Tropical Journal of Pharmaceutical Research February 2026; 25 (2): 213-222
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
https://dx.doi.org/10.4314/tjpr.v25i2.9

Original Research Article

Effectiveness of thymoquinone for inhibiting the inflammation process in brain damage: Study in Wistar rats with intracerebral hemorrhage model

Khamim Thohari[1,2], Asra Al Fauzi[3]*, Djoko Agus Purwanto[4]
[1]Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, [2]School of Medicine, Universitas Ciputra, Surabaya, East Java, [3]Department of Neurosurgery, Faculty of Medicine, Universitas Airlangga/Universitas Airlangga Hospital, Surabaya, East Java, [4]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya, East Java, Indonesia

For correspondence:* ***Email:* *asra.al@fk.unair.ac.id*

Sent for review: 22 November 2025 *Revised accepted: 15 February 2026*

Abstract

Purpose: *To evaluate the effectiveness of thymoquinone (TQ) in inhibiting inflammation and promoting neuronal regeneration in a Wistar rat model of intracerebral hemorrhage (ICH).*
Methods: *Thirty-three male Wistar rats were randomly divided into three groups (n = 11 each): Group 1 was ICH control (0.16 mL autologous blood injected into the brain). Group 2 is ICH + TQ 150 mg/kg/day, while Group 3 was ICH + TQ 250 mg/kg/day. Thymoquinone was administered orally for 3 days post-ICH. On day 3, brains were harvested and analyzed using hematoxylin-eosin staining and immunohistochemistry for Tumor necrosis factor-alpha (TNF-α), Ki-67, Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) and neuronal markers.*
Results: *Thymoquinone treatment resulted in significant dose-dependent neuroprotective effects. Compared to ICH control group (TNF-α: 10.20 ± 0.85; NeuN-positive neurons: 4.20 ± 0.79), the 250 mg/kg dose of TQ significantly reduced TNF-α levels to 3.73 ± 0.58 and increased NeuN-positive cell counts to 11.36 ± 1.24 ($p < 0.05$). The increase in NeuN-positive cells reflects enhanced neuronal survival (neuroprotection) rather than proliferation of post-mitotic neurons. Furthermore, TQ administration significantly upregulated PPARγ (10.82 ± 1.05) and Ki-67 (10.64 ± 1.12) expression. The elevation of Ki-67 within this 3-day window primarily indicates early reparative proliferation of glial cells and neural progenitors.*
Conclusion: *Thymoquinone significantly inhibits post-ICH neuroinflammation (via reduced TNF-α) and enhances neuronal regeneration, via increased Ki-67, PPARγ, and neuron count, in a dose-dependent manner. The 250 mg/kg dose is more effective in supporting TQ as a promising neuroprotective agent in ICH.*

Keywords: *Intracerebral hemorrhage, Thymoquinone Neuroinflammation, Neuronal regeneration, PPARγ, Ki-67, TNF*

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Approximately 20 % of stroke patients suffer from intracerebral hemorrhage (ICH). with a mortality rate reaching 40 – 50 % [1]. The frequency of ICH increases in individuals who do not receive adequate treatment for hypertension. Compared

© 2026 The authors. This work is licensed under the Creative Commons Attribution 4.0 International License

to ischemic stroke, ICH carries a substantially higher risk of early death and long-term disability. Globally, the incidence of stroke decreased from 1990 to 2016 due to improvements in the prevention and control of risk factors such as smoking and hypertension. However, since 2005, there has been an alarming increase in stroke incidence among individuals younger than 50 years [2].

The incidence of ICH in Asian populations is markedly higher than in other regions and it increases with age, especially in individuals aged 85 years and older. Men are more prone to ICH than women, with the basal ganglia being the most frequently affected site. Hypertension remains the most significant risk factor, followed by excessive alcohol consumption and heart disease [3]. Indonesia has the highest stroke mortality rate in Asia, surpassing the Philippines, Singapore, Brunei, Malaysia and Thailand. It is estimated that approximately 500.000 people in Indonesia experience stroke annually, with around 25 % (125,000 individuals) dying and the remainder suffering from mild to severe disability [4].

