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Acute Post-Ischemic Neuroprotection By Metformin And Vitamin E: A Complementary Strategy Against Cerebral Ischemia-Reperfusion Injury

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ABSTRACT:

Introduction:

Cerebral ischemia-reperfusion (IR) injury is a leading cause of long-term neurological disability, with limited post-stroke pharmacological options. Targeting energy metabolism and oxidative stress may offer complementary neuroprotection. This study evaluated the acute post-stroke efficacy of Metformin (MET) and Vitamin E (Vit-E), individually and in combination.

Materials and Methods:

Global cerebral ischemia was induced in male rats via bilateral common carotid artery occlusion (BCCAO) for 15 minutes followed by 3 days of reperfusion. Animals were divided into Sham, IR, MET (200 mg/kg, i.m.), Vit-E (100 mg/kg, i.m.), and MET + Vit-E groups. Treatments were administered once daily for 3 days post-IR. Behavioral performance, infarct size, oxidative stress markers (LPO, SOD, GSH), and BBB integrity (Evans Blue assay) were assessed.

Results:

MET and Vit-E, particularly in combination, significantly reduced infarct area, lipid peroxidation, and Evans Blue extravasation. The combination therapy showed enhanced antioxidant defense (SOD, GSH) compared to monotherapy. Behavioral tests showed cognitive improvement though it was not statistically significant during the 3-day treatment protocol.

Conclusion:

Post-stroke coadministration of MET and Vit-E confers complementary neuroprotection by targeting metabolic dysfunction and oxidative stress. These findings support a dual-mechanism strategy for managing cerebral IR injury and warrant further investigation with extended treatment durations.

Keywords: Ischemia-reperfusion injury; Post-stroke treatment; Neuroprotection; Oxidative stress; Metformin; Vitamin-E.

INTRODUCTION:

Stroke is a complex and devastating neurological disorder that ranks among the leading causes of mortality and long-term disability globally [1, 2]. Ischemic stroke, the most prevalent form, results from the obstruction of cerebral blood flow, triggering a cascade of deleterious cellular and molecular events [3]. Although timely restoration of blood flow is essential to salvage ischemic penumbra, the reperfusion process itself paradoxically contributes to secondary brain injury through mechanisms collectively termed ischemia-reperfusion (IR) injury [4].

The current therapeutic options for stroke management remain strikingly limited. The tissue plasminogen activator (tPA), that restore blood flow, is effective only in that narrow therapeutic window (≤4.5 hours) and carries a significant risk of hemorrhagic transformation. Thus limits its clinical use to a small fraction of eligible patients [4, 5]. Recently, novel agents such as sovateltide (IRL-1620), an endothelin-B receptor

agonist, has emerged with potential to promote neurogenesis, angiogenesis, and anti-apoptotic effects [6]. Although sovateltide showed promise by modulating oxidative stress and apoptosis, its exact role in reperfusion injury remains unclear. Moreover, it too had narrow therapeutic window that affects its broader clinical utility [7]. As a result, a vast majority of stroke survivors are left without effective pharmacological options beyond acute thrombolysis. This scenario highlights an urgent need for safe, effective, and widely applicable therapies, particularly one applicable to post-stroke injury recovery.

Addressing secondary injury processes after stroke is critical not only to limit infarct progression but also to support long-term neurological recovery. For patients who survive the initial ischemic insult, the quality of recovery determines functional independence, caregiver burden, and overall quality of life [8, 9]. Therefore, therapeutic strategies aimed at post-stroke neuroprotection could fill a major gap in current clinical care [10], especially for patients outside the thrombolytic window or those who experience incomplete reperfusion.

Among the emerging therapeutic approaches, targeting cerebral metabolism has gained momentum due to its pivotal role in maintaining neuronal viability [11]. Neurons are highly energy-dependent cells, and ischemic conditions rapidly deplete ATP, impair mitochondrial function, and activate cell death cascades[11, 12]. AMP-activated protein kinase (AMPK), a key cellular energy sensor, becomes activated under metabolic stress and plays a critical role in restoring energy homeostasis, enhancing mitochondrial function, and regulating autophagy and inflammation [13, 14]. Pharmacological activation of AMPK has shown promising neuroprotective effects in various preclinical models of stroke and neurodegeneration, making it a compelling metabolic target [15, 16].

Parallel to metabolic dysfunction, oxidative stress is one of the most prominent contributors to ischemic brain injury. Reperfusion leads to the excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), resulting in lipid peroxidation, protein oxidation, and DNA damage [17, 18]. Neurons are particularly susceptible to oxidative damage due to their high oxygen consumption and relatively weak endogenous antioxidant defenses. Antioxidant therapy, therefore, represents an essential axis of neuroprotection [19]. However, mono-target antioxidant interventions have yielded inconsistent results in clinical settings, likely due to the multifactorial nature of oxidative injury in stroke.

Given the complexity of stroke pathology, a combined therapeutic approach that addresses both energy imbalance and oxidative stress may offer a more effective strategy. Such a dual-targeted approach is not only rational but also necessary to disrupt the vicious cycle of metabolic failure and oxidative damage that perpetuates neuronal loss after ischemia-reperfusion.

Metformin (MET), an oral antidiabetic agent, has pleiotropic effects beyond glycemic control. Notably, MET is a potent activator of AMPK and modulator of mitochondrial function, and attenuate neuroinflammation [14, 20]. It makes MET as a promising candidate for neuroprotective intervention. Meanwhile, Vitamin E (Vit-E), a lipid-soluble antioxidant, neutralizes lipid peroxyl radicals and protect cellular membrane from peroxidation [21]. Evidences suggests that Vit-E enhances endothelial nitric oxide synthase (eNOS) activity, thereby increasing nitric oxide bioavailability [22, 23]—critical factor to facilitate AMPK activation [15, 24] and improving cerebrovascular function. Since physiological levels of NO are known to support AMPK phosphorylation and activity, Vit-E may potentially enhance MET's neuroprotective efficacy via nitric oxide-dependent pathways.

Despite these promising properties, the combined application of MET and Vit-E in the acute post-stroke phase has not been adequately explored. Their distinct but complementary mechanisms—AMPK-mediated metabolic regulation by MET and direct ROS scavenging by Vit-E—make them a rational combination for counteracting ischemia-reperfusion injury. Furthermore, the post-stroke treatment paradigm aligns with the real-world clinical scenario, where early intervention after ischemic onset is crucial yet often delayed beyond the thrombolytic window.

