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Review Article

Potent and broad-spectrum efficacy of thymoquinone in preclinical multi-organ ischemia-reperfusion injury a systematic review

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Abstract: Objectives: Ischemia-reperfusion injury (IRI) is a major clinical challenge. Thymoquinone (TQ) has shown promise in preclinical models. This study aims to systematically review and synthesize the preclinical evidence for the multi-organ protective efficacy of TQ against IRI and to evaluate its translational potential. Methods: A systematic literature search of six electronic databases (PubMed, EBSCO, Web of Science, CNKI, VIP, and Wanfang) was conducted to identify relevant animal studies published between January 2000 and January 2024. Studies investigating TQ intervention in animal IRI models were included based on predefined criteria. Results: A total of 40 studies, involving 1,858 animals, met the inclusion criteria. The evidence demonstrated significant TQ-mediated protection across a wide range of organs, including the heart, brain, kidneys, liver, intestines, and reproductive systems. The primary protective mechanisms consistently identified were the attenuation of oxidative stress, suppression of inflammation, and modulation of apoptosis and autophagy. These effects were mediated through key signaling pathways such as TLR4/NF-κB, MAPK, and Bcl-2/Bax. Conclusions: This systematic review consolidates robust preclinical evidence supporting TQ as a potent, broad-spectrum protective agent against multi-organ IRI. However, its clinical translation is currently hindered by challenges, most notably its poor bioavailability and the absence of human clinical trials. Future research must focus on developing optimized delivery systems and conducting rigorous clinical validation to harness its therapeutic potential.

Keywords: Thymoquinone, ischemia-reperfusion injury, multi-organ protection, preclinical systematic review, therapeutic potential, bioavailability

Introduction

Ischemia-reperfusion injury (IRI) is a critical clinical problem that drives significant organ dysfunction [1, 2]. This paradoxical damage occurs in diverse settings, from organ transplantation and myocardial infarction to trauma and vascular surgery, affecting vital organs like the heart, brain, kidneys, and liver [3-6]. The core pathophysiology - a destructive cascade of oxidative stress, inflammation, and programmed cell death - consistently outpaces current therapeutic strategies [4]. While promising techniques such as machine perfusion [8] and ischemia-free transplantation [7, 8] exist, their limited applicability highlights the urgent need for new, broadly effective pharmacological agents.

The search for effective IRI therapies has been challenging, with many promising drugs failing in clinical translation [9]. A primary reason for these failures is a strategic mismatch: the drugs were often designed for a single target, while the disease itself is a complex, multi-pathway process. This “one-target, one-drug” approach struggles against the redundant and interconnected nature of the injury cascade. Blocking a single pathway is often insufficient when multiple others contribute to the damage. This high-

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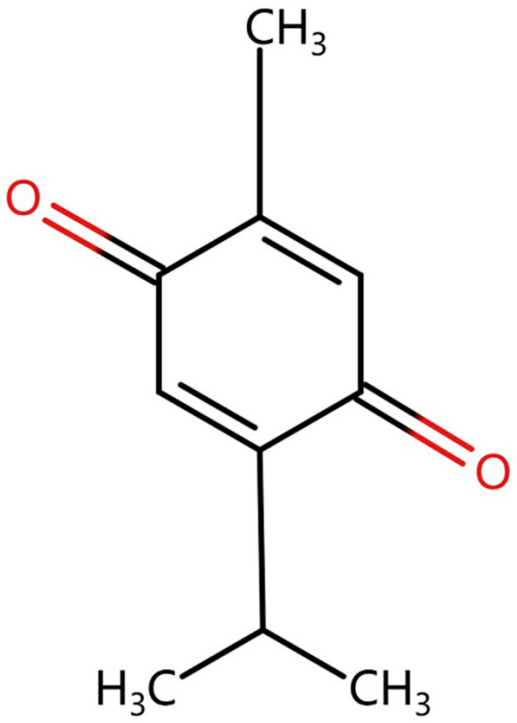

Figure 1. Chemical structure of TQ.

lights a fundamental dilemma in IRI drug development and points toward the need for agents with pleiotropic, or multi-target, capabilities [10].

This need for a multi-target agent has led to renewed interest in Thymoquinone (TQ) (**Figure 1**), the main bioactive compound in *Nigella sativa*. Derived from a plant used for centuries in traditional medicine for conditions like inflammation and diabetes [11-16], TQ possesses a combination of potent anti-inflammatory, antioxidant, and cell-death-regulating properties [14, 15, 17-20]. These activities directly counter the core pathological drivers of IRI. It is this inherent pleiotropy that makes TQ a compelling solution to the strategic mismatch that has hampered the development of single-target drugs.

Although previous narrative reviews have discussed TQ's general pharmacology [21, 22], a systematic synthesis of its protective effects across multiple organs in IRI has been needed. The current literature is a mosaic of single-organ studies, which has prevented a clear, evidence-based overview of TQ's efficacy spectrum and shared mechanisms. This review addresses that gap by synthesizing 24 years of preclinical evidence (2000-2024). Our analysis defines the breadth of TQ's protective actions, clarifies its common and organ-specific mechanisms, and critically assesses the path toward clinical use.

Materials and methods

Search strategy

We performed a systematic literature search of six databases: PubMed, EBSCO, Web of Science, the China National Knowledge Infrastructure (CNKI), the China Science and Technology Periodical Database (VIP), and the Wanfang Data Knowledge Service Platform (WF). The search was completed on January 31, 2024, to identify animal studies published between January 2000 and January 2024. The following search string was used: ((((Thymoquinone) OR (Polythymoquinone)) OR (Thymolquinone)) OR (Thymoquinon)) AND (((ischemia-reperfusion injury) OR (Reperfusion Injuries)) OR (Reperfusion Damage)).

Study selection

Two reviewers (Xiaolin Wang and Bangjiang Fang) independently managed the study selection process. They first screened titles and abstracts to remove irrelevant articles. Next, they examined the full texts of the remaining articles to ensure they met all inclusion criteria: (1) original research on animal models of ischemia-reperfusion injury (IRI); (2) thymoquinone (TQ) used as the intervention; (3) outcomes reported on the protective effects of TQ; and (4) published in English or Chinese. Studies were excluded if they were non-experimental (e.g., reviews, case reports), did not use TQ, had unobtainable full texts, or contained incomplete data. Any disagreements on inclusion were resolved by discussion or consultation with a third reviewer (Zhiming Liu).

