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Poly-Herbal Neuroprotection: Pharmacological mitigation of Ischemic Damage and BBB disruption in a rat model of tMCAO

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ABSTRACT

Background
Cerebral ischemia due transient middle cerebral artery occlusion (tMCAO) induced substantial neuronal injury, compromises the integrity of the Blood –Brain Barrier (BBB), and result in marked neurological impairments. The present study evaluates the neuroprotective potential of a poly-herbal mixture in mitigating ischemic brain injury and promoting functional recovery.

Methods
A well-established tMCAO model was employed in rats to induce focal cerebral ischemia. Experimental groups included healthy controls, untreated tMCAO animals, and tMCAO animals treated with the poly-herbal mixture. Infarct volume was quantified, BBB integrity assessed via Evans blue dye extravasation, and neurological function evaluated through multiple behavioural parameters including grid hold test, limb symmetry, forepaw outstretching, body proprioception, and vibrissae touch response.

Results
The tMCAO group exhibited a significant increase in infarct volume ($27.79 \pm 3.37\%$) associated to healthy controls ($0.00 \pm 0.00\%$, $p < 0.001$). Treatment with the poly-herbal mixture significantly reduced infarct volume ($6.15 \pm 3.70\%$, $p < 0.01$), with no statistically significant difference from healthy controls. BBB disruption in the tMCAO group was confirmed by elevated Evans blue dye concentration (7.93 ± 0.67), while treated animals showed significantly reduced levels (3.55 ± 0.37, $p < 0.0001$), indicating partial restoration of BBB integrity.
Neurological scores significantly improved with treatment across all behavioural parameters. Grid hold test scores increased from 0.61 ± 0.25 (tMCAO) to 2.11 ± 0.17 (treated group). Improvements were similarly observed in limb symmetry (2.17 ± 0.18), forepaw outstretching (2.33 ± 0.44), body proprioception (2.39 ± 0.23), and response to vibrissae touch (2.66 ± 0.23), all with $p < 0.0001$.

Conclusion
The poly-herbal mixture demonstrated robust neuroprotective effects in the tMCAO model by significantly reducing infarct volume, partially restoring BBB integrity, and improving motor and sensory functions. These findings support the therapeutic potential of poly-herbal strategies for ischemic stroke and warrant further investigation.

Keywords: Cerebral Ischemia, Bbb, Neurology, Inflammation, Infraction.

1. INTRODUCTION

Cerebral ischemia characterized by a substantial reduction in cerebral flow (CBF) to brain tissue, continuous to be a leading contributor to global contributor to global mortality and long term neurological disability. Following ischemic

onset, a complex cascade of pathophysiological mechanism is initiated, encompassing excitotoxic neurotransmitters release, oxidative stress, and disruption of the blood brain barrier, robust inflammatory responses and apoptotic as well as necrotic neuronal death. Together these processes several impair neural function and contribution to progressive brain injury (Lo et al., 2003). Due to its high metabolic demands, the brain is especially vulnerable to any disruption in blood supply. A reduction in CBF below 25ml/100 g of brain tissue per minute significantly lower than the physiological norm of approximately 55ml/100 g /min rapidly precipitates a critical energy crisis characterized by ATP Depletion. This energy failure impairs ion pump function, resulting in the loss of ionic gradients, membrane depolarization, and excessive intracellular calcium accumulation. These disruption initiate excitotoxic cascade, mitochondrial dysfunction, oxidative stress, and ultimately lead to neuronal injury and cell death (Durukan & Tatlisumak, 2007)(Traystman, 2003a). When blood flow drops further, particularly under 15 mL/100 g/min, critical cellular functions such as ion exchange, membrane potential maintenance, and synaptic activity cease, leading to irreversible damage (Traystman, 2003a).

The pathophysiology of cerebral ischemia is multifarious and multifactorial, involving a series of unified processes including energy depletion, excitotoxicity, oxidative stress, and inflammation (Braeuninger & Kleinschnitz, 2009). Injured neurons release molecular distress signals that activate surrounding glial cells—particularly astrocytes and microglia—provoking a neuroinflammatory response. This response subsidises to BBB interruption, cerebral oedema, and the infiltration of peripheral immune cells, all of which aggravate neuronal injury (Harukuni et al., n.d.)(Iadecola & Anrather, 2011). Reperfusion, while essential for restoring blood flow, paradoxically exacerbates injury through the generation of reactive oxygen species (ROS) exacerbates cellular injury to further apoptosis and cellular degeneration (Fan & Lei, n.d.). Central mediators of these mechanisms include Toll-like receptor 4 (TLR4) signalling and regulatory non-coding RNAs, both of which have gained increasing attention as therapeutic targets (Yang et al., 2023).

