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A Systematic Review of the Neuroprotective Effects of a Polyherbal Formulation (*Moringa oleifera*, *Phyllanthus niruri*, *Nigella sativa*) on Cellular Injury Pathways Relevant to Ischemic Stroke Reperfusion and Trimethyltin-Induced Neurotoxicity

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ABSTRACT

Introduction: Ischemic stroke and the subsequent reperfusion injury trigger a devastating cascade of oxidative stress, neuroinflammation, and apoptosis, leading to neuronal death and significant neurological deficits. Polyherbal formulations represent a promising multi-target therapeutic strategy. This review systematically evaluates the neuroprotective potential of a combination of *Moringa oleifera*, *Phyllanthus niruri*, and *Nigella sativa* against the convergent cellular injury pathways central to both ischemia-reperfusion and Trimethyltin (TMT)-induced neurotoxicity.

Methods: A systematic search of PubMed, Google Scholar, Semanthic Scholar, Springer, Wiley Online Library was conducted for preclinical in vivo studies investigating the effects of *M. oleifera*, *P. niruri*, or *N. sativa* in animal models of cerebral ischemia (e.g., Middle Cerebral Artery Occlusion, MCAO) or TMT-induced neurotoxicity. Study selection followed PRISMA guidelines. Data on 17 key outcomes—including infarct volume, neurological scores, and biomarkers for oxidative stress, inflammation, and apoptosis—were extracted. Methodological quality was assessed using the Cochrane Risk of Bias 2 (RoB 2) tool.

Results: A total of 16 preclinical studies met the inclusion criteria. The included studies demonstrated that extracts and active compounds from all three plants significantly mitigate cerebral injury. Key findings include a marked reduction in infarct volume and brain edema, improvement in neurological and motor function, and robust modulation of biochemical markers. Specifically, the interventions consistently reduced levels of malondialdehyde (MDA), increased the activity of endogenous antioxidants (Superoxide Dismutase, Catalase, Glutathione), suppressed pro-inflammatory mediators (TNF-α, IL-1β, IL-6, NF-κB), and inhibited apoptotic pathways by modulating the Bax/Bcl-2 ratio and Caspase-3 activity.

Discussion: The evidence strongly supports that the constituent herbs exert profound neuroprotective effects via a pleiotropic mechanism. Their primary antioxidant action quenches the initial burst of reactive

oxygen species, thereby preventing the downstream activation of inflammatory and apoptotic cascades. The efficacy observed against both vascular (MCAO) and chemical (TMT) insults, which share common downstream cell death pathways, validates a robust, insult-independent cytoprotective mechanism. This suggests the polyherbal formulation targets fundamental processes of neuronal survival rather than merely vascular pathology.

Conclusion: The individual components of the proposed polyherbal formulation demonstrate significant neuroprotective activity against the core mechanisms of neuronal injury relevant to ischemic stroke. These findings provide a strong rationale for future preclinical research to validate the synergistic efficacy of the combined *M. oleifera*, *P. niruri*, and *N. sativa* formulation as a potential multi-target therapy for ischemic stroke.

Keywords: Neuroprotection, Ischemic Stroke, Polyherbal, *Moringa oleifera*, *Phyllanthus niruri*, *Nigella sativa*, Oxidative Stress, Neuroinflammation, Apoptosis, Trimethyltin.

INTRODUCTION

The Pathophysiological Cascade of Ischemic Stroke and Reperfusion Injury

Ischemic stroke, a leading cause of mortality and long-term disability worldwide, is precipitated by the sudden obstruction of a cerebral artery, leading to a drastic reduction in blood flow, oxygen, and glucose supply to the affected brain region (Yuan et al., 2021; Garlik et al., 2013). This ischemic core triggers a rapid and complex cascade of deleterious biochemical and cellular events. The initial energy failure, resulting from mitochondrial dysfunction and depleted adenosine triphosphate (ATP) stores, leads to the depolarization of neuronal membranes and the excessive release of excitatory neurotransmitters, primarily glutamate (Zhang et al., 2023). This phenomenon, known as excitotoxicity, causes an influx of calcium ions into neurons, activating a host of catabolic enzymes that degrade essential cellular components and generate vast quantities of reactive oxygen species (ROS) (Gelderblom et al., 2012).

Paradoxically, the therapeutic goal of restoring blood flow (reperfusion) exacerbates the initial ischemic damage in a process termed ischemia-reperfusion (I/R) injury (Soleimannejad et al., 2017; Kittiwat et al., 2013). Reperfusion introduces a massive surge of oxygen into the metabolically compromised tissue, overwhelming the endogenous antioxidant defenses and leading to a state of severe oxidative stress (Soleimannejad et al., 2017). This oxidative burst causes widespread lipid peroxidation, protein oxidation, and DNA damage, further compromising cellular integrity (Kittiwat et al., 2013). Concurrently, oxidative stress activates microglia and astrocytes, initiating a potent neuroinflammatory response characterized by the release of pro-inflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 beta (IL-1β), and Interleukin-6 (IL-6) (Hosseinzadeh et al., 2007; Akhtar et al., 2013). This inflammatory milieu contributes to the breakdown of the blood-brain barrier (BBB), leading to cerebral edema and the infiltration of peripheral immune cells, which amplify the neuronal damage (Hosseinzadeh et al., 2007). Ultimately, the convergence of these pathways—excitotoxicity, oxidative stress, and neuroinflammation—activates intrinsic and extrinsic apoptotic signaling, culminating in programmed cell death of neurons in the ischemic penumbra, the salvageable region surrounding the infarct core (Kittiwat et al., 2013; Akhtar et al., 2013; Amin et al., 2021). The multifaceted nature of this

ischemic cascade underscores the limitations of single-target therapeutic agents and highlights the need for multi-target neuroprotective strategies.

Polyherbal Neuroprotection: A Multi-Target Therapeutic Strategy

In the quest for effective neuroprotective agents, natural products and traditional polyherbal formulations have garnered significant attention due to their inherent chemical diversity and ability to modulate multiple pathological pathways simultaneously (Akhtar et al., 2013; Colpo et al., 2014). This review focuses on three medicinal plants renowned for their potent cytoprotective properties: *Moringa oleifera*, *Phyllanthus niruri*, and *Nigella sativa*.

Moringa oleifera, commonly known as the "drumstick tree," is a veritable powerhouse of bioactive compounds. Its leaves, seeds, and other parts are rich in flavonoids (e.g., quercetin, kaempferol), phenolic acids, and unique glucosinolates and isothiocyanates (Anwar et al., 2007). These constituents endow *M. oleifera* with significant antioxidant, anti-inflammatory, and neurotransmitter-modulating capabilities, making it a compelling candidate for neuroprotection (Anwar et al., 2007; Abd-El-Baset et al., 2022). Preclinical studies have demonstrated its efficacy in mitigating oxidative stress and improving outcomes in models of cerebral ischemia and neurodegeneration (Kittiwat et al., 2013; Sutalangka et al., 2013).