Data extraction and data analysis

Using a pre-designed standardized form, two reviewers independently extracted data from each included study. Key information collected included: animal model details (species, IRI induction method, ischemia/reperfusion duration), intervention specifics (TQ dose, administration route and timing, control drugs), and outcome data (organ studied, main findings,

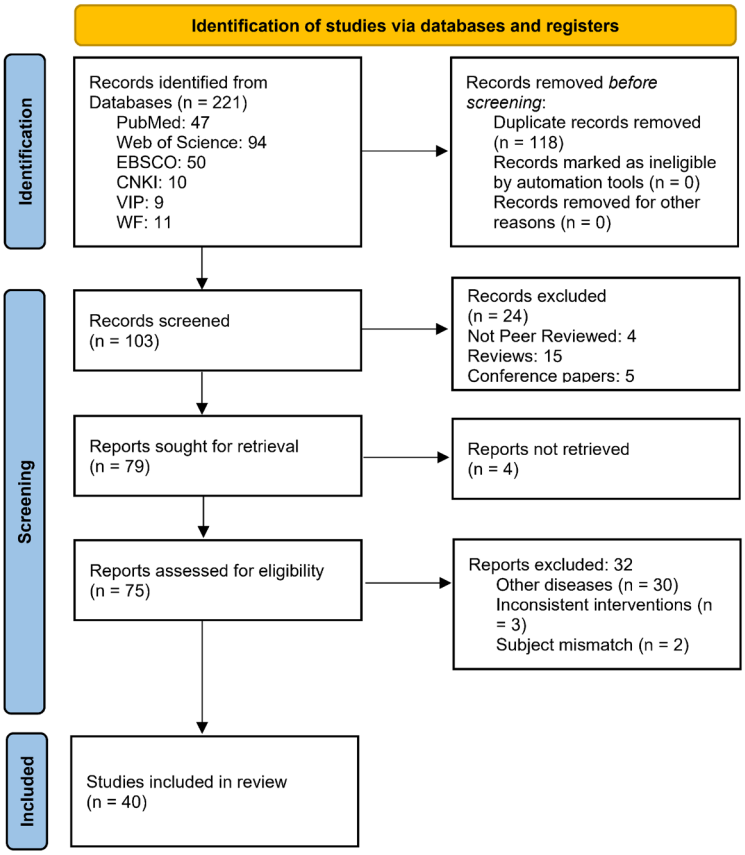

Figure 2. PRISMA flowchart of screening and including studies.

and identified mechanisms or signaling pathways).

Due to significant heterogeneity in animal models, TQ administration protocols, and experimental designs across the studies, a quantitative meta-analysis was not feasible. Instead, we employed a narrative synthesis to summarize the findings. Data were organized into tables to clearly present the characteristics and results of each study. We then descriptively categorized TQ's protective effects and mechanisms by organ system to provide a comprehensive qualitative assessment.

The outcome measures were predefined and categorized into primary and secondary outcomes. Primary outcome measures were direct indicators of organ function and tissue injury. These included, but were not limited to, serum biomarkers of organ function (e.g., alanine aminotransferase [ALT] and aspartate aminotransferase [AST] for liver; blood urea nitrogen [BUN] and creatinine [Cr] for kidney), measurements of infarct size (for heart and brain), and histopathological damage scores. Secondary outcome measures were biomarkers and parameters related to the underlying mechanisms of TQ's protective effects. These encompassed markers of oxidative stress (e.g., malondialdehyde [MDA], superoxide dismutase [SOD]), inflammation (e.g., tumor necrosis factor-alpha [TNF-α], interleukin-6 [IL-6]), apoptosis (e.g., Caspase-3 activity, Bcl-2/Bax ratio), and autophagy (e.g., LC3II/p62 ratio).

Results

Literature search results

Our initial search across the six databases yielded 221 articles. After removing 118 duplicates, 103 unique articles (92 in English, 11 in Chinese) proceeded to the screening stage. We first reviewed titles and abstracts, excluding 24 records that were reviews, dissertations, or non-peer-reviewed articles. Subsequently, we assessed the full texts of the remaining 79 articles. From these, 39 were further excluded because they investigated conditions other than IRI, involved inconsistent interventions, or had mismatched study subjects. This selection process, detailed in the PRISMA flowchart (**Figure 2**), resulted in a final cohort of 40 studies that met our inclusion criteria [23-62].

Characteristics of included studies

The 40 included studies primarily focused on the liver (n=11) and kidney (n=8), indicating these organs are the most extensively studied in the context of TQ's protective effects against IRI (**Figure 3**). Collectively, these studies involved 1,858 animals, with Wistar rats (21 studies) and Sprague-Dawley rats (13 studies) being the most common models. Research also covered the heart, brain, intestines, reproductive systems, spinal cord, and skeletal muscle.

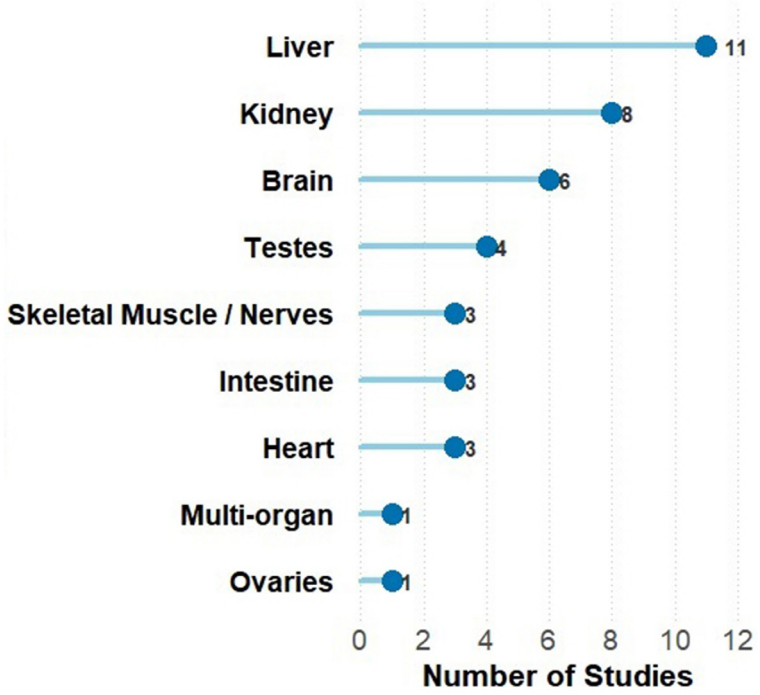

Figure 3. Distribution of included preclinical studies by organ system.