Current therapeutic interventions remain limited in scope and efficacy. Recombinant tissue plasminogen activator (rtPA) remains the only FDA approved thrombolytic therapy for acute ischemic stroke: however its clinical use is restricted to a narrow therapeutic window 3 to 4.5 hours after symptom onset , and only a limited percentage of patients are able to receive treatment within this critical timeframe (Emberson et al., 2014) (Powers et al., 2018). Other available treatments—including antiplatelet and anticoagulant medications, as well as neuroprotective agents like edaravone—offer limited benefits and fail to comprehensively address the multifaceted nature of ischemic brain injury (Fan & Lei, n.d.). This highlights the urgent need for novel therapies that not only mitigate neuronal damage but also extend the window of intervention and promote recovery through multi-modal action.

Natural compounds and poly-herbal mixtures have emerged as promising therapeutic alternatives due to their ability to modulate diverse pathological pathways. In the present study, we explore the neuroprotective potential of a poly-herbal combination comprising curcumin (75 mg/kg) from *Curcuma longa* (turmeric), aloe emodin (3.25 mg/kg) from *Aloe vera*, and trigonelline (25 mg/kg) from *Trigonella foenum-graecum*. This mixture was assessed in a rat model of tMCAO, a well-established experimental paradigm for mimicking focal cerebral ischemia(Chu et al., 2008)(Feigin et al., 2024). These phytochemicals have individually demonstrated antioxidant, anti-inflammatory, and neuroprotective properties, making their combined use a compelling strategy for ischemic stroke management(Subedi & Gaire, 2021).

Our experimental data reveal that the poly-herbal mixture significantly reduced cerebral infarct volume and markedly limited the permeability of the BBB, as demonstrated via decreased Evans blue dye extravasation. Treated animals also exhibited significantly improved neurological outcomes, with higher scores on the grid-walking test assessing key motor and sensory parameters, including symmetry of movement, forepaw extension, body proprioception, and vibrissae touch response. At the molecular level, the mixture enhanced antioxidant defence mechanism by up-regulating superoxide dismutase (SOD) and nuclear respiratory factor-1, while concurrently reducing level of malondialdehyde and neuronal nitric oxide synthase. Further, the treatment attenuated the expression of pro-inflammatory cytokines and endothelial adhesion molecules, mitigated apoptotic process, and facilitated neuroregenration through the promotion of axonal and dendritic repair (Fan & Lei, n.d.). Collectively, these findings suggest that the poly-herbal combination may provide broad-spectrum neuroprotection and offer a novel multi-targeted approach for the treatment and recovery of cerebral ischemia.

2. MATERIAL AND METHODS

2.1. Animal Grouping and Experimental Design

In this study adult Sprague Dawley (SD) rats weighing between 250- 310 grams were utilized. Randomly allocated into three experimental groups (n=6per group); (i) Healthy control, (ii) tMCAO, (iii) tMCAO treated with Poly-herbal mixture. Both male and female rats were included to minimize gender bias in the experimental outcomes.

Anaesthesia was induced intrperiotonally (*i.p.*) using thiopental sodium at a dosage of 5 mg/kg, following previously established protocols(Kozler & Pokorný, 2003) (Sookplung et al., 2023). The Poly-Herbal mixture, prepared in a sterile saline solution, was administered *i.p*. at two critical time points: During the induction tMCAO and after 2 hour to maintain occlusion, after which it was gently withdrawn to permit reperfusion (Maheshwari et al., n.d.).

Rats be situated held in groups of three within standard polypropylene cages under a controlled environment. The animal facility maintained a 10% air exhaust through the air conditioning system, with temperature and humidity regulated at 25 ± 3 °C and 60 ± 5%, respectively. A 12-hour light/dark cycle was upheld throughout the experimental period. Standard laboratory rodent diet and water were provided *ad libitum*.

All experimental procedures were conducted in strict accordance with institutional ethical guidelines and received approval from the Institutional Animal Ethics Committee (IAEC).

2.2. Transient Middle Cerebral Artery Occlusion (tMCAO)(Chu et al., 2008) (Maheshwari et al., n.d.): Transient focal cerebral ischemia was induced using a modified intraluminal filament technique, adapted from the method described by (Traystman, 2003b). A midline incision was made along the ventral surface of the neck to expose the left common carotid artery (CCA). The CCA was carefully followed to its bifurcation into the external carotid artery (ECA) and internal carotid artery (ICA). To facilitate access to the junction of the ECA and ICA, the occipital artery was ligated and severed.

The ECA was double-ligated using sterile cotton sutures, and the intervening segment was excised to allow for filament insertion. A sterile 3-0 nylon monofilament (0.35 mm in diameter), flame-rounded Filament, with its tip coated in poly-L-lysin to promote adhesion, was introduced through the ECA and advanced 19-21 mm into the ICA. Advancement continued until mild resistance was encountered, signifying successful occlusion of middle cerebral artery (MCA). The filament was then secured, and the surgical site was sutured closed. It remained in place for two hours to ensure stable occlusion, after which it was gently withdrawn to initiate reperfusion.

