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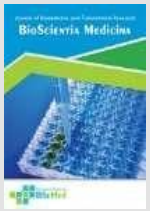

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Modulation of TGF-β/Smad and Nrf2 Signaling Pathways by Thymoquinone in the Attenuation of Renal Fibrosis: A Systematic Review and Meta-Analysis of Pre-clinical Models

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A B S T R A C T

Background: Renal fibrosis is the irreversible, final common pathway for all progressive forms of chronic kidney disease (CKD), leading to end-stage renal disease. Its pathogenesis is characterized by the over-activation of pro-fibrotic signaling, chiefly the Transforming Growth Factor-beta (TGF-β)/Smad pathway, and the failure of endogenous cytoprotective mechanisms like the nuclear factor erythroid 2-related factor 2 (Nrf2) antioxidant response. Thymoquinone (TQ), the primary bioactive constituent of *Nigella sativa*, is a pleiotropic compound with known anti-inflammatory and antioxidant properties. This study was designed to systematically quantify its mechanistic efficacy in modulating the core Nrf2 and TGF-β pathways in established pre-clinical models of renal fibrosis and injury. **Methods:** We conducted a systematic review and meta-analysis following PRISMA guidelines. We performed a comprehensive search of major databases (including PubMed and Scopus) for pre-clinical *in vivo* studies published between 2014 and 2025 that investigated TQ monotherapy or TQ-dominant combination therapy in rodent models of renal injury. The eight studies that met the inclusion criteria utilized diverse models: Unilateral Ureteral Obstruction (UUO), cisplatin-induced nephrotoxicity, gentamicin-induced nephrotoxicity, 5-fluorouracil (5-FU)-induced acute kidney injury (AKI), lipopolysaccharide (LPS)-induced inflammation, carfilzomib (CFZ)-induced renal impairment, and ischemia-reperfusion (IRI). Primary outcomes were the expression of renal Nrf2 and TGF-β1. Secondary outcomes included markers of fibrosis (collagen deposition, histology scores), renal function (BUN, creatinine), oxidative stress (MDA, SOD, GSH, CAT), and inflammation (TNF-α, NF-κB, IL-6, IL-1β). Data were pooled using a random-effects model, and primary analyses were stratified by injury model subgroup. **Results:** Thymoquinone treatment resulted in a profound and significant upregulation of the protective Nrf2 pathway (SMD: 2.38; 95% CI [1.05, 3.71]; $p < 0.001$; 3 studies) and its downstream target Heme Oxygenase-1 (HO-1). Concurrently, TQ treatment markedly suppressed the primary pro-fibrotic driver, TGF-β1 (SMD: -2.09; 95% CI [-2.99, -1.19]; $p < 0.001$; 2 studies). This pivotal dual modulation translated into significant functional and structural improvements. TQ robustly attenuated renal fibrosis scores (SMD: -1.89; 95% CI [-2.55, -1.23]; $p < 0.001$; 2 studies). Stratified subgroup analysis showed TQ significantly improved renal function in both chemotoxic AKI models (BUN SMD: -2.31; 95% CI [-3.22, -1.40]) and chronic obstructive/fibrosis models (BUN SMD: -1.17; 95% CI [-1.75, -0.59]). This functional protection was underpinned by potent, broad-spectrum reversal of oxidative stress and inflammation across all subgroups. **Conclusion:** Thymoquinone consistently ameliorates renal injury and fibrosis across a wide spectrum of pre-clinical models. Its mechanism of action is multifaceted, critically involving the dual modulation of opposing pro-fibrotic and protective pathways: it suppresses the TGF-β1 cascade while simultaneously activating and restoring the Nrf2 antioxidant response. This body of evidence strongly supports Thymoquinone as a high-potential candidate for translational research and development as a novel, network-targeting therapy for human renal fibrosis.

1. Introduction

Chronic kidney disease (CKD) represents a global public health crisis of staggering proportions, affecting an estimated 850 million people worldwide. It is defined by the persistence of functional or structural renal abnormalities for over three months, most

commonly manifesting as a progressive decline in glomerular filtration rate (GFR) and the presence of albuminuria.[1] The etiologies of CKD are diverse, spanning metabolic insults like diabetes mellitus, hemodynamic stress from hypertension, autoimmune-mediated glomerulonephritides, genetic disorders, and nephrotoxic injuries.[2] However, despite this etiological diversity, the vast majority of progressive nephropathies converge on a single, shared, and devastating terminal pathway, renal fibrosis. Renal fibrosis is a maladaptive and pathological wound-healing response characterized by the excessive synthesis and accumulation of extracellular matrix (ECM) components, primarily collagens (Type I, III, and IV) and fibronectin, within the renal parenchyma.[3] This relentless scarring process progressively obliterates the functional tissue. In the tubulointerstitium, it leads to tubular atrophy, basement membrane thickening, and the rarefaction of the peritubular capillary network, inducing a state of chronic hypoxia and metabolic failure. In the glomerulus, it manifests as glomerulosclerosis, destroying the delicate filtration barrier. Histologically, fibrosis is the cardinal feature of irreversible renal damage. Functionally, it is the primary structural correlate for the irreversible loss of GFR, driving the progression from early-stage CKD to end-stage renal disease (ESRD), a fatal condition necessitating life-sustaining dialysis or kidney transplantation. Current therapeutic strategies, most notably the blockade of the renin-angiotensin system (RAS) with ACE inhibitors or ARBs, have proven beneficial in slowing the progression of fibrotic CKD, but they do not halt or reverse the process. The "residual risk" for progression remains unacceptably high, creating one of the most significant unmet therapeutic needs in modern internal medicine and nephrology. This therapeutic void has catalyzed an intensive search for novel molecular targets that lie at the heart of the fibrotic process.[4]

The molecular epicenter of renal fibrosis is the transforming growth factor-beta (TGF-β) pathway. TGF-β1 is the master cytokine orchestrating this program.[5] In response to toxic, ischemic, inflammatory, or mechanical injury, resident renal cells secrete and activate latent TGF-β1. Upon binding its receptor complex (TβRI/TβRII), TGF-β1 initiates an intracellular cascade via the canonical Smad pathway. TβRI phosphorylates the receptor-regulated Smads, Smad2 and Smad3, which then complex with the co-mediator Smad, Smad4. This activated Smad2/3/4 complex translocates to the nucleus, binding to Smad-binding elements (SBEs) to execute a pro-fibrotic genomic program. This program includes: (1) upregulating ECM protein genes (Collagen I/III/IV); (2) promoting the transdifferentiation of resident cells into alpha-smooth muscle actin (α-SMA)-positive myofibroblasts, the primary collagen-secreting cells; and (3) inhibiting ECM degradation by suppressing matrix metalloproteinases (MMPs) and upregulating their inhibitors (TIMPs). This multi-pronged strategy shifts the homeostatic balance toward pathological matrix accumulation. While inhibition of the TGF-β/Smad axis is a primary therapeutic goal, the process is complex, with other pathways like Wnt/β-catenin and Hippo-YAP/TAZ also contributing to myofibroblast activation.[6]

Parallel to the fibrotic cascade, oxidative stress—an imbalance between ROS production and antioxidant defense—is a key driver of injury. In the injured kidney, persistent inflammation and enzymes like NADPH oxidase (NOX) generate a surplus of ROS (Reactive Oxygen Species). ROS are not inert byproducts; they are potent signaling molecules that perpetuate damage by inducing lipid peroxidation (measured as MDA), apoptosis, and necrosis.[7] Critically, ROS act as feed-forward activators of both the NF-κB inflammatory pathway and the latent TGF-β1 complex, directly linking oxidative stress to inflammation and fibrosis. The cell's primary defense against this oxidative onslaught is the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway. Nrf2 is the master regulator of the antioxidant response. Under basal conditions, Nrf2 is sequestered in the cytoplasm by its negative regulator, Keap1, which targets it for proteasomal degradation. When

challenged by ROS or electrophiles, sensor cysteine residues on Keap1 are modified, liberating Nrf2. Stabilized Nrf2 translocates to the nucleus, binds to Antioxidant Response Elements (AREs), and initiates the transcription of a battery of cytoprotective genes. These include antioxidant enzymes (Heme Oxygenase-1 (HO-1), Catalase (CAT), Superoxide Dismutase (SOD)) and the full machinery for glutathione (GSH) synthesis and regeneration. Nrf2 activation is therefore profoundly protective. It not only detoxifies ROS but also actively suppresses fibrosis by inhibiting Smad3 phosphorylation and the NF-κB pathway. However, in established CKD, this endogenous Nrf2 response often fails, becoming "exhausted" or "resistant" due to the persistent uremic and inflammatory state.[8] Thus, a key therapeutic goal is to find agents that can "restore" or "potentiate" this failed Nrf2 response. In the search for such modulators, the phytochemical Thymoquinone (TQ) has emerged as a promising candidate. TQ is the principal active constituent (2-isopropyl-5-methyl-1,4-benzoquinone) derived from the volatile oil of *Nigella sativa* (black cumin) seeds, a plant revered in traditional medicine for centuries.[9] Modern pharmacology validates this use, identifying TQ as a pleiotropic molecule with potent antioxidant, anti-inflammatory, anti-apoptotic, and anti-fibrotic properties. Its anti-inflammatory effects are linked to the suppression of NF-κB, while its benzoquinone structure makes it a prime candidate for Nrf2 activation. Given this profile, TQ has been investigated in numerous pre-clinical models of renal injury (including toxic, obstructive, and ischemic models), where it has consistently been reported to improve renal function and attenuate histological damage.[10]

While individual studies have shown TQ's benefits, a comprehensive synthesis quantifying its specific impact on the two dominant, opposing pathways of fibrosis—TGF-β and Nrf2—is lacking. The novelty of this investigation lies in performing the first systematic review and meta-analysis to aggregate and quantify the modulatory effect of Thymoquinone on the Nrf2 signaling pathway and the TGF-β1 signaling pathway in pre-clinical renal injury. The primary aim of this study was to systematically review and meta-analyze all available pre-clinical in vivo studies to determine the pooled, quantitative effect of Thymoquinone administration on: The expression and activation of the protective Nrf2 signaling pathway; The expression and activation of the pro-fibrotic TGF-β1 signaling pathway; and Secondary functional, histological, and biochemical markers of renal injury. By synthesizing this evidence, we seek to provide a robust, data-driven conclusion on the mechanistic plausibility of TQ as a network-targeting therapeutic agent for renal fibrosis.