Most studies induced IRI through vascular ligation. TQ was typically administered via oral gavage or intraperitoneal injection, though dosages and administration timing varied significantly. Some experiments also included a positive control group for comparison. The specific characteristics of each included study are summarized in **Table 1**.

Protective effects and mechanisms of TQ against IRI in various organs or tissues

Liver: Eleven preclinical studies included in this review investigated the protective effects of TQ against liver IRI [23-33]. In the majority of these studies, liver IRI was induced by clamping the portal triad or specific hepatic lobes to achieve approximately 70% hepatic ischemia, with ischemia durations ranging from 30 to 90 minutes, followed by reperfusion periods of 30 minutes to 24 hours. TQ dosage varied from 10 to 50 mg/kg, administered primarily via oral gavage or intraperitoneal injection, typically prior to the ischemic insult.

Notably, one study utilized a self-nano-emulsifying drug delivery system (SNEDDS) for TQ administration [33], and another employed dexmedetomidine as a positive control [27].

Collectively, these studies demonstrated that TQ intervention significantly mitigated IRI-induced liver histopathological damage, improved liver function markers (e.g., ALT, AST), enhanced endogenous antioxidant enzyme activity, reduced the expression of pro-inflammatory cytokines, and modulated pathways related to cell death. The reported hepatoprotective mechanisms were multifaceted, including the attenuation of oxidative and nitrosative stress, suppression of inflammatory responses, reduction of endoplasmic reticulum stress, regulation of mitochondrial function, anti-apoptotic effects (e.g., modulation of Bcl-2/Bax ratio), and inhibition of TRPM channels leading to decreased Ca^{2+} influx. Key signaling pathways implicated in these protective actions included Wnt/β-catenin/p53, ERK-MAPK, p38-MAPK, TLR-4/TNF-α/NF-κB/IL-6, LKB1/AMPK, TRPM2/6/7/8, GRP78/CHOP/Caspase-12, JAK2/STAT3, and the nitric oxide signaling pathway.

Brain: Six included studies investigated the neuroprotective effects of TQ in animal models of brain IRI [37-42]. Cerebral ischemia was predominantly induced using transient middle cerebral artery occlusion (tMCAO) or bilateral common carotid artery occlusion (BCCAO) models, with ischemic durations varying from 10 minutes to 2 hours, followed by reperfusion periods ranging from 24 hours to 7 days. TQ dosages in these studies ranged from 3 to 10 mg/kg, administered mainly via intraperitoneal injection or oral gavage, often commencing at the onset of reperfusion. Some studies incorporated phenytoin or edaravone as positive controls. Treatment with TQ consistently demonstrated significant neuroprotective outcomes, including improved neurological behavioral scores, enhanced motor function, reduction in cerebral infarct size, alleviation of neuronal damage, and decreased cerebral edema. These beneficial effects were attributed to multiple mechanisms, notably TQ's antioxidant and anti-apoptotic properties, its ability to modu-

Table 1. Characteristics of preclinical animal studies investigating the protective effects of thymoquinone (TQ) in Ischemia-Reperfusion Injury (IRI)

Animal Species	Ischemia Model	Ischemia Time	Reperfusion Time	TQ Dosage	TQ Administration Timing	Administration Route	Control Drug	Target Tissue	Key Findings	Mechanism of Action	Molecular Signaling Pathway	Reference
C57BL/6 mice	Hepatic ischemia (70%): clamping left and middle lobes	90 min	4 h	40 mg/kg	1 h pre-ischemia	IP	None	Liver	↓TNF-α, ↓MDA, ↑antioxidant enzymes, ↓liver injury	↓inflammation, ↓oxidative stress	Wnt/β-catenin/p53	[23]
Wistar rats	Hepatic ischemia (70%): portal vein clamping	45 min	1 h	20 mg/kg/d	10 days pre-ischemia	IP	Dexmedetomidine	Liver	↑total antioxidant capacity, ↓histopathological damage	↓oxidative stress, ↓inflammation	None	[27]
Wistar rats	Partial hepatic ischemia: clamping hepatic artery and portal vein branches	60 min	24 h	30 mg/kg/d	10 days pre-ischemia	Oral	None	Liver	↓transaminase activity, ↓liver injury, ↓ER stress, ↓mitochondrial apoptosis	Antioxidant, anti-inflammatory, ↓ER stress, regulate mitochondrial function, anti-apoptotic	ERK-MAPK, p38-MAPK	[30]
Wistar rats	Hepatic ischemia (70%): clamping left and middle lobes	60 min	24 h	10 mg/kg/d	10 days pre-ischemia	Oral (SNEDDS)	None	Liver	↑hepatocyte integrity, ↓inflammatory cytokines	↑antioxidant capacity, ↓inflammation, ↓apoptosis	TLR-4/TNF-α/NF-κB/IL-6; Bcl-2/Bax	[33]
Sprague-Dawley rats	Hepatic ischemia (70%): clamping left and middle lobes	45 min	24 h	50 mg/kg/d	7 days pre-ischemia	Oral	None	Liver	↓ALT/AST, ↓Bax, ↓Caspase-3/9	Regulate oxidative stress-induced apoptosis	Bcl-2/Bax, LKB1/AMPK	[26]
Wistar rats	Partial hepatic ischemia: portal vein clamping	45 min	1 h	50 mg/kg/d	10 days pre-ischemia	IP	None	Liver	↓TRPM2/6/7/8 gene expression, ↓oxidative stress markers	Inhibit TRPM channel-mediated calcium influx	TRPM2/6/7/8	[28]
Wistar rats	Partial hepatic ischemia + partial hepatectomy: clamping hepatic artery and portal vein branches	60 min	24 h	30 mg/kg/d	10 days pre-ischemia	Oral	None	Liver	↓tissue damage, ↑ATP, ↓ER stress, ↓apoptosis	↑antioxidant capacity, improve mitochondrial function	GRP78/CHOP/Caspase-12	[31]
C57BL/6 mice	Hepatic ischemia (70%): clamping left and middle lobes	90 min	4 h	50 mg/kg	1 h pre-ischemia	IP	None	Liver	↓ROS, ↓MDA, ↑CAT/GPx/SOD activity	Inhibit oxidative stress	None	[24]
Sprague-Dawley rats	Hepatic ischemia (70%): portal vein clamping	45 min	1 h	50 mg/kg/d	10 days pre-ischemia	Oral	None	Liver	↓Caspase-8/9/3 activity, ↓mitochondrial damage, ↓cytochrome C release, ↓DNA fragmentation	Anti-mitochondrial oxidative stress, anti-apoptotic, anti-inflammatory	Bcl-2/Bax, NF-κB	[32]
C57BL/6 mice	Hepatic ischemia (70%): clamping left and middle lobes	90 min	4 h	50 mg/kg	1 h pre-ischemia	IP	None	Liver	↓IL-6, ↓TNF-α, ↓IL-1β, ↓JAK2/STAT3 phosphorylation	Inhibit inflammation	JAK2/STAT3	[25]