The present study designed to evaluate the post-stroke neuroprotective ability of MET, Vit-E, and their coadministration in a well-established rat model of global cerebral ischemia induced by bilateral common carotid artery occlusion (BCCAO) followed by reperfusion. We hypothesized that the combination therapy would provide neuroprotection by targeting complementary pathophysiological mechanisms.

MATERIAL AND METHODS:

Materials:
MET was received as a gift sample from Emcure Pharmaceuticals, Pune, Maharashtra. Vit-E (alpha-tocopherol) was procured from Sigma-Aldrich. All other chemicals and reagents used were of analytical grade.

Animals:
Adult Wistar rats (200–250 g) of either sex were procured from the central animal house facility. The study protocol was approved by the Institutional Animal Ethical Committee (Protocol No. PH/IAEC/VNS/2K22/02) and adhered to the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Animals were housed under controlled conditions (24 °C, 12 h light/dark cycle) with free access to standard pellet diet and water.

Induction of Ischemia-Reperfusion Injury:
Global cerebral ischemia was induced by the bilateral common carotid artery occlusion (BCCAO) method with slight modifications to the procedure described by Iwasaki et al. [25] Rats were anesthetized with ketamine (60 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), placed supine, and their body temperature maintained at 37 °C. A midline neck incision was made, and both common carotid arteries (CCAs) were carefully isolated from surrounding tissues and the vagus nerve. Occlusion was achieved by ligating both CCAs for 15 minutes. In the sham group, the CCAs were exposed but not ligated. Reperfusion was initiated by removing the ligatures and continued for 3 days.

Experimental Design:
Animals were randomly divided into five groups; each group comprises 24 rats. Each group further subdivided into four subsets (n=6) based on the outcome measures:

Sham: Surgery without ligation (vehicle-treated)
IR: BCCAO-induced ischemia and reperfusion (vehicle-treated)
MET: Metformin (200 mg/kg, i.m. once daily for 3 days post-IR)
Vit-E: Vitamin E (100 mg/kg, i.m. once daily for 3 days post-IR)
MET + Vit-E: Co-administration of Metformin and Vitamin E (200 mg/kg and 100 mg/kg respectively, i.m. for 3 days post-IR)

Subsets: Each group further subdivided into four subsets and each subsets comprises six rats (n=06)

Set I: Neurological score, behavioral tests, infarct analysis
Set II: Oxidative stress markers (LPO, GSH)
Set III: Blood-brain barrier permeability
Set IV: Histopathological analysis

Neurological Scoring:
On day 3 post-reperfusion, neurological function was evaluated using a modified neurological scoring system describe by Li et. al. [26]. The score ranges from 0 to 14, (Table 1) where higher scores indicate more severe neurological deficits.

Table 1: Neurological score

Behavioural assessment	Score
A. Motor tests :	
1. Raising the rat via the tail :	
a. Flexion of forelimb	1
b. Flexion of hindlimb	1
c. Head moving more than 10° to the vertical axis within 30 seconds	1
2. Placing the mouse on the floor :	
a. Inability to walk straight	1

b. Circling toward the paretic side	1
c. Falling down to the paretic side	1
3. Abnormal movements :	
a. Immobility and staring	1
b. Tremor (wet-dog-shakes)	1
c. Myodystony, irritability, seizures, myoclonus	1
B. Sensory tests :	
1. Visual and tactile placing (limb placing test to detect visual and superficial sensory) Moving the mouse laterally toward the table:	1
a. Reaching the table slowly with limbs or cannot place at all	
2. Proprioceptive test (deep sensory) : Pushing the paw against the table edge to stimulate limb muscles	1
3. Losing the resistance	1
C. Reflexs : (blunt or sharp stimulation)	
a. Absence of Pinna reflex (a head shake when touching the auditory meatus)	1
b. Corneal reflex (an eye blink when lightly touching the cornea with cotton)	1
c. Startle reflex (a motor response to a brief loud noise from snapping a clipboard paper)	1

Y-Maze Test for Spontaneous Alternation:
Spontaneous alternation behavior was assessed using a three-arm Y-maze describe in Wahl et al.[27],. Rats were trained for 5 days prior to BCCAO. On day 3 post-injury, animals were allowed to explore the maze for 8 minutes. Alternation percentage was calculated as:
% alteration: [Total number of alterations]/[number of arms entered]*100

Transfer Latency on Elevated Plus Maze (EPM):
Transfer latency (TL) was measured as the time taken by the animal to move from an open arm to a closed arm on the EPM. Rats were trained before BCCAO and tested again on day 3 post-injury. A cutoff time of 90 seconds was used [28].

Novel Object Recognition Test (NORT):
NORT was performed based on previously described protocols in Panta et. al.,[29] to assess recognition memory. Rats were trained for 5 days before BCCAO surgery. Following training, BCCAO was performed and on day 3 of reperfusion, animals were re-exposed to the test. The discrimination ratio was calculated using the formula [30].
Discrimination ratio: [Time spent with novel object]-Time spent with familiar object] / [total time spent with bot object]

Brain Infarction Analysis:
Brains were sectioned into 2 mm coronal slices and stained with 2% TTC (2, 3, 5-triphenyltetrazolium chloride) for 30 minutes at room temperature. Sections were fixed in 10% formalin overnight. Infarct volume was quantified using ImageJ software [31]. The total infarct area was determined by summing the individual areas across all sections. This total infarct area was then multiplied by the thickness of the brain slice to compute the infarct volume per brain in cubic millimeters (mm^3). It was then converted into percentage infarct by dividing the total infarct area by the total brain area and multiplying by 100

Oxidative Stress Assessment:
Brain tissues were homogenized in 10% TCA. Lipid peroxidation (LPO) was estimated by measuring Malondialdehyde (MDA) using thiobarbituric acid reactive substances (TBARS) at 532 nm [32]. Reduced glutathione (GSH) content was determined using 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) reagent at 412 nm [33]. Superoxide dismutase (SOD) activity in brain tissue homogenates was quantified based on its ability to inhibit pyrogallol autoxidation. The reaction was monitored spectrophotometrically at 420 nm for 5 minutes [34]. Protein levels were quantified using the Lowry method [35].

Blood-Brain Barrier (BBB) Permeability:
BBB integrity was assessed by Evans Blue dye extravasation. On day 3 post-injury, rats were injected with 4% Evans Blue (0.1 ml, tail vein). Following perfusion, brains were homogenized, incubated with TCA, and centrifuged. The supernatant was analyzed at 610 nm and quantified against a standard curve [36].
Histological Analysis:
Formalin-fixed brains were embedded in paraffin, and 5 µm sections were cut and stained with 1% cresyl violet. Neuronal changes in hippocampus of brain were observed under a microscope.