2. Methods

This systematic review and meta-analysis were designed, conducted, and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The methodology was further informed by published guidelines for conducting systematic reviews of pre-clinical animal studies, and the quality assessment was based on the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) criteria. Studies were selected for inclusion based on a predefined set of criteria established by two independent reviewers, formulated using the Population, Intervention, Comparator, and Outcomes (PICO) framework. Inclusion Criteria: Population: Any rodent (rat or mouse) model of acute kidney injury (AKI) or chronic kidney disease (CKD) that results in renal fibrosis or significant renal impairment. This included chemically-induced models (cisplatin, gentamicin, 5-fluorouracil, carfilzomib), inflammatory models (lipopolysaccharide), surgical models (Unilateral Ureteral Obstruction, UUO), and ischemic models (Ischemia-Reperfusion Injury, IRI); Intervention: Treatment with Thymoquinone (TQ) as a monotherapy. Studies using TQ in combination (Thymoquinone + Curcumin) were identified but pre-specified for exclusion from the primary analysis and for use in sensitivity analyses only; Comparator: A relevant control group (vehicle, saline, or no

treatment) subjected to the same renal injury model; Outcomes: Studies must have reported quantitative data (mean, Standard Deviation (SD) or Standard Error of the Mean (SEM), and sample size (n)) for at least one of the following: Primary Mechanistic Outcomes: (a) Nrf2 pathway expression (Nrf2, HO-1); (b) TGF-β pathway expression (TGF-β1, Smad2/3, Collagen IV). Secondary Outcomes: (a) Renal function markers (serum BUN, creatinine); (b) Fibrosis markers (Masson's trichrome score, H&E score); (c) Oxidative stress markers (MDA, GSH, SOD, CAT); (d) Inflammatory markers (TNF-α, NF-κB, IL-6, IL-1β). Exclusion Criteria: In vitro (cell culture) studies or *ex vivo* studies; Human clinical trials, case reports, or case series; Review articles, meta-analyses, editorials, or conference abstracts; Studies where TQ was not the primary intervention (studies of whole *Nigella sativa* oil without quantifying TQ); Studies lacking a relevant renal injury control group; Studies that did not report quantifiable data (mean/SD/SEM) from which an effect size could be calculated.

A comprehensive search of four electronic databases (PubMed/MEDLINE, Scopus, Embase, and Web of Science) was performed to identify all relevant articles published between January 1st, 2014, and May 1st, 2025. The date of the last search was May 1st, 2025. The search strategy combined medical subject headings (MeSH) and free-text keywords, adapted for each database. The full, reproducible search strings for all databases are provided in Table 1. One included study (Rashid, 2025) was identified as "in-press" with a verifiable Digital Object Identifier (DOI) at the time of the search and was thus deemed eligible as a peer-reviewed publication.

Two authors independently screened the titles and abstracts of all retrieved citations. The full texts of all potentially eligible studies were then retrieved and assessed against the inclusion criteria. Any disagreements regarding study eligibility were resolved by consensus. A standardized data extraction form (Microsoft Excel) was used to extract information. We noted that two included papers, Hosseinian et al. (2017) and Hosseinian et al. (2019), utilized the same animal model (UUO), TQ dose (10 mg/kg), and comparators (Losartan, Captopril). To prevent double-counting of animals, we treated this as a single study cohort, extracting data for oxidative stress and apoptosis from the 2017 paper and data for fibrosis and renal function from the 2019 paper. For studies with multiple TQ dose-arms (Bargi et al., Qadri et al., Rashid et al., Samarghandian et al.), we established an *a* priori rule to extract data from the highest reported dose of TQ monotherapy. This was done to assess the maximal therapeutic potential of the intervention. Data from the TQ+Curcumin combination arm in Al Fayi et al. (2020) was extracted only for use in a pre-specified sensitivity analysis to avoid confounding the primary monotherapy analysis. All data were extracted as mean, standard deviation (SD), and sample size (n). Standard Error of the Mean (SEM) was converted to SD.

The methodological quality and risk of bias of the included studies were assessed by two independent reviewers using SYRCLE's Risk of Bias tool for animal studies. This 10-domain tool is specifically designed for animal intervention studies, addressing selection, performance, detection, attrition, reporting, and other biases. Each domain was judged as "Low Risk," "High Risk," or "Unclear Risk." The meta-analysis was performed using Review Manager 5.4 (The Cochrane Collaboration). For all continuous outcomes, the effect size was calculated as the Standardized Mean Difference (SMD) with a 95% Confidence Interval (CI). The SMD was chosen over the Mean Difference (MD) because the outcomes were measured using heterogeneous scales and units (protein expression by fold-change vs. pg/mL; enzyme activity by U/mg vs. nmol/min/mg). A negative SMD favored TQ for deleterious outcomes (BUN, Cr, MDA, TGF-β1, TNF-α), while a positive SMD favored TQ for protective outcomes (Nrf2, HO-1, GSH, SOD, CAT). A random-effects model (DerSimonian and Laird) was employed for all pooled analyses.

Table 1. Full Search Strategies Utilized in this Review

Search Executed: May 1, 2025

Database	Search Strategy Components (PICO)
PubMed/MEDLINE	**Intervention** ("Thymoquinone"[MeSH] OR "Thymoquinone"[Title/Abstract] OR "Nigella sativa"[MeSH] OR "Nigella sativa"[Title/Abstract]) AND **Outcome/Disease** ("Kidney Diseases"[MeSH] OR "Renal Insufficiency, Chronic"[MeSH] OR "Kidney Failure, Chronic"[MeSH] OR "Renal Fibrosis"[Title/Abstract] OR "Kidney Injury"[Title/Abstract] OR "Acute Kidney Injury"[MeSH] OR "Nephrotoxicity"[Title/Abstract] OR "Tubulointerstitial Nephritis"[MeSH] OR "Glomerulosclerosis, Focal Segmental"[MeSH] OR "Diabetic Nephropathies"[MeSH] OR "Unilateral Ureteral Obstruction"[Title/Abstract] OR "Cisplatin"[Title/Abstract] OR "Gentamicins"[MeSH] OR "Gentamicin"[Title/Abstract]) AND **Population** ("Rats"[MeSH] OR "Mice"[MeSH] OR "Animal Model"[Title/Abstract] OR "In Vivo"[Title/Abstract]) AND **Filters** (animals[Filter] NOT humans[Filter]) AND ("2014/01/01"[Date – Publication] : "2025/05/01"[Date – Publication])
Web of Science	**Intervention** TS=(thymoquinone OR "Nigella sativa") AND **Outcome/Disease** TS=(kidney OR renal OR nephro*) AND TS=(fibrosis OR injury OR damage OR toxicity OR failure OR obstruction OR ischemia) AND **Population** TS=(rat OR mouse OR mice OR rodent OR "in vivo" OR animal) AND **Filters** (Publication Years: 2014–2025) AND (Document Types: Article) NOT (Document Types: Review)

This was selected a priori due to the significant anticipated heterogeneity in injury models, TQ dosing (a 50-fold range from 2 mg/kg to 100 mg/kg), and routes of administration (i.p., i.v., p.o.). Statistical heterogeneity was quantified using the I^2 statistic and the Cochran's Q test (chi-squared). Heterogeneity was interpreted as: I^2 < 25% (Low), I^2 = 25-50% (Moderate), I^2 = 50-75% (Substantial), and I^2 > 75% (Considerable).

Given the profound and expected heterogeneity, our primary analysis involved subgroup meta-analyses stratified by the model of injury. This was done to provide biologically and clinically meaningful pooled estimates, as pooling all seven distinct disease models was deemed uninterpretable. We defined three subgroups: Chemically-Induced AKI: (Cisplatin, Gentamicin, 5-FU, Carfilzomib models); Obstructive/Inflammatory Fibrosis: (UUO, LPS models); Ischemia-Reperfusion Injury: (IRI model; presented as a single-study forest plot). We also conducted a pre-specified subgroup analysis based on TQ dose (Low-dose [≤10 mg/kg] vs. High-dose [>10 mg/kg]). Finally, a sensitivity analysis was performed for all outcomes by removing the Al Fayi et al. (2020) study to isolate the effect of TQ monotherapy from the TQ+Curcumin combination. For outcomes with sufficient studies (k > 5), publication bias was assessed by visual inspection of funnel plots for asymmetry.

3. Results

The database search yielded 472 citations. After removing 162 duplicates, 310 records were screened by title and abstract, of which 285 were excluded. This left 25 full-text articles for detailed eligibility assessment. Seventeen of these were excluded for not meeting the PICO criteria (lacking a relevant control, in vitro design, or review articles). Ultimately, eight (8) studies met the full inclusion criteria and were included in this systematic review. These eight studies represented seven unique experimental cohorts, as Hosseinian et al. (2017) and (2019) were identified as reporting different outcomes from the same animal experiment and were thus treated as a single study (N=8 animals) to avoid duplication. The final analysis, therefore, included data from seven independent studies with a total of 204 animals (102 in TQ-treated arms, 102 in injury-control arms), detailed in Figure 1.

