REVIEW

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Thymoquinone Modulates Gene Expression Associated with Apoptosis in Colorectal Cancer: A Preclinical Systematic Review and Meta-Analysis of *BAX, BCL2,* and *CASP3*

Muhammad Evy Prastiyanto[1], Kuncara Nata Waskita[2], Rina Nurmaulawati[2], Nur Rahmawati Wijaya[3], Sofa Farida[3], Devi Safrina[3], Aditya Dwi Permana Putra[3], Siti Hamidatul Aliyah[4], Rantika Silfarohana[5], Mohammad Miftakhus Sholikin[6], Rizal Maarif Rukmana[3]*

Abstract

Objective: Colorectal cancer (CRC) continues to be a significant global health issue. Thymoquinone (TQ), a bioactive component of Nigella sativa, has shown anticancer capabilities by inducing apoptosis. This systematic review and meta-analysis aim to assess the impact of TQ on the levels of pro-apoptotic (*BAX, CASP3*) and anti-apoptotic (*BCL2*) markers in colorectal cancer cells. **Methods:** An extensive literature search was performed in Scopus, BASE, PubMed, and Web of Science for articles published from 2004 to 2025, adhering to PRISMA guidelines. Eligible in vitro and in vivo studies provided numerical data on gene or protein expression levels for *BAX*, *BCL2*, and *CASP3*, along with standard deviations. Effect sizes (g) were computed using a random-effects model, and heterogeneity and publication bias were evaluated. **Result:** A total of ten qualifying studies were incorporated. The meta-analysis indicated that TQ significantly ($p < 0.001$) increased *BAX* mRNA (g = 3.901) and protein levels (g = 4.232), decreased *BCL2* mRNA (g = -3.680) and protein levels (g = -3.328), and markedly upregulated *CASP3* mRNA (g = 5.669) and protein levels (g = 6.336). Subgroup analyses revealed consistent effects across CRC cell lines (HT29, SW480, SW620, HCT-15, and HCT116). Heterogeneity varied from low to moderate, and publication bias was low or not significant. **Conclusion:** The findings demonstrate that TQ exerts pro-apoptotic effects in colorectal cancer (CRC) models through the upregulation of *BAX* and *CASP3* and the downregulation of *BCL2*. This suggests its potential therapeutic relevance rather than definitive biomarker utility. Nevertheless, further in vivo studies and early-phase clinical investigations are required to clarify its translational significance and to explore its possible implications for treatment responsiveness.

Keywords: *BAX- BCL2- CASP3-* Colorectal carcinoma- Thymoquinone

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Introduction

Colorectal cancer (CRC) has emerged as a significant public health issue, being identified as the second leading cause of cancer-related mortality worldwide, with over 1.93 million new cases and 916,000 deaths in 2020 [1, 2]. CRC elevation among those below 50 years of age is strikingly alarming in developed countries, where CRC is linked to a sedentary, obese, and heavily processed food diet [3, 4]. The current hypothesis respecting the increased prevalence of CRC associates it with a shift towards more sedentary lifestyles and a rise in animal-based food consumption, leading to overweight and obesity [5, 6]. It is not only Western countries that are undergoing these lifestyle changes, but developing countries have also been experiencing an increase in CRC cases in the past ten years [7]. This increasing burden underscores the necessity for innovative, accessible treatment alternatives, including pharmaceuticals derived from natural ingredients with potential anticancer efficacy [8].

Thymoquinone (TQ), a major bioactive ingredient of black cumin (Nigella sativa) is one of the most notable

*[1]Department of Medical Laboratory Technology, Faculty of Health and Nursing Science, Universitas Muhammadiyah Semarang, Semarang, 50273, Indonesia. [2]The College of Health Sciences Bhakti Husada Mulia, Taman Praja Street No. 25, Taman district, Madiun, East Java, Indonesia. [3]Research Center for Pharmaceutical Ingredients and Traditional Medicine, Research Organization for Health, National Research and Innovation Agency (BRIN), Bogor, 16911, Indonesia. [4]Center for Biomedical Research, Research Organization for Health, National Research and Innovation Agency (BRIN), Bogor, 16911, Indonesia. [5]Research Center for Vaccine and Drug, Research Organization for Health, National Research and Innovation Agency (BRIN), Bogor, 16911, Indonesia. [6]Research Center for Animal Husbandry, National Research and Innovation Agency, Bogor, 16911, Indonesia. *For Correspondence: riza007@brin.go.id*

phytochemicals. Unlike traditional chemotherapies which frequently lead to severe side effects due to off-target damage, TQ acts through multiple pathways with relative safety profile [9, 10]. For example, TQ can modulate multiple apoptosis pathways providing an adjunctive and/or synergistic mechanism[11, 12]. In colorectal cancer (CRC) models, TQ equals or trumps 5-fluorouracil (5-FU) by revving up *BAX* (pro-death)/downregulating *BCL2* (anti-death), all without the toxicity of conventional chemo drugs, even yields less chemo drug resistance compared to standard protocols[10]. In comparison to other plant extracts as curcumin, TQ induces a more extensive caspase-3 activity in HT-29 [11]. It was known to have undergone investigation, particularly regarding how it exerts its anti-inflammatory and anticancer effects, which have been noted in traditional medicinal practices [13]. It was reported that TQ has a complex mechanism of action. It acts on pathways that signal cancer development and progression [14]. More recently, the focus of TQ research has been on determining its mechanisms of action, particularly its anticancer effects, which have been found to act on several pathways responsible for cancer cell proliferation, survival, and metastasis [8, 15]. There is ample literature on TQ as a crude extract or as a purified fraction or isolated compound regarding its ability to inhibit the progression of cancer by several mechanisms, including cell cycle arrest, anti-angiogenesis, and apoptosis [16, 17].

Apoptosis is a form of programmed cell death that plays a crucial role in cancer cells. Its dysfunction causes cancer cells to grow uncontrollably [18, 19]. In the apoptotic signalling pathway, caspase-3 plays a crucial role as the primary executioner of apoptosis [20] completing the biochemical and morphological alterations necessary for programmed cell death [21]. The *BCL2* (B-cell lymphoma 2) gene functions as an anti-apoptotic protein via the mitochondrial pathway. *BCL2* associates suppress pro-apoptotic proteins like *BAX*, thereby obstructing the initiation of apoptosis [22]. The *BAX* (*BCL2*-associated X protein) gene promotes apoptosis via the mitochondrial pathway. Elevated *BAX* expression expedites the initiation of apoptosis in cells [23, 24].