Statistical Analysis:
Results are presented as mean ± SEM. One-way ANOVA followed by Tukey's post hoc test was used for most comparisons. Neurological scores were analyzed using the non-parametric Kruskal-Wallis test and reported as median (25–75% quartile range). A p-value < 0.05 was considered statistically significant. GraphPad Prism 5.0 was used for analysis.

RESULTS:
Neurological outcomes:
The neurological score of the IR group was significantly increased ($p < 0.05$) compared to the sham group, indicating substantial neurological injury induced by BCCAO. In contrast, post-stroke co-administration of MT and Vit-E significantly restricted the neurological score compared to the IR group. The median score (25%–75% interquartile range) of the IR group was 10.0 (10.00–11.00), whereas a significantly lower median score of 0.5 (0.00–2.00) was observed in the co-administration group ($p < 0.05$). Treatment with MT alone resulted in a non-significant reduction to 2.5 (2.00–3.25), while Vit-E alone significantly improved the neurological score to 1.5 (0.00–3.00) compared to the IR group ($p < 0.05$) shown in Figure 1A.
Post stroke treatment enhancing % alteration response:
The IR group exhibited a significant decrease ($p < 0.05$) in spontaneous alternation response (16.27 ± 5.93%) on the Y-maze compared to the Sham group (65.38± 11.06) shown in Figure 2B. Treatment with either MT alone (38.45± 14.27) or Vit-E (24.51 ± 8.43) for three days post-stroke led to an improvement in alternation performance. Notably, co-administration of MT and Vit-E resulted in a greater improvement (52.70 ± 16.21) compared to either treatment alone. However, none of the treatment groups achieved a statistically significant improvement when compared to the IR group.
Improvement in transfer latency following coadministration:
IR injury significantly prolonged the transfer latency (51.00± 7.32 seconds; $p < 0.05$) in the elevated plus maze compared to the Sham group (15.67 ± 1.54 seconds), indicating post-ischemic cognitive deficits. Acute post-injury treatment with either MT (20.17± 1.27 seconds), Vit-E (19.33 ± 1.38 seconds), or their coadministration (12.67 ± 1.28 seconds) significantly reduced transfer latency compared to the IR group. Notably, coadministration of MT and Vit-E resulted in a greater reduction in transfer latency than either treatment alone shown in Figure 1C.
Effect of simultaneous administration on Discrimination Ratio in NORT:
Simultaneous administration of MT and Vit-E significantly improved recognition memory, as evidenced by an increased discrimination ratio (0.64 ± 0.15) in the novel object recognition test (NORT). In contrast, the IR group showed a significant ($p < 0.05$) reduction in the discrimination ratio (0.38 ± 0.17; $p < 0.05$) compared to the Sham group (0.96 ± 0.03) shown in Figure 1D. Treatment with either MT (0.57 ± 0.12) or Vit-E alone (0.36 ± 0.11) also showed trends toward improvement in the discrimination ratio, though less pronounced than the combined treatment.

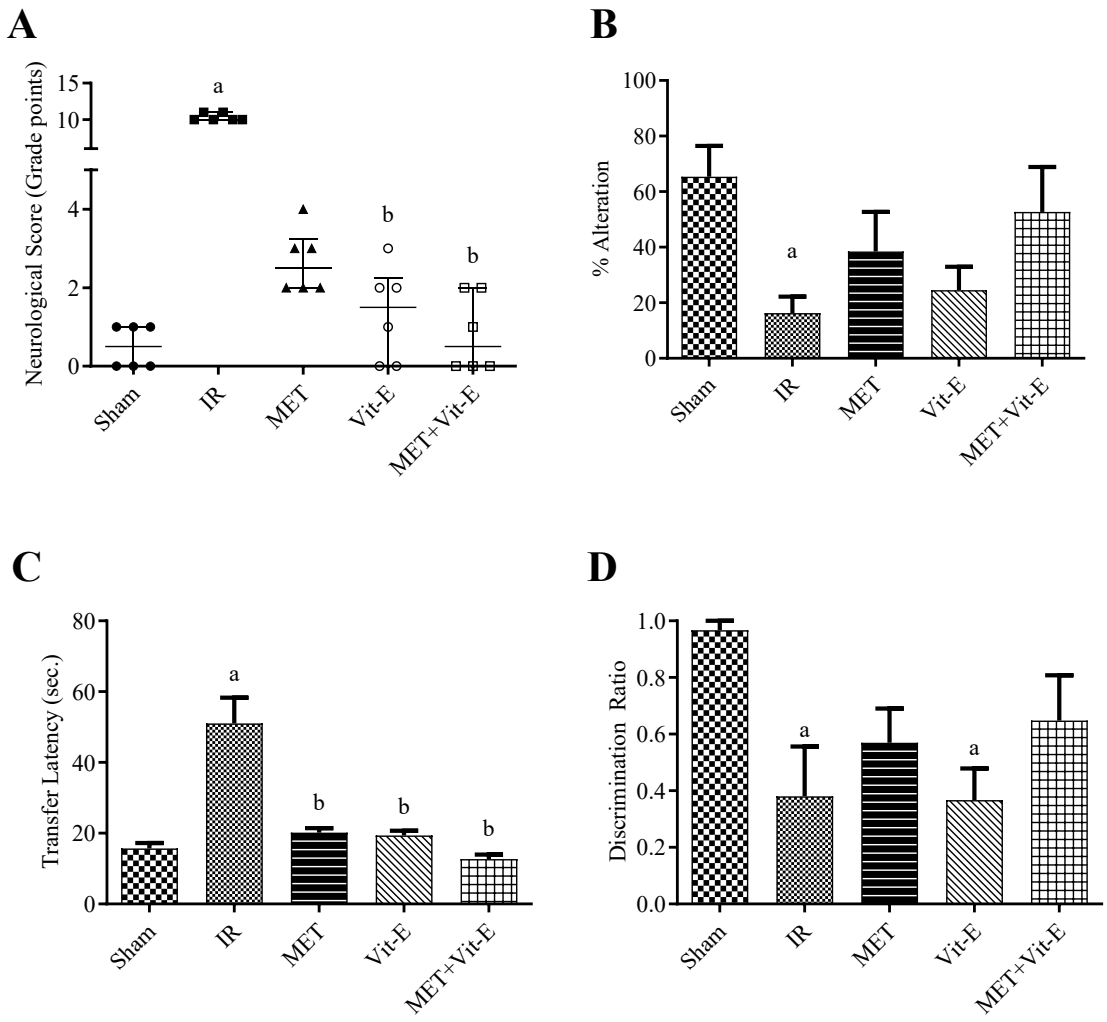

Figure 1: (A) Neurological score; (B) % Alteration response on Y-maze; (C) Transfer latency on EPM; (D): Discrimination ratio on NORT.