Wistar rats	Hepatic ischemia (70%): clamping left and middle lobes	30 min	30 min	20 mg/kg/d	10 days pre-ischemia	Oral	None	Liver	Alleviated liver damage (biochemical & architecture), mitigated inflammatory infiltration	Ameliorate oxidative stress, nitrosative stress, and inflammatory responses	Nitric oxide signaling pathway	[29]
Wistar rats	Bilateral common carotid artery occlusion (BCCAO)	10 min	7 days	5 mg/kg/d	5 days pre- to 7 days post-reperfusion	Oral	None	Brain	↓neuronal death, ↓MDA, ↑GSH, ↑antioxidant enzymes	Antioxidant, inhibit lipid peroxidation	None	[40]
Sprague-Dawley rats	Transient middle cerebral artery occlusion (tMCAO)	2 h	24 h	5 mg/kg	Immediately post-reperfusion	IP	Edaravone	Brain	↑neurobehavioral score, ↓infarct area, ↓brain edema, regulate energy metabolism molecules	Antioxidant, anti-inflammatory, regulate energy metabolism	None	[41]
Sprague-Dawley rats	Transient middle cerebral artery occlusion (tMCAO)	2 h	24 h	5 mg/kg	Immediately post-reperfusion	IP	Edaravone	Brain	Regulate glucose, citrate, succinate; ↑ADP, AMP, GSH	Regulate energy metabolism, antioxidant	None	[39]
Wistar rats	Bilateral common carotid artery occlusion (BCCAO)	30 min	24 h	3 mg/kg	Immediately post-reperfusion	IP	None	Brain	↓apoptotic cells, ↓astrocyte activation, ↓TMAO	Antioxidant, anti-apoptotic	None	[37]
C57BL/6 mice	Middle cerebral artery occlusion/reperfusion (MCAO)	60 min	3 days	5 mg/kg/d	5 days pre- to 3 days post-reperfusion	IP	TAK-242 (TLR4 inhibitor)	Brain	↑M2 microglia polarization, ↓infarct volume, ↑motor function	Regulate microglia activation, anti-inflammatory	TLR4/NF-κB, Hif-1α	[42]
Male NMRI rats	Four-vessel occlusion (4-VO)	20 min	72 h	10 mg/kg	Immediately post-reperfusion	IP	Phenytoin	Brain	Significant ↓hippocampal MDA, inhibit lipid peroxidation	Antioxidant, inhibit lipid peroxidation	None	[38]
Sprague-Dawley rats	Bilateral renal pedicle clamping	30 min	4 h	10 mg/kg/d	10 days pre-ischemia	Oral	None	Kidney	↓MDA, restore SOD/GST activity; ↓CYP3A1 and SSAT gene expression	Antioxidant, regulate metabolic enzyme gene expression	None	[51]
Sprague-Dawley rats	Unilateral renal pedicle clamping	60 min	1 h	10 mg/kg	24 h and 1 h pre-ischemia	Oral	None	Kidney	↑renal function, ↓oxidative stress and inflammation markers	Antioxidant, anti-inflammatory	None	[55]
Wistar rats	Bilateral renal pedicle clamping	45 min	24 h	10 mg/kg/d	10 days pre-ischemia	IV	None	Kidney	↓BUN/Cr, ↓HIF-1α and inflammatory factors, ↓oxidative stress	Antioxidant, anti-inflammatory, inhibit hypoxia signaling	None	[53]
Wistar rats	Unilateral renal pedicle clamping	60 min	24 h	10 mg/kg/d	Continuous for 3 weeks	Oral	None	Kidney	Synergistically ↑renal function, ↑antioxidant enzymes	Synergistic antioxidant	None	[54]
Sprague-Dawley rats	Unilateral renal pedicle clamping	45 min	24 h	40 mg/kg	Single pre-treatment	IP	None	Kidney	↓BUN/Cr, ↓JAK2/STAT3 phosphorylation, ↓apoptosis proteins	Anti-oxidative stress, anti-inflammatory	JAK2/STAT3/p53	[50]
Wistar rats	Unilateral renal pedicle clamping	35 min	6 days	10 mg/kg/d	10 days pre-ischemia	Oral	None	Kidney	↑renal blood flow and filtration, ↓KIM-1/NGAL/TNF-α/TGF-β1	Inhibit inflammatory fibrosis	None	[52]