Previous studies have shown that TQ treatment stimulates the upregulation of *BAX* and caspase-3 genes and the downregulation of *BCL2* in colon cancer cells. However, the significance of these gene responses varies widely depending on the type of colon cancer cells [25, 26]. Variations in gene expression responses are determined by differences in treatment concentration, treatment duration, and expression levels (mRNA or protein expression) [27, 28]. Additional research is required to ascertain the impact of TQ on the gene expression of *BAX*, *BCL2*, and *CASP3* at varying levels (mRNA or protein) in CRC, including calculating effect size with Hedges' g values and investigating its effects across different cell lines. Moreover, researchers have yet to investigate the translational significance of these molecular changes, especially their potential as predictive biomarkers for treatment response. Rectifying this deficiency is crucial to enhancing the preclinical evidence foundation and informing the design of early-phase clinical trials. Thus, novel and reliable prognostic markers were much needed to individualize CRC treatment, and the development of the nomogram groups based on multi-omics data has led to significantly more accurate risk and outcome predictions [29]. This work aims to perform a systematic review and meta-analysis of preclinical evidence to assess the effects of thymoquinone on the expression of *BAX*, *BCL2*, and *CASP3* in colorectal cancer models.

Materials and Methods

Study Design and Search Strategy

The systematic review and meta-analysis methodologies conform to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) requirements [30]. All study protocols have been registered in the International Prospective Register of Systematic Reviews database with registration number CRD420251110795. The article search was conducted in indexed databases such as Scopus, BASE, PubMed, and WoS, covering the years 2004–2025. The search queries used were ("colorectal cancer" OR "colon cancer" OR "rectal cancer") AND ("thymoquinone extract" OR thymoquinone) AND ("*BAX*" OR "*BCL2*" OR "*CASP3*"). The search was conducted using the PICO approach, encompassing the population (colon cancer cells), intervention (thymoquinone or thymoquinone extract), comparator (colon cancer cells without thymoquinone treatment or thymoquinone extract), and outcome (*BAX*, *BCL2*, and *CASP3* gene and protein expression). The specifics of the study's search conduct are illustrated in Figure 1.

Criteria for Inclusion and Exclusion

This study looked at how TQ affect the gene expression of *BAX*, *BCL2*, and *CASP3* in colon cancer. The inclusion criteria for this study were studies focused on the effect of TQ on colon cancer, studies focused on target genes *BAX*, *BCL2*, and *CASP3*, target gene and control gene expression data must be numerical, and target gene and control gene expression data must have a standard deviation. The study did not include research on the effects of TQ on anything other than colon cancer, genes other than *BAX*, *BCL2*, and *CASP3*, studies that provided qualitative data instead of numerical data for gene expression, and studies where the target gene data lacked a standard deviation. When studies presented both gene (mRNA) and protein expression results for the same apoptotic markers, these data were regarded as separate biological endpoints and examined independently. Gene expression data were prioritized for inclusion in the primary analysis, consistent with the emphasis of this evaluation on transcriptional regulation. When available, protein expression data were used as supplementary outcomes to assess the translational consistency of thymoquinone's effects at the functional level.

Data Extraction

The information taken from the chosen articles included the author's name and publication year, the type of treatment TQ, the kind of study (in vitro or in

vivo), the animal model or type of colon cancer cells, how long the treatment lasted, the method used to check gene expression (qRT-PCR and Western blotting), the concentration of the treatment, and the results for gene expression (*BAX*, *BCL2*, and *CASP3* at the mRNA and protein level). The PRISMA diagram (Figure 1) provides a detailed view of the literature search, screening, eligibility, and included articles.

Analysis Data, Software, and Bias

Effect sizes were calculated separately for each apoptotic marker (*BAX*, *BCL2*, and *CASP3*) and expression level (mRNA and protein). To avoid dependency bias, outcomes from the same study were not pooled. Instead, mRNA and protein expression data were treated as distinct outcomes and analyzed separately. Each study contributed only one effect size per outcome per analysis. When multiple experimental conditions were reported for the same outcome, a single representative dataset was selected based on the most common condition.

The effect of TQ on *BAX*, *BCL2*, and *CASP3* gene expression in colon cancer cells was analysed using the Hedges effect size model with random effects. Effect size (g) values, $g > 0.8$ indicate that thymoquinone can strongly increase or decrease gene expression [31]. This statistical approach, which is similar to what has been used in meta-analyses as a means of validating multivariable prognostic models, also helps incorporate differing preclinical results into one single and more credible pooled effect [29]. Meta-analysis was performed using OpenMEE software; outcome studies are depicted in a forest plot. The points to the right or left of the vertical line (number 0) indicate the pooled effect size value of the increase or decrease in target gene expression due to thymoquinone treatment [32]. The individual effect size is shown by the square in the plot, and the line that connects the squares indicates the upper and lower 95% confidence intervals (CI) for the effect size [33]. Heterogeneity was assessed independently for each outcome-specific meta-analysis.

Heterogeneity was obtained using Cochran's Q test and the inconsistency index (I^2). According to Higgins and Thompson's categorisation, low heterogeneity was defined as I^2 values below 25%, medium heterogeneity as I^2 values between 25% and 50%, and high heterogeneity as I^2 values 50% and above [34]. Publication bias was assessed using fail-safe number (FSN) [35]. The FSN is compared with the Rosenthal tolerance level = 5 (k) + 10, where k = the number of recorded experiments. If the FSN is greater than the Rosenthal tolerance level, publication bias is small, and the meta-analysis results are stable [32].

Subgroup analysis was performed on different types of colon cancer cell lines: HT29, SW480, SW620, DLD-1, HCT-15, and HCT116. The grouping aimed to see the effect size of TQ treatment on target gene expression in each cell line. Subgroup analysis was performed using OpenMEE software, with effect size calculated using a random-effects model and Hedges' g effect size. The study results are represented as a forest plot and 95% CI, including upper and lower limits. Inconsistent index was used to assess the heterogeneity between the studies.

Results

Study Characteristics and Publication Bias

This study obtained 112 articles from four journal search databases, of which 64 were duplicates and therefore excluded. Forty-eight articles were screened based on title, abstract, and keywords, and 30 were excluded. All 18 original articles were downloadable, and eight articles were excluded from the full-text feasibility study (Figure 1). Data extraction was performed on 10 articles that met the meta-analysis criteria. Table 1