Postoperative Care and Treatment Protocol

Immediately following the surgical procedure, each animal received 1 ml of sterile saline subcutaneously (administered as 0.5 ml on each flank) to prevent dehydration and maintain circulatory volume (Feigin et al., 2024)(O'Neill & Clemens, 2001). Rats in the tMCAO control group were given a saline vehicle, whereas those in the treatment group were administered the poly- herbal mixture following the regimen outline earlier.

2.3. Assessment of Infarct Volume (Maheshwari et al., n.d.) (Lin et al., 1993)

24 hours following tMCAO, animals were euthanized using an intra-peritoneal injection thiopental sodium at a dosage of 5 mg/kg. Following this, intra-cardiac perfusion was performed with 100ml of 1% heparinized normal saline to clear the vasculature. The brains were quickly extracted, rinsed in ice-cold saline, and briefly placed in a deep freezer at approximately -18^{0}C for 10 minutes to aid in tissue slicing. For morphometric analysis, brains were sectioned coronally at a thickness of 2mm using a custom-made blade cutter. Seven serial section from each brain were collected and incubated in a 2% solution of 2, 3, 5-triphenyltetrazolium chloride (TTC) to visualize infarcted areas. Viable brain tissue was stained red, while infracted regions remained pale or white. The section were then fixed overnight in 10% phosphate-buffered formalin. (Bederson et al., 1986) (Lin et al., 1993).

Images of the stained brain slices be situated captured using a mobile camera under a validated zoom setting from a predetermined height (fixed 1foot) to ensure consistency across samples. Infarct area measurements were performed using ImageJ software, and volumetric analysis was conducted by integrating the infarct and total hemisphere areas across all seven sections.

To account for oedema-induced distortion of in the ipsilateral hemisphere, the infract volume was calculated indirectly using the following formula:

Infarct Volume = Total volume of the contralateral hemisphere − Volume of the non-infarcted area in the ipsilateral hemisphere (Lin et al., 1993) (Swanson et al., 1990) (Maheshwari et al., n.d.).

The following formula the infract volume is expressed as a percentage of the contralateral hemisphere volume using the following formula:

$$\textbf{\% infarction} = \frac{\text{Total indirect volume of infarction in the ipsilateral hemisphere}}{\text{Total volume of the respective contralateral hemisphere}} \times 100$$

2.3.1. Histological assessment

TTC Staining was performed by – seven 2 mm slice. These section were incubated in a 2% TTC solution in saline for 30 minutes at 37^{0}c, followed by fixation in a 10% buffered formalin solution. TTC stains viable brain tissue res, while infracted tissue remain unstained and appears white. (Traystman, 2003c) (Schäbitz et al., 1997).TTC stained brain section were then photographed for further analysis.

2.4. Evans Blue Dye Extravasation and Quantification

(Kozler & Pokorný, 2003) (Michalicova et al., 2017) (Manaenko et al., 2011)**:** To evaluate BBB integrity, a 2% solution of Evans blue dye in normal saline (2 mL/Kg) was administered via *i.p*. before the experimental impact. The dye was allowed to circulate for 24 hours to ensure adequate binding to plasma protein. After the circulation period, animal were anesthetized with thiopental sodium, and transcardial perfusion was performed using ice-cold heparinized phosphate-buffered saline to clear the residual dye from the circulation.

The brain was then removed and separated into right and left hemispheres. Tissue sample were placed in stoppered glass tubes containing 2mL of 0.5 N KOH and incubated overnight at 37^{0}C to facilitate dye extraction.

After incubation, 2.5mL of a mixed solution consisting of 4 N H_3PO_4 and acetone (3:15) was added to each tube. The sample were vortexed for one minute and centrifuged at 3000 rpm for 15 minutes at 25^0C to separate the dye from the tissue.
The absorbance of the blue colored layer was measured spectrophotometrically at 620 nm, and Evans blue concentration was determined from a standard curve to quantify the extent of dye extravasation, serving as an indicator of BBB disruption.

2.5. ***Behaviour assessment***(Garcia et al., 1995a) (Schäbitz et al., 1997)
Ischemic stroke often results in profound brain injury and leads to various functional impairments, particularly motor deficits (Carmichael, 2005). The severity of these impairments, which ultimately shapes the long-term neurological outcome, can fluctuate rapidly within a narrow time window of several hours following the onset of ischemia (Sujata Wankhede, 2025) (Biller et al., 1990) (Minnerup et al., 2012). Therefore, early therapeutic intervention represent the most rational and effective approach to minimize neuronal damage and enhance functional recovery.
To accurately assess the impact of early interventions in preclinical stroke models, it is essential to move beyond histological evaluations and incorporate sensitive, time-dependent behavioural assessments (Balkaya et al., 2013). However, the current repertoire of behavioural testing paradigms remains relatively limited, particularly in the acute phase following stroke. One major challenge is the confounding influence of anaesthesia and surgical procedures, which can significantly affect early neurobehavioral performance and obscure true functional deficits(Veizovic et al., 2001). Therefore, developing reliable and reproducible behavioural assessment tools that capture early functional changes remains a critical need in stroke research.