Data were represented as median with interquartile range (n=6) for neurological score and analyzed by Kruskal Wallis non-parametric test. While, for % alteration response, transfer latency, and discrimination ratio data were expressed as Mean ± SEM and analyzed by one-way ANOVA followed by Tukey's test. Where, [a] significant ($p<0.05$) difference from Sham; [b] significant ($p<0.05$) difference from IR; [c] significant ($p<0.05$) difference from MET; [d] significant ($p<0.05$) difference from Vit-E.

Coadministration limiting brain damage:

BCCAO-induced IR injury led to significant cellular damage in the brain, as evidenced by a significantly ($p < 0.05$) higher infarct area in the IR group (31.32 ± 2.03) compared to the Sham group (7.59 ± 0.53) (Figure 2). Acute post-injury treatment with either MT (18.20± 1.12) or Vit-E (17.14 ± 1.24) significantly ($p < 0.05$) reduced brain infarction compared to the IR group. Notably, co-administration of MT and Vit-E not only significantly decreased the infarct area compared to the IR group but also showed a greater reduction than either treatment alone, indicating superior neuroprotective effects with combined therapy (Figure 3A).

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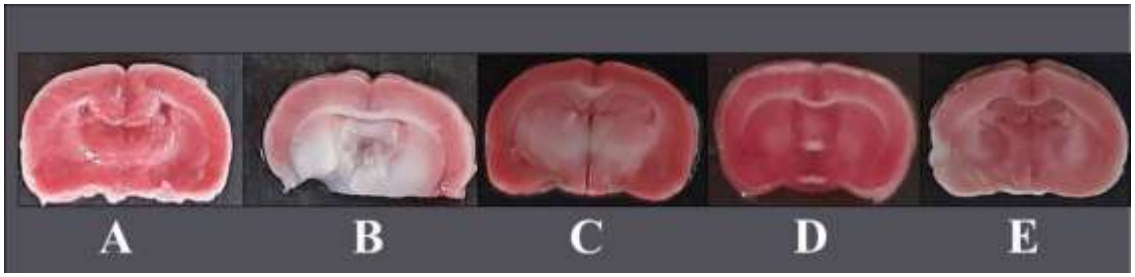

Figure 2: Evaluation of Cerebral Infarction by TTC Staining After Ischemia-Reperfusion
Red regions indicate viable (non-infarcted) brain tissue, while white areas represent infarcted regions. Where **A:** Sham; **B:** IR; **C:** MET; **D:** Vit-E; **E:** MET+Vit-E

Preservation of Blood-Brain Barrier Integrity by MET and Vit-E:
IR injury significantly disturbed BBB integrity, as evident from marked increase in Evans Blue dye extravasation in the IR group (7.61 ± 0.70) compared to the Sham group (1.69 ± 0.35). Acute post-stroke treatment with either MET (1.90 ± 0.48) or Vit-E (2.40 ± 0.19) effectively attenuated BBB disruption, thus limiting dye extravasation (Figure 3B). Notably, coadministration of MET and Vit-E (1.88 ± 0.19) resulted in comparable protection, suggesting that both agents, alone or in combination, preserved BBB integrity following IR injury.

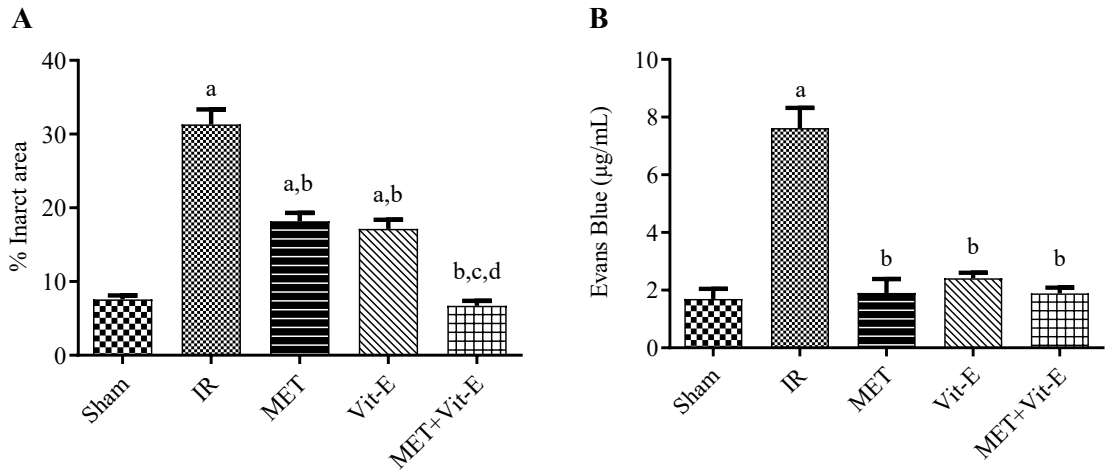

Figure 3: (A) % Infarct area (B) Blood brain barrier (BBB) permeability.

Data were represented as Mean ± SEM and analyzed by one-way ANOVA followed by Tukey's test. Where, [a] significant ($p<0.05$) difference from Sham; [b] significant ($p<0.05$) difference from IR; [c] significant ($p<0.05$) difference from MET; [d] significant ($p<0.05$) difference from Vit-E.

Attenuation of Oxidative Stress by MET and Vit-E:
IR induced substantial oxidative stress, as evidenced by elevated LPO and depleted endogenous antioxidant defences. In the IR group, LPO levels were significantly ($p < 0.05$) increased (14.05± 1.05) compared to the Sham group (5.25 ± 0.50). Post-stroke treatment with MET alone (13.10± 0.98) did not significantly reduce LPO levels compared to the IR group. However, Vit-E treatment (7.49 ± 0.57; $p < 0.05$ vs IR) and especially its coadministration with MET (5.41 ± 0.85; $p < 0.05$ vs IR) led to a significant reduction in LPO. The combined treatment not only had better outcomes than IR and MET groups but also demonstrated a trend of greater efficacy compared to Vit-E alone, suggesting that Vit-E may potentiate MET's antioxidant effect (Figure 4C).