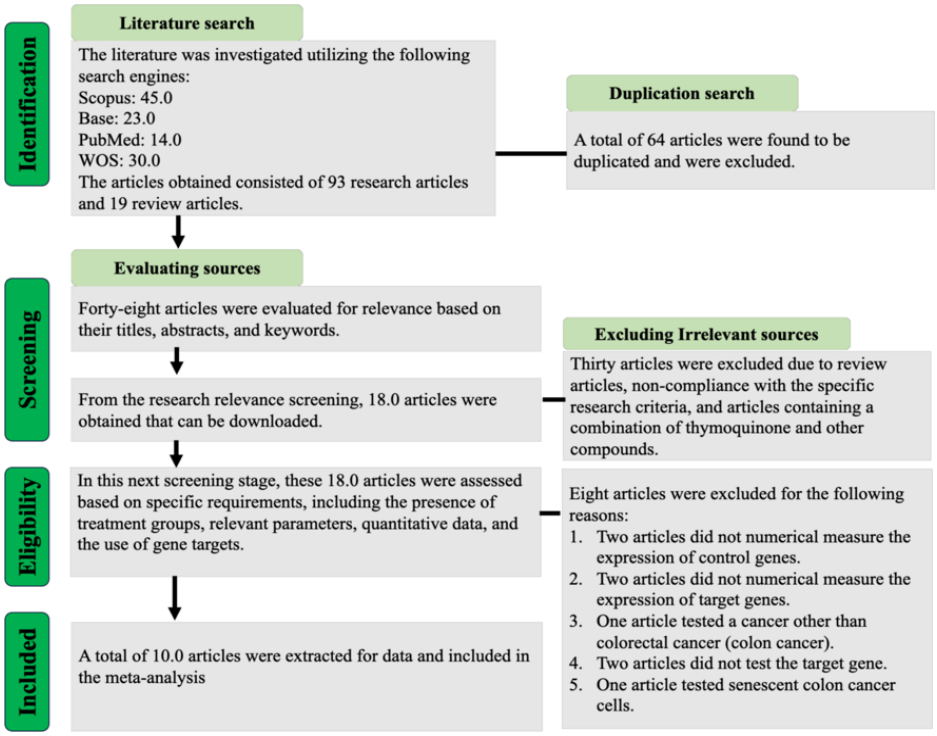

Figure 1. The PRISMA Flowchart Illustrates the Search Methodology

presents a comprehensive overview of the data extraction outcomes, encompassing the dosage administered, the duration of exposure, and the experimental model employed.

We performed a publication bias assessment on all target genes (*BAX*, *BCL2*, and *CASP3*) for mRNA and protein expression, respectively. The results showed that all target genes for all expression levels (mRNA and protein) had a fail-safe N value greater than the Rosenthal tolerance level (Table 2). A high fail-safe N value indicates that publication bias in this study was low, resulting in highly stable meta-analysis results.

The risk of bias (RoB) assessment revealed that most domains were rated low risk or with some concerns, while fewer were high or unclear risk, especially regarding randomization and blinding (Figure 2). The qualitative evaluation based on the RoB assessment showed most domains were low risk or presented some concerns, indicating generally acceptable methodological quality. However, some domains were rated high risk or unclear, particularly those related to randomization, blinding, and selective outcome reporting. These findings suggest limitations in methodological reporting and a possible risk of selective publication of significant results. Despite moderate internal validity, publication bias cannot be entirely excluded and is acknowledged as a limitation.

The RoB of the study was evaluated using a domain-based approach adapted from the SYRCLE Risk of Bias tool. This tool is widely recommended for preclinical and animal-based experimental studies. It was selected because it systematically assesses key sources of bias relevant to experimental oncology research, including selection bias, performance bias, detection bias, attrition bias, and reporting bias. In this study, RoB was assessed across eight methodological domains (D1–D8), covering aspects such as random sequence generation, baseline comparability, allocation concealment, blinding of interventions and outcome assessment, incomplete outcome data, selective reporting, and other potential sources of bias. Each domain was classified as low risk, some concerns, high risk, or unclear risk, in accordance with established guidelines. Detailed individual and summary RoB visualizations are presented in Figure 2.

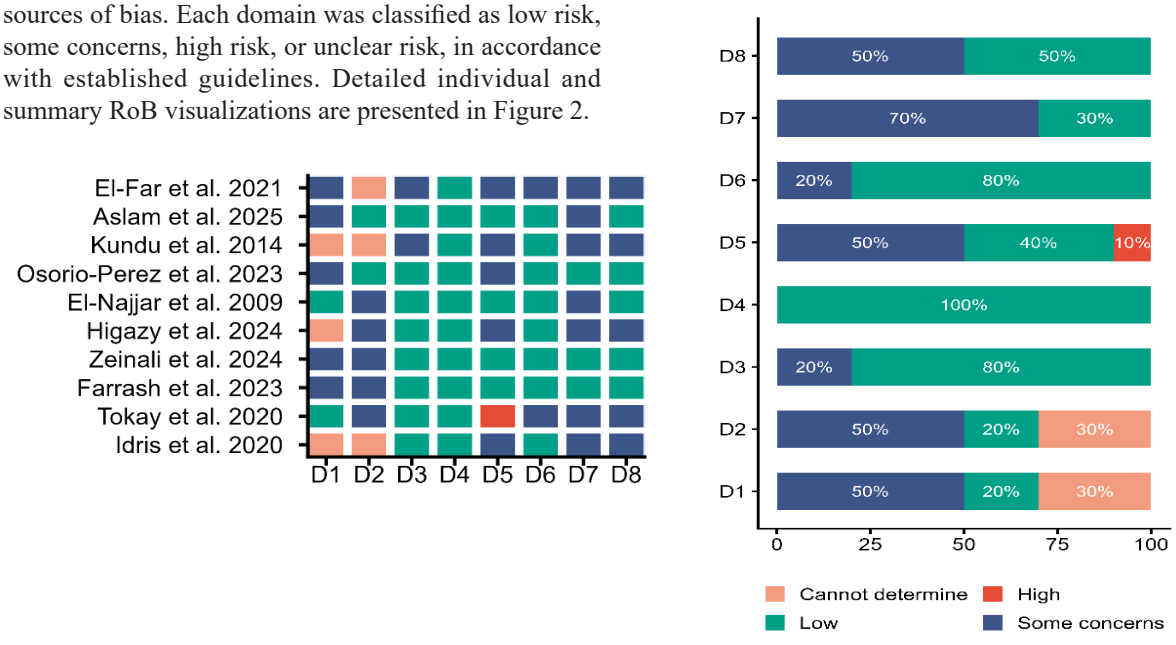

Figure 2. Risk of Bias (RoB) Assessment of Included Preclinical Studies

Effect of Thymoquinone on mRNA Expression in Colorectal Cancer

The results of the meta-analysis of thymoquinone's effects on mRNA expression in colon cancer are shown in the forest plot in Figure 3. The results showed that TQ significantly increased the expression of *BAX* and *CASP3* mRNAs, with effect sizes of 3.901 and 5.669, respectively (Figure 3A and 3C). In addition, the forest plot in Figure 3B showed that TQ significantly decreased *BCL2* mRNA expression, with an effect size of -3.680. Substantial heterogeneity was observed across the included studies. For *BAX* expression, the pooled analysis demonstrated high heterogeneity ($I^2 = 79.46\%$). Similarly, the analysis of *CASP3* expression showed moderate to high heterogeneity ($I^2 = 76.71\%$). In contrast, the pooled analysis for *BCL2* expression revealed low to moderate heterogeneity ($I^2 = 36.41\%$). Subgroup analysis was performed to examine the effect of TQ across cell lines. Figure 4A is a forest plot showing that TQ significantly increased *BAX* mRNA expression in colon cancer cell lines (HT29, SW480, and SW620). Subgroup analysis showed that TQ significantly increased *CASP3* mRNA expression in the HT29, SW480, and SW620 cell lines (Figure 4C). Figure 4B shows that TQ significantly decreased the expression of *BCL2* mRNA in HT29 and SW480 cell lines.