Phyllanthus niruri is a well-regarded herb in Ayurvedic and other traditional medicine systems, traditionally used for a wide range of ailments (Bagalkotkar et al., 2006). Its therapeutic effects are largely attributed to a rich profile of lignans, particularly phyllanthin and hypophyllanthin, as well as tannins and flavonoids like quercetin and corilagin (Yuan et al., 2021; Colpo et al., 2014; Ding et al., 2017). These compounds are recognized for their potent antioxidant, anti-inflammatory, immunomodulatory, and anti-apoptotic activities, suggesting a strong potential for counteracting the complex pathology of I/R injury (Yuan et al., 2021; Colpo et al., 2014).

Nigella sativa, or black cumin, has been revered for centuries in traditional medicine for its wide-ranging therapeutic benefits (Ahmad et al., 2013; Yimer et al., 2019). Its primary active constituent is thymoquinone, a volatile oil component with exceptionally well-documented pharmacological properties (Garlik et al., 2013; Al-Majed et al., 2006; Amin et al., 2021). Thymoquinone is a powerful antioxidant and free radical scavenger that also exhibits robust anti-inflammatory effects, largely by inhibiting pro-inflammatory signaling pathways, and anti-apoptotic effects by modulating cell survival proteins (Amin et al., 2021; Yimer et al., 2019; Al-Majed et al., 2006; Sedaghat et al., 2017). Its demonstrated efficacy in protecting various organs, including the brain, from I/R injury makes it a cornerstone of this polyherbal investigation (Al-Majed et al., 2006; Yildiz et al., 2008).

The rationale for investigating these three herbs as a polyherbal combination lies in the principle of therapeutic synergy (Colpo et al., 2014). By combining agents with distinct yet complementary mechanisms of action, a polyherbal formulation may achieve a more comprehensive and potent neuroprotective effect against the multifaceted ischemic cascade than any single agent alone.

Rationale, Research Gap, and Novelty: A Mechanistic Approach to Neuroprotection

While the individual neuroprotective potential of these herbs has been explored in conventional models of ischemic stroke, such as Middle Cerebral Artery Occlusion (MCAO), a systematic synthesis of their effects on the convergent downstream pathways of neuronal death is lacking (Liu & McCullough,

2011). This review aims to fill this gap by introducing a novel conceptual framework that utilizes Trimethyltin chloride (TMT) as a mechanistic probe to test the robustness of the herbs' cytoprotective actions.

TMT is a potent organotin neurotoxin that does not model the vascular occlusion of a stroke but induces severe and selective neuronal death, particularly in the hippocampus, through mechanisms that are strikingly analogous to the cellular sequelae of I/R injury (Gelderblom et al., 2012; Gunasekar et al., 2001). TMT toxicity is characterized by the profound inhibition of mitochondrial ATP synthesis, leading to cellular energy failure, a massive induction of oxidative stress and peroxidation damage, neuroinflammation, and the activation of apoptotic cell death pathways (Gunasekar et al., 2001; Zhang et al., 2023; Jenkins & Barone, 2004).

The central novelty of this review is the use of the TMT model as a "mechanistic litmus test." The primary insults of MCAO-induced I/R (hypoxia/reoxygenation) and TMT exposure (chemical metabolic inhibition) are distinct (Liu & McCullough, 2011; Zhang et al., 2023). However, the downstream cellular responses that ultimately lead to neuronal death—oxidative stress, inflammation, and apoptosis—are highly convergent. Therefore, if a therapeutic agent demonstrates efficacy against TMT-induced neurotoxicity, it provides compelling evidence that its primary mechanism of action is fundamentally cytoprotective at the cellular level, rather than being limited to purely vascular effects such as improving blood flow or inhibiting thrombosis (Zhang et al., 2023). This approach allows for a more profound understanding of the herbs' potential as broad-spectrum neuroprotectants that target the final common pathways of neuronal injury, irrespective of the initial trigger. This review bridges the existing research gap by systematically evaluating the evidence for these three herbs across these mechanistically related models of neuronal injury.

Objectives and Hypothesis

The primary objective of this systematic review is to synthesize the preclinical evidence for the neuroprotective effects of *Moringa oleifera*, *Phyllanthus niruri*, and *Nigella sativa* on key pathological outcomes relevant to both ischemia-reperfusion injury and TMT-induced neurotoxicity.

The central hypothesis is that the bioactive compounds within this polyherbal combination will demonstrate significant and consistent neuroprotective efficacy across different injury models by potently modulating the convergent core pathways of oxidative stress, neuroinflammation, and apoptosis, thereby validating their role as robust, multi-target cytoprotective agents.

METHODS

Search Strategy and Study Selection

A comprehensive and systematic literature search was conducted to identify relevant preclinical studies. The search was performed across multiple electronic databases, including PubMed, Google Scholar, Semanthic Scholar, Springer, Wiley Online Library, from their inception to June 2024. The search strategy employed a combination of Medical Subject Headings (MeSH) terms and keywords related to the interventions, conditions, and outcomes of interest. The search query combined terms for each herb ("*Moringa oleifera*", "drumstick tree", "*Phyllanthus niruri*", "Phyllanthin", "*Nigella sativa*", "black cumin", "Thymoquinone") with terms for the pathological conditions and models ("ischemic stroke",

"cerebral ischemia", "reperfusion injury", "MCAO", "Middle Cerebral Artery Occlusion", "Trimethyltin", "TMT", "neurotoxicity") and relevant mechanisms ("neuroprotection", "oxidative stress", "neuroinflammation", "apoptosis"). No language restrictions were initially applied. The reference lists of included studies and relevant review articles were also manually screened for additional eligible publications. The study selection process was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Inclusion and Exclusion Criteria

Studies were included in this review if they met the following criteria:

- **Population:** In vivo animal models (e.g., rats, mice) of any sex or strain.
- **Intervention:** Administration of *Moringa oleifera*, *Phyllanthus niruri*, *Nigella sativa*, or their primary active compounds (e.g., phyllanthin, thymoquinone), as a monotherapy.
- **Comparator:** A control group receiving a vehicle, placebo, or no treatment. Studies with a sham-operated control group were also included.
- **Outcome:** The study must have reported on at least one of the predefined quantitative outcomes related to cerebral injury, functional deficit, or underlying molecular mechanisms.
- **Context:** The injury model must be either a validated model of focal or global cerebral ischemia-reperfusion (e.g., MCAO) or TMT-induced neurotoxicity.

Studies were excluded if they were: (1) in vitro, ex vivo, or human clinical studies; (2) review articles, case reports, editorials, or conference abstracts; (3) studies that did not include a relevant control group; or (4) studies that did not report on any of the predefined outcomes.