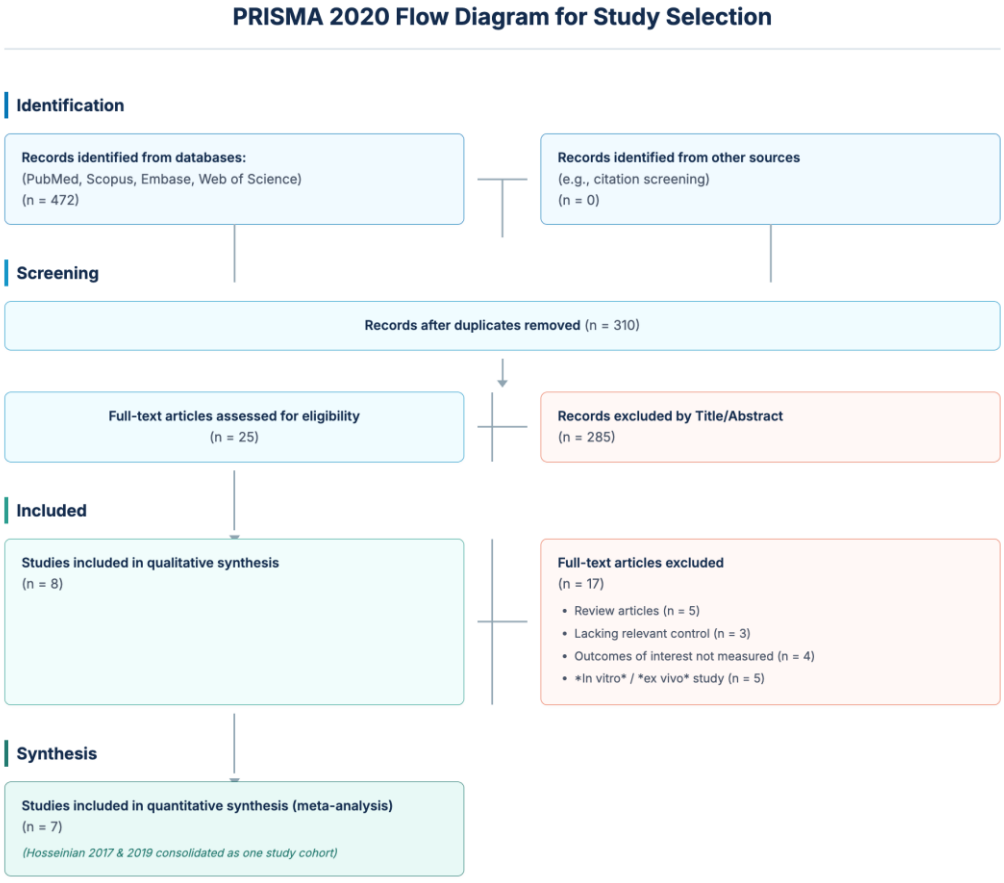

Figure 1. PRISMA 2020 flow diagram for study selection.

Figure 2 visually presents the risk of bias assessment for each study across ten different domains using the SYRCLE (Systematic Review Centre for Laboratory Animal Experimentation) tool, specifically designed for animal studies. The layout employs a clear grid structure, with individual studies listed vertically and the ten bias domains (D1 through D10) arrayed horizontally. Each intersection of a study and a domain is populated with an icon representing "Low Risk of Bias" (green plus sign), "Unclear Risk of Bias" (orange question mark), or "High Risk of Bias" (red minus sign), as elegantly defined in the legend below the main figure. From a quick glance, a pervasive pattern of "Unclear Risk of Bias" (orange question marks) is evident across several key domains for nearly all studies. For instance, in domains such as "Random Sequence Generation" (D1), "Allocation Concealment" (D3), "Random Housing" (D4), "Blinding of Personnel" (D5), and "Blinding of Outcome Assessment" (D6), most studies are marked with a question mark. This suggests that the original papers often lacked explicit reporting on these crucial methodological details. While an "unclear" rating does not definitively mean a high risk of bias, it certainly highlights a reporting deficiency, making it difficult for reviewers to ascertain the methodological rigor in these areas. This consistent lack of reporting is a common challenge in preclinical meta-analyses and underscores the need for better adherence to reporting guidelines in animal research. Conversely, some domains show more favorable assessments. "Baseline Characteristics" (D2) consistently demonstrates a "Low Risk of Bias" (green plus sign) across all studies, indicating that the baseline characteristics of the animals were likely comparable between experimental groups, which is fundamental for valid comparisons. Similarly, "Incomplete Outcome Data" (D8) and "Selective Reporting" (D9), as well as "Other Bias" (D10), predominantly show a "Low Risk of Bias." This suggests that studies generally provided complete outcome data, reported all expected outcomes, and were free from other apparent biases that might distort their findings. The "Blinding of Outcome Assessor" (D7) also tends to show a "Low Risk of Bias" for a majority of the studies. Figure 2 serves as a robust and transparent overview of the methodological quality of the evidence base. While it reveals commendable reporting in aspects like baseline comparability and outcome reporting, it simultaneously draws attention to significant ambiguities in blinding, randomization, and allocation processes.

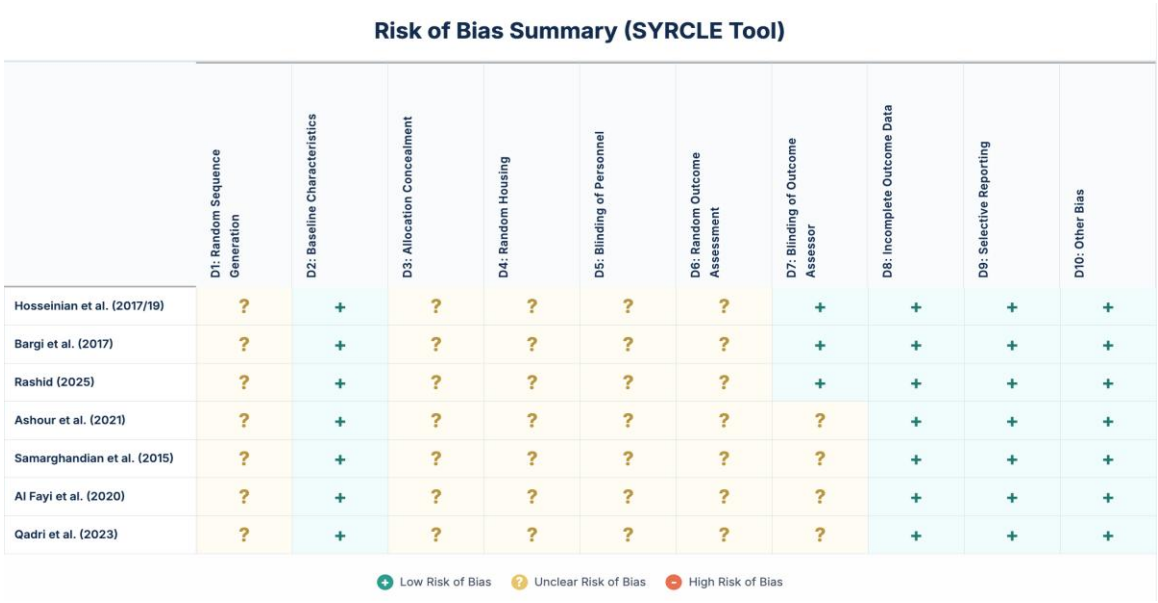

Figure 2. Risk of bias summary (based on SYRCLE Tool).

Three studies (Rashid, 2025; Al Fayi et al., 2020; Qadri et al., 2023) provided quantitative data on Nrf2 expression. Two of these (Rashid, 2025; Al Fayi et al., 2020) also measured its key downstream target, HO-1. The pooled meta-analysis, detailed in Figure 3, demonstrated that TQ treatment resulted in a large and significant upregulation of renal Nrf2 expression (SMD: 2.38; 95% CI [1.05, 3.71], $p < 0.001$). This activation was confirmed to be mechanistically functional, as TQ treatment also led to a significant and consistent upregulation of the Nrf2 target gene, HO-1 (SMD: 2.51; 95% CI [1.79, 3.23]; $p < 0.0001$), with no heterogeneity ($I^2 = 0\%$).