Effect of Thymoquinone on Protein Expression in Colorectal Cancer

Consistent with the results of the mRNA expression meta-analysis, the meta-analysis of TQ effects on protein expression showed increased *BAX* and *CASP3* protein levels and decreased *BCL2* protein levels (Figure 5). Thymoquinone significantly increased *BAX* and *CASP3* protein expression, with effect sizes of 4.232 and 6.336, respectively (Figures 5A and 5C). The forest plot results in Figure 5B showed that TQ significantly decreased *BCL2* protein expression with an effect size of -3.328. The study results showed variations in heterogeneity. *BAX* protein

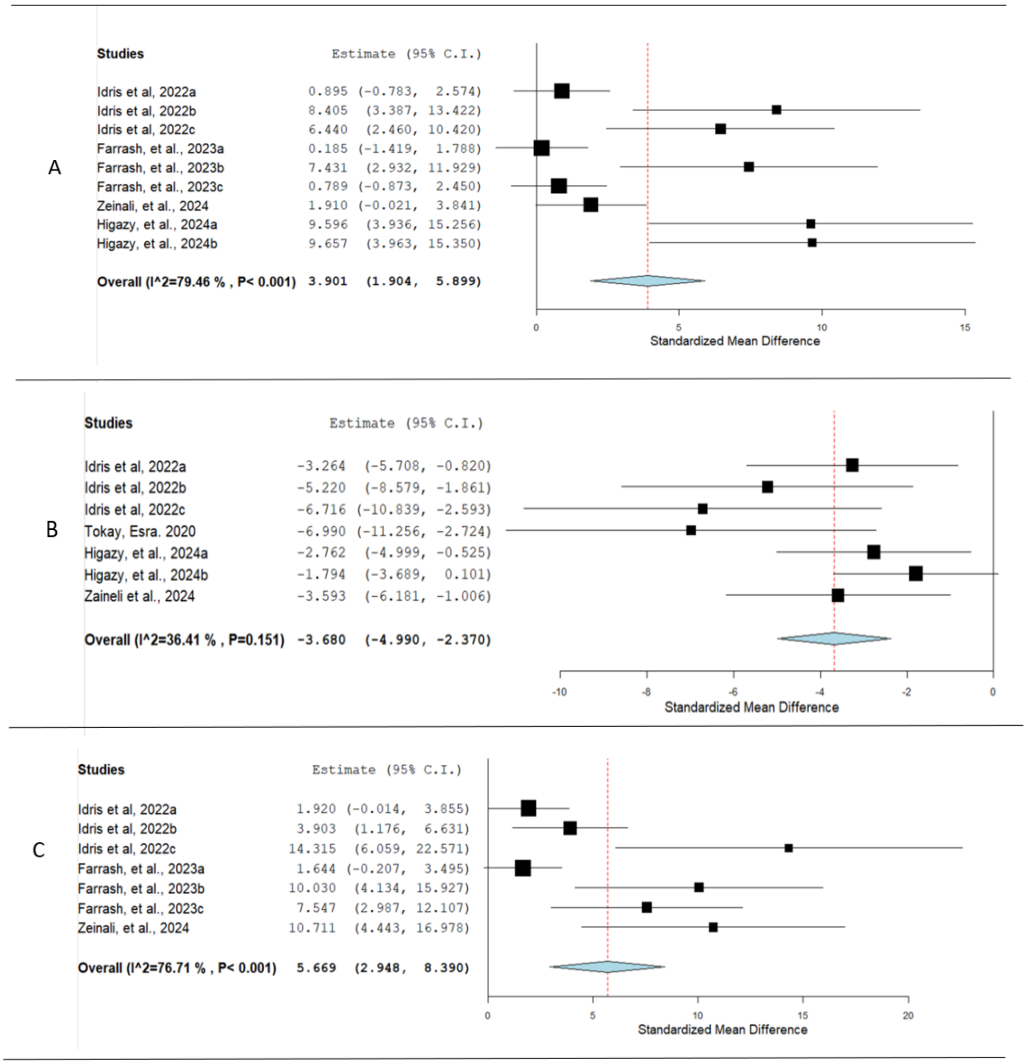

Figure 3. Forest Plot Summarizing the Meta-Analysis Results on the Effect of Thymoquinone on mRNA Expression levels; A: *BAX* mRNA expression study; B: *BCL2* mRNA expression study; C: *CASP3* mRNA expression study.

expression showed high heterogeneity (I^2 = 76.39%), *CASP3* protein expression showed high heterogeneity (I^2 = 76.16%), and *BCL2* protein expression showed moderate heterogeneity (I^2 = 52.84%). To illustrate the specific response of each cell line to TQ treatment, subgroup analysis was performed. Figure 6A shows that TQ significantly increased *BAX* protein expression in various HT29, SW480, and SW620 cell lines. In line with *BAX*, *CASP3* protein expression also increased significantly after TQ administration across HT29, SW480, SW620, DLD-1, and HCT-116 cell lines (Figure 6C). Figure 6B shows that TQ significantly decreased *BCL2* protein expression in HT29, SW480, and SW620 cell lines.

Discussion

The meta-analysis of studies [25–27, 36], showed TQ significantly increased *BAX* mRNA expression, indicating thymoquinone induces apoptosis by upregulating *BAX*, a key intrinsic pathway protein. Substantial heterogeneity stemmed from varying effect sizes. Previous studies [22, 36] reported substantial effects, whereas [26, 36] documented more moderate effects. Such variability is likely attributable to differences in experimental protocols, thymoquinone concentrations, or cell culture conditions.

Subgroup analysis by cancer cell type revealed: significant pooled effect with high heterogeneity in HT29 [27] strong significant pooled effect in SW480 [25, 26] inconclusive pooled result in SW620 () due to wide confidence interval [25]. Overall, modulation of apoptosis-related genes (*BAX*, *CASP3*, and *BCL2*) in colorectal cancer cell lines suggests that TQ has potential as a chemotherapeutic candidate for CRC, warranting further mechanistic investigation.