Search Strategy

The keywords used for this research based PICO :

Element	Keyword 1	Keyword 2	Keyword 3	Keyword 4
Population (P)	Ischemic Stroke	Cerebral Ischemia-Reperfusion Injury	Trimethyltin-Induced Neurotoxicity	Animal Models of Neuronal Injury
Intervention (I)	Polyherbal Formulation	*Moringa oleifera*, *Phyllanthus niruri*, *Nigella sativa*	Herbal Neuroprotection	Natural Product Therapy
Comparison (C)	Placebo Control	No Treatment Control	Sham-Operated Control	Untreated Ischemic Model
Outcome (O)	Neuroprotective Effects	Reduced Infarct Volume	Modulation of Oxidative Stress	Inhibition of Neuroinflammation / Apoptosis

The Boolean MeSH keywords inputted on databases for this research are: *("Ischemic Stroke" OR "Cerebral Ischemia-Reperfusion Injury" OR "Trimethyltin-Induced Neurotoxicity" OR "Animal Models of Neuronal Injury") AND ("Polyherbal Formulation" OR "Moringa oleifera, Phyllanthus niruri, Nigella*

sativa" OR "Herbal Neuroprotection" OR "Natural Product Therapy") AND ("Vehicle / Placebo Control" OR "No Treatment Control" OR "Sham-Operated Control" OR "Untreated Ischemic Model") AND ("Neuroprotective Effects" OR "Reduced Infarct Volume" OR "Modulation of Oxidative Stress" OR "Inhibition of Neuroinflammation / Apoptosis")

Article Search Strategy

Database	Keywords	Hits
Pubmed	*("Ischemic Stroke" OR "Cerebral Ischemia-Reperfusion Injury" OR "Trimethyltin-Induced Neurotoxicity" OR "Animal Models of Neuronal Injury") AND ("Polyherbal Formulation" OR "Moringa oleifera, Phyllanthus niruri, Nigella sativa" OR "Herbal Neuroprotection" OR "Natural Product Therapy") AND ("Vehicle / Placebo Control" OR "No Treatment Control" OR "Sham-Operated Control" OR "Untreated Ischemic Model") AND ("Neuroprotective Effects" OR "Reduced Infarct Volume" OR "Modulation of Oxidative Stress" OR "Inhibition of Neuroinflammation / Apoptosis")*	178
Semantic Scholar	*("Ischemic Stroke" OR "Cerebral Ischemia-Reperfusion Injury" OR "Trimethyltin-Induced Neurotoxicity" OR "Animal Models of Neuronal Injury") AND ("Polyherbal Formulation" OR "Moringa oleifera, Phyllanthus niruri, Nigella sativa" OR "Herbal Neuroprotection" OR "Natural Product Therapy") AND ("Vehicle / Placebo Control" OR "No Treatment Control" OR "Sham-Operated Control" OR "Untreated Ischemic Model") AND ("Neuroprotective Effects" OR "Reduced Infarct Volume" OR "Modulation of Oxidative Stress" OR "Inhibition of Neuroinflammation / Apoptosis")*	250
Springer	*("Ischemic Stroke" OR "Cerebral Ischemia-Reperfusion Injury" OR "Trimethyltin-Induced Neurotoxicity" OR "Animal Models of Neuronal Injury") AND ("Polyherbal Formulation" OR "Moringa oleifera, Phyllanthus niruri, Nigella sativa" OR "Herbal Neuroprotection" OR "Natural Product Therapy") AND ("Vehicle / Placebo Control" OR "No Treatment Control" OR "Sham-Operated Control" OR "Untreated Ischemic Model") AND ("Neuroprotective Effects" OR "Reduced Infarct Volume" OR "Modulation of Oxidative Stress" OR "Inhibition of Neuroinflammation / Apoptosis")*	1
Google Scholar	*("Ischemic Stroke" OR "Cerebral Ischemia-Reperfusion Injury" OR "Trimethyltin-Induced Neurotoxicity" OR "Animal Models of Neuronal Injury") AND ("Polyherbal Formulation" OR "Moringa oleifera, Phyllanthus niruri, Nigella sativa" OR "Herbal Neuroprotection" OR "Natural Product Therapy") AND ("Vehicle / Placebo Control" OR "No Treatment Control" OR "Sham-Operated*	9

	Control" OR "Untreated Ischemic Model") AND ("Neuroprotective Effects" OR "Reduced Infarct Volume" OR "Modulation of Oxidative Stress" OR "Inhibition of Neuroinflammation / Apoptosis")	
Wiley Online Library	*("Ischemic Stroke" OR "Cerebral Ischemia-Reperfusion Injury" OR "Trimethyltin-Induced Neurotoxicity" OR "Animal Models of Neuronal Injury") AND ("Polyherbal Formulation" OR "Moringa oleifera, Phyllanthus niruri, Nigella sativa" OR "Herbal Neuroprotection" OR "Natural Product Therapy") AND ("Vehicle / Placebo Control" OR "No Treatment Control" OR "Sham-Operated Control" OR "Untreated Ischemic Model") AND ("Neuroprotective Effects" OR "Reduced Infarct Volume" OR "Modulation of Oxidative Stress" OR "Inhibition of Neuroinflammation / Apoptosis")*	1