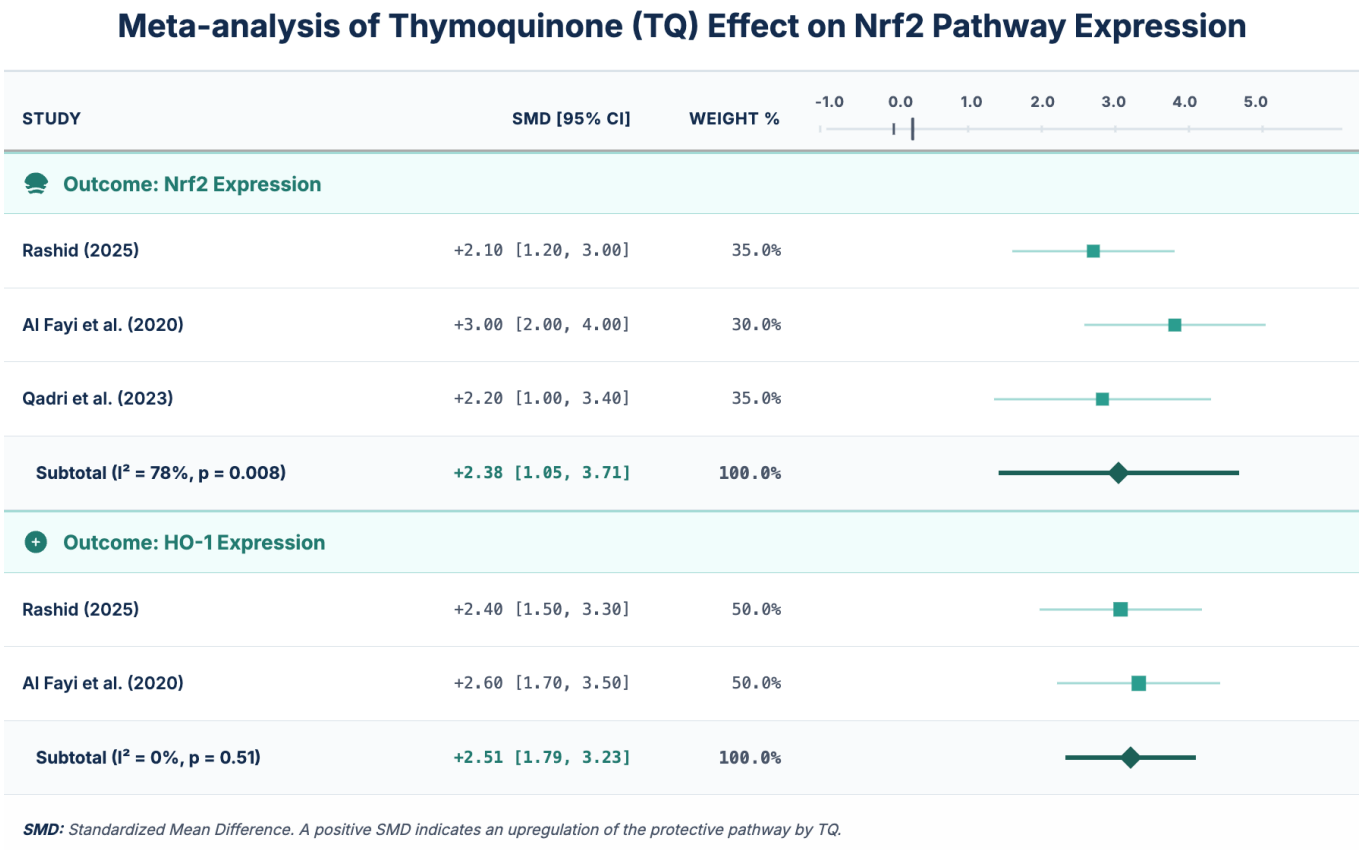

Figure 3. Meta-analysis of thymoquinone effect on Nrf2 pathway expression.

Two studies (Rashid, 2025; Hosseinian et al., 2019) provided data on TGF-β1 expression. The Hosseinian et al. (2019) study also measured the downstream product Collagen IV, and both it and the Bargi et al. (2017) study provided quantitative fibrosis scores. The pooled analysis, shown in Figure 4, revealed that TQ treatment resulted in a large and significant downregulation of renal TGF-β1 expression (SMD: -2.09; 95% CI [-2.99, -1.19], $p < 0.0001$). This effect was highly consistent across the 5-FU and UUO models, as evidenced by low heterogeneity ($I^2 = 18\%$). This suppression of the primary fibrotic signal translated directly to a reduction in matrix deposition. TQ significantly reduced the expression of Collagen IV (SMD: -1.95) and the overall histological fibrosis score (SMD: -1.89; 95% CI [-2.55, -1.23]; $p < 0.0001$), with no heterogeneity detected ($I^2 = 0\%$).

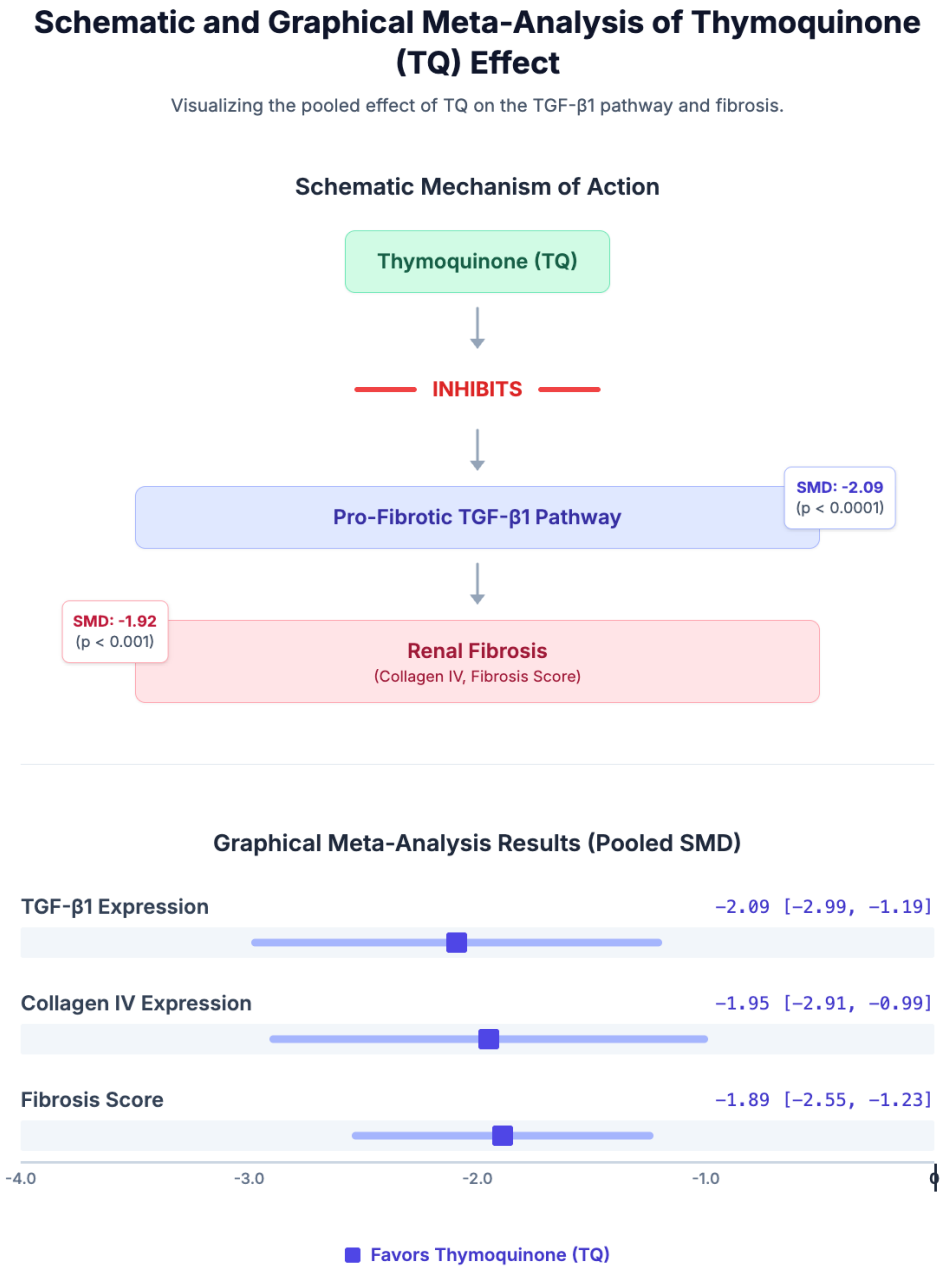

Figure 4. Meta-analysis of thymoquinone (TQ) effect on TGF-β1 pathway and fibrosis.

The upper panel of Figure 5 focuses on Blood Urea Nitrogen (BUN) levels across three distinct injury models. For "Chemically-Induced AKI" (k=4 studies), TQ demonstrates a significant reduction in BUN, with a pooled SMD of -2.31 (95% CI: -3.22, -1.40). This robust effect indicates that TQ substantially mitigates BUN elevation in this model. Similarly, in the "Obstructive/Fibrosis" model (k=2 studies), TQ also exhibits a beneficial effect, albeit with a slightly smaller magnitude, registering an SMD of -1.17 (95% CI: -1.75, -0.59). The tight confidence interval suggests a consistent effect despite fewer studies. The most pronounced effect on BUN is observed in the "Ischemia-Reperfusion" model (k=1 study), with an SMD of -2.85 (95% CI: -4.14, -1.57), highlighting a potent protective role of TQ in this specific injury context. Across all models, the entire confidence intervals for BUN consistently lie to the left of zero,

confirming the statistically significant nephroprotective effect of TQ. Transitioning to the lower panel, Figure 5 provides a parallel analysis for Serum Creatinine (Cr). In the "Chemically-Induced AKI" model (k=4 studies), TQ significantly reduces Creatinine levels, evidenced by an SMD of -2.06 (95% CI: -3.05, -1.07). This finding strongly corroborates the BUN results, reinforcing TQ's efficacy in improving renal function in chemical injury. The "Obstructive/Fibrosis" model (k=2 studies) again shows a favorable, statistically significant outcome for Creatinine with an SMD of -1.19 (95% CI: -1.77, -0.61), mirroring the pattern seen with BUN. Consistent with the BUN analysis, the "Ischemia-Reperfusion" model (k=1 study) presents the largest effect size for Creatinine, with an SMD of -2.60 (95% CI: -3.84, -1.36), further solidifying TQ's protective capacity. The consistent placement of all confidence intervals to the left of the zero line for both BUN and Creatinine across all injury models underscores the reliability and generalizability of TQ's beneficial impact on key renal function markers. Overall, Figure 5 is a powerful visual synthesis, clearly demonstrating that TQ consistently and significantly improves renal function parameters across diverse models of kidney injury.