2.5.1. ***Grid hold test***(Garcia et al., 1995b)***:***
To evaluate forelimb motor function, each rat was gently positioned on a wire grid and then lifted by the tail. The animal's ability to grasp the grid was assessed, with particular attention to the strength and coordination of the forelimbs. The scoring was based on the following criteria: **Score 3**: The rat grasped the grid firmly with both forelimbs. **Score 2**: The rat initially held the grid with both forelimbs but released the right forelimb, maintaining grip only with the left. **Score 1**: The rat grasped the grid solely with the left forelimb. **Score 0**: The rat failed to grasp the grid with the left forelimb. This assessment provides a simple yet effective measure of unilateral motor impairment commonly observed following ischemic injury.

2.5.2. ***Symmetry in the Movement of Four Limbs***
To evaluate neurological function and limb coordination, each rat was gently lifted by the tail, allowing it to hang freely in the air. Symmetry of limb movement was closely observed, particularly in relation to potential unilateral deficits. Scoring was performed according to the subsequent criteria: **Score 3**: All four limbs are extended symmetrically. **Score 2**: limbs on the left side extend less fully or more slowly compared to those on the right. **Score 1**: Minimal movement was observed in the left limbs. **Score 0**: No movement was observed in the left forelimb. This test serves as a reliable indicator of post-stroke motor asymmetry and functional impairment.

2.5.3. Forepaw Outstretching
To assess forelimb coordination and motor strength, each rat was gently held by the tail and positioned so that its forelimbs made contact with the edge of a flat surface while the hind limbs remained suspended. As the animal attempted to walk forward using its forelimbs, symmetry in limb extension and walking pattern was observed. Scoring was based on the following criteria: **Score 3**: Both forelimbs were fully outstretched, and the rat walked symmetrically on its forepaws. **Score 2**: The left forelimb extended less than the right, with noticeable impairment in forepaw locomotion. **Score 1**: Minimal movement was observed in the left forelimb. **Score 0**: No movement was observed in the left forelimb. This test provides valuable insight into unilateral motor deficits following cerebral ischemia.

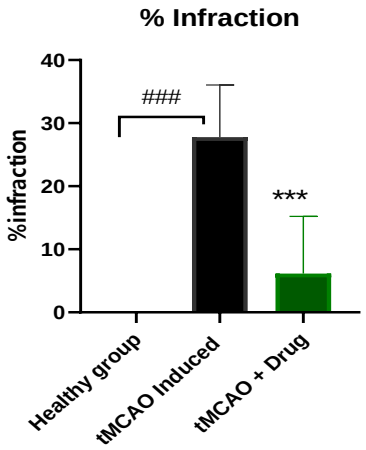
2.5.4. ***Body Proprioception***
To evaluate tactile sensory function and lateralized response to external stimuli, each rat was gently touched on both sides of the body using a blunt stick. The animal's reaction to the stimulus was observed and scored as follows: **Score 3**: The rat responded promptly by rotating its head toward the stimulus on both sides, indicating symmetrical sensory perception. **Score 2**: The rat showed a noticeably delayed or reduced response to stimulation on the left side compared to the right. **Score 1**: No observable response was elicited when the left side was stimulated. This test helps in detecting sensory deficits, particularly those resulting from unilateral brain injury.

2.5.5. ***Response to Vibrissae Touch***
To assess sensory responsiveness through the facial whiskers, a blunt stick was gently brushed against the vibrissae on each side of the rats face to assess the sensory response. The stimulus was applied from behind the animal to avoid visual cues and ensure a purely tactile response. Reactions were scored as follows: **Score 3**: The rat exhibited a prompt and symmetrical response, such as head turning or startle reflex, to stimulation on both sides. **Score 2**: The rat showed a delayed or reduced response to stimulation on the left side equated to the right. **Score 1**: No response was perceived when