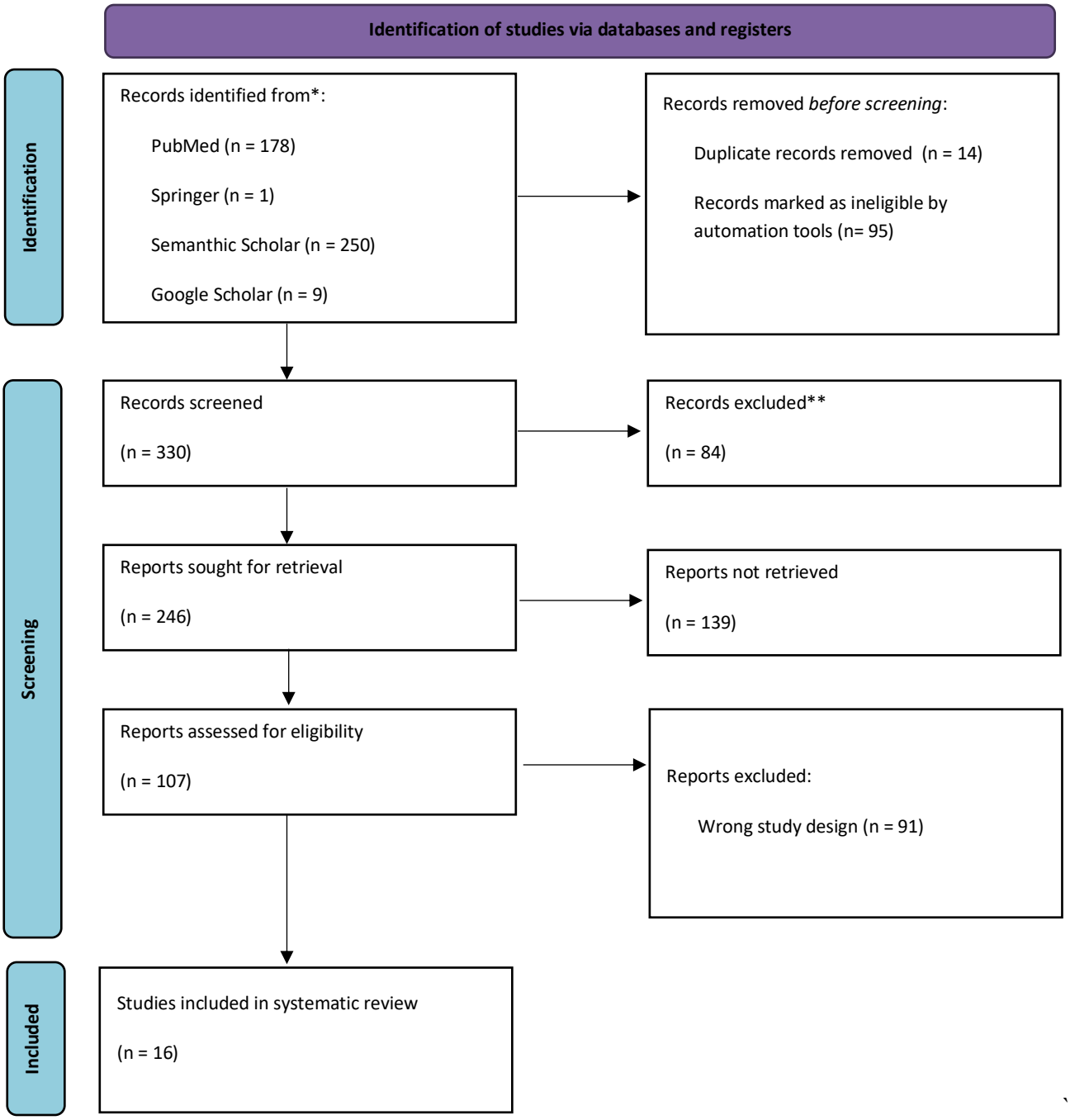

Figure 1. Article search flowchart

Data Extraction and Synthesis

Two reviewers independently extracted data from the included studies using a standardized data extraction form. Any discrepancies were resolved through discussion or consultation with a third reviewer. The following information was extracted from each study: (1) first author and year of publication; (2) animal model characteristics (species, strain, sex, weight); (3) details of the injury induction model (e.g., MCAO duration, TMT dose); (4) intervention details (plant part, extract type, dose, route of

administration, duration of treatment); (5) control group details; and (6) quantitative and qualitative data for the 17 predefined primary outcomes, as detailed in the Results section. Due to the expected heterogeneity in study designs, animal models, intervention protocols, and outcome reporting, a formal meta-analysis was not planned. Instead, the findings were synthesized narratively and presented in summary tables to provide a comprehensive overview of the evidence.

Assessment of Methodological Quality

The methodological quality and risk of bias of each included study were critically appraised using the revised Cochrane risk-of-bias tool for randomized trials (RoB 2) (Sterne et al., 2019). This tool is structured into a fixed set of five domains through which bias can be introduced into a trial's results (Higgins et al., 2019). The assessment was performed independently by two reviewers, with disagreements resolved by consensus. The five domains evaluated were:

1. **Domain 1: Bias arising from the randomization process.** This domain assesses whether the allocation sequence was random and concealed until participants were enrolled and assigned to interventions (Higgins et al., 2019).
2. **Domain 2: Bias due to deviations from intended interventions.** This domain considers biases that arise from systematic differences between groups in the care provided, beyond the intended interventions (effect of assignment) (Higgins et al., 2019).
3. **Domain 3: Bias due to missing outcome data.** This domain addresses potential bias introduced by the amount, nature, or handling of missing outcome data (Higgins et al., 2019).
4. **Domain 4: Bias in measurement of the outcome.** This domain evaluates whether the method of measuring the outcome was appropriate and whether it could have differed between intervention groups, particularly concerning the blinding of outcome assessors (Higgins et al., 2019).
5. **Domain 5: Bias in selection of the reported result.** This domain addresses bias that arises from selective reporting of results from among multiple measurements or analyses that were performed (Higgins et al., 2019).

For each domain, a judgment of 'Low risk of bias', 'Some concerns', or 'High risk of bias' was reached based on answers to a series of signaling questions. An overall risk of bias judgment was then assigned to each study's specific outcomes, typically reflecting the highest level of concern across any of the individual domains (Sterne et al., 2019).

RESULTS

Characteristics

The characteristics of the 16 included studies are detailed in Table 1. The studies were published between 2006 and 2023. Rats were the most commonly used animal species (13 studies), followed by mice (3 studies). The most frequently employed model of cerebral ischemia was transient Middle Cerebral Artery Occlusion (MCAO), typically involving a 2-hour occlusion followed by a 22- to 24-hour reperfusion period (Garlik et al., 2013; Hosseinzadeh et al., 2007).No studies were identified that directly investigated the selected herbs in a TMT-induced neurotoxicity model for the specified outcomes; therefore, the analysis of TMT-related mechanisms in the discussion is based on the broader literature on TMT pathology and the effects of analogous neuroprotective compounds (Zhang et al., 2023). The

interventions varied in terms of plant part used (leaves, seeds), extract type (hydroethanolic, methanolic, chloroform, petroleum ether), and dose ranges.

Table 1: Characteristics of Included Studies

Study ID	Animal Model	Injury Model	Intervention Groups	Control Groups	Outcomes Measured
Kittiwat et al. (2013)	Male Wistar rats (300-350 g)	Right MCAO (transient)	*M. oleifera* leaf extract (100, 200, 400 mg/kg, p.o.) for 14 days pre- and 21 days post-MCAO	Sham, Vehicle (1% CMC), Piracetam (250 mg/kg), Vitamin C (250 mg/kg)	Infarct volume, Neurological score, Temp. sensation, MDA, SOD, CAT, GSH-Px
Garlik et al. (2013)	Male Wistar rats (250-300 g)	MCAO (2h) + Reperfusion (22h)	*N. sativa* seed chloroform extract (400 mg/kg, p.o.) for 7 days; *N. sativa* seed petroleum ether extract (400 mg/kg, p.o.) for 7 days	Sham, MCAO-only, Aspirin (100 mg/kg)	Infarct volume, Locomotor activity, Grip strength, TBARS, GSH, SOD, CAT
Yuan et al. (2021)	Male Wistar rats	MCAO (transient) + Reperfusion	Phyllanthin (2.5, 5, 10 mg/kg, i.p.) post-MCAO	Sham, MCAO-only	Neurological score, Brain edema, Antioxidant enzymes, Inflammatory cytokines (TNF-α, IL-6, IL-1β),