A similar significance pattern was observed for SOD activity. IR markedly suppressed SOD levels (6.29 ± 0.58) relative to Sham (16.05 ± 0.39; $p < 0.05$). MET treatment alone (7.27 ± 0.44) did not produce a statistically significant improvement compared to IR. In contrast, Vit-E (8.98 ± 0.29; $p < 0.05$ vs IR) and especially the MET + Vit-E combination (11.64 ± 0.52; $p < 0.05$ vs IR and MET) significantly enhanced SOD activity (Figure 4A). The coadministration group also showed a statistically significant elevation compared to MET alone, underscoring the superior antioxidant response achieved with combination therapy.

GSH levels followed a comparable pattern. The IR group showed a marked reduction (3.87 ± 0.73) compared to Sham (17.63 ± 1.22; $p < 0.05$). Unlike the LPO and SOD patterns, MET alone significantly improved GSH levels (12.41 ± 1.42; $p < 0.05$ vs IR), as did Vit-E (11.15 ± 1.85) and the combination group (16.32 ± 1.16; $p < 0.05$ vs IR). Although the combination group showed the highest restoration of GSH levels among the treatments (Figure 4B), the improvement was not statistically significant compared to the individual MET or Vit-E groups, indicating a trend rather than an additive effect.

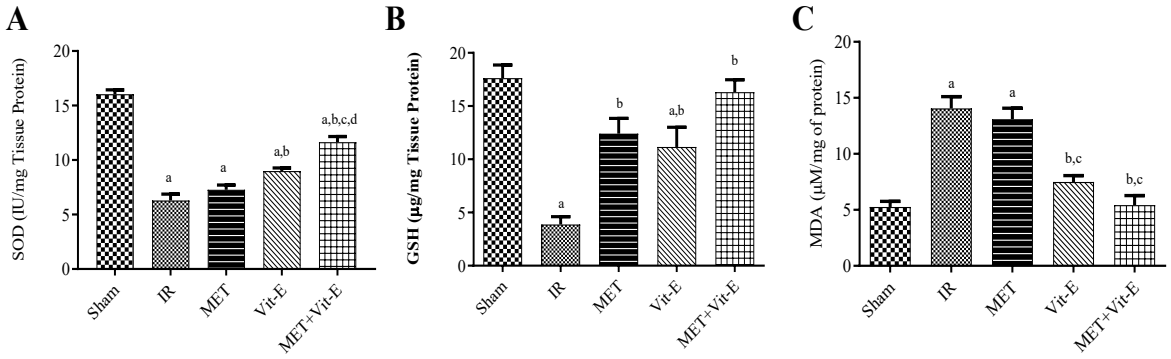

Figure 4: (A) Superoxide dismutase activity (B) Glutathione activity; (C): Lipid peroxidation
Data were represented as Mean ± SEM and analyzed by one-way ANOVA followed by Tukey's test. Where, [a] significant ($p<0.05$) difference from Sham; [b] significant ($p<0.05$) difference from IR; [c] significant ($p<0.05$) difference from MET; [d] significant ($p<0.05$) difference from Vit-E.

Histopathological evaluation of brain hippocampal area:

In the Sham group, the CA1 pyramidal neurons exhibited normal histological features with well-organized layers, distinct cell bodies, and prominent nuclei, indicating preserved neuronal architecture demonstrated by arrow heads (Figure 5A). The IR group showed marked neuronal damage, characterized by shrunken pyramidal neurons, condensed (pyknotic) nuclei, and disrupted cellular arrangement indicated by black arrows (Figure 5B), indicating significant ischemic injury.

In MET and Vit-E treated groups, neuronal preservation was evident with reduced pyknosis and less nuclear shrinkage compared to the IR group, although mild cellular disruption remained observable exhibited as arrow head (Figure 5C and 5D). The Combination-treated group demonstrated near-complete preservation of CA1 neuronal structure, with intact cell morphology, clear nuclei, and minimal signs of degeneration shown as arrow heads, closely resembling the Sham group (Figure 5E).