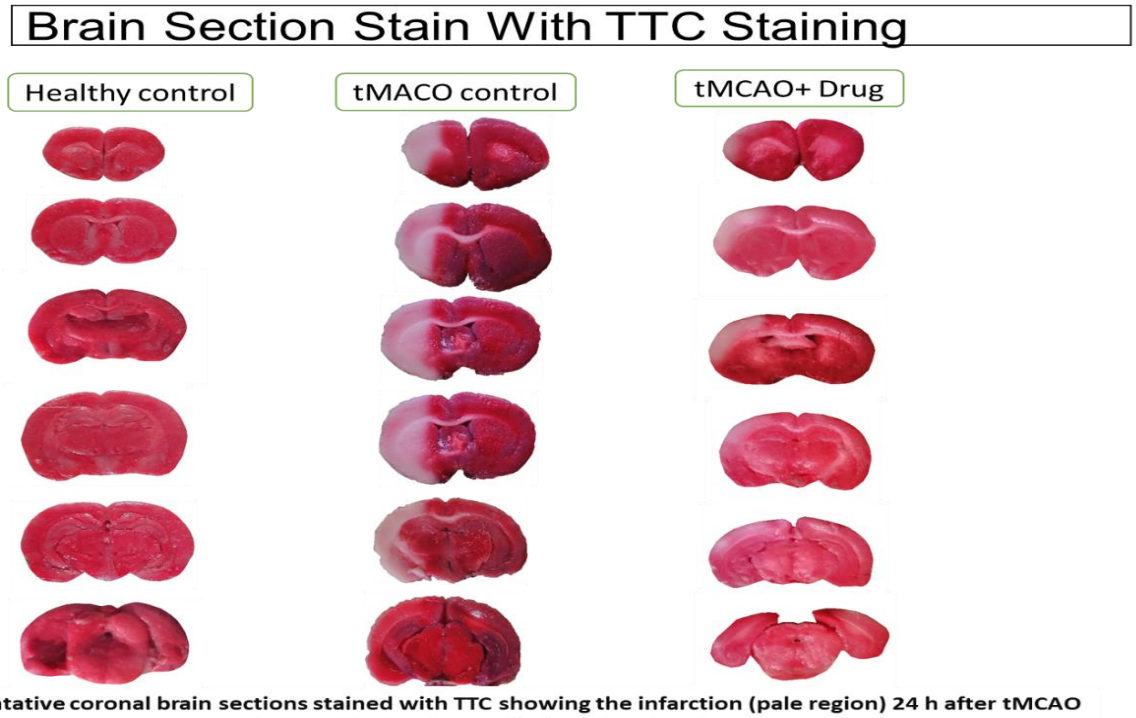
the left side was stimulated. This test serves as a sensitive measure of sensorimotor impairment, particularly for detecting subtle unilateral deficits following cerebral ischemia.

3. RESULT AND DISCUSSION:

3.1. ***Effect of Poly Herbal on brain infarct volume after tMCAO Reperfusion:*** Poly-herbal Treatment Significantly Reduces Infarct Volume Following Ischemic Stroke Mean percentage infarct volumes observed were: **Healthy Control**: 0.00 ± 0.00%, **tMCAO**: 27.79 ± 3.37%, **tMCAO + Drug**: 6.15 ± 3.70%. One-way ANOVA revealed an extremely significant difference among the groups (**F = 25.49, p < 0.00001**). When analysed by one sample t test, multi comparison test **tMCAO vs. Healthy**: Significant increase in infarct volume (mean: 27.79 ± 3.37%, **p < 0.001**) denoted ### indicating substantial ischemic damage. **tMCAO + Drug vs. tMCAO**: Significant reduction in infarct volume (mean: 6.15 ± 3.70%, **p =0.0004)** denoted as *****,** showing significant protection against ischemic injury**.** Statistical analysis confirmed a exceedingly significant alteration among groups **(p < 0.00001) (figure no. 3.1A & 3.1 B).** These results highlight the therapeutic potential of early poly-herbal intervention in minimizing brain infarction and preserving neurological function following ischemic stroke.



(figure no. 3.1.A) Percentage infraction tMCAO induced cerebral ischemia, was analyzed by one sample T test, . values are expressed as mean +/- S.E.M. (n=6). as compaired Healthy Vs. tMCAO group significance P<0.001denote ###, in comparision in tMCAO Vs. tMCAO + drug group significance P=0.0004 denotes ***.



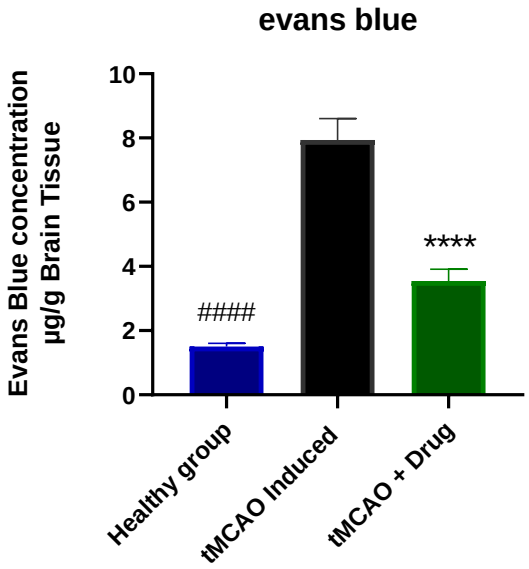
Representative coronal brain sections stained with TTC showing the infarction (pale region) 24 h after tMCAO induced ischemia reperfusion injury in rats.