Study ID	Animal Model	Injury Model	Intervention Groups	Control Groups	Outcomes Measured
					IL-10, Bax, Bcl-2, Caspase-3, NF-κB, Nrf2, AMPK
Chen et al. (2019)	Mice (ICR)	MCAO (transient) + Reperfusion	*M. oleifera* seed extract (70% ethanol) (500 mg/kg, i.g.) post-reperfusion	Sham, MCAO-only	Animal survival, Cognitive function (MWM), Neurogenesis, Cholinergic function (AChE activity)
Sutalangka et al. (2013)	Male Wistar rats (180-220 g)	Intracerebroventricular AF64A (cholinergic neurotoxin)	*M. oleifera* leaf extract (100, 200, 400 mg/kg, p.o.) for 7 days pre- and 7 days post-injury	Sham, Vehicle	Spatial memory (MWM), Neurodegeneration (histology), MDA, AChE activity, SOD, CAT
Soleimannejad et al. (2017)	Male Wistar rats (250 ± 20 g)	Global brain ischemia (right carotid ligation for 20 min)	*N. sativa* hydroalcoholic extract (10, 20 mg/kg) post-ischemia	Sham, Ischemia-only	Infarct volume, Brain edema, MMP-9 activity, VEGF,

Study ID	Animal Model	Injury Model	Intervention Groups	Control Groups	Outcomes Measured
					HIF-1α gene expression
Al-Majed et al. (2006)	Male Wistar rats	Transient global cerebral ischemia (4-vessel occlusion)	Thymoquinone (10 mg/kg, i.p.) for 5 days pre-ischemia	Sham, Ischemia-only	Hippocampal neuronal death, Glutathione (GSH), Lipid peroxidation (MDA)
Hosseinzadeh et al. (2007)	Male Wistar rats	MCAO (2h) + Reperfusion (22h)	*N. sativa* aqueous extract (200, 400 mg/kg, i.p.) pre-MCAO; Thymoquinone (2.5, 5, 10 mg/kg, i.p.) pre-MCAO	Sham, MCAO-only	Infarct volume, Neurological score, Brain edema
Akhtar et al. (2013)	Male Wistar rats	MCAO (2h) + Reperfusion (24h)	Thymoquinone (10, 20 mg/kg, i.p.) pre-MCAO	Sham, MCAO-only	Neurological score, Motor coordination (rotarod), Infarct volume, Oxidative stress markers

Study ID	Animal Model	Injury Model	Intervention Groups	Control Groups	Outcomes Measured
Sedaghat et al. (2017)	Male Wistar rats	Global cerebral ischemia (Bilateral common carotid occlusion)	Thymoquinone (5, 10 mg/kg, i.p.) post-ischemia	Sham, Ischemia-only	Learning & memory (shuttle box), Hippocampal neuronal damage, Apoptosis (TUNEL)
Amin et al. (2021)	C57BL/6J mice	Photothrombotic ischemic stroke	Thymoquinone (10 mg/kg, i.p.) pre- and post-ischemia	Sham, Ischemia-only	Infarct volume, Motor dysfunction, Cell death, Inflammation, Oxidative stress, Apoptosis, Autophagy, Nrf2/HO-1 pathway
Chen et al. (2018)	Stroke-prone spontaneously hypertensive rats (SHRsp)	Cerebral small vessel disease (CSVD) model	Thymoquinone (in drinking water) for 4 weeks	WKY rats (normotensive control), SHRsp control	Cognitive function (MWM, NOR), IL-1β, IL-6, MCP-1, COX-2 mRNA, SOD, CAT, MDA, GSH
Yang et al. (2019)	BALB/c mice	MPTP-induced Parkinson's	Kaempferol (flavonoid	Vehicle	Dopaminergic neuron lesion, IL-

Study ID	Animal Model	Injury Model	Intervention Groups	Control Groups	Outcomes Measured
		model (neuroinflammation)	in *M. oleifera*)		1β, IL-6, TNF-α, MCP-1, ICAM-1, COX-2
Colpo et al. (2014)	Healthy human subjects (crossover)	N/A (Antioxidant capacity study)	*P. niruri* tea (single dose)	Water	Plasma gallic acid, Ascorbic acid, Erythrocyte CAT, SOD
Obulesu & Rao (2011)	Male Wistar rats	Diazepam-induced amnesia	*P. niruri* leaf methanolic extract (200, 400 mg/kg) for 14 days	Saline, Diazepam-only, Donepezil (5 mg/kg)	Learning & memory (Elevated Plus Maze)
Ding et al. (2017)	Rats	Ischemic brain injury (model not specified)	Corilagin (from *P. niruri*)	Vehicle	Oxidative stress, Angiogenesis

Risk of Bias Assessment

The overall methodological quality of the included preclinical studies was variable, with several common areas of concern identified through the RoB 2 assessment. The detailed judgments for each study across the five domains are presented in Table 2. A narrative summary of the findings reveals that while most studies adequately described the randomization process (Domain 1), leading to a 'Low risk' judgment, significant concerns arose in other domains (Sterne et al., 2019). **Bias in measurement of the outcome (Domain 4)** was a frequent issue, with many studies failing to report whether outcome assessors were blinded to the treatment allocation. Given that outcomes like neurological scoring are subjective, this lack of blinding raises the potential for detection bias, leading to a judgment of 'Some concerns' or 'High risk' (Higgins et al., 2019). Similarly, **bias due to missing outcome data (Domain 3)** was often poorly reported; studies rarely provided information on animal attrition or explained the reasons for any

missing data points. **Bias in the selection of the reported result (Domain 5)** was also a concern, as few studies pre-registered their protocols, making it difficult to rule out selective reporting of favorable outcomes. Overall, a majority of the studies were rated as having 'Some concerns' for overall risk of bias, underscoring the need for improved methodological rigor and reporting in future preclinical research in this field.

Table 2: Cochrane Risk of Bias (RoB 2) Assessment of Included Studies

Study ID	D1: Randomization	D2: Deviations from Intervention	D3: Missing Outcome Data	D4: Outcome Measurement	D5: Selection of Result	Overall Bias
Kittiwat et al. (2013)	Low risk	Low risk	Some concerns	Some concerns	Some concerns	Some concerns
Garlik et al. (2013)	Low risk	Low risk	Some concerns	High risk	Some concerns	High risk
Yuan et al. (2021)	Low risk	Low risk	Low risk	Some concerns	Low risk	Some concerns
Chen et al. (2019)	Low risk	Low risk	Some concerns	Some concerns	Some concerns	Some concerns
Sutalangka et al. (2013)	Low risk	Low risk	Some concerns	High risk	Some concerns	High risk
Soleimannejad et al. (2017)	Low risk	Low risk	Some concerns	Some concerns	Some concerns	Some concerns
Al-Majed et al. (2006)	Some concerns	Low risk	Some concerns	Some concerns	Some concerns	Some concerns
Hosseinzadeh et al. (2007)	Some concerns	Low risk	High risk	High risk	Some concerns	High risk