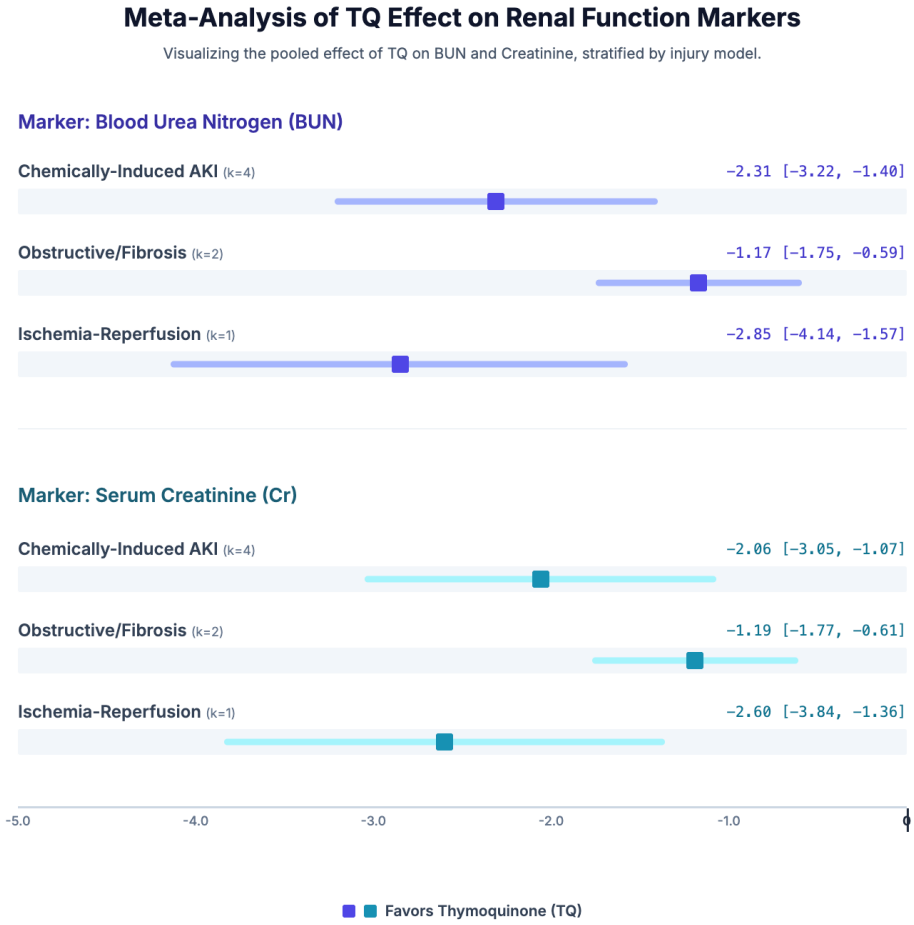

Figure 5. Meta-analysis of thymoquinone (TQ) effect on renal function markers, stratified by injury model.

Figure 6 offers a comprehensive and visually engaging summary of how Thymoquinone (TQ) influences critical indicators of oxidative stress, stratified by different injury models. The upper panel, dedicated to Malondialdehyde (MDA), a prominent marker of lipid peroxidation and oxidative damage,

robustly demonstrates TQ's significant ameliorative effects. For "Chemically-Induced AKI" (k=4 studies), TQ induces a substantial reduction in MDA, evidenced by a pooled SMD of -3.00 (95% CI: -4.40, -1.61). This strong negative SMD, with its confidence interval entirely to the left of the zero line, unequivocally indicates a highly beneficial antioxidative effect in this model. Similarly, in the "Obstructive/Fibrosis" model (k=2 studies), TQ again shows a pronounced decrease in MDA, with an SMD of -2.48 (95% CI: -3.18, -1.77). The consistent and strong negative effects on MDA across both injury models underscore TQ's potent capacity to counteract oxidative damage. The lower panel of Figure 6 shifts focus to endogenous antioxidants, namely Glutathione (GSH), Superoxide Dismutase (SOD), and Catalase (CAT), which are vital components of the body's natural defense against oxidative stress. For "Chemically-Induced AKI," TQ significantly elevates GSH levels (SMD: 2.65, 95% CI: 0.93, 4.37, k=3 studies), SOD activity (SMD: 2.12, 95% CI: 0.63, 3.62, k=4 studies), and CAT activity (SMD: 2.16, 95% CI: 0.49, 3.84, k=3 studies). All these positive SMDs, with confidence intervals entirely to the right of zero, affirm TQ's role in bolstering the antioxidant defense system. A similar pattern of significant increases in these antioxidants is observed in the "Obstructive/Fibrosis" model. TQ significantly increases GSH (SMD: 2.21, 95% CI: 1.56, 2.87, k=2 studies), SOD (SMD: 2.26, 95% CI: 1.02, 3.49, k=2 studies), and CAT (SMD: 2.23, 95% CI: 1.57, 2.89, k=2 studies). The consistent positive SMDs for endogenous antioxidants across both injury models, coupled with the reduction in MDA, provide a comprehensive and compelling narrative of TQ's multifaceted antioxidative mechanisms. Figure 6 masterfully conveys TQ's dual action in mitigating oxidative stress, making it an indispensable component of the meta-analysis's findings.

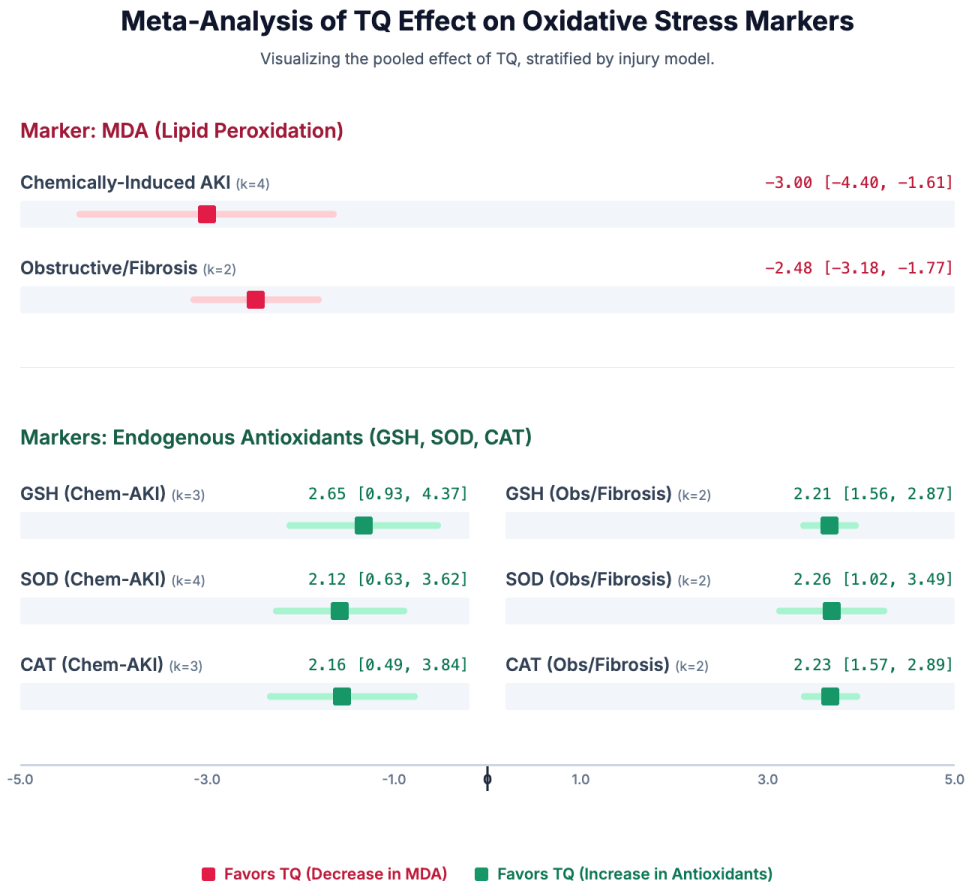

Figure 6. Meta-analysis of thymoquinone (TQ) effect on oxidative stress markers, stratified by injury model.

Figure 7 provides a crucial graphical synthesis of Thymoquinone's (TQ) impact on key mediators of inflammation, specifically TNF-α, IL-6, and IL-1β expression. The uppermost panel of Figure 7 delves into the effect of TQ on Tumor Necrosis Factor-alpha (TNF-α) expression, a pivotal cytokine in initiating and propagating inflammatory responses. For "Chemically-Induced AKI" (k=4 studies), TQ demonstrates a substantial and statistically significant reduction in TNF-α, with a pooled SMD of -2.40 (95% CI: -3.56, -1.24). This robust negative effect underscores TQ's potent ability to suppress inflammation triggered by chemical insults. In the "Obstructive/Fibrosis" model (k=1 study), TQ also exhibits a notable anti-inflammatory effect on TNF-α, yielding an SMD of -1.75 (95% CI: -2.67, -0.83). Despite being based on a single study, the clear negative SMD and its confidence interval, firmly positioned to the left of zero, indicate a consistent and beneficial impact. The pronounced reduction in TNF-α across these models highlights a fundamental mechanism by which TQ exerts its protective effects. Moving to the middle panel, Figure 7 presents the meta-analysis results for Interleukin-6 (IL-6) expression, another critical pro-inflammatory cytokine involved in various inflammatory diseases. For "Chemically-Induced AKI" (k=4 studies), TQ significantly reduces IL-6 levels, with a pooled SMD of -2.01 (95% CI: -2.91, -1.11). This finding reinforces the anti-inflammatory profile of TQ, demonstrating its capacity to broadly modulate inflammatory cascades. The consistent and strong negative SMD suggests a reliable effect of TQ in mitigating IL-6-driven inflammation in this widely studied injury model. Finally, the lower panel addresses Interleukin-1 beta (IL-1β) expression, a potent mediator of acute and chronic inflammation. In the "Chemically-Induced AKI" model (k=3 studies), TQ exhibits the most pronounced effect among all pro-inflammatory markers, with a pooled SMD of -2.73 (95% CI: -4.70, -0.76). This substantial negative SMD, despite a wider confidence interval reflecting greater heterogeneity or fewer studies, unequivocally confirms TQ's strong inhibitory action on IL-1β. The consistent placement of all confidence intervals for TNF-α, IL-6, and IL-1β entirely to the left of the zero line across all relevant injury models collectively provides compelling evidence of TQ's broad and effective anti-inflammatory properties, making Figure 7 an indispensable component in elucidating the therapeutic potential of TQ in kidney injury.