Figure no. 3.1 B

Figure 3.1 A: Percentage infraction Volume, Values are articulated as Mean ± SEM (n=6), analyzed by one sample t test value expressed as ### (p<0.001) as compare Healthy vs tMCAO, *** (p=0.0004) as compare tMCAO vs tMCAO + Drug. ***Figure no 3.1 B:*** represent coronal brain section stained with TTC showing the Infraction (pale region) 24 hours after tMCAO induced ischemia reperfusion injury rats.

3.2. Evans Blue Dye Extravasation and Quantification

Quantitative investigation of Evans Blue dye infiltration revealed significant differences in blood–brain barrier (BBB) permeability, estimated following tMCAO. In this calculation having in micro gram Evans blue in per gram brain tissue. **Healthy control Mean Blue level = 1.51 ± 0.10**, tMCAO group mean Evans Blue Level = 7.93 ± 0.67, tMCAO + Drug mean Evans Blue Level = 3.55 ± 0.37. One-way ANOVA analysis presented a **greatly significant difference** between groups (**F = 328.42**, **p < 0.0001**). Analysed by 2 way ANOVA ordinary multi comparison test, tMCAO **vs Healthy**: Significant increase in Evans Blue, confirming BBB breakdown. **tMCAO + Drug vs tMCAO**: (**p < 0.0001**) denote ****, Significant reduction in Evans Blue, indicating partial restoration of BBB integrity. **Healthy vs tMCAO + Drug**: **(p < 0.0001)** denote **####,** Levels remained higher than healthy controls, but significantly lower than untreated ischemic rats dye accumulation, suggesting a protective effect on the BBB ***(figure no. 3.2)***. These findings support the hypothesis that early therapeutic intervention can preserve BBB integrity, likely contributing to reduced secondary injury and improved functional outcomes.



(figure no. 3.2,) Evans Blue Dye, BBB intigriti Test,data analyzed by 2Way ANOVA Odnary Multipe Compairision test Value expresed as Mean ± S.E.M. (n=6). #### show comparision between Healthy Vs tMCAO, Significance (P<0.0001), **** s comparision between tMCAO Vs tMCAO + Drug significance (P<0.0001)

3.3. *Behaviour assessment for poly-herbal treatment:*

Behavioural evaluations following tMCAO-induced cerebral ischemia revealed significant functional deficits across multiple parameters, including neurological scores, grid hold, limb symmetry, forepaw outstretching, body proprioception, and vibrissae response. Rats treated with the poly-herbal mixture demonstrated marked improvements in all behavioural domains paralleled to the untreated tMCAO group. Remarkably, the treatment group presented better grip strength in the grid hold test, enhanced limb symmetry and extension, and more consistent proprioceptive and tactile responses. These findings suggest that the poly-herbal intervention effectively mitigated ischemia-induced neurobehavioral deficits, supporting its potential role in early functional recovery.

3.3.1. *Grid hold test*

Behavioural scores were significantly affected by ischemic injury and treatment. The **Healthy group** maintained a consistent score of **3.0 ± 0.0**, indicating unimpaired function. In contrast, the **tMCAO group** exhibited a marked decline (**0.61 ± 0.25**), reflecting severe neurological deficits. Treatment with the **poly-herbal mixture** substantially improved scores to **2.11 ± 0.17**, suggesting a protective effect against ischemic damage. One-way ANOVA revealed statistically significant differences among groups (**F (2, 15) = 285.36, p < 0.0001**). In the 2 way ANOVA ordinary multiple comparison test value conveyed as mean **± S. E. M. (n=6), as matched to Healthy vs. tMCAO (p<0.0001**) denote ####, as equalled to tMCAO vs. tMCAO + Drug (**p<0.0001**) denote ****, (***figure no. 3.3.1***). Post hoc interpretation supports that the poly-herbal treatment significantly improved behavioural performance compared to the untreated tMCAO group.

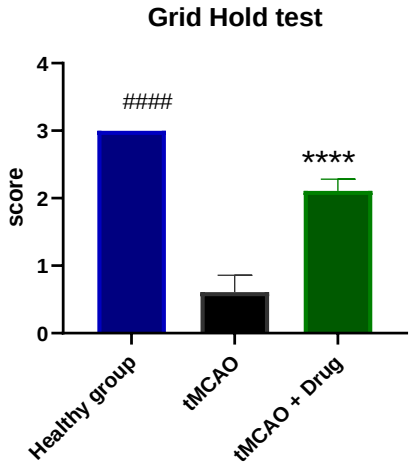


figure no 3.3.1, Neurological parameter, behaviour grid hold test score data was analyzed by 2way ANOVA Ordinary Multiple comparions test Values are expressed as mean +/- S.E.M.(n=6). as copmaired Healthy Vs. tMCAO group significance P<0.0001 denote ####, in tMCAO Vs. tMCAO + drug group significance P<0.0001, denote ****.