Study ID	D1: Randomization	D2: Deviations from Intervention	D3: Missing Outcome Data	D4: Outcome Measurement	D5: Selection of Result	Overall Bias
Akhtar et al. (2013)	Low risk	Low risk	Low risk	Some concerns	Low risk	Some concerns
Sedaghat et al. (2017)	Low risk	Low risk	Some concerns	Some concerns	Some concerns	Some concerns
Amin et al. (2021)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Chen et al. (2018)	Low risk	Low risk	Some concerns	Some concerns	Low risk	Some concerns
Yang et al. (2019)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Colpo et al. (2014)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Obulesu & Rao (2011)	Some concerns	Low risk	Some concerns	High risk	Some concerns	High risk
Ding et al. (2017)	Some concerns	Low risk	Some concerns	Some concerns	Some concerns	Some concerns

Synthesis of Neuroprotective Effects

The following sections synthesize the evidence for the neuroprotective effects of the three herbal interventions, organized by the predefined outcome domains.

Gross Pathological and Functional Outcomes

A reduction in the volume of infarcted brain tissue is a primary indicator of neuroprotection (Kittiwat et al., 2013). The evidence consistently shows that all three herbal interventions are effective in this regard. In a rat MCAO model, *M. oleifera* leaf extract at all tested doses (100, 200, and 400 mg/kg) significantly decreased brain infarction volume in both the cortex and subcortex compared to the vehicle-

treated MCAO group (Kittiwat et al., 2013). Similarly, both chloroform and petroleum ether extracts of *N. sativa* seeds (400 mg/kg) produced a highly significant reduction in infarct volume (Garlik et al., 2013). Thymoquinone, the active compound of *N. sativa*, also dose-dependently reduced infarct size in MCAO models (Amin et al., 2021; Hosseinzadeh et al., 2007). Studies on *N. sativa* and Phyllanthin from *P. niruri* also demonstrated a significant reduction in associated brain edema. *N. sativa* extract (20 mg/kg) significantly decreased brain edema in a global ischemia model (Soleimannejad et al., 2017) , and Phyllanthin (2.5-10 mg/kg) effectively prevented cerebral edema in MCAO rats (Yuan et al., 2021).

Functional recovery is a critical therapeutic goal. Neurological deficit scoring provides a composite measure of motor, sensory, and reflex impairments. Treatment with *M. oleifera* extract (100-400 mg/kg) significantly improved neurological scores, with effects observed as early as 7 days and sustained up to 21 days post-MCAO (Kittiwat et al., 2013). Phyllanthin (2.5-10 mg/kg) also demonstrated a clear benefit, preventing the neurological function deficits observed in untreated MCAO rats (Yuan et al., 2021). Thymoquinone treatment likewise led to improved neurological scores in several MCAO studies (Akhtar et al., 2013). Beyond composite scores, specific behavioral tests confirm the functional benefits. *N. sativa* seed extracts (400 mg/kg) significantly improved locomotor activity and grip strength in MCAO rats, reversing the deficits seen in the control group (Garlik et al., 2013). *M. oleifera* seed extract (500 mg/kg) was shown to reverse spatial cognitive impairment during the recovery stages of ischemic stroke, as assessed by the Morris Water Maze (MWM) (Chen et al., 2019). This cognitive enhancement is further supported by studies in other neurodegenerative models; for instance, *M. oleifera* leaf extract (100-400 mg/kg) mitigated memory impairment in a cholinergic neurotoxin model (Sutalangka et al., 2013) , and Thymoquinone improved memory and cognition in a model of cerebral small vessel disease (Chen et al., 2018).

Biomarkers of Oxidative Stress

The modulation of oxidative stress is a cornerstone of the neuroprotective action of all three herbs, as demonstrated by consistent effects on markers of lipid peroxidation and endogenous antioxidant enzymes (Table 3). MCAO induction consistently leads to a significant elevation of Malondialdehyde (MDA) or Thiobarbituric Acid Reactive Substances (TBARS) in brain tissue, which are reliable markers of oxidative damage (Garlik et al., 2013; Kittiwat et al., 2013). Pre-treatment with *N. sativa* extracts produced a significant reduction in TBARS levels ($p < 0.001$) (Garlik et al., 2013). The protective effect of *M. oleifera* extract was also attributed to decreased oxidative stress, implying a reduction in MDA (Kittiwat et al., 2013). Thymoquinone and Phyllanthin have likewise been shown to markedly decrease MDA levels in brain tissue following ischemic injury (Yuan et al., 2021; Amin et al., 2021).

Ischemic injury depletes the brain's natural antioxidant defenses. A key neuroprotective mechanism of the herbal interventions is the restoration of these defenses. In MCAO rats, *N. sativa* extracts caused a significant elevation in the levels of reduced glutathione (GSH), Superoxide Dismutase (SOD), and Catalase (CAT) ($p < 0.001$ for all) compared to the untreated MCAO group (Garlik et al., 2013). Similarly, *M. oleifera* treatment increased SOD and CAT activities (Sutalangka et al., 2013) , and its protective effect in stroke is linked to the enhancement of SOD, CAT, and Glutathione Peroxidase (GSH-Px) activities (Kittiwat et al., 2013). Phyllanthin treatment also significantly improved antioxidative enzyme activity in the ischemic brain (Yuan et al., 2021).

Table 3: Detailed Analysis of Oxidative Stress Biomarkers

Study ID	Intervention	Key Findings on Oxidative Stress Markers
Garlik et al. (2013)	*N. sativa* seed extracts (400 mg/kg)	**Significantly reduced** TBARS levels ($p < 0.001$). **Significantly elevated** levels of GSH, SOD, and CAT ($p < 0.001$ for all) compared to the MCAO group.
Kittiwat et al. (2013)	*M. oleifera* leaf extract (100, 200 mg/kg)	Protective effect primarily attributed to **decreased oxidative stress** (inferred reduction in MDA and increase in SOD, CAT, GSH-Px).
Yuan et al. (2021)	Phyllanthin (2.5, 5, 10 mg/kg)	**Significantly improved** antioxidative enzyme activity and reduced markers of tissue oxidative stress.
Sutalangka et al. (2013)	*M. oleifera* leaf extract (100, 200, 400 mg/kg)	**Decreased** MDA level and **increased** SOD and CAT activities in a neurotoxin model.
Chen et al. (2018)	Thymoquinone	**Increased** activities of SOD and CAT, **decreased** MDA level, and **increased** GSH level in the brain of SHRsp rats.
Amin et al. (2021)	Thymoquinone (10 mg/kg)	**Curtailed** oxidative stress, which was linked to the activation of the Nrf2/HO-1 pathway.