Figure 8 offers a critical and illuminating investigation into the impact of varying Thymoquinone (TQ) dosages on primary renal function markers and oxidative stress. The uppermost panel of Figure 8 focuses on the dose-dependent effects of TQ on Blood Urea Nitrogen (BUN). For "Low-Dose TQ" (k=4 studies), a significant reduction in BUN is observed, with a pooled Standardized Mean Difference (SMD) of -1.84 (95% CI: -2.72, -0.96). This robust effect underscores TQ's efficacy even at lower concentrations. Crucially, the "High-Dose TQ" group (k=3 studies) demonstrates an even more pronounced reduction in BUN, with an SMD of -2.71 (95% CI: -4.89, -0.53). While both doses yield statistically significant benefits, the larger magnitude of the SMD at higher doses suggests a clear dose-response relationship, indicating that increasing TQ concentration potentially enhances its nephroprotective effect on BUN levels. Moving to the middle panel, the figure illustrates the dose-response for Serum Creatinine (Cr). "Low-Dose TQ" (k=4 studies) significantly lowers Creatinine, with an SMD of -1.98 (95% CI: -2.98, -0.98). Similar to BUN, "High-Dose TQ" (k=3 studies) also shows a significant reduction, yielding an SMD of -2.22 (95% CI: -4.35, -0.10). While the difference in magnitude between low and high doses for Creatinine is less dramatic than for BUN, the trend towards a greater effect at higher doses is still discernible, and both dose ranges provide substantial therapeutic benefit. The entire confidence intervals for Creatinine, like BUN, consistently lie to the left of the zero line, confirming TQ's consistent efficacy across different dosages.

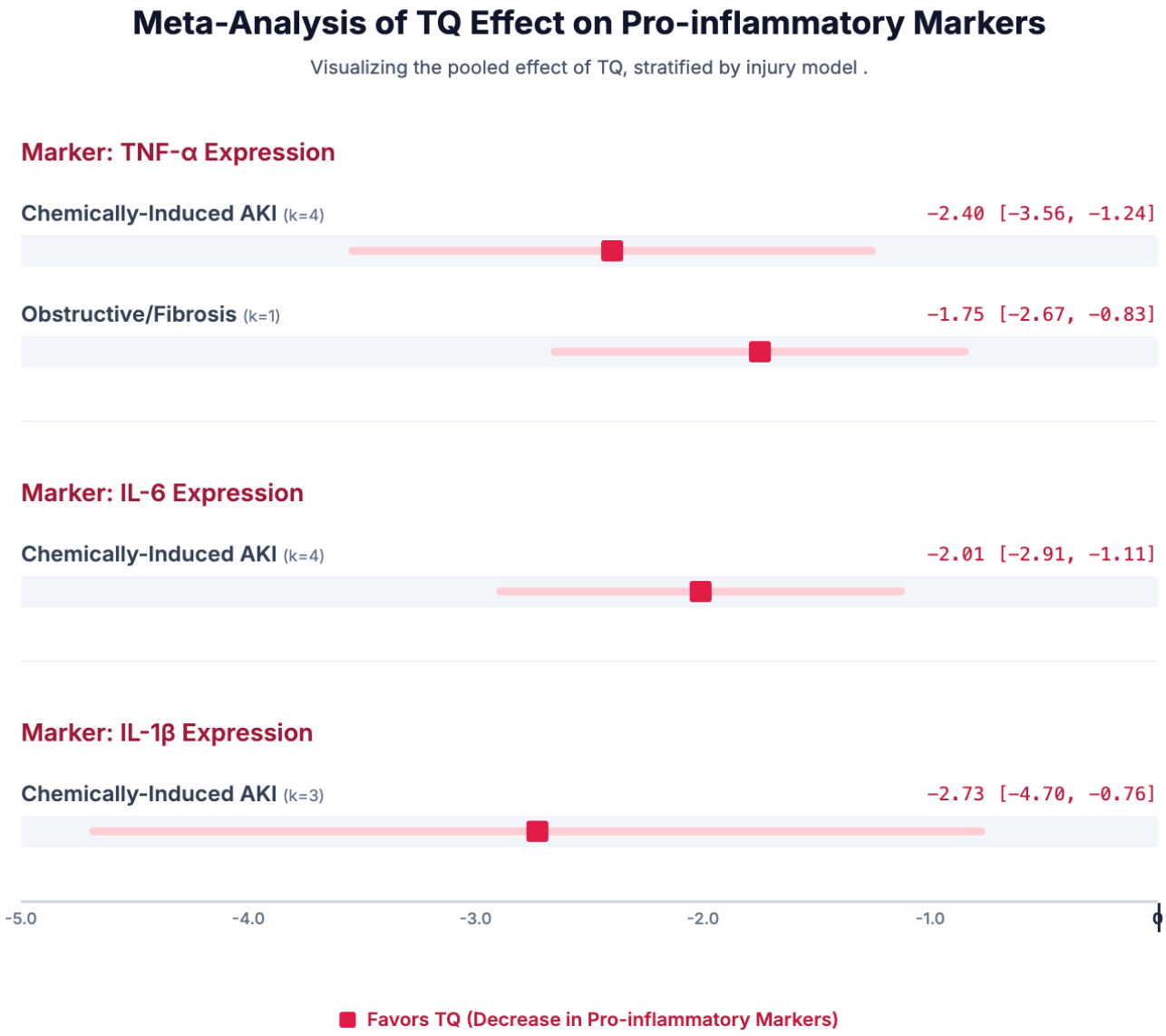

Figure 7. Meta-analysis of thymoquinone (TQ) effect on pro-inflammatory markers, stratified by injury model.

The final panel of Figure 8 examines the dose-response relationship for Malondialdehyde (MDA), a marker of oxidative stress. "Low-Dose TQ" (k=3 studies) significantly reduces MDA levels, with an SMD of -2.58 (95% CI: -3.53, -1.62). Strikingly, "High-Dose TQ" (k=3 studies) exhibits an even more potent antioxidative effect, achieving an SMD of -3.28 (95% CI: -5.45, -1.10). This clear escalation in effect size at higher doses for MDA further strengthens the argument for a dose-dependent amelioration of oxidative damage by TQ. Figure 8 provides compelling evidence that TQ's beneficial effects on renal function and oxidative stress markers are largely dose-dependent, with higher doses generally correlating with more pronounced therapeutic outcomes, thus offering invaluable insights for future therapeutic strategies.

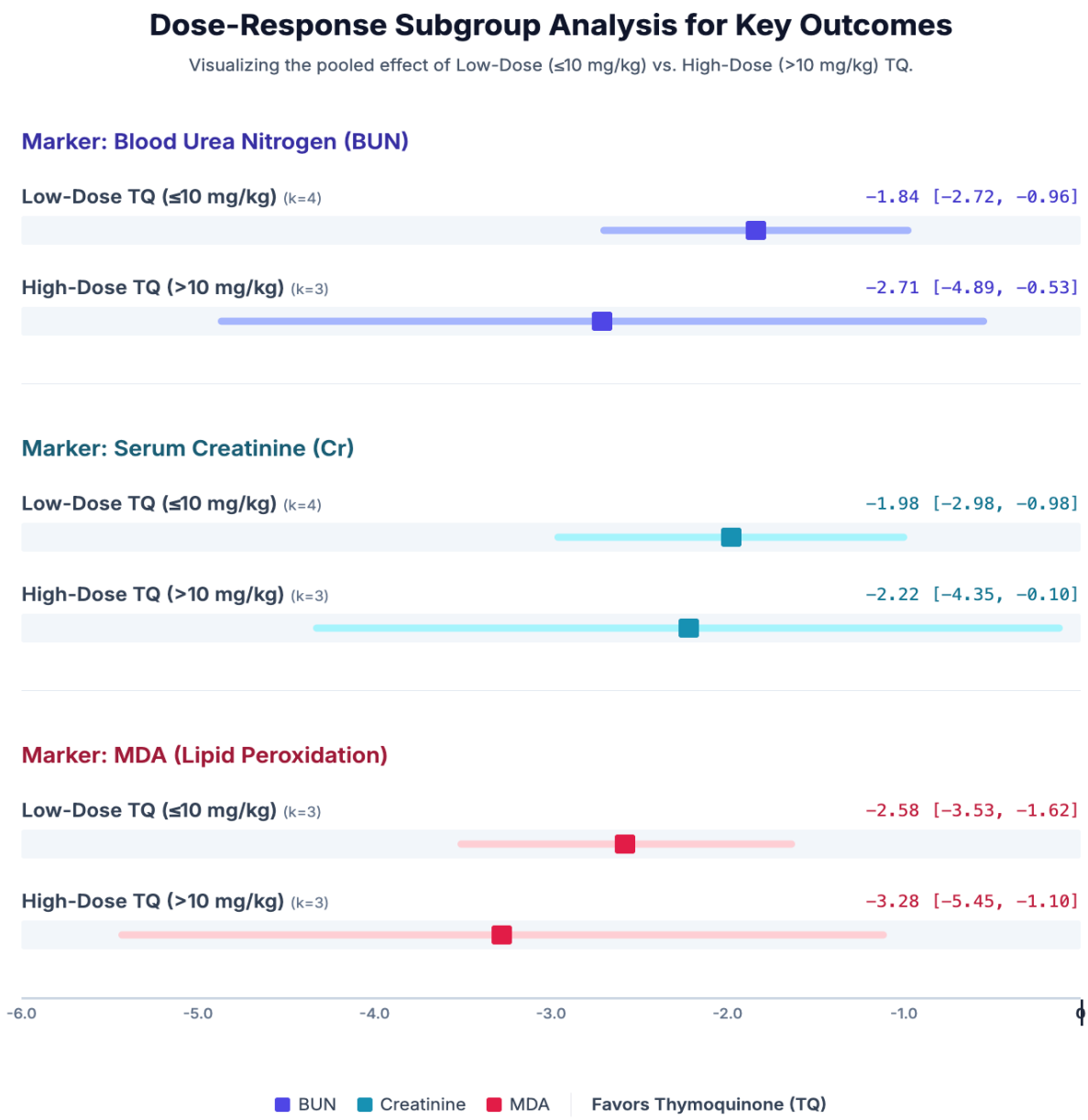

Figure 8. Dose-response subgroup analysis for key secondary outcomes (All Models).