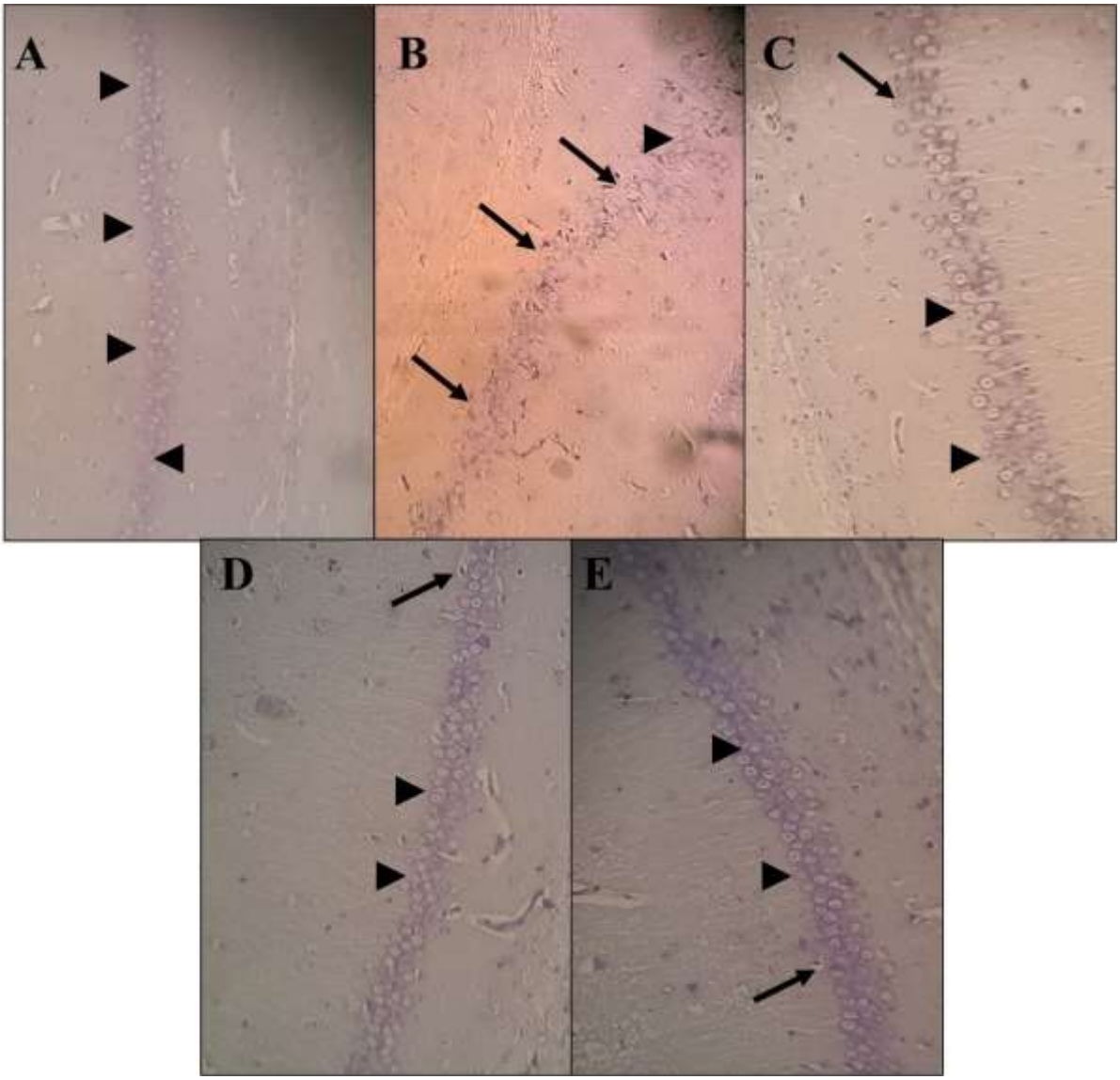

Figure 5: Histological evaluation of ca1 region in rat hippocampus following IR and treatment interventions

Where **A:** Sham; **B:** IR; **C:** MET; **D:** Vit-E; **E:** MET+Vit-E
Representative cresyl violet-stained sections of the hippocampal CA1 region from different groups (400× magnification). The Sham group shows normal pyramidal neurons with clear cytoplasm and centrally located nuclei (arrowheads). The IR group displays severe neuronal damage with shrunken cells and pyknotic nuclei (arrows). MET and Vit-E groups show partial neuronal preservation. The Combination-treated group MET + Vit-E exhibits marked neuroprotection, closely resembling the Sham group (arrowheads).

DISCUSSION:
The present study provides compelling evidence that acute post-stroke coadministration of MET and Vit-E offers significant neuroprotection in a rat model of cerebral IR injury. While both agents individually conferred beneficial effects, their combination demonstrated superior efficacy in several outcome parameters, including infarct size reduction, oxidative stress modulation, and BBB preservation. These

findings underscore the therapeutic potential of a dual-target strategy addressing both metabolic and oxidative components of post-stroke injury.

One of the central mechanisms underlying MET's neuroprotective effect is the activation of AMPK, a master regulator of cellular energy homeostasis [24, 37]. Under ischemic conditions, neurons experience severe energy depletion due to impaired ATP production. AMPK activation has been shown to shift cellular metabolism toward energy conservation by inhibiting anabolic processes and promoting mitochondrial biogenesis, autophagy, and stress adaptation [13, 15, 38]. In this study, MET-treated animals exhibited improved histological outcomes and restoration of GSH levels, suggesting that AMPK-mediated metabolic reprogramming may have contributed to enhanced neuronal survival and mitigation of secondary damage.

Complementing this, Vit-E exerted its protective effect primarily through antioxidant mechanisms. As a lipid-soluble compound, Vit-E localizes to cellular membranes and effectively scavenges lipid peroxyl radicals, thereby preventing lipid peroxidation [23]—a hallmark of oxidative injury in cerebral IR [39]. In the current study, significant reductions in MDA levels, a biomarker of lipid peroxidation, were observed in Vit-E and combination treatment groups. This antioxidant action likely contributed to the observed reduction in infarct area and improvement in SOD activity, indicating enhanced endogenous defense against ROS.

The combination of MET and Vit-E produced a greater reduction in oxidative stress markers (LPO, SOD, and GSH) and infarct size than either agent alone. This enhanced effect can be attributed to their complementary modes of action: MET targeting cellular energy metabolism via AMPK activation[40], and Vit-E directly mitigating oxidative injury by neutralizing ROS and peroxynitrite [41]. Given the multifactorial pathology of stroke, this dual mechanism likely allowed broader control over both upstream metabolic stress and downstream oxidative damage, leading to more robust neuroprotection.

Another possible explanation for the observed synergism lies with of nitric oxide mediated recovery may be by vasodilation and energy homeostasis[42, 43]. Physiological levels of nitric oxide are known to enhance AMPK phosphorylation and activity, thereby supporting energy restoration pathways during ischemic stress[15, 16]. Vit-E has been reported to enhance endothelial nitric oxide synthase (eNOS) activity and increase nitric oxide bioavailability [22, 23]. This raises the possibility that Vit-E may have facilitated AMPK activation by MET, indirectly amplifying its energy-regulating effects. While direct measurement of nitric oxide was not conducted in this study, the pattern of results—especially the combination's superior effect on SOD and LPO—supports this hypothesis and warrants further exploration.

Behavioral assessments, including Y-maze alternation, NORT, and EPM, showed trends toward improvement in all treatment groups, with coadministration again yielding the best, though not always statistically significant, outcomes. These findings suggest that short-term antioxidant and metabolic support can influence cognitive recovery, but a 3-day treatment window may be insufficient to fully reverse post-ischemic cognitive deficits. Previous literature suggests that long-term neuronal remodelling and synaptic plasticity—essential for behavioral recovery—often require extended therapeutic support.

Preservation of BBB integrity, as indicated by reduced Evans Blue extravasation, was another important finding. Both MET and Vit-E, individually and in combination, significantly protected the BBB, possibly through complementary pathways—MET via reduction of endothelial metabolic stress, and Vit-E by limiting oxidative damage to tight junction proteins[44–46]. Since BBB disruption contributes to vasogenic edema and secondary inflammation, its preservation is crucial for limiting lesion expansion and promoting neurological stability.

Despite these promising outcomes, the study has notable limitations. First, we did not measure AMPK phosphorylation, nitric oxide levels, or inflammatory markers such as TNF-α and IL-6, which would have offered deeper mechanistic insights into the observed neuroprotection. Second, the short duration of treatment (3 days), while sufficient to demonstrate biochemical and histological improvement, may not capture the full extent of functional recovery. Third, cholinergic dysfunction, an important contributor to post-stroke cognitive decline, was not assessed via acetylcholinesterase or cholinergic receptor markers.