Figure no. 3.3.1

3.3.2. *Symmetry in the Movement of Four Limbs*

In the assessment of limb movement symmetry, the **Healthy group** maintained a perfect score of **3.00 ± 0.00**, indicating normal symmetrical forelimb movement. The **tMCAO group** displayed a significant reduction in score (**0.61 ± 0.25**), highlighting the impact of ischemic injury on motor coordination. Following treatment with the **poly-herbal mixture**, scores significantly improved to **2.17 ± 0.18**, reflecting partial restoration of motor symmetry. One way ANOVA shown statistically significant alteration among groups (**F (2, 15) = 276.9, p < 0.0001**). In the 2way ANOVA ordinary multi comparison test value are stated as mean **± S. E. M. (n=6) as matched to Healthy Vs. tMCAO (P<0.0001) denote ####**, tMCAO vs. tMCAO + Drug (P=0.0001) denote ***, (***figure 3.3.2***), post interpretation support that the poly- herbal treatment significantly improved behavioural performance compared with tMCAO group.

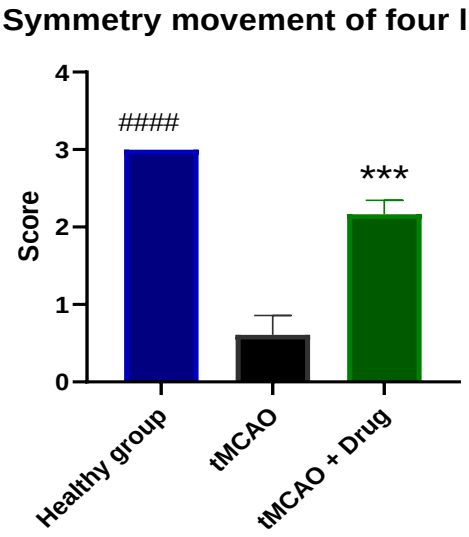


Figure no 3.3.2, Neurological parameter, behaviour symmetry movemnet of four limbs score, data anlyzed by 2way ANOVA ordinary multiple comparisons test, value are expressed as mean +/- S.E.M., (n=6). in comparision Healthy Vs. tMCAO group significance P<0.0001 denote ####, when compared tMCAO Vs. tMCAO + drug significance P=0.0001, denot ***.

Figure no. 3.3.2

3.3.3. Forepaw Outstretching

The **Healthy group** exhibited normal forepaw outstretching behaviour, with a consistent score of **3.00 ± 0.00**, indicating no motor impairment. In distinction, the **tMCAO group** established a marked reduction in scores (**0.72 ± 0.36**), reflecting significant impairment in motor coordination following ischemic injury. Notably, animals in the **tMCAO + Drug** group showed significant improvement, achieving an average score of **2.33 ± 0.44**, suggesting a protective effect of the poly-herbal treatment on forelimb motor function. Statistical investigation using one-way ANOVA discovered a highly

significant variance among groups (**p < 0.0001**). In the 2way ANOVA ordinary Multi comparison test value are uttered as mean **± S. M. E. (n=6) as associated to Healthy Vs. tMCAO (P<0.0001**) denote ####, as compared tMCAO + Drug (**P=0.0001**) denote **, (***figure no. 3.3.3***), indicating that the drug treatment substantially mitigated ischemia-induced deficits.

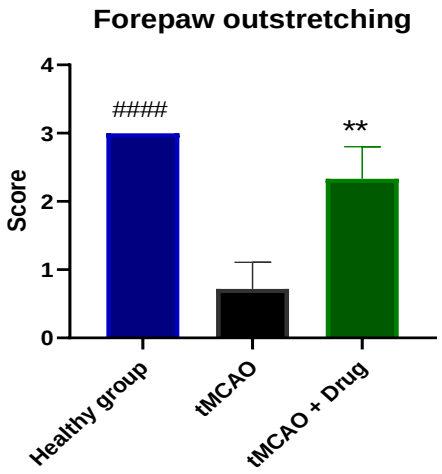


figure no. 3.3.3, Neurological parameter, behaviour forpaw outstreching of four limbs score, was analyzed by 2way ANOVA odinary multiple comparision test, values expressed as mean +/- S.E.M. (n=6), in compared Healthy Vs. tMCAO, significance P<0.0001 denote ####, when compared tMCAO Vs. tMCAO+Drug significance P=0.0017 denote **,

Figure no 3.3.3

3.3.4. *Body Proprioception*

Body proprioception was assessed across all experimental groups to evaluate post-ischemic motor coordination and balance. The **Healthy group** maintained full proprioceptive function with a consistent score of **3.00 ± 0.00**. In the **tMCAO group**, scores significantly declined to **1.27 ± 0.24**, indicating notable deficits in body position awareness following cerebral ischemia. Treatment with the poly-herbal mixture in the **tMCAO + Drug** group resulted in a marked improvement, with animals scoring **2.39 ± 0.23**, demonstrating a significant recovery in proprioceptive performance. Statistical exploration (one-way ANOVA) confirmed a significant variance between groups (**F (2, 15) =111.5 p < 0.0001**). 2way ANOVA ordinary multiple comparison test, value are stated as mean **± S. E. M. (n=6). As compared Healthy vs. tMCAO, (#### P<0.0001), as compared tMCAO Vs. tMCAO + Drug (** P = 0.0028) (*Figure no. 3.3.4*),** suggesting that the treatment effectively restored proprioceptive function impaired by ischemic insult.