The human *BAX* gene on chromosome 19q contains six exons and four variants. Genetic alterations in its coding and promoter regions disrupt *BAX* expression, impairing apoptosis and promoting tumour suppressor loss and chemotherapy resistance in cancer [37]. Single-nucleotide polymorphisms (SNPs) in the *BAX* gene at positions −125 (G→A) and −248 have been linked to altered mRNA and protein expression, contributing to cancer progression and chemoresistance [38]. *BAX* drives mitochondria-dependent apoptosis by translocating to the mitochondrial outer membrane, releasing cytochrome c,

Table 1. Characteristics of Studies Included in the Systematic Review and Meta-Analysis of TQ, which Modulates Apoptosis through the Expression of *BAX, BCL2,* and *CASP3* at the mRNA and Protein Levels

Studies	Treatment	Experiment	Evaluate cell studies	Treatment duration	Methods	Dose	Expression	
							mRNA	Protein
Idris, et al., 2022 [21]	Thymoquinone	In vitro	HT29, SW480, and SW620 cell lines	12 h	qRT-PCR and Western blotting	75 µM	*BAX, BCL2*, and *CASP3*	*BAX*, *BCL2*, and *CASP3*
Tokay, esra., 2020 [31]	Thymoquinone	In vitro	HT29 cell lines	48 h	qRT-PCR	150 µM	*BAX* and *BCL2*	-
Farrash, et al., 2023 [32]	Thymoquinone	In vitro	HT29, SW480, SW620 cell lines	12 h	qRT-PCR and Western blotting	75 µM	*BAX* and *CASP3*	*BAX* and *CASP3*
Zeinali, et al., 2024 [33]	Thymoquinone	In vitro	SW480 cell lines	48 h	qRT-PCR and Western blotting	40 µM	*BAX*, *BCL2*, *CASP3*	*BAX*, Cas-9, Cas-3, γ-H2AX; Bcl-2
Higazy, et al., 2024 [23]	Thymoquinone extract	In vitro	HT29 cell lines	48 h	qRT-PCR	928.3 µg/ml and 465 µg/ml	*BAX* and *BCL2*	-
El-Najjar, et al., 2010 [24]	Thymoquinone	In vitro	DLD-1 cell lines	24 h, 48 h	Western blotting	40 µM	-	*CASP3*
Osorio-perez, et al., 2023 [34]	Thymoquinone	In vitro	HCT-15 cell lines	24 h	qRT-PCR	82.59 µM	*BCL2*	-
Kundu, et al., 2014 [35]	Thymoquinone	In vitro	HCT116 cell lines	24 h	Western blotting	10 µM, 25 µM, and 50 µM	-	*CASP3*
Aslam, et al., 2025 [36]	Thymoquinone	In vitro	HT29, SW480, SW620 cell lines	12 h	Western blotting	75 µM	-	*BAX*, *BCL2*, and *CASP3*
El-far, et al., 2021 [37]	Thymoquinone	In vitro	HCT116 cell lines	24 h	Flow cytometry	30 µM	-	*BAX* and *BCL2*

Table 2. Publication Bias Results are Based on the Calculation of the Fail-Safe N Value and the Rosenthal Tolerance Level

Type of expression	k	Publication bias	
		Fail-safe N	Rosenthal tolerance level
BAX mRNA levels	9	147	55
BCL2 mRNA levels	7	128	45
CASP3 mRNA levels	7	139	45
BAX protein levels	10	224	60
BCL2 protein levels	7	111	45
CASP3 Protein levels	14	610	80

k, number of recorded studies; FSN, publication bias evaluated using the FSN test. If the FSN value is greater than the Rosenthal tolerance level, the publication bias in this study is low or not significant.

and activating caspases, often via p53. qRT-PCR showed TQ upregulates *BAX* while downregulating *BCL2*, BCL-XL, ATG-7, ATG-12, and LC3-II in HT-29 cells, favoring apoptosis over autophagy [39].

This meta-analysis found TQ significantly increased *BAX* protein expression in colorectal cancer cells (pooled g=4.232, 95% CI: 2.377-6.086; p<0.001), consistent with elevated *BAX* mRNA. These findings reinforce TQ's role as an intrinsic apoptosis modulator via *BAX*. Studies [25, 26, 40, 41] showed an interesting pattern: most studies reported positive effects, although the effects varied widely and were quite significant (heterogeneity I^2=76.39%). Specifically, previous research results [25, 40] reported strong effects (g > 8), while other research results [25, 41] reported a more minimal effect with confidence intervals encompassing zero.

Subgroup analysis by cancer cell type showed distinct responses to TQ. HT29 cells exhibited a moderate effect (g=1.669, I^2=65.16%), while SW480 (g=5.674, I^2=0%) and SW620 (g=7.849, I^2=0%) cells displayed consistent, robust effects. In contrast, HCT116 cells showed small, insignificant effects. These results indicate TQ's effectiveness in upregulating *BAX* expression varies by colorectal cancer cell subtype, with strongest responses in SW480 and SW620 cells [41].

Under basal conditions, *BAX* remains inactive in the cytoplasm through interactions with antiapoptotic proteins such as BCL-XL [42]. Upon receiving death signals, including BH3-only proteins or t-Bid, *BAX* undergoes conformational activation, translocates to the mitochondrial outer membrane, and promotes mitochondrial outer membrane permeabilization (MOMP), thereby amplifying the apoptotic cascade [43].

This meta-analysis demonstrates that TQ significantly downregulates *BCL2* expression at the mRNA level, with a pooled effect size (g) of -3.680 (95% CI: -4.990 to -2.370, P < 0.001), confirming a robust overall effect. Results were consistent across all studies, showing no positive effects and moderate heterogeneity (I^2 = 36.41%), reinforcing TQ's reliable anti-apoptotic action.

BCL2 serves as a key apoptosis regulator, primarily promoting cancer cell survival [44]. TQ-mediated *BCL2* downregulation offers therapeutic potential in malignancies, as evidenced by enhanced apoptosis (e.g., elevated *BAX*/*BCL2* ratio) in colorectal cancer cells, particularly HCT116 model, alone or with imatinib [22]. These findings support TQ's role in sensitizing cancer cells to programmed cell death, highlighting its value as