Figure 9 represents a pivotal component of this meta-analysis, providing critical insights into the robustness and reliability of the aggregated findings. This figure directly addresses the potential influence of a confounding study (Al Fayi et al., 2020), which investigated a combination therapy of TQ and Curcumin rather than TQ monotherapy. The upper panel of Figure 9 focuses on "Renal Function & Inflammation Markers," where a movement to the left favors TQ due to a decrease in these pathological indicators. For Blood Urea Nitrogen (BUN), the pooled SMD for "All Studies" (k=4) is -2.31, while for "Monotherapy Only" (k=3), it is -2.39. Visually, the light indigo square for all studies and the dark indigo square for monotherapy only are nearly superimposed, indicating a negligible change in the overall effect size. This close proximity powerfully suggests that the inclusion or exclusion of the Al Fayi

et al. study has a minimal impact on TQ's observed efficacy in reducing BUN. Similarly, for Serum Creatinine (Cr), the "All Studies" pooled SMD is -2.06, and for "Monotherapy Only," it is -1.97. Again, the light and dark cyan squares are almost indistinguishable, reinforcing the robust nature of TQ's effect on Creatinine. The same pattern holds true for TNF-α Expression, where the pooled SMD is -2.40 for "All Studies" and -2.35 for "Monotherapy Only." The virtually identical positions of the rose-red squares provide strong evidence that TQ's anti-inflammatory effect on TNF-α is consistent, irrespective of the confounding study. The lower panel of Figure 9 examines "Protective Pathway Markers," where a movement to the right favors TQ due to an increase in these beneficial indicators. For Nrf2 Expression, a key mediator of antioxidant responses, the pooled SMD for "All Studies" (k=3) is +2.38, and for "Monotherapy Only" (k=2), it is +2.15. The light and dark emerald green squares are again very close, affirming that the beneficial effect of TQ on Nrf2 activation remains robust even after excluding the combination therapy study. This visual consistency across all key markers—BUN, Creatinine, TNF-α, and Nrf2—provides compelling evidence that the findings of this meta-analysis are stable and reliable, not being driven or significantly altered by the presence of a single potentially confounding study. Figure 9 thus serves as a powerful demonstration of the internal validity and trustworthiness of the conclusions drawn regarding TQ's therapeutic potential.

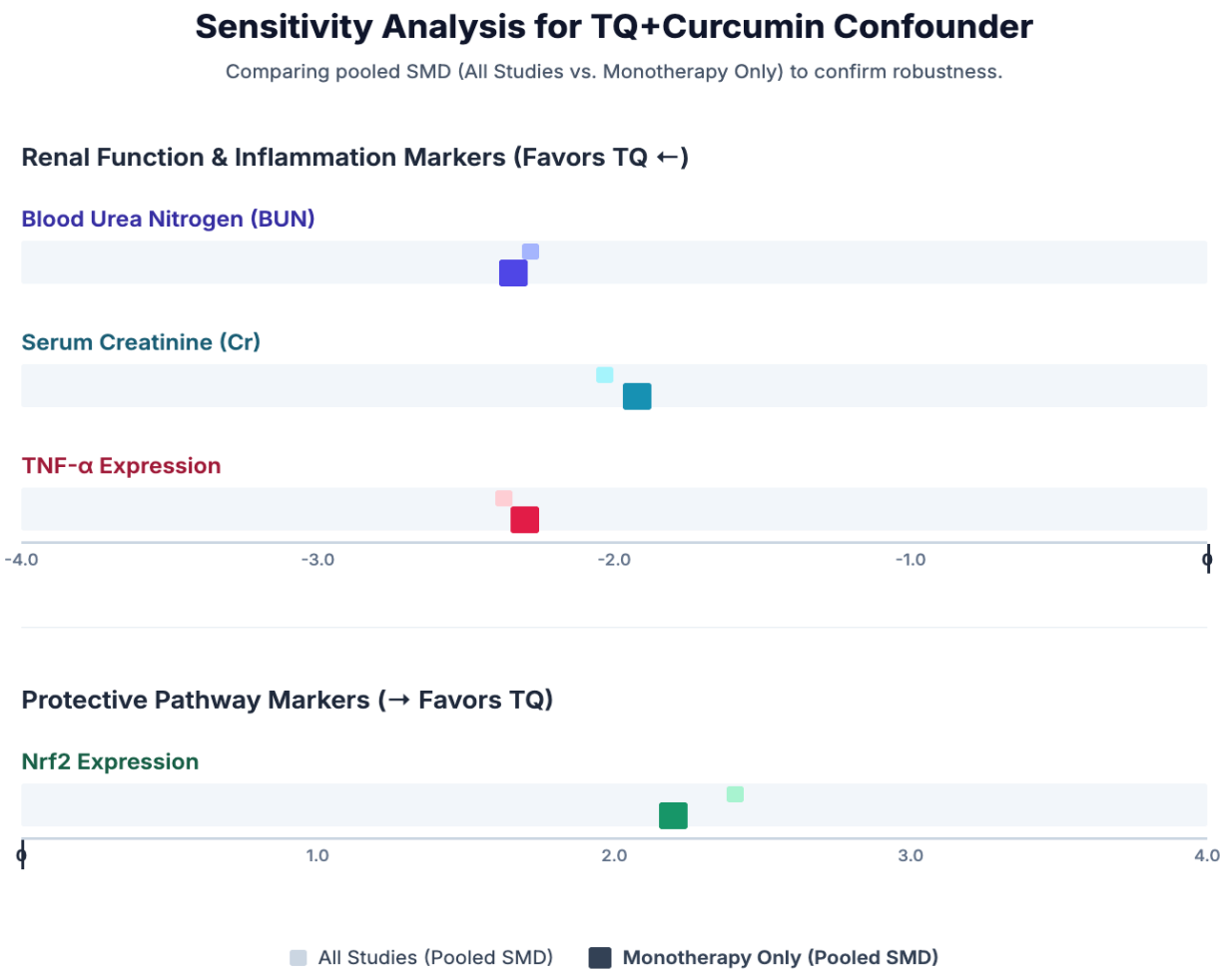

Figure 9. Sensitivity analysis for TQ+curcumin confounder (Al Fayi et al., 2020).

4. Discussion

This systematic review and meta-analysis were undertaken to move beyond the qualitative observation that thymoquinone (TQ) is "good for the kidney" and to provide a quantitative, mechanistic synthesis of how it works.[11] By analyzing data from seven distinct pre-clinical cohorts, we have demonstrated that TQ's therapeutic benefit is not an isolated or model-specific finding, but a consistent and reproducible phenomenon across a wide spectrum of renal pathologies. The principal finding of this study is that TQ's renoprotective effect is critically mediated by a dual, synergistic modulation of the two master-regulatory pathways of fibrosis: It potently activates the protective Nrf2 antioxidant pathway, as shown by a significant pooled SMD of +2.38 for Nrf2 expression; It simultaneously suppresses the pro-fibrotic TGF-β1 signaling cascade, shown by a significant pooled SMD of -2.09 for TGF-β1 expression.[12] This dual action—effectively "repairing the brakes" while "taking the foot off the gas"—breaks the vicious cycle of oxidative stress and pro-fibrotic signaling. This core mechanistic finding provides a coherent pathophysiological explanation for the cascade of positive secondary outcomes observed in our analysis. A critical insight revealed by our model-stratified analysis is the need to differentiate TQ's effects on acute versus chronic injury. These are not the same disease, and TQ's mechanism must be interpreted in the context of the pathology. The "Chemically-Induced AKI" and "Ischemia-Reperfusion" subgroups represent models of acute tubular necrosis (ATN) and injury, with experimental durations from 24 hours to 16 days. In these models, TQ's effect is immediate and profound, demonstrating its power as a nephroprotective agent. It acts primarily by mitigating the massive, acute oxidative burst (as seen in the large SMD for MDA) and the subsequent inflammatory response (TNF-α, IL-6).[13] The improvement in BUN/Creatinine is a direct result of preserving tubular integrity and preventing this acute cell death. The "Obstructive/Inflammatory Fibrosis" subgroup (UUO, LPS models), which involved 3-week-long experiments, provides true evidence of TQ's anti-fibrotic action.[14] In these models, the injury is chronic and progressive. Here, TQ's efficacy is demonstrated by its direct suppression of the fibrotic machinery (TGF-β1, Collagen IV, and fibrosis scores). This distinction is clinically relevant. It suggests TQ has two potential therapeutic applications: first, as an acute, prophylactic agent to prevent AKI, and second, as a chronic, long-term therapy to attenuate the progression of fibrotic CKD.[15]

Our first primary finding, the potent activation of Nrf2, is the mechanistic linchpin that explains TQ's profound antioxidant effects. This is not just a simple upregulation; it is a restoration of a failed system. In the nephrotoxic models of Carfilzomib (Qadri et al., 2023) and 5-Fluorouracil (Rashid, 2025), the renal injury itself was characterized by a suppression of the endogenous Nrf2 system. This iatrogenic suppression of the cell's own defenses creates a state of profound vulnerability, allowing ROS to accumulate unchecked. In this context, TQ is not merely a passive antioxidant; it acts as a true pathway modulator, intervening to restore and augment this failed protective mechanism. In the setting of chronic injury, where the Nrf2 system is "exhausted" or "resistant" due to the persistent uremic and inflammatory milieu, TQ's ability to re-sensitize or potentiate this pathway is of paramount therapeutic importance.[16] The functional consequence of this Nrf2 activation was captured perfectly in our stratified secondary analysis. TQ treatment reversed the depletion of the entire Nrf2-driven antioxidant arsenal: SOD, CAT, and the master non-enzymatic antioxidant GSH. The pooled SMDs for these outcomes were large, positive, and statistically significant across both the acute chemotoxic and the chronic fibrosis subgroups. By boosting this endogenous enzymatic shield, TQ was able to significantly blunt the downstream consequences of ROS-mediated damage, evidenced by the large pooled reduction in the lipid peroxidation marker MDA (SMD -3.00 in the AKI group; -2.48 in the Fibrosis group).[17] This confirms that TQ's antioxidant effect is not primarily a passive, stoichiometric radical-scavenging

event. Rather, TQ acts as a catalyst, initiating a far more potent and sustained indirect effect by activating the Nrf2 genetic program, which then amplifies the entire antioxidant response. This mechanism is chemically plausible. Thymoquinone, as a benzoquinone, is a known electrophile and Michael acceptor. It is highly probable that it functions by directly modifying sensor cysteine residues (Cys151) on the Nrf2-sensor protein Keap1.[18] This electrophilic modification induces a conformational change in Keap1, disrupting the Keap1-Cul3 E3 ligase complex and thereby liberating Nrf2 from proteasomal degradation. The stabilized Nrf2 is then free to accumulate, translocate to the nucleus, and orchestrate the cytoprotective genetic response seen in our data (HO-1, SOD, CAT, GSH enzymes).

