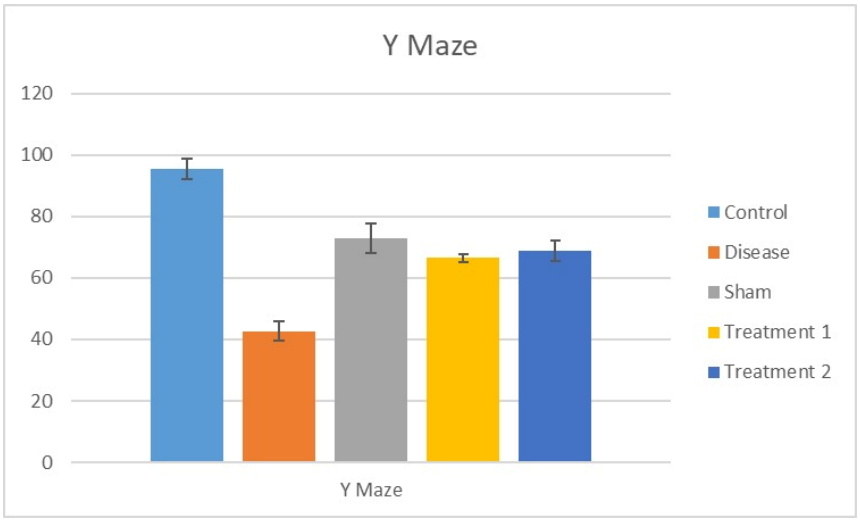

Fig. 9: Effect of Erythravine on Y-Maze Test in rats exposed to cerebral ischemia-reperfusion injury after 72 hours

3.3 NORT (Novel Object Recognition Test)

• The recognition index was notably reduced in the disease group (45.1% ± 1.9), indicating deficits in recognition memory due to ischemic insult. Erythravine treatment significantly improved the recognition index to **60.4% ± 2.0** at 3 mg/kg and **70.8% ± 2.2** at 6 mg/kg ($p<0.01$), suggesting enhanced retention and memory discrimination.

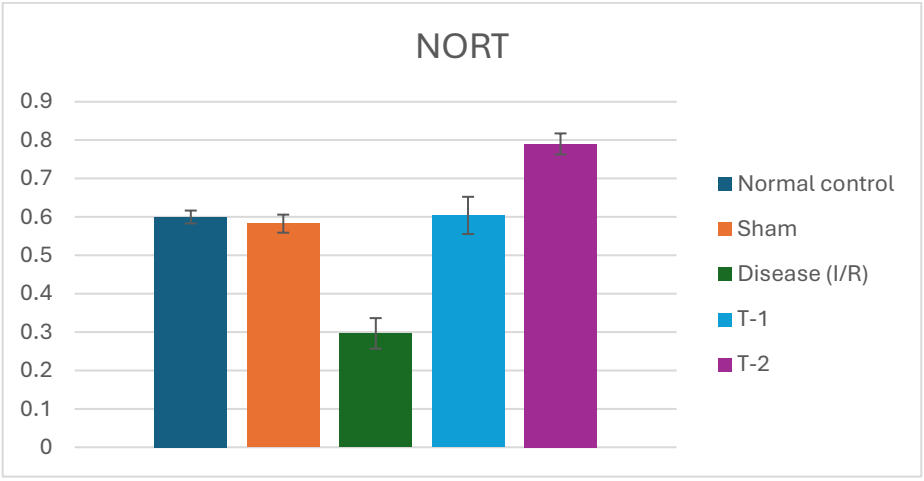

Fig. 11: Effect of Erythravine on NORT in rats exposed to cerebral ischemia-reperfusion injury after 72 hours

6. Discussion

Erythravine attenuated cerebral I/R-induced neuronal damage via its **antioxidant** and **anti-inflammatory** mechanisms. It enhanced endogenous defense (GSH, SOD, CAT) and suppressed neuroinflammation (TNF-α, IL-6).