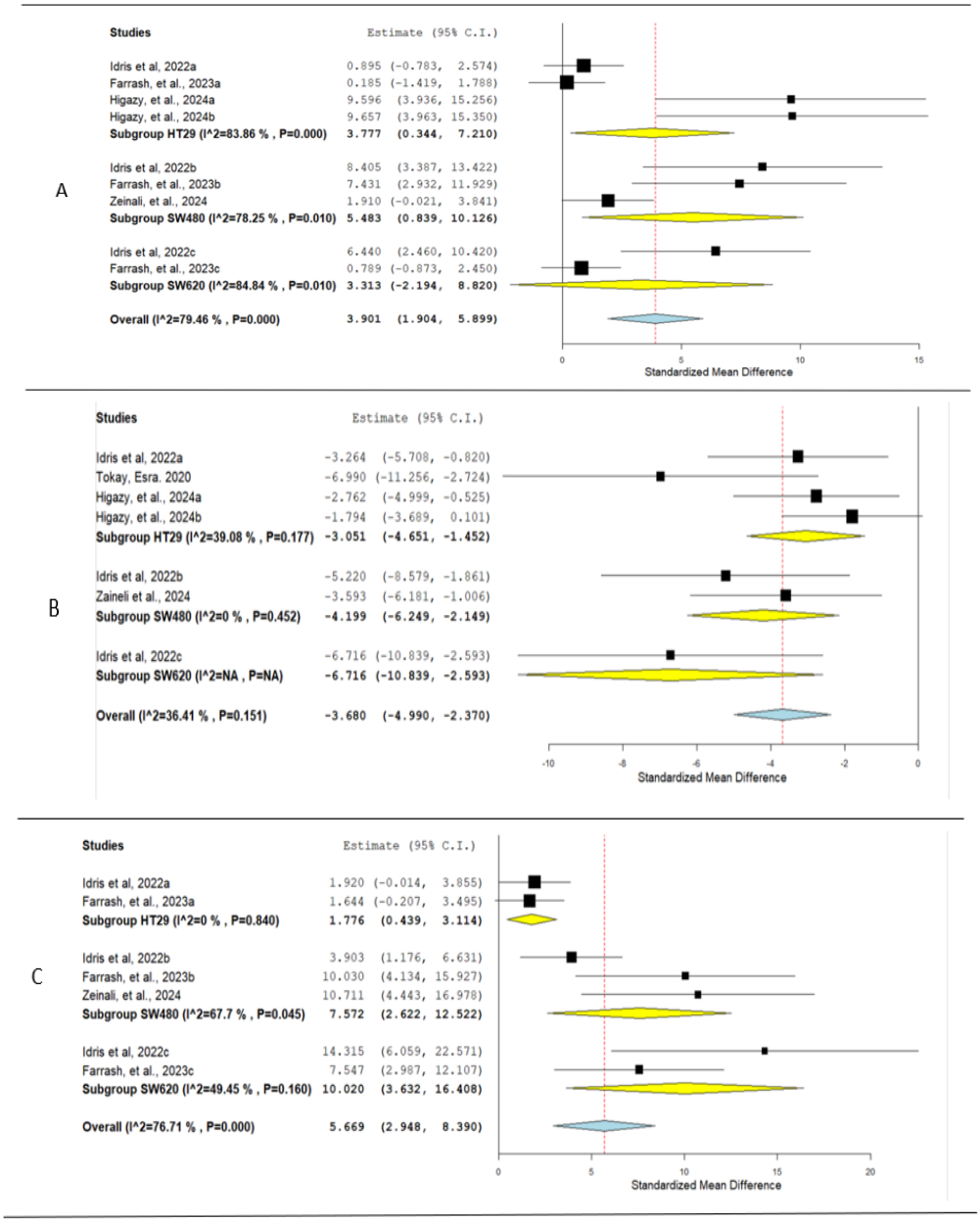

Figure 4. Subgroup Meta-Analysis Results of Thymoquinone Affecting mRNA Expression, Grouped by Cell Line Type; A: *BAX* mRNA expression study; B: *BCL2* mRNA expression study; C: *CASP3* mRNA expression study.

an adjunctive anticancer agent.

These findings align with prior mechanistic studies. TQ induces apoptosis in HCT116 cells by inhibiting STAT3 signaling, downregulating *BCL2* and BCL-XL, and activating caspase cascades [45]. In 5-fluorouracil-resistant colorectal cancer stem cells, TQ reduced *BCL2* mRNA, promoted apoptosis, and inhibited proliferation [46]. These results contextualize the meta-analysis within a broader mechanistic framework.

Despite robust effects, the analysis has limitations: few included studies, variability in cell lines, TQ doses, treatment durations, and quantification methods (explaining moderate heterogeneity), and focus on mRNA changes without protein-level or functional apoptosis validation. Future studies should standardize methods, extend to in vivo and clinical models.

This meta-analysis demonstrates consistent, significant *BCL2* mRNA downregulation across colorectal cancer cell lines (HT29, SW480, SW620), with all subgroups showing negative effect sizes (g) and 95% CIs excluding zero. SW620 cells exhibited the strongest suppression (g=–6.716, 95% CI: –10.839 to –2.593), followed by SW480 (g=–4.199, 95% CI: –6.249 to –2.149) and HT29 (g=–3.051, 95% CI: –4.651 to –1.452).

The pronounced SW620 effect likely reflects metastatic cells' greater apoptosis resistance, as SW620 originates from lymph node metastasis unlike primary tumor-derived HT29/SW480. *BCL2* downregulation suggests TQ enhances intrinsic (mitochondrial) apoptosis, aligning with prior reports of TQ's pro-apoptotic activity in cancer models.

HT29 subgroup showed moderate heterogeneity (I^2=39.08%), likely due to variations in dosage, exposure time, or gene expression methods across studies. SW480