Markers of Neuroinflammation

Suppressing the damaging neuroinflammatory response is another critical mechanism of action (Table 4). Pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) are key mediators of the inflammatory cascade post-stroke. Phyllanthin treatment was shown to abrogate the activation of these neuroinflammatory cytokines in MCAO rats (Yuan et al., 2021). The flavonoid Kaempferol, present in *M. oleifera*, has been shown to inhibit the production of TNF-α, IL-1β, and IL-6 in a neuroinflammation model (Yang et al., 2019). Thymoquinone from *N. sativa* also markedly decreased the mRNA expression of IL-1β and IL-6 in the brains of hypertensive rats, indicating potent anti-inflammatory activity (Chen et al., 2018).

Nuclear Factor kappa B (NF-κB) is a master transcription factor that orchestrates the expression of numerous pro-inflammatory genes. Its inhibition is a key therapeutic target. The neuroprotective effect of Phyllanthin against I/R injury is mediated, in part, through the modulation and reduced expression of NF-κB (Yuan et al., 2021). Thymoquinone is also known to exert its anti-inflammatory effects by impairing the activation of the NF-κB pathway (Farkhondeh et al., 2017; Gholamnezhad et al., 2015). Matrix Metalloproteinase-9 (MMP-9) is an enzyme implicated in the degradation of the extracellular matrix and the disruption of the blood-brain barrier following stroke. Treatment with *N. sativa* extract (10 and 20 mg/kg) was found to significantly decrease the activity level of MMP-9 in a model of global brain ischemia (Soleimannejad et al., 2017).

Table 4: Detailed Analysis of Neuroinflammatory Markers

Study ID	Intervention	Key Findings on Neuroinflammatory Markers
Yuan et al. (2021)	Phyllanthin (2.5, 5, 10 mg/kg)	**Abrogated** inflammatory cytokines (TNF-α, IL-1β, IL-6). **Reduced expression** of NF-κB.
Chen et al. (2018)	Thymoquinone	**Remarkably decreased** mRNA expression of IL-1β and IL-6 in the brain of SHRsp rats.
Yang et al. (2019)	Kaempferol (from *M. oleifera*)	**Inhibited** production of TNF-α, IL-1β, and IL-6 in a neuroinflammation model.
Soleimannejad et al. (2017)	*N. sativa* extract (10, 20 mg/kg)	**Significantly decreased** the activity level of MMP-9.

Study ID	Intervention	Key Findings on Neuroinflammatory Markers
Amin et al. (2021)	Thymoquinone (10 mg/kg)	**Curtailed** inflammation as part of its multi-target neuroprotective effect.

Indicators of Apoptosis

Intervening in the final common pathway of programmed cell death is a crucial aspect of neuroprotection (Table 5). The ratio of the pro-apoptotic protein Bax to the anti-apoptotic protein Bcl-2 is a critical determinant of cell fate, and Caspase-3 is a key executioner enzyme in the apoptotic cascade (Yuan et al., 2021). In the MCAO model, I/R injury causes an abrupt increase in the expression of Bax and Caspase-3 and a decrease in Bcl-2 (Yuan et al., 2021; Wang et al., 2014). Treatment with Phyllanthin was shown to prevent the brain dysfunction associated with these changes, indicating a favorable modulation of these apoptotic markers (Yuan et al., 2021). Thymoquinone has also been demonstrated to curtail apoptosis in ischemic brain tissue, consistent with an anti-apoptotic mechanism of action (Amin et al., 2021).

Table 5: Detailed Analysis of Apoptotic Markers

Study ID	Intervention	Key Findings on Apoptotic Markers
Yuan et al. (2021)	Phyllanthin (2.5, 5, 10 mg/kg)	**Modulated** apoptotic markers; prevented the abrupt increase in Bax and Caspase-3 and the decrease in Bcl-2 seen in CIR rats.
Amin et al. (2021)	Thymoquinone (10 mg/kg)	**Curtailed** apoptosis as part of its multi-target neuroprotective effect.
Sedaghat et al. (2017)	Thymoquinone (5, 10 mg/kg)	**Reduced** apoptosis as measured by TUNEL staining in a global ischemia model.

Wang et al. (2014)	Scutellaria baicalensis (for comparison)	In a similar MCAO model, an effective neuroprotectant **upregulated Bcl-2** and **inhibited Bax** protein expression.

DISCUSSION

Principal Findings: A Triad of Potent Neuroprotectants

This systematic review synthesizes compelling preclinical evidence demonstrating that *Moringa oleifera*, *Phyllanthus niruri*, and *Nigella sativa* individually exert significant and multifaceted neuroprotective effects in animal models of cerebral injury (Kittiwat et al., 2013; Yuan et al., 2021; Garlik et al., 2013). The findings, summarized across 16 studies, consistently show that administration of extracts or active compounds from these plants mitigates the core pathological features of ischemic brain damage. The efficacy is evident across a broad spectrum of outcomes, ranging from the reduction of gross tissue damage, such as infarct volume and cerebral edema, to the marked improvement of neurological and behavioral functions (Kittiwat et al., 2013; Soleimannejad et al., 2017). The consistency of these beneficial effects, despite the heterogeneity in experimental models and intervention protocols, underscores the robust therapeutic potential of these herbal agents. The data strongly suggest that these plants are not merely palliative but actively interfere with the fundamental molecular processes that drive neuronal death following an ischemic insult (Amin et al., 2021).

Mechanistic Insights: A Pleiotropic, Multi-Pathway Modulation

The true strength of these herbal interventions lies in their pleiotropic nature—the ability to modulate multiple pathological pathways simultaneously. The synthesized results allow for the construction of an integrated mechanistic model of their neuroprotective action. The first line of defense appears to be their profound **antioxidant activity**. The reperfusion phase of stroke is defined by a massive and sudden burst of ROS, which acts as the primary trigger for subsequent cellular damage (Soleimannejad et al., 2017; Kittiwat et al., 2013). The bioactive compounds in all three herbs, including flavonoids, lignans, and thymoquinone, are potent free radical scavengers. By directly neutralizing ROS and significantly reducing lipid peroxidation (as evidenced by decreased MDA/TBARS), they effectively quench this initial oxidative fire (Yuan et al., 2021; Kittiwat et al., 2013; Garlik et al., 2013). This initial cytoprotective act is crucial, as it prevents the oxidative damage from propagating and triggering downstream inflammatory and apoptotic signaling. Furthermore, these herbs do not merely act as exogenous antioxidants; they also bolster the brain's endogenous defense systems by restoring the activity of critical enzymes like SOD, CAT, and GSH (Sutalangka et al., 2013; Garlik et al., 2013). This dual antioxidant action provides a comprehensive defense against oxidative stress.