In conclusion, the findings of this study suggest that acute post-stroke coadministration of MET and Vit-E offers a promising therapeutic approach by targeting two key pathological mechanisms of stroke—energy dysregulation and oxidative stress. While MET activates AMPK to conserve energy and support neuronal survival, Vit-E neutralizes damaging ROS and enhances nitric oxide availability, potentially augmenting MET's efficacy. Their combination thus represents a multifaceted strategy that could be especially valuable when conventional thrombolytic therapies are not viable. Future research should aim to validate the involvement of the nitric oxide–AMPK axis, explore inflammatory pathways, and assess the impact of extended treatment durations on long-term functional outcomes. This integrative approach may pave the way for the development of more effective, accessible post-stroke neurotherapeutics.

CONCLUSION:

The present study demonstrates that acute post-stroke treatment with MET and Vit-E confers significant neuroprotection in a rat model of cerebral ischemia-reperfusion injury. Individually, both agents reduced oxidative stress, preserved blood-brain barrier integrity, and improved histological and behavioural outcomes. Notably, their combination offered broader protective effects, suggesting complementary mechanisms involving AMPK activation, antioxidant defence, and nitric oxide modulation.

Although the benefits of combination therapy did not always reach statistical significance over individual treatments, particularly within a short treatment window, the trends suggest potential for enhanced efficacy with extended administration. These findings support the rationale for combinatorial metabolic-antioxidant therapies in stroke management and warrant further investigation into chronic treatment protocols and molecular mechanisms.

Conflict of interests:

All author declares no conflict of interests

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REFERENCE:

1. Feigin VL, Brainin M, Norrving B, et al (2025) World Stroke Organization: Global Stroke Fact Sheet 2025. Int J Stroke 20:132–144. https://doi.org/10.1177/17474930241308142
2. Kuriakose D, Xiao Z (2020) Pathophysiology and treatment of stroke: Present status and future perspectives. Int J Mol Sci 21:1–24. https://doi.org/10.3390/ijms21207609
3. Zhang G, Wang Q, Jiang B, et al (2024) Progress of medicinal plants and their active metabolites in ischemia-reperfusion injury of stroke: a novel therapeutic strategy based on regulation of crosstalk between mitophagy and ferroptosis. Front Pharmacol 15:1–20. https://doi.org/10.3389/fphar.2024.1374445
4. Kawadkar M, Mandloi AS, Saxena V, et al (2021) Noscapine alleviates cerebral damage in ischemia-reperfusion injury in rats. Naunyn Schmiedebergs Arch Pharmacol 394:669–683. https://doi.org/10.1007/s00210-020-02005-x
5. Lansberg MG, Bluhmki E, Thijs VN (2009) Efficacy and safety of tissue plasminogen activator 3 to 4.5 hours after acute ischemic stroke: a meta-analysis. Stroke 40:2438–2441. https://doi.org/10.1161/STROKEAHA.109.552547.Efficacy
6. Ranjan AK, Gulati A (2022) Sovateltide Mediated Endothelin B Receptors Agonism and Curbing Neurological Disorders. Int J Mol Sci 23:. https://doi.org/10.3390/ijms23063146
7. Gulati A, Agrawal N, Vibha D, et al (2021) Safety and Efficacy of Sovateltide (IRL-1620) in a Multicenter Randomized Controlled Clinical Trial in Patients with Acute Cerebral Ischemic Stroke. CNS Drugs 35:85–104. https://doi.org/10.1007/s40263-020-00783-9
8. Pluta R, Januszewski S, Czuczwar SJ (2022) Post-ischemic neurodegeneration of the hippocampus resembling alzheimer's disease proteinopathy. Int J Mol Sci 23:. https://doi.org/10.3390/ijms23010306
9. Tian J, Wang Y, Guo L, Li S (2023) Association of Income with Post-Stroke Cognition and the Underlying Neuroanatomical Mechanism. Brain Sci 13:. https://doi.org/10.3390/brainsci13020363
10. Jiang Y, Liu Z, Liao Y, et al (2022) Ischemic stroke: From pathological mechanisms to neuroprotective strategies. Front Neurol 13:. https://doi.org/10.3389/fneur.2022.1013083
11. Radenovic L (2024) Exploring therapeutical targets and innovative treatments for ischemic stroke: a comprehensive review. Explor Neuroprotective Ther 4:459–484.