Sprague-Dawley rats	Bilateral renal pedicle clamping	45 min	24 h	40 mg/kg	Pre-ischemic preconditioning	IP	None	Kidney	↑mitochondrial function (↑ATPase, ↓MDA), ↓NO/LD	Mitochondrial protection, antioxidant	None	[49]
Sprague-Dawley rats	Unilateral renal pedicle clamping	45 min	24 h	40 mg/kg	Pre-ischemic preconditioning	IP	None	Kidney	↓apoptosis index, ↓caspase-3/Bax/TNF-α/IL-1β	Anti-apoptotic, anti-inflammatory	None	[48]
Wistar rats	Abdominal aorta clamping (infra-renal)	45 min	2 h	20 mg/kg	Pre-ischemic preconditioning	IP	None	Kidney, Liver, Heart, Lung	↓TOS/OSI, ↓multi-organ histopathology	Systemic antioxidant	None	[56]
Wistar rats	Left coronary artery ligation	30 min	2 h	10 mg/kg	20 min pre-ischemia	IP	None	Heart	↓infarct size, inhibit reperfusion ventricular arrhythmias	Antioxidant, anti-inflammatory	None	[57]
Sprague-Dawley rats	Langendorff isolated heart perfusion	45 min	1 h	2.5, 5, 10 μmol/L (perfusion)	5 min post-isolation	Perfusion	SIRT1 inhibitor sirtinol	Heart	↑left ventricular function, ↓myocardial infarct size, ↓mitochondrial oxidative damage	Antioxidant	SIRT1 signaling pathway	[59]
Wistar rats	Langendorff isolated heart perfusion	30 min	1 h	10 mg/kg	20 min pre-ischemia	IP	Autophagy inhibitor chloroquine (CQ)	Heart	↑cardiac function, ↓myocardial infarct size, ↓myocardial enzyme activities	Activate autophagy, inhibit apoptosis	LC3II/p62 autophagy pathway	[58]
Sprague-Dawley rats	Superior mesenteric artery (SMA) clamping	60 min	2 h	50 mg/kg	During reperfusion	IP	None	Small intestine	↓histopathological damage, ↓apoptosis index, regulate MDA/SOD, regulate Bax/Bcl-2/caspase-3	Antioxidant, anti-apoptotic	Bax/Bcl-2, caspase-3 pathway	[34]
Wistar rats	Superior mesenteric artery (SMA) clamping	60 min	24 h	50 mg/kg/d	3 days pre- to entire reperfusion	Oral	None	Small intestine	Restore intestinal contraction, ↓MDA, restore GSH, ↓MPO activity, ↓TNF-α/IL-1β	Antioxidant, anti-inflammatory	None	[36]
Wistar rats	Superior mesenteric artery (SMA) clamping	60 min	2 h	50 mg/kg	30 min pre-ischemia	IP	Melatonin	Small intestine	↓MDA/SOD/GSH-Px, ↓apoptosis	Antioxidant, anti-apoptotic	None	[35]
C57BL/6 mice	Left unilateral testicular 720° torsion	120 min	4 h	10 mg/kg	30 min pre-reperfusion	IP	None	Testis	↓MDA, ↓TOS, ↓OSI; improved histology	Anti-oxidative stress, reduced oxidative damage	None	[46]
Wistar rats	Left unilateral testicular 720° torsion	2 h	30 min	50 mg/kg	30 min pre-ischemia	IP	None	Testis	↓MDA, ↓SOD activity; ↓apoptosis index; ↓Caspase-3 and Bax	Antioxidant, anti-apoptotic	None	[45]
Sprague-Dawley rats	Left unilateral testicular 720° torsion	4 h	4 h	50 mg/kg	40 min pre-reperfusion	Oral	None	Testis	↑seminiferous tubule diameter & score; ↓apoptosis; ↑PCNA; ↓MDA, ↑SOD, CAT, GPx	Antioxidant, anti-apoptotic, promote cell proliferation	None	[47]
Wistar rats	Left unilateral testicular 720° torsion	1 h	4 h	20 mg/kg	30 min pre-reperfusion	IP	CA1, CA2 combined antioxidants	Testis	↓MDA, ↓iNOS, ↓eNOS; ↑TAS activity; APAF-1 reduction weaker than combined	Antioxidant, regulate NO synthesis, partially anti-apoptotic	APAF-1 apoptosis pathway	[44]

Wistar rats	Bilateral ovarian vessel clamping	3 h	3 h	20/40 mg/kg	30 min pre-ischemia or intraoperatively	IP	None	Ovary	↓IL-6, ↓MDA; ↑CAT, GPx; ↓histology damage & Caspase-3	Antioxidant, anti-inflammatory, anti-apoptotic	None	[43]
Wistar rats	Femoral artery and vein clamping	2 h	1 h	20, 40, 80 mg/kg	Single dose 1 h pre-reperfusion	IP	None	Skeletal muscle system	Significant ↑EMG amplitude, ↓MDA, ↑total SH content & antioxidant capacity	Antioxidant, anti-inflammatory	None	[60]
Sprague-Dawley rats	Left hindlimb ischemia (tourniquet)	45 min	90 min	25 mg/kg/d	7 days pre-ischemia	IP	α-tocopherol	Sciatic nerve/Gastrocnemius muscle	↓nerve and muscle edema, ↓myolysis	Antioxidant, anti-inflammatory, anti-apoptotic	None	[62]
Wistar rats	Abdominal aorta clamping (infrarenal to bifurcation)	30 min	24 h	10 mg/kg/d	Continuous from 7 days pre-ischemia	IP	Methylprednisolone	Spinal cord	↑neurological function, protect nerve tissue, maintain neuron number	Antioxidant, anti-inflammatory, anti-apoptotic	None	[61]

IP: Intraperitoneal (injection route); IV: Intravenous (injection route); SNEDDS: Self Nano-Emulsifying Drug Delivery System; ER: Endoplasmic Reticulum; ↑: Increase; ↓: Decrease; TNF-α: Tumor Necrosis Factor alpha; MDA: Malondialdehyde; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; BUN: Blood Urea Nitrogen; Cr: Creatinine; HIF-1α: Hypoxia-Inducible Factor 1-alpha; ROS: Reactive Oxygen Species; CAT: Catalase; GPx: Glutathione Peroxidase; SOD: Superoxide Dismutase; IL-6: Interleukin-6; IL-1β: Interleukin-1 beta; JAK2/STAT3: Janus Kinase 2/Signal Transducer and Activator of Transcription 3; GSH: Glutathione; ADP: Adenosine Diphosphate; AMP: Adenosine Monophosphate; TMAO: Trimethylamine N-oxide; TLR4: Toll-like Receptor 4; NF-κB: Nuclear Factor kappa B; Hif-1α: Hypoxia-inducible factor 1-alpha; CYP3A1: Cytochrome P450 family 3 subfamily A member 1; SSAT: Spermidine/spermine N1-acetyltransferase; KIM-1: Kidney Injury Molecule-1; NGAL: Neutrophil gelatinase-associated lipocalin; TGF-β1: Transforming Growth Factor beta 1; NO: Nitric Oxide; LD: Lactate Dehydrogenase; TOS: Total Oxidant Status; OSI: Oxidative Stress Index; EMG: Electromyography; SH: Sulfhydryl; iNOS: Inducible Nitric Oxide Synthase; eNOS: Endothelial Nitric Oxide Synthase; TAS: Total Antioxidant Status; APAF-1: Apoptotic Peptidase Activating Factor 1; PCNA: Proliferating Cell Nuclear Antigen; MPO: Myeloperoxidase.

late energy metabolism, and its capacity to regulate microglial activation. The TLR4/NF-κB and Hif-1α signaling pathways were identified as key molecular pathways involved in mediating TQ's neuroprotective actions.