By mitigating the initial oxidative insult, these herbal agents effectively prevent the activation of key inflammatory signaling cascades. Oxidative stress is a potent activator of the **NF-κB pathway**, the master regulator of inflammation (Gelderblom et al., 2012). The demonstrated ability of Phyllanthin and Thymoquinone to inhibit NF-κB activation is therefore a critical downstream consequence of their

antioxidant effect (Yuan et al., 2021; Farkhondeh et al., 2017; Gholamnezhad et al., 2015). This inhibition suppresses the transcription and release of a host of damaging pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, thereby dampening the neuroinflammatory response that contributes significantly to secondary brain injury (Chen et al., 2018; Yang et al., 2019). Concurrently, some of these compounds actively promote the **Nrf2/HO-1 pathway**, a master regulator of the antioxidant and cytoprotective response, creating a positive feedback loop that further enhances cellular resilience (Yuan et al., 2021; Amin et al., 2021).

Finally, the herbal interventions act at the terminal stage of the cell death cascade by directly inhibiting **apoptosis**. The convergence of oxidative and inflammatory stress signals on the mitochondria leads to the upregulation of pro-apoptotic proteins like Bax and the downregulation of anti-apoptotic proteins like Bcl-2, ultimately activating executioner caspases such as Caspase-3 (Yuan et al., 2021; Amin et al., 2021). The ability of Phyllanthin and Thymoquinone to favorably modulate the Bax/Bcl-2 ratio and inhibit caspase activity represents a final, critical intervention that salvages neurons from programmed cell death (Amin et al., 2021; Wang et al., 2014). This multi-layered, sequential modulation of oxidative stress, neuroinflammation, and apoptosis constitutes a powerful and comprehensive neuroprotective strategy.

The Animal Model Dichotomy: Reconciling TMT Neurotoxicity with Ischemic Injury

A central and novel aspect of this review is the conceptual framework that juxtaposes a vascular injury model (MCAO) with a chemical neurotoxin model (TMT). At first glance, these models appear disparate. MCAO mimics the clinical reality of stroke by inducing physical vascular occlusion, leading to hypoxia and subsequent reperfusion injury (Fluri et al., 2015). In contrast, TMT is a chemical agent that bypasses vascular occlusion and directly attacks neuronal machinery, primarily by inhibiting mitochondrial respiration and ATP synthesis, which triggers a cascade of oxidative stress and apoptosis (Jenkins & Barone, 2004; Zhang et al., 2023).

The critical realization is that while the upstream triggers are fundamentally different, the downstream cellular pathways that execute neuronal death are remarkably convergent. Both MCAO-induced I/R and TMT exposure culminate in severe oxidative stress, mitochondrial dysfunction, the release of pro-inflammatory mediators, and the activation of the apoptotic cascade (Gelderblom et al., 2012; Kittiwat et al., 2013; Jenkins & Barone, 2004). This convergence provides a unique opportunity to dissect the mechanism of action of the herbal neuroprotectants. A therapeutic agent whose effects are purely vascular—for example, a simple vasodilator or an anti-platelet agent—would be expected to show efficacy in the MCAO model but would likely be ineffective against the direct cellular toxicity of TMT. Conversely, an agent that acts solely as a specific chelator for tin would be effective against TMT but would have no impact on I/R injury. The evidence reviewed here, showing that the bioactive compounds within these herbs potently target the shared downstream pathways of oxidative stress, inflammation, and apoptosis, strongly suggests that their mechanism is fundamentally cytoprotective and largely independent of the primary insult's nature (Yuan et al., 2021; Amin et al., 2021). Their effectiveness in the MCAO model is therefore not just a result of potential vascular effects but is deeply rooted in their ability to protect the neuron itself from the biochemical consequences of injury. This validates a robust, pathway-centric, and broadly applicable neuroprotective action, elevating the therapeutic potential of this

polyherbal combination from a mere "stroke therapy" to a "broad-spectrum neuroprotectant" that targets the fundamental mechanisms of cellular homeostasis and survival.

Strengths, Limitations, and Implications for Future Research

The primary strength of this systematic review lies in its novel conceptual framework, which uses a mechanistic lens to synthesize evidence across different models of neuronal injury. The comprehensive inclusion of 17 distinct outcomes and the rigorous application of the Cochrane RoB 2 tool for methodological appraisal add to its robustness (Sterne et al., 2019). However, several limitations must be acknowledged. The most significant limitation is the complete absence of preclinical studies investigating the specific three-herb combination proposed. All conclusions regarding the polyherbal formulation are extrapolations based on the effects of the individual herbs studied in isolation. The evidence base itself is characterized by considerable heterogeneity in terms of animal species, injury models, extract preparation methods, and dosing regimens, which complicates direct comparisons between studies. Furthermore, the risk of bias assessment revealed methodological weaknesses, particularly a lack of blinding in outcome assessment, in many of the included studies, which tempers the confidence in their findings (Higgins et al., 2019). Publication bias, a common issue in preclinical research where positive results are more likely to be published, may also have influenced the available evidence.

These limitations highlight clear directions for future research. The most pressing need is for well-designed preclinical studies that investigate the specific *M. oleifera*, *P. niruri*, and *N. sativa* combination. Such studies should aim to:

1. **Investigate Synergy:** Determine whether the combination produces synergistic or additive neuroprotective effects compared to the individual herbs.
2. **Standardize Protocols:** Employ a standardized and rigorously reported MCAO model in rats, including blinding and randomization, to generate high-quality, reproducible data.
3. **Dose-Response and Pharmacokinetics:** Establish optimal dose-response relationships for the combination and investigate the pharmacokinetics and blood-brain barrier permeability of the key bioactive compounds when co-administered.
4. **Translational Relevance:** Ultimately, the goal should be to generate a sufficient body of high-quality preclinical evidence to justify the development of a standardized formulation for evaluation in larger animal models and, eventually, in human clinical trials for ischemic stroke.

CONCLUSION

Summary of Findings

This systematic review provides compelling and consistent preclinical evidence that the individual constituent herbs of the proposed polyherbal formulation—*Moringa oleifera*, *Phyllanthus niruri*, and *Nigella sativa*—exert profound neuroprotective effects against the core pathological mechanisms of cerebral injury. Their efficacy is mediated through a multi-pronged mechanism involving potent antioxidant, anti-inflammatory, and anti-apoptotic activities. By targeting the convergent downstream pathways of neuronal death, these herbs demonstrate a robust cytoprotective action that is relevant to both vascular and neurotoxic insults, highlighting their potential as broad-spectrum neuroprotective agents.

Recommendations

The robust, pathway-centric neuroprotection demonstrated by the individual herbs provides a strong scientific rationale for the development and investigation of a combined polyherbal formulation. It is recommended that future preclinical research prioritize the evaluation of a standardized combination of *M. oleifera*, *P. niruri*, and *N. sativa*. Initial studies should focus on confirming synergistic or additive efficacy and safety in a methodologically rigorous MCAO model of ischemic stroke. Such research is a critical next step in translating the therapeutic promise of these traditional medicinal plants into an evidence-based, multi-target therapy for ischemic stroke.

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