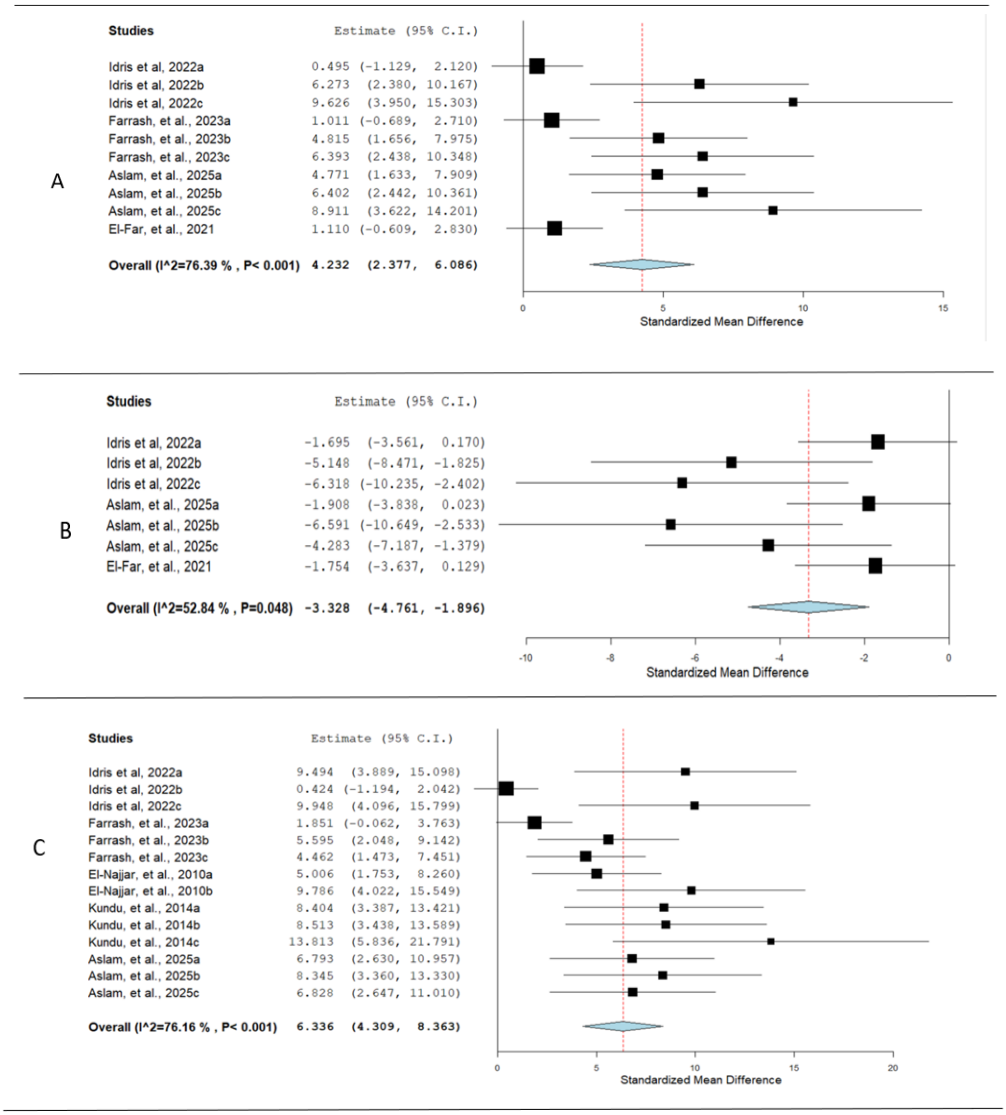

Figure 5. Forest Plot Summarizing the Meta-Analysis Results on the Effect of Thymoquinone on Protein Expression levels; A: *BAX* protein expression study; B: *BCL2* protein expression study; C: *CASP3* protein expression study.

exhibited no heterogeneity (I^2=0%), indicating consistent results, while SW620 heterogeneity was unevaluable due to single-study inclusion, representing an analysis limitation. These findings position TQ as a promising adjuvant for colorectal cancer, especially metastatic stages, with robust *BCL2* suppression in aggressive cell lines overcoming apoptosis resistance. Further validation requires protein-level confirmation (e.g., Western blot), in vivo studies, and clinical translation.

This meta-analysis shows TQ significantly downregulates *BCL2* protein expression across models (SMD=–3.328; 95% CI: –4.761 to –1.896; P<0.05). Despite moderate-high heterogeneity (I^2=52.84%), most studies reported negative effect sizes, confirming consistent protein-level inhibition.

Molecularly, TQ induces apoptosis via *BCL2*/BCL-XL suppression, *BAX* upregulation, *CASP3*/9 activation, and PARP cleavage in colorectal cancer lines (HCT116, HT29). Similar *BCL2* reduction occurs in breast cancer xenografts, correlating with tumor growth inhibition [45, 47]. These results provide mechanistic validation for TQ's pro-apoptotic effects and support meta-analytic findings [48].

The observed effect of TQ on *BCL2* protein expression is consistent with preceding in vitro investigations. Previous studies [25, 40] reported effect sizes of -6.318 and -6.591, respectively, indicating significant suppression in the data. Mechanistic studies confirm TQ induces apoptosis in colorectal cancer cells by inhibiting anti-apoptotic signaling and activating pro-apoptotic pathways [43, 49], with similar *BCL2*/BCL-XL reduction and JAK2/STAT3 inhibition in renal carcinoma Caki-1 cells [50]. These consistencies across several in vitro cell lines support the robustness of TQ's effect within the preclinical study.

This meta-analysis has limitations including moderate-high heterogeneity (I^2=52.84%) from variations in protein quantification, dosing, exposure times, and cell types. Some studies showed non-significant results (CIs crossing zero), underscoring the need for standardized protocols [25, 41]. It lacks direct correlation between protein expression and functional apoptosis or in vivo validation.