Intestine: Three *in vivo* studies included in this review assessed the effects of TQ on intestinal IRI [34-36]. In these studies, intestinal ischemia was induced by clamping the superior mesenteric artery (SMA) for 60 minutes, followed by reperfusion periods of 2 or 24 hours. A TQ dosage of 50 mg/kg was utilized, though TQ exposure times varied among the studies. One investigation [35] employed melatonin as a positive control. TQ administration was consistently shown to alleviate pathological damage to intestinal tissue in rats subjected to IRI and improve intestinal function. These protective effects were primarily attributed to TQ's antioxidant, anti-inflammatory, and anti-apoptotic mechanisms. For instance, the anti-apoptotic effects in one study were linked to the modulation of the Bax/Bcl-2 ratio and caspase-3 activity [34].

Kidney: Eight reviewed studies investigated the renoprotective effects of TQ in animal models of renal IRI [48-56]. Renal ischemia was typically induced by unilateral or bilateral clamping of the renal pedicle for durations of 30 to 60 minutes, with subsequent reperfusion periods ranging from 1 hour to 6 days. TQ dosages varied from 10 to 40 mg/kg. TQ treatment demonstrated significant mitigation of renal IRI, evidenced by reductions in serum creatinine and blood urea nitrogen levels, decreased renal tubular damage, alleviation of renal interstitial edema, and improvements in renal blood flow and glomerular filtration rate. The underlying mechanisms for these protective effects were multifaceted, encompassing anti-apoptotic, anti-inflammatory, and antioxidant actions, as well as regulation of metabolic enzyme gene expression, mitochondrial protection, and attenuation of renal fibrosis. For example, specific studies highlighted the involvement of pathways such as JAK2/STAT3 in mediating these renoprotective effects.

Heart: Three reviewed studies specifically examined TQ's cardioprotective role in myocardial IRI [57-59]. These investigations utilized both *ex vivo* Langendorff isolated heart perfusion systems and *in vivo* models involving left anterior descending coronary artery ligation to induce cardiac ischemia-reperfusion. Ischemic durations ranged from 30 to 45 minutes, with a subsequent reperfusion period of 1 hour. In the isolated perfused heart model, TQ was administered via perfusion at concentrations of 2.5, 5, and 10 μmol/L for 5 minutes, while *in vivo* studies employed intraperitoneal TQ injection at a dose of 10 mg/kg, 20 minutes prior to ischemia. Notably, the autophagy inhibitor chloroquine (CQ) and the SIRT1 inhibitor sirtinol were used as comparators or mechanistic tools in some of these studies. The findings consistently demonstrated that TQ treatment reduced myocardial infarct size, improved cardiac function, decreased the release of myocardial enzymes, and suppressed cardiac arrhythmias. These cardioprotective effects were attributed to TQ's antioxidant and anti-inflammatory properties, as well as its capacity to activate autophagy by modulating the LC3II/p62 pathway, and to inhibit apoptosis, and these beneficial actions also implicated the SIRT1 signaling pathway.

Testes: Four reviewed studies specifically investigated the protective effects of TQ against testicular IRI [44-47]. Testicular ischemia was induced by 720° torsion of the left testicle, followed by detorsion for reperfusion. The duration of ischemia ranged from 1 to 4 hours, with subsequent reperfusion periods lasting from 30 minutes to 4 hours. TQ dosages were reported as 20 or 50 mg/kg in rats and 10 mg/kg in mice, with administration occurring either 30 to 40 minutes prior to reperfusion or 30 minutes before the onset of ischemia. One study utilized a combination of CA1 and CA2 antioxidants as a positive control. The findings from these studies indicated that TQ treatment reduced testicular tissue damage, improved seminiferous tubule diameter, and enhanced spermatogenesis scores. The protective mechanisms identified included antioxidant effects, regulation of nitric oxide (NO) synthesis, anti-apoptotic activity (potentially involving the APAF-1 apoptotic pathway), and promotion of cell proliferation.

Ovaries: One study included in this review specifically examined the effects of TQ on ovarian IRI [43]. In this study, ischemia was induced by clamping bilateral ovarian vessels for 3 hours, followed by a 3-hour reperfusion period. TQ was

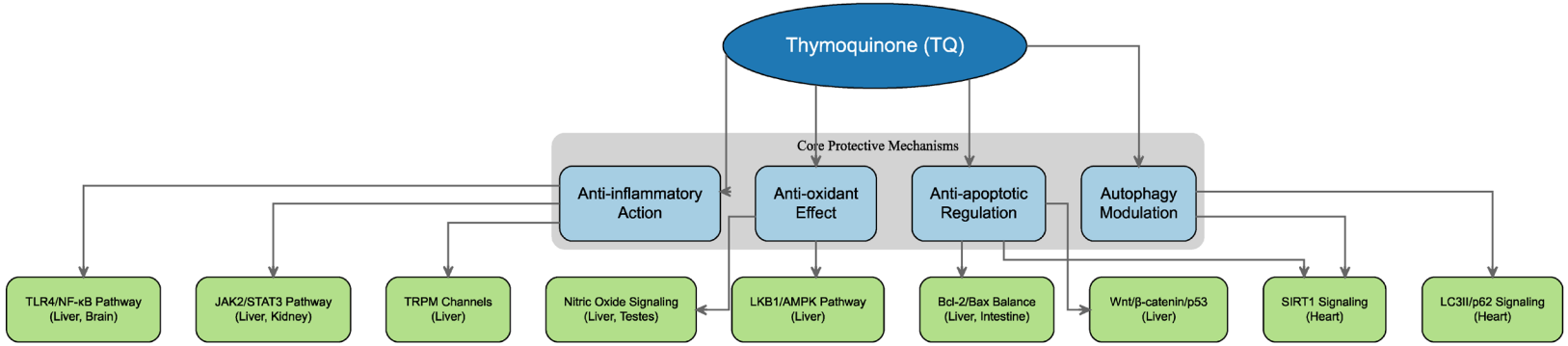

Figure 4. Hierarchical network of Thymoquinone's protective mechanisms. This diagram illustrates the multi-level mechanisms of Thymoquinone (TQ) in protecting against ischemia-reperfusion injury. The network flows from the core compound (TQ) to its primary protective actions (Level 1: Core Protective Mechanisms) and further branches into the specific molecular signaling pathways identified in the literature (Level 2: Key Signaling Pathways). The organ systems in which each pathway was predominantly reported are indicated in parentheses.