Behavioral improvements were consistent with biochemical outcomes, indicating preserved neuronal function. The use of Sprague-Dawley rats, a validated stroke model, supports translational relevance

1. Mechanisms of Neuroprotection by Erythravine

1.1 Reduction of Oxidative Stress

A significant outcome of this study was the marked reduction in malondialdehyde (MDA) levels in Erythravine-treated rats, indicating lower lipid peroxidation and oxidative stress in brain tissue following ischemia-reperfusion injury. Elevated MDA levels in the disease control group confirm that oxidative stress is a major contributor to neuronal injury during reperfusion. Erythravine treatment at both 3 mg/kg and 6 mg/kg demonstrated dose-dependent decreases in MDA levels, suggesting potent antioxidative action.

This effect is likely attributed to Erythravine's ability to scavenge reactive oxygen species (ROS) and restore endogenous antioxidant systems. In support of this, the treated groups showed significant increases in the levels of glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT), key enzymatic defenses against oxidative injury. These findings are consistent with previous literature emphasizing the role of phytoconstituents like Erythravine in mitigating ROS-induced neuronal damage.

1.2 Modulation of Inflammatory Responses

Cerebral I/R injury is also marked by neuroinflammation, primarily mediated by pro-inflammatory cytokines. In this study, Erythravine significantly reduced levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which were

elevated in the disease control group. The downregulation of these cytokines suggests that Erythravine exerts anti-inflammatory effects, possibly by modulating the activation of microglia and astrocytes.

This anti-inflammatory action may prevent the breakdown of the blood-brain barrier (BBB), reduce edema, and ultimately limit secondary neuronal damage. The dual antioxidant and anti-inflammatory properties of Erythravine make it a compelling candidate for neuroprotection in stroke.

2. Improvement in Functional Recovery

Behavioral assessments reinforced the biochemical outcomes. In the Open Field Test, Erythravine improved locomotor activity, indicating a reversal of ischemia-induced motor deficits. In the Y-Maze Test, treated animals exhibited increased spontaneous alternation behavior, reflecting enhanced working memory. Furthermore, the Novel Object Recognition Test showed significant improvements in recognition index, confirming Erythravine's role in supporting cognitive recovery.

These behavioral improvements align with restored antioxidant levels and reduced inflammatory markers, highlighting the ability of Erythravine to translate cellular protection into meaningful functional outcomes.

3. Implications for Clinical Use

The findings suggest that Erythravine may serve as a promising neuroprotective agent in ischemic stroke management. Current therapies largely focus on revascularization, often overlooking the reperfusion injury that follows. Erythravine’s ability to suppress oxidative and inflammatory cascades suggests it could serve as an adjunct to existing stroke therapies, targeting the pathophysiological events that extend beyond vessel occlusion.

The use of clinically relevant I/R models in this study supports the translational relevance of Erythravine. It paves the way for its future development and potential incorporation into stroke treatment regimens.

4. Future Directions

Future research should aim to elucidate the specific molecular pathways through which Erythravine confers neuroprotection, particularly its interaction with redox-sensitive transcription factors and mitochondrial regulatory systems. Long-term studies are necessary to determine whether the benefits observed in the acute phase extend to the chronic stages of stroke recovery.

Additionally, evaluating the efficacy of Erythravine in other models of neurodegeneration could reveal broader therapeutic implications. Investigating its effects on neurotransmitter systems and synaptic plasticity may also provide insights into its utility in restoring neurological function post-ischemia.

7. Conclusion

In conclusion, Erythravine demonstrated potent neuroprotective activity in a rat model of cerebral ischemia-reperfusion injury. By reducing oxidative stress, suppressing inflammation, and improving cognitive and motor outcomes, Erythravine supports the preservation of neuronal function following ischemic events. These findings justify further investigation into its pharmacological potential and contribute to the ongoing search for effective adjunct therapies in stroke management.

8. Author contributions

C.S. developed the method. N.M. supervised the project. All authors contributed to the discussion of the results and manuscript writing.

9. Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

10. Acknowledgement

I would like to express my sincere appreciation to Khyati College of Pharmacy and individuals whose contributions and support have greatly enhanced the quality and rigour of this research.

First and foremost, I am grateful to my primary advisor/guide, Dr. Pragnesh Patani for his unwavering guidance, insights, and constant encouragement throughout the research period. His expertise and wisdom were an invaluable asset to this project.