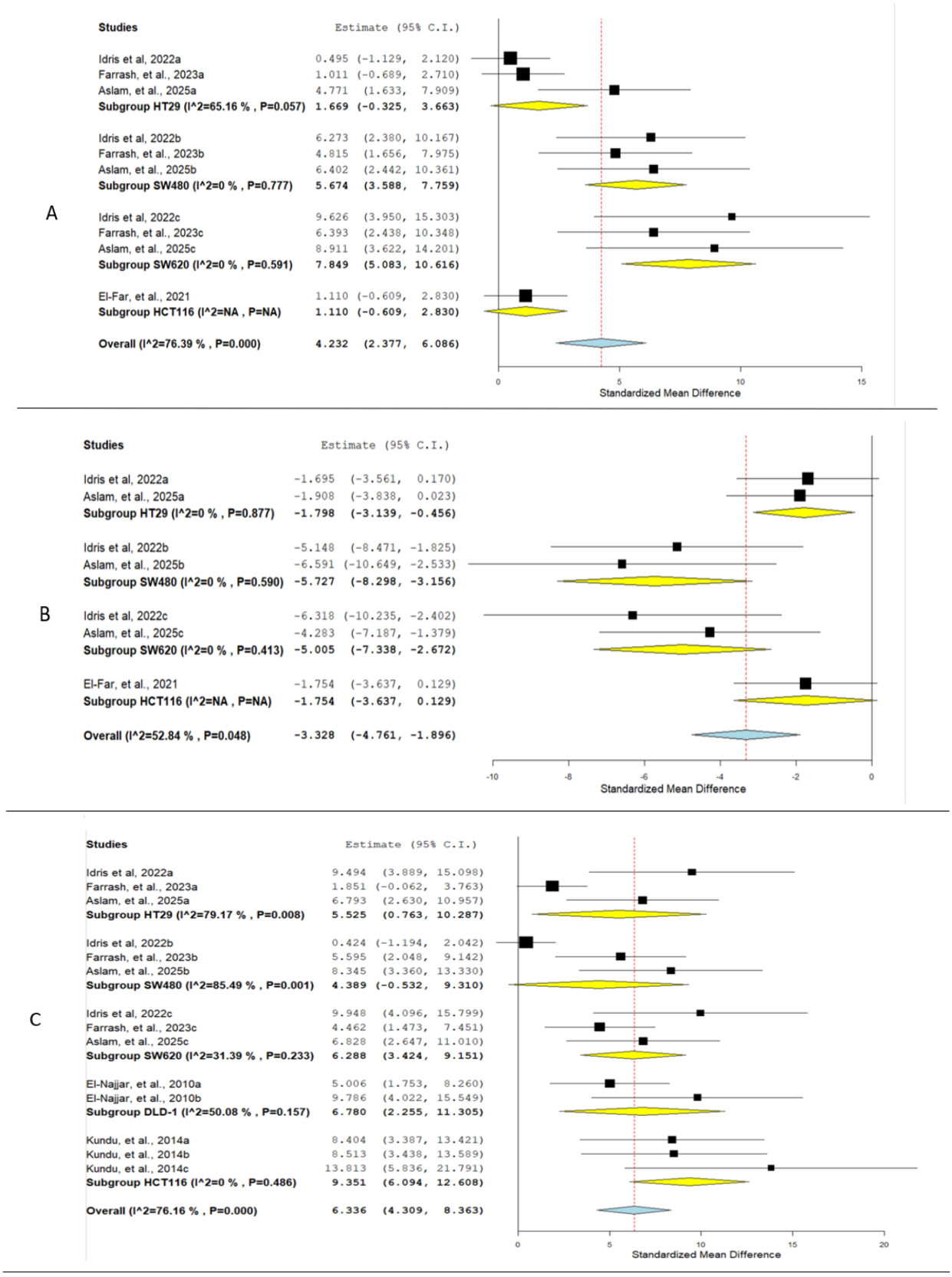

Figure 6. Subgroup Meta-Analysis Results of Thymoquinone Affecting Protein Expression, Grouped by Cell Line Type; A: *BAX* protein expression study; B: *BCL2* protein expression study; C: *CASP3* protein expression study.

Future studies should assess caspase activation, annexin V/TUNEL assays, dose-response relationships, and animal orclinical models for translational relevance.

This meta-analysis suggests that TQ treatment significantly reduces *BCL2* protein expression in various colorectal cancer cell lines, yielding an overall standardized mean difference (g) of –3.328 (95% CI: –4.761 to –1.896). The effect was uniform across

subgroups, including HT29, SW480, SW620, and HCT116, exhibiting minimal heterogeneity within these groups ($I^2 = 0\%$). This demonstrates the significant pro-apoptotic effect of TQ through the modulation of the anti-apoptotic gene *BCL2*.

The *BCL2* protein is essential in inhibiting apoptosis via the mitochondrial pathway, positioning it as a significant therapeutic target in various cancers, such as colorectal cancer. Overexpression of this factor has been linked to chemoresistance and an unfavourable prognosis [51]. Consequently, the observed downregulation of *BCL2* in this analysis suggests that TQ may enhance apoptotic sensitivity in preclinical colorectal cancer models.

SW480 subgroup showed the strongest effect (g=–5.727), followed by SW620 and HT29, likely reflecting cell line-specific factors like mitochondrial dependency, baseline *BCL2* levels, or TP53 status. Prior studies confirm this: demonstrated TQ reduced *BCL2* while increasing *BAX* in HT29 cells [52], promoting intrinsic apoptosis; reported similar apoptosis induction and *BCL2* downregulation in breast cancer models [53].

Moderate overall heterogeneity (I^2=52.84%) was observed, but absent within subgroups, indicating cell type significantly contributes to variability. Standardizing cell lines and treatment parameters could enhance future research consistency. Persistent *BCL2* downregulation across colorectal cancer lines confirms TQ's robust pro-apoptotic activity, supporting its potential as a targeted cancer therapy adjunct.

TQ induces apoptosis in colon cancer models by upregulating *CASP3* expression/activity, with *CASP3*/PARP cleavage enhancing chemosensitivity in HCT116 cells [54]. It primarily activates intrinsic pathways via CASP9/*CASP3*, correlating with cell death [43]. Gene analyses show TQ elevates *CASP3* mRNA, counteracting reduced expression in colon tumors and restoring apoptosis [55, 56].

Meta-analyses of research on TQ demonstrate that it significantly enhances *CASP3* protein expression in colon cancer cells, thereby promoting apoptosis through intrinsic pathways. Treatment with TQ alone or in combination with 5-fluorouracil (5-FU) resulted in a greater upregulation of *CASP3* compared to single-agent treatment, suggesting a potential chemosensitizing mechanism at the molecular level. The coordinated upregulation of *BAX* and *CASP3*, coupled with the downregulation of *BCL2*, observed in this meta-analysis aligns with the activation of the intrinsic mitochondrial apoptosis pathway. An elevated *BAX*/*BCL2* ratio facilitates mitochondrial outer membrane permeabilization, leading to the release of cytochrome c into the cytosol and subsequent apoptosome formation. This process triggers caspase-9 activation, culminating in the activation of executioner caspases such as *CASP3*, ultimately resulting in programmed cell death [57, 58]. Given that dysregulation of mitochondrial apoptosis is a hallmark of colorectal cancer, the modulation of this pathway by thymoquinone underscores its mechanistic relevance in preclinical colorectal cancer models. Overall, these findings suggest that TQ exhibits potential therapeutic relevance in preclinical models of colon cancer by enhancing apoptosis and demonstrating synergistic effects with conventional chemotherapeutic agents [36, 47]. Notably, TQ has been shown to enhance the experimental efficacy of 5-fluorouracil in vitro and in vivo, potentially through modulation of apoptosis-related and drug-resistance pathways. However, these observations are derived from preclinical studies, and further translational and clinical investigations are required to determine their relevance in clinical settings[12].

This meta-analysis suggests that thymoquinone may have potential adjuvant effects in preclinical colorectal cancer models. The pro-apoptotic effects of TQ, as quantitatively reported in previous studies, appear more consistently observed across CRC models compared with findings reported for other phytochemicals such as curcumin and resveratrol, although variability in experimental conditions precludes definitive conclusions [9]. It demonstrates the ability of thymoquinone to upregulate pro-apoptotic genes (*BAX* and *CASP3*) and downregulate the anti-apoptotic gene *BCL2*. These molecular effects are consistent with broader therapeutic strategies that seek to enhance anticancer efficacy by modulating apoptotic pathways. However, further translational and clinical validation is necessary to confirm these findings.

Author Contribution Statement

M.E. Prastiyanto: study conception/design, data analysis, materials, funding. K.N. Waskita & R. Nurmaulawati: administrative/logistic support, materials, data collection. N.R. Wijaya, S. Farida, D. Safrina, A.D.P. Putra: article drafting. S.H. Aliyah: critical revision. R. Silfarohana: drafting, administrative support. M.M. Sholikin: conception/design, data analysis, statistics, approval. R.M. Rukmana: conception/design, analysis, revision, approval, materials, funding, data collection.

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Data Availability

Data will be made available on request to the corresponding author.

Study Registration

This study was registered by PROSPERO (CRD420251110795).

Ethics approval and Consent to Participate

Since it is a meta-analysis and systematic review, no ethical permission was required for the study.

Conflict of Interest

There is no conflict of interest.

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