Figure 5. Organ-mechanism matrix of Thymoquinone's protective effects.

administered at doses of 20 or 40 mg/kg. The findings indicated that TQ treatment mitigated ovarian tissue damage and helped maintain normal follicular development following IRI. These protective effects were attributed to TQ's antioxidant, anti-inflammatory, and anti-apoptotic mechanisms.

Other tissues and multi-organ IRI: TQ's protective capacity was also demonstrated in IRI models involving several other tissues. One study employing femoral artery and vein clamping in rats to induce skeletal muscle IRI found that TQ intervention protected muscle tissue from IRI-induced damage and improved skeletal muscle motor function post-injury [60]. Another investigation induced partial spinal cord ischemia-reperfusion in rats by clamping the abdominal aorta (distal to the renal arteries down to the bifurcation); TQ administration in this model protected spinal cord integrity, preserved the number of viable neurons, and resulted in improved motor function outcomes [61]. Additionally, a study using a tourniquet applied to the proximal left thigh in rats to induce sciatic nerve and femoral muscle ischemia showed that TQ intervention led to reduced edema in both nerve and muscle tissues and reduced myolysis [62]. Across these diverse tissue models, the observed protective effects of TQ were consistently associated with its antioxidant, anti-inflammatory, and anti-apoptotic mechanisms.

Significantly, in a multi-organ IRI model created by infrarenal clamping of the abdominal aorta, TQ intervention was found to reduce histopathological damage across multiple systemic organs, including the kidney, liver, heart, and lung [56]. This widespread protective effect was mechanistically linked to TQ's systemic antioxidant properties.

Discussion

The collective preclinical evidence systematically reviewed herein establishes Thymoquinone (TQ) as a uniquely potent and broad-spectrum agent against multi-organ IRI. Its therapeutic promise does not reside in a single mechanism, but in its profound ability to engage a complex network of interconnected biological pathways. We have synthesized these multifaceted mechanisms into a hierarchical network model (**Figure 4**), which will serve as the conceptual framework for this discussion. This model posits that TQ's efficacy is built upon four core protective pillars - anti-inflammation, anti-oxidation, anti-apoptosis, and autophagy modulation - which are orchestrated through a series of key signaling pathways, as illustrated in **Figure 6**.

A deeper analysis of this mechanistic framework reveals a fascinating duality in TQ's mode of action: a foundation of universally applicable core principles complemented by a layer of highly specific, targeted interventions. As visualized in the evidence map (**Figure 5**), the foundational pillars of anti-inflammation, anti-oxidation, and anti-apoptosis represent the "universal language" of TQ, spoken consistently

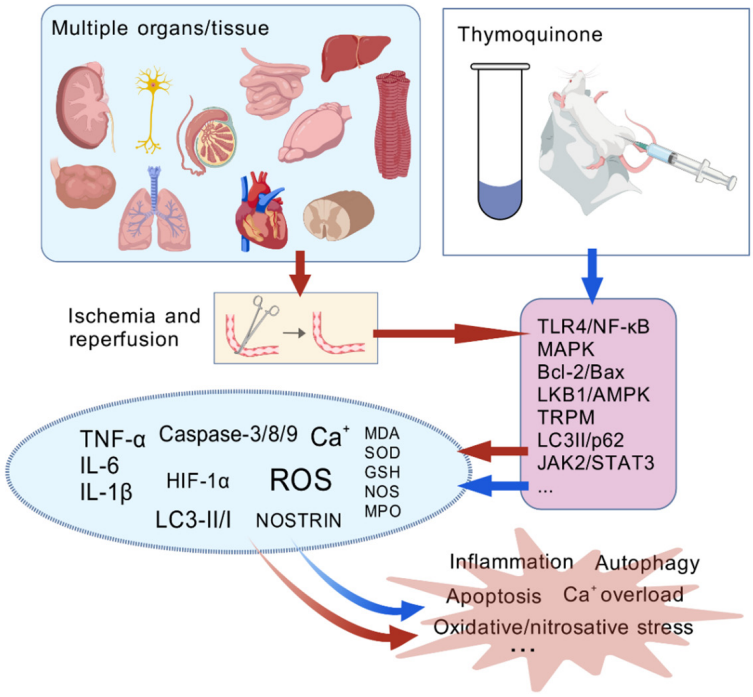

Figure 6. Schematic illustration of the protective mechanisms of Thymoquinone (TQ) against ischemia-reperfusion injury (IRI) in multiple organs/tissues (Created with BioGDP.com). Ischemia and reperfusion in multiple organs/tissues leads to a cascade of detrimental events including the overproduction of Reactive Oxygen Species (ROS), release of pro-inflammatory cytokines (e.g., TNF-α, IL-6, IL-1β), activation of caspases (e.g., Caspase-3/8/9), calcium (Ca^{2+}) overload, increased malondialdehyde (MDA), and altered levels of antioxidant enzymes (e.g., SOD, GSH) and other mediators like HIF-1α, LC3-II/I, NOSTRIN, NOS, and MPO. Thymoquinone intervention (represented by administration to an animal model) counteracts these IRI-induced pathological processes. TQ achieves this by modulating key intracellular signaling pathways, such as TLR4/NF-κB, MAPK, Bcl-2/Bax, LKB1/AMPK, TRPM, LC3II/p62, and JAK2/STAT3. The modulation of these pathways ultimately leads to the attenuation of inflammation, regulation of autophagy, suppression of apoptosis, reduction of Ca^{2+} overload, and mitigation of oxidative/nitrosative stress, thereby conferring protection against IRI across various organs. This legend is based on the visual elements of **Figure 3** and the mechanistic discussion in the manuscript, e.g.

across nearly all affected organ systems. This demonstrates TQ's remarkable capacity to counteract the core, shared pathologies of IRI. In stark contrast, specific signaling pathways appear to function as "local dialects", employed only in particular organ contexts. For instance, the modulation of SIRT1 and LC3II/p62 signaling was reported exclusively in the heart [58, 59], while the engagement of TRPM channels was a prominent finding in liver studies [28]. This observed specificity may reflect either true, organ-dependent biological nuances in cellular response or a potential focus bias within different research subfields. Disentangling these possibilities is a crucial task for future mechanistic studies.