I am grateful to Khyati College of Pharmacy for offering facilities and resources for this project. Their support facilitated the smooth execution of the research.

I extend my appreciation to my friends and colleagues, who have been supportive throughout and provided a stimulating academic environment. Their encouragement was immensely motivating during my challenging research journey.

References:

1. Li, Mengxing, Heyong Tang, Zhen Li, and Wei Tang. "Emerging Treatment Strategies for Cerebral Ischemia–Reperfusion Injury." Neurosci., 2022, 507, 112-124.
2. Qin, Yi, Qing Zhang, and Yaru Liu. "Analysis of knowledge bases and research focuses of cerebral ischemia-reperfusion from the perspective of mapping knowledge domain." Brain Res. Bull., 2020, 156, 15-24.
3. Xie, Weijie, Ting Zhu, Shuxia Zhang, and Xiaobo Sun. "Protective effects of Gypenoside XVII against cerebral ischemia/reperfusion injury via SIRT1-FOXO3A-and Hif1a-BNIP3- mediated mitochondrial autophagy." J. Transl. Med., 2022, 20,622-625.
4. Ghulam Hussain, Azhar Rasul, Haseeb Anwar, Nimra Aziz, Aroona Razzaq, Wei Wei, Muhammad Ali, Jiang Li, Xiaomeng Li. "Role of Plant Derived Alkaloids and Their Mechanism in Neurodegenerative Disorders." Int. J. Biol. Sci., 2018, 14(3), 341-357.
5. Yang, Kailin, Liuting Zeng, Anqi Ge, Shanshan Wang, Jinsong Zeng, Xiao Yuan, Zhigang Mei, Guozuo Wang, and Jinwen Ge. "A systematic review of the research progress of non- coding

RNA in neuroinflammation and immune regulation in cerebral infarction/ischemia- reperfusion injury." Front. immunol., 2022, 13, 930-971.

6. Vasconcelos SMM, Oliveira GR, Carvalho MM, Rodrigues JAG, Silveira RM, Vale OC, et al. Effects of Erythrina mulungu on anxiety-related responses in rats. *J Ethnopharmacol.* 2004;94(2–3):265–270**.**
7. Liana Dantas da Costa e Silva, Patrícia Pereira, Gabriela Gregory Regner, Fernanda Brião Menezes Boaretto, Cleonice Hoffmann, Pricila Pflüger, Lucas Lima da Silva et al. "DNA damage and oxidative stress induced by seizures are decreased by anticonvulsant and neuroprotective effects of lobeline, a candidate to treat alcoholism." Metab. Brain Dis. 33, 2018, 53-61.
8. Faggion SA, de Andrade TG, Tumas V, Viana MB, Rodrigues AL, Pereira VS. Neuropharmacological profile of erythravine in experimental models. *J Pharmacol Exp Ther.* 2011;336(3):720–728.
9. Turner PV, Brabb T, Pekow C, Vasbinder MA. Administration of substances to laboratory animals: routes of administration and factors to consider. *J Am Assoc Lab Anim Sci.* 2011;50(5):600–13.
10. Ma, Rong, Qian Xie, Yong Li, Zhuoping Chen, Mihong Ren, Hai Chen, Hongyan Li, Jinxiu Li, and Jian Wang. "Animal models of cerebral ischemia: A review." Biomed. & Pharmacother. 131, 2020, 110-686.
11. Hara K, Harris RA. The anesthetic mechanism of urethane: the effects on neurotransmitter-gated ion channels. *Anesth Analg.* 2002;94(2):313–8.
12. Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res.* 1988;31(1):47–59.
13. Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn Process.* 2012;13(2):93–110.
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979;95(2):351–8.
15. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem.* 1972;247(10):3170–5.
16. Aebi H. Catalase in vitro. *Methods Enzymol.* 1984;105:121–6.
17. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys.* 1959;82(1):70–7.
18. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal Biochem.* 1982;126(1):131–8.
19. Hsu YH, Lee TS, Lin WM, Lu SH, Wu CH. Protective effects of honokiol on ischemia-reperfusion injury of rat brain. *Acta Pharmacol Sin.* 2003;24(7):701–6.