https://doi.org/10.37349/ent.2024.00094
12. Majumder D (2024) Ischemic Stroke: Pathophysiology and Evolving Treatment Approaches. Neurosci Insights 19:1–8. https://doi.org/10.1177/26331055241292600
13. Hasanvand A (2022) The role of AMPK-dependent pathways in cellular and molecular mechanisms of metformin: a new perspective for treatment and prevention of diseases. Inflammopharmacology 30:775–788. https://doi.org/10.1007/s10787-022-00980-6
14. Ashabi G, Khodagholi F, Khalaj L, et al (2014) Activation of AMP-activated protein kinase by metformin protects against global cerebral ischemia in male rats: Interference of AMPK/PGC-1α pathway. Metab Brain Dis 29:47–58. https://doi.org/10.1007/s11011-013-9475-2
15. Jia J, Cheng J, Ni J, Zhen X (2015) Neuropharmacological Actions of Metformin in Stroke. Curr Neuropharmacol 13:389–394
16. Li J, Benashski SE, Venna V, McCullough LD (2010) Effect of Metfomrin in Experimental Stroke. Stroke 41:2645–2652. https://doi.org/10.1161/STROKEAHA.110.589697.Effects
17. Qin C, Yang S, Chu YH, et al (2022) Signaling pathways involved in ischemic stroke: molecular mechanisms and therapeutic interventions. Signal Transduct Target Ther 7:. https://doi.org/10.1038/s41392-022-01064-1
18. Woodruff TM, Thundyil J, Tang SC, et al (2011) Pathophysiology, treatment, and animal and cellular models of human ischemic stroke. Mol Neurodegener 6:11. https://doi.org/10.1186/1750-1326-6-11
19. Wu L, Xiong X, Wu X, et al (2020) Targeting Oxidative Stress and Inflammation to Prevent Ischemia-Reperfusion Injury. Front Mol Neurosci 13:1–13. https://doi.org/10.3389/fnmol.2020.00028
20. Ashabi G, Khalaj L, Khodagholi F, et al (2015) Pre-treatment with metformin activates Nrf2 antioxidant pathways and inhibits inflammatory responses through induction of AMPK after transient global cerebral ischemia. Metab Brain Dis 30:747–754. https://doi.org/10.1007/s11011-014-9632-2
21. Ogunmiluyi OE, Naiho AO, Emojevwe VO, et al (2024) Zinc or / and Vitamin E Supplementation Mitigates Oxidative Stress , Neuroinflammation , Neurochemical Changes and Behavioural Deficits in Male Wistar Rats Exposed to Bonny Light Crude Oil. J Toxicol 2024:. https://doi.org/10.1155/jt/9317271
22. Green D, O'Driscoll G, Rankin JM, et al (1998) Beneficial effect of vitamin E administration on nitric oxide function in subjects with hypercholesterolaemia. Clin Sci 95:361–367. https://doi.org/10.1042/CS19970304
23. Nguyen TTU, Yeom JH, Kim W (2021) Beneficial effects of vitamin e supplementation on endothelial dysfunction, inflammation, and oxidative stress biomarkers in patients receiving hemodialysis: A systematic review and meta-analysis of randomized controlled trials. Int J Mol Sci 22:. https://doi.org/10.3390/ijms222111923
24. Li J, Benashski SE, Venna VR, McCullough LD (2010) Effects of metformin in experimental stroke. Stroke 41:2645–2652. https://doi.org/10.1161/STROKEAHA.110.589697
25. Iwasaki Y, Ito S, Suzuki M, et al (1989) Forebrain ischemia induced by temporary bilateral common carotid occlusion in normotensive rats. J Neurol Sci 90:155–165. https://doi.org/10.1016/0022-510X(89)90098-1
26. Li Y, Chopp M, Chen J, et al (2000) Intrastriatal transplantation of bone marrow nonhematopoietic cells improves functional recovery after stroke in adult mice. J Cereb Blood Flow Metab 20:1311–1319. https://doi.org/10.1097/00004647-200009000-00006
27. Wahl F, Allix M, Plotkine M, Boulu RG (1992) Neurological and behavioral outcomes of focal cerebral ischemia in rats. Stroke 23:267–272. https://doi.org/10.1161/01.STR.23.2.267
28. Sharma AC, Kulkarni SK (1992) Evaluation of learning and memory mechanisms employing elevated plus-maze in rats and mice. Prog Neuropsychopharmacol Biol Psychiatry 16:117–125. https://doi.org/10.1016/0278-5846(92)90014-6
29. Panta A, Montgomery K, Nicolas M, et al (2020) Mir363-3p Treatment Attenuates Long-Term Cognitive Deficits Precipitated by an Ischemic Stroke in Middle-Aged Female Rats. Front Aging Neurosci 12:1–12. https://doi.org/10.3389/fnagi.2020.586362
30. Sivakumaran MH, Mackenzie AK, Callan IR, et al (2018) The Discrimination Ratio derived from Novel Object Recognition tasks as a Measure of Recognition Memory Sensitivity, not Bias. Sci Rep 8:1–12. https://doi.org/10.1038/s41598-018-30030-7
31. Bederson JB, Pitts LH, Germano SM, et al (1986) Evaluation of 2, 3, 5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats. Stroke 17:1304–1308. https://doi.org/10.1161/01.STR.17.6.1304
32. Draper HH, Hadley M (1990) Malondialdehyde determination as index of lipid Peroxidation. Methods

Enzymol 186:421–431. https://doi.org/10.1016/0076-6879(90)86135-I
33. Moron MS, Depierre JW, Mannervik B (1979) Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. BBA - Gen Subj 582:67–78. https://doi.org/10.1016/0304-4165(79)90289-7
34. Marklund S, Marklund G (1974) Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. Eur J Biochem 47:469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x
35. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275. https://doi.org/10.1016/s0021-9258(19)52451-6
36. Gürsoy-Özdemir Y, Bolay H, Saribaş O, Dalkara T (2000) Role of endothelial nitric oxide generation and peroxynitrite formation in reperfusion injury after focal cerebral ischemia. Stroke 31:1974–1981. https://doi.org/10.1161/01.STR.31.8.1974
37. Ronnett G V, Ramamurthy S, Kleman AM, et al (2009) AMPK in the Brain: Its Roles in Energy Balance and Neuroprotection. J Neurochem 109:17–23. https://doi.org/10.1111/j.1471-4159.2009.05916.x.AMPK
38. Jiang S, Li T, Ji T, et al (2018) AMPK: Potential therapeutic target for ischemic stroke. Theranostics 8:4535–4551. https://doi.org/10.7150/thno.25674
39. Li W, Yang S (2016) Targeting oxidative stress for the treatment of ischemic stroke: Upstream and downstream therapeutic strategies. Brain Circ 2:153. https://doi.org/10.4103/2394-8108.195279
40. Alshehri GH, Al-kuraishy HM, Alsfouk BA, et al (2025) The AMPK/GDF15 Axis: A Novel Target for the Neuroprotective Effects of Metformin in Ischemic Stroke. Mol Neurobiol. https://doi.org/10.1007/s12035-025-05126-7
41. Al-Sowayan NS, Abdullah Almarzougi R (2024) Vitamin E reduces oxidative stress in brains of male albino male rats undergoing immobilization. Saudi J Biol Sci 31:103900. https://doi.org/10.1016/j.sjbs.2023.103900
42. Wang Y, Hong F, Yang S (2022) Roles of Nitric Oxide in Brain Ischemia and Reperfusion. Int J Mol Sci 23:1–13. https://doi.org/10.3390/ijms23084243
43. Garry PS, Ezra M, Rowland MJ, et al (2015) The role of the nitric oxide pathway in brain injury and its treatment - From bench to bedside. Exp Neurol 263:235–243. https://doi.org/10.1016/j.expneurol.2014.10.017
44. Liu Y, Tang G, Li Y, et al (2014) Metformin attenuates blood-brain barrier disruption in mice following middle cerebral artery occlusion. J Neuroinflammation 11:177. https://doi.org/10.1186/s12974-014-0177-4
45. Kadry H, Noorani B, Bickel U, et al (2021) Comparative assessment of in vitro BBB tight junction integrity following exposure to cigarette smoke and e-cigarette vapor: a quantitative evaluation of the protective effects of metformin using small-molecular-weight paracellular markers. Fluids Barriers CNS 18:1–15. https://doi.org/10.1186/s12987-021-00261-4
46. Haghnejad Azar A, Oryan S, Bohlooli S, Panahpour H (2017) Alpha-Tocopherol Reduces Brain Edema and Protects Blood-Brain Barrier Integrity following Focal Cerebral Ischemia in Rats. Med Princ Pract 26:17–22. https://doi.org/10.1159/000450648