Positioning these findings within the existing scientific discourse is crucial for validating their significance. While previous narrative reviews have eloquently summarized the general pharmacological virtues of TQ [21, 22], our systematic review provides a fundamentally different and more rigorous level of evidence. By employing a comprehensive, reproducible search strategy and a predefined analytical framework, this work transcends general commentary to deliver the first holistic, evidence-based synthesis of TQ's efficacy specifically across the multi-organ landscape of IRI. It is through this systematic lens that the nuanced patterns of 'universal' versus 'organ-specific' mechanisms (**Figure 5**) could be discerned - an insight not achievable through narrative overviews. Therefore, this review does not merely reaffirm TQ's promise; it fundamentally reframes it, providing a structured, pan-organ evidence map that validates its broad-spectrum potential and offers a more granular understanding of its therapeutic architecture.

The strength of these conclusions must be critically appraised in the context of the methodological landscape of the included preclinical studies. A qualitative assessment of the evidence base reveals that the overall quality, particularly in terms of reporting rigor, is suboptimal. A significant proportion of studies failed to provide sufficient detail to judge the risk of bias across critical domains, such as random sequence generation, allocation concealment, and blinding of outcome assessment, as recommended by the SYRCLE tool. This widespread lack of methodological transparency may contribute to an overestimation of TQ's

true effect size and underscores an urgent need for more stringent adherence to reporting standards like the ARRIVE guidelines. Furthermore, while a formal statistical subgroup analysis was precluded by heterogeneity, our qualitative exploration noted that studies employing intraperitoneal injection tended to report more robust effects than those using oral gavage, likely reflecting differences in bioavailability. These methodological considerations do not invalidate the consistent protective signal observed, but they highlight the necessity for future, higher-quality preclinical trials to definitively ascertain the magnitude and optimal conditions of TQ's efficacy.

Despite the robust protective signal identified, the translational journey of TQ from preclinical promise to clinical reality is confronted by a formidable barrier: its challenging pharmacokinetics. The most significant obstacle is TQ's poor aqueous solubility, which leads to low and erratic oral bioavailability [63, 64]. This fundamental limitation means that the effective doses observed in animal models may not be readily achievable in humans with simple formulations, posing a major impediment to its clinical development. However, this is not an insurmountable impasse but a clear call for innovation. The path to breakthrough lies in advanced drug delivery systems. Indeed, one study included in this review provided a compelling proof-of-concept, demonstrating that a self-nano-emulsifying drug delivery system (SNEDDS) markedly enhanced TQ's bioavailability and hepatoprotective efficacy [33]. This, along with emerging nanotechnology platforms (e.g., nanoparticles, liposomes) and chemical modifications designed to improve pharmacokinetics [65], represents the critical next step. Overcoming the bioavailability challenge is the pivotal event that will unlock the therapeutic potential systematically evidenced in this review, paving the way for meaningful clinical evaluation.

Finally, it is imperative to frame these insights within the inherent limitations of this review. Our findings are derived exclusively from preclinical animal models, and the significant gap to human clinical reality cannot be understated; clinical efficacy remains entirely unproven. Secondly, as previously discussed, this review opted for a qualitative appraisal of the evidence base's methodological quality rather than a formal, study-by-study risk of bias assessment. This approach, while pragmatic, means the potential impact of bias from individual studies on our synthesized conclusions cannot be precisely quantified. The substantial heterogeneity in models and protocols also precluded a quantitative meta-analysis, limiting our conclusions to a narrative synthesis. Lastly, the potential for publication bias, which favors positive findings, may influence the overall landscape of the available evidence. These limitations collectively underscore that while this review provides the most comprehensive preclinical evidence map to date, its conclusions should be interpreted as a strong, hypothesis-generating foundation rather than definitive proof of clinical utility.

In conclusion, this systematic review has constructed the most comprehensive preclinical evidence map to date, establishing TQ as a formidable candidate for mitigating multi--organ IRI. The path forward, however, is a dual carriageway that demands simultaneous progress on two fronts. First, the preclinical research community must elevate its methodological rigor to provide higher-quality, more reliable data that can robustly inform clinical trial design. Concurrently, a concerted effort from pharmacologists and formulation scientists is required to dismantle the critical bioavailability barrier through innovative drug delivery systems. Successfully navigating this dual path will be the pivotal step in transforming Thymoquinone from a molecule of immense preclinical promise into a tangible therapeutic reality for patients worldwide.

Conclusion

This systematic review consolidates the extensive preclinical evidence for Thymoquinone (TQ), establishing it not merely as a promising molecule, but as a compelling strategic answer to the therapeutic dilemma outlined in our introduction. Where single-target agents have faltered against the multifaceted pathology of ischemia-reperfusion injury (IRI), the broad-spectrum efficacy of TQ validates the necessity of a pleiotropic, multi-target approach. We have not only mapped its protective footprint across a diverse range of organs but also synthesized its complex mechanistic architecture, critically

appraised the quality of the evidence upon which this knowledge rests, and identified the principal barrier - poor bioavailability - that separates this preclinical promise from clinical reality.

Therefore, the path forward is one of constructing a robust translational bridge, built upon two indispensable pillars. The first pillar is a foundation of superior preclinical science; future studies must be designed with uncompromising methodological rigor to provide the high-quality evidence necessary to confidently design human trials. The second is the pillar of pharmaceutical innovation, where the challenge of bioavailability must be met with advanced drug delivery systems. Building this bridge is the logical and necessary next chapter in this scientific narrative. It represents the collective effort required to translate a compelling biological rationale, systematically validated herein, into a therapeutic reality for patients suffering from the devastating consequences of IRI.

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Disclosure of conflict of interest

None.

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