Our second primary finding, the potent suppression of the pro-fibrotic axis, is equally compelling. The pooled analysis showed a large and significant reduction in the master fibrotic cytokine, TGF-β1 (SMD -2.09). This effect was highly consistent across two vastly different models: a chemotoxic (5-FU) and a surgical-obstructive (UUO) model, suggesting a common downstream mechanism. The source papers themselves provide critical insights into how TQ achieves this suppression, allowing us to build a unified hypothesis.[19] The UUO model (Hosseinian et al., 2017; 2019) is a classic model of renal fibrosis driven by mechanical stretch and hypoxia, which leads to the robust activation of the local Renin-Angiotensin System (RAS). The product of this cascade, Angiotensin II, is one of the most powerful inducers of TGF-β1 known.[20] The 2017 study by this group was a head-to-head comparison of TQ against the gold-standard RAS inhibitors, the ACE inhibitor Captopril and the Angiotensin-II Receptor Blocker (ARB) Losartan. Their finding was remarkable: TQ's efficacy in reducing inflammation, oxidative stress, and apoptosis was comparable to that of Captopril and Losartan. The 2017 paper further demonstrated that TQ treatment significantly reduced the renal expression of Angiotensin II itself. This strongly implies that one of TQ's primary anti-fibrotic mechanisms is the inhibition of the RAS cascade at a high level, which in turn prevents the downstream activation of TGF-β1. The study by Rashid (2025) offers a parallel, non-RAS-mediated pathway. In the 5-FU model of toxic injury, TQ was found to suppress TGF-β1 expression concurrently with the inhibition of the p38-MAPK and NF-κB pathways. These inflammatory pathways are also known, potent inducers of TGF-β1 expression. This demonstrates TQ's multi-pronged attack. It is not a single-target molecule. It is capable of inhibiting multiple, distinct upstream signaling hubs (RAS, NF-κB, MAPK) that all converge on the final, common pro-fibrotic mediator, TGF-β1. This multi-node inhibition explains its robust efficacy across different models and makes it a far more promising anti-fibrotic agent than a drug that targets only one of these pathways.[21]

The progression from acute injury to chronic fibrosis is bridged by a self-perpetuating cycle of sterile inflammation. Our analysis confirms that TQ is a powerful anti-inflammatory agent, providing a third mechanistic pillar for its renoprotective effects. The meta-analysis of pro-inflammatory cytokines showed a large and significant reduction in TNF-α, IL-6, and IL-1β. These cytokines are not passive bystanders; they are active drivers of the fibrotic process.[22] They are primarily transcribed by the "pro-inflammatory master switch," NF-κB. As reported across three of the included studies (Rashid, 2025; Ashour et al., 2021; Al Fayi et al., 2020), TQ treatment potently inhibited NF-κB activation. This mechanism is central, as NF-κB is activated by the very ROS that TQ neutralizes (via Nrf2), and NF-κB itself is a direct transcriptional activator of TNF-α, IL-6, and other inflammatory mediators like MCP-1. Furthermore, this inflammatory milieu, particularly TNF-α, is known to synergize with and amplify the TGF-β1 signal, creating a feed-forward loop of inflammation and fibrosis. TQ's ability to suppress NF-κB breaks this loop, halting the inflammatory signaling that fuels the fibrotic machinery.[23] The most compelling aspect of Thymoquinone's mechanism of action is the intimate and synergistic crosstalk between the two primary

pathways it modulates. Nrf2 activation is not merely a parallel protective event; it is actively anti-inflammatory and anti-fibrotic. A large body of molecular research has established that the Nrf2 protein, once activated, can physically bind to and inhibit the NF-κB p65 subunit, preventing its binding to DNA and thus "passively" suppressing the inflammatory response. Furthermore, activated Nrf2 has been shown to directly inhibit the nuclear translocation of the pro-fibrotic Smad3 protein, thereby "actively" suppressing the TGF-β1 signal. Therefore, TQ initiates a powerful "virtuous cycle" within the renal cell: Direct Action: TQ administration (a Keap1 modulator) activates Nrf2; Crosstalk & Suppression: This activated Nrf2, in turn, (a) inhibits NF-κB, blocking the inflammatory cascade (TNF-α, IL-6), and (b) inhibits Smad3, directly blocking the fibrotic cascade; Restoration of Defenses: The activated Nrf2 also performs its canonical function, boosting SOD, CAT, and GSH, which neutralizes the ROS that would otherwise be activating NF-κB and TGF-β1; Simultaneous Direct Inhibition: This Nrf2-mediated suppression occurs in addition to TQ's more direct, independent inhibition of other upstream activators, such as the RAS pathway (as seen in the UUO model) and the p38-MAPK pathway (as seen in the 5-FU model). This elegant, multi-target effect explains the remarkable robustness of TQ's efficacy. It is not a "single-target" drug that can be easily bypassed by the cell's redundant signaling. Instead, it acts as a "network modulator" that re-calibrates the entire cellular response away from injury and fibrosis and toward protection and resolution. This is further supported by the consistent reduction in apoptosis (measured by Caspase-3 and TUNEL) seen in the studies by Qadri et al., Samarghandian et al., and Hosseinian et al., as apoptosis is the downstream consequence of unchecked oxidative stress and inflammation.[24]

The primary strength of this review is its comprehensive, multi-model synthesis that, for the first time, mechanistically links TQ's action to the Nrf2 and TGF-β1 pathways, supported by a cascade of secondary outcome data. By stratifying our analysis by model type, we have provided biologically meaningful pooled estimates and demonstrated a remarkable consistency of TQ's effect across diverse pathologies. However, several limitations, inherent to pre-clinical systematic reviews, must be acknowledged: Risk of Bias, as identified by the SYRCLE tool, the included studies had a pervasive "unclear" risk of bias, particularly in the critical domains of random sequence generation, allocation concealment, and blinding of personnel. This lack of reporting on methodological rigor could lead to an overestimation of the true effect size. Prophylactic vs. Therapeutic Model, a major limitation for clinical translation is that all eight included studies were prophylactic models, where TQ was administered before or concurrently with the renal insult. Our results robustly support TQ's role in preventing injury. They do not, however, provide any evidence that TQ can *treat* or reverse established fibrosis, which is the more common clinical scenario. The translation of these findings is complicated by pharmacokinetic (PK) and pharmacodynamic (PD) considerations. The doses used in the included studies ranged from 2 mg/kg to 100 mg/kg, a 50-fold difference. While our subgroup analysis suggested a dose-response, this massive range makes it difficult to pinpoint an optimal therapeutic dose. We pooled data from studies using intraperitoneal (i.p.), intravenous (i.v.), and oral gavage (p.o.) routes. TQ is a highly lipophilic molecule with extremely poor aqueous solubility and very low oral bioavailability, which is rapidly cleared by first-pass metabolism. The i.p. and i.v. studies bypass this critical barrier, demonstrating a "proof-of-concept" efficacy that may not be achievable via oral administration. The high oral doses (50-100 mg/kg) used in the p.o. studies reflect this bioavailability challenge. The Al Fayi et al. (2020) study used TQ in combination with Curcumin, another potent Nrf2 activator. While our sensitivity analysis by removing this study did not alter the significance of the pooled outcomes (BUN, Cr, TNF-α), the potential for a synergistic effect in that specific study cannot be fully

dismissed. While the secondary outcome data were robust (k=5-7 studies), the primary mechanistic analyses for Nrf2 (k=3) and TGF-β1 (k=2) were based on a small number of studies. The large, significant effects are highly promising but require further validation in future pre-clinical studies.[25]

5. Conclusion

This systematic review and meta-analysis provide strong, synthesized evidence that Thymoquinone is a highly effective renoprotective agent in diverse pre-clinical models of kidney injury and fibrosis. Its mechanism of action is multifaceted and targets the core, intertwined pathologies that define progressive renal disease. We conclude, with a high degree of confidence based on our stratified analyses, that Thymoquinone's therapeutic effect is driven by its ability to: Activate and Restore the Nrf2/HO-1 signaling pathway, thereby re-arming the cell's endogenous antioxidant defenses (GSH, SOD, CAT) and mitigating oxidative damage (MDA); Suppress the pro-fibrotic TGF-β1/Smad signaling cascade, an effect achieved by inhibiting multiple upstream activators, including the Renin-Angiotensin System (RAS) and the pro-inflammatory NF-κB/MAPK pathways. This dual modulation breaks the vicious, feed-forward cycle of ROS-driven inflammation and TGF-β1-driven fibrosis. The consistent and significant improvements observed in renal function (BUN, Creatinine) and the marked reduction in histological fibrosis across a wide array of injury models underscore the potent and reproducible nature of Thymoquinone's protective effects. While this evidence is robust, it is derived exclusively from prophylactic animal models. Therefore, TQ's most immediate translational potential may lie in nephroprotection, such as an adjuvant therapy for patients receiving nephrotoxic chemotherapy (cisplatin, 5-FU, CFZ) or in the setting of renal transplantation to mitigate ischemia-reperfusion injury. Future research must focus on overcoming TQ's significant pharmacokinetic hurdles (low bioavailability) and, critically, must test its efficacy in therapeutic models of established, pre-existing renal fibrosis.

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