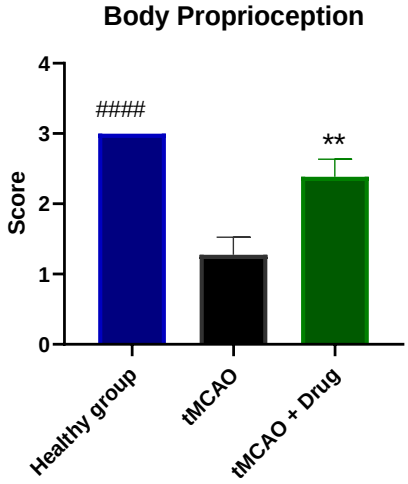


figure no. 3.3.4, Behaviour score (neurological parameter) Body Proprioception data was analyzed by two-way ANOVA ordinary multiple comparisons test. Values are expressed as mean ± S.E.M. (n=6). ####P<0.0001 as compared to healthy vs tMCAO animals.,**P=0.0028, as compared to tMCAO vs tMACO + Drug group

Figure no. 3.3.4

3.3.5. *Response to Vibrissae Touch:*

The vibrissae touch response test was used to evaluate somatosensory function following cerebral ischemia. Rats in the **Healthy group** consistently responded with a full score of **3.00 ± 0.00**, indicating intact sensorimotor integration. The

tMCAO group presented a significant decline in responsiveness, by means of an average score of **1.27 ± 0.24**, reflecting impaired sensory perception due to ischemic damage. Treatment with the poly-herbal mixture in the **tMCAO + Drug** group led to a substantial recovery, with scores improving to **2.66 ± 0.23**, approaching normal levels. One-way ANOVA discovered a statistically significant variance among groups **(F (2, 15) = 141.1**, **p < 0.0001**), in the 2way ANOVA ordinary multiple comparisons test, value communicated as mean **± S.E.M., (n=6), as compared Healthy Vs. tMCAO , significance #### P< 0.0001, as compared tMCAO Vs. tMCAO + Drug, significance *** P = 0.0003, (*figure no. 3.3.5*),** confirming the efficacy of the treatment in restoring vibrissae-evoked sensory responses.

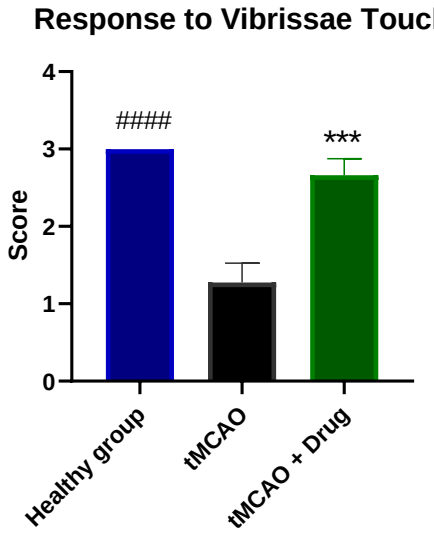


figure no. 3.3.5, Behaviour score (neurological parameter) Response to Vibrissae Touch data was analyzed by two-way ANOVA ordinary multiple comparisons test. Values are expressed as mean ± S.E.M. (n=6). ####P<0.0001 as compared to healthy vs tMCAO animals.,***P=0.0003, as compared to tMCAO vs tMACO + Drug group

Figure no. 3.3.5

4. CONCLUSION

This study strong evidence demonstrating the neuroprotective efficacy of a poly-herbal mixture in a rodent model of tMCAO, a widely recognized model for investigation focal cerebral ischemia. Treatment with the poly-herbal combination bring about in a statistically significant decline in infarct volume, indicating its capacity to attenuate ischemic brain injury. Furthermore, the treatment preserved BBB integrity, as demonstrated by a marked reduction in Evans blue dye extravasation into brain tissue, signifying decreased BBB permeability.

Behavioural assessments further reinforced the therapeutic efficacy of the mixture. Treated animals exhibited significantly improved neurological outcomes, as reflected in elevated composite neurology scores. Improvements were consistently observed across a range of functional parameters, including grid hold performance, symmetrical limb movement, forepaw outstretching, body proprioception, and vibrissae touch response. These findings suggest that the poly-herbal intervention not only provides structural neuroprotection but also promotes functional recovery following ischemic insult.

Collectively, these findings indicate that the poly-herbal treatment exerts multifaceted neuroprotective effects in ischemic stroke by mitigating infarction, preserving BBB function, and improving motor-sensory outcomes. These results warrant further mechanistic studies and clinical translation to explore its therapeutic potential in human ischemic stroke.

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