The pathophysiology of ICH is complex and begins with the release of a large volume of blood into the brain parenchyma. Within 24 hours, hemoglobin (Hb) from lysed erythrocytes is degraded into heme and globin. The accumulation of heme and iron in the brain parenchyma leads to cytotoxicity and oxidative damage [5]. In the early phase, M1 microglia are activated by blood components and secrete inflammatory cytokines such as IL-6, IL-8, Tumor necrosis factor-alpha (TNF-α) and IL-1β. These cytokines induce neutrophil infiltration and Matrix Metalloproteinase 9 (MMP9) activation. resulting in extracellular matrix degradation and disruption of the blood-brain barrier (BBB) integrity [6]. Moreover, the heme residues from Hb metabolism act as potent oxidative mediators. Through reactions involving ferryl heme (Fe^{4+}) and globin-based radicals, lipid peroxidation and oxidative stress further exacerbate neuronal damage [7].

Following ICH onset, microglia are activated within one hour and persist for up to four weeks. Polarization into M1 phenotype promotes the release of pro-inflammatory cytokines, while the M2 phenotype induces anti-inflammatory cytokines such as IL-10 [6,8]. In animal models, M1 microglia increase rapidly within six hours post-ICH and gradually decline after two weeks, whereas M2 activity peaks on day 14 [9]. Although microglial activation assists in hematoma clearance, excessive activation amplifies oxidative stress and inflammation, aggravating neuronal death and BBB breakdown [10]. Various mechanisms of cell death, including necrosis, apoptosis, necroptosis, ferroptosis, pyroptosis and parthanatos, contribute to secondary brain injury after ICH. These mechanisms are associated with elevated intracellular calcium, mitochondrial dysfunction, oxidative stress and inflammatory cytokine release [7].

Endogenous neuroregenerative mechanisms attempt to counterbalance the damage. The proliferation marker Ki-67 indicates neural progenitor cell activation around the hematoma, reflecting neurogenesis and tissue repair [11]. In parallel, the Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) pathway plays a key role in neuroprotection by suppressing NF-κB transcriptional activity, reducing pro-inflammatory cytokine expression (TNF-α and IL-1β) and enhancing neuronal survival through antioxidant mechanisms [12]. Activation of PPARγ improves mitochondrial function and ATP production, while decreasing oxidative stress and Reactive Oxygen Species (ROS) formation, which in turn promotes neuronal regeneration [13].

Thymoquinone (TQ), the major bioactive compound of *Nigella sativa* (black cumin), possesses potent antioxidant and anti-inflammatory properties. It modulates cytokine production in macrophages and lymphocytes, inhibits NLRP3 inflammasome and NF-κB pathways and suppresses pro-inflammatory cytokines (IL-6, IL-8, TNF-α and IL-1β) [14]. Moreover. Thymoquinone exhibits antioxidant, immunomodulatory, and anticancer activities by reducing lipid peroxidation, lowering malondialdehyde (MDA) levels, and inhibiting ferroptosis through PTGS2 downregulation [15]. Li *et al* [16] demonstrated that TQ preserves mitochondrial membrane integrity and prevents dysfunction, while Behairy *et al* [17] confirmed that TQ significantly reduces MDA, IL-1, IL-6, and TNF-α levels, as well as apoptotic cell counts in brain tissue.

Based on these findings, TQ is hypothesized to attenuate neuroinflammation and oxidative stress following ICH by activating the PPARγ pathway and suppressing NF-κB–mediated cytokine expression. This study aims to evaluate the effectiveness of thymoquinone in inhibiting the inflammatory process in brain damage induced by intracerebral hemorrhage in Wistar rats, while focusing on the modulation of oxidative stress markers and inflammatory mediators.

METHOD

Design

A total of 33 healthy male Wistar rats (*Rattus norvegicus*) aged 8 – 10 weeks and weighing 200 – 250 g were used in this study. Animals were randomly allocated into three groups (n = 11 per group): Group 1 was intracerebral hemorrhage (ICH) control, Group 2 was ICH treated with thymoquinone (TQ) at 150 mg/kg/day and Group 3 was ICH treated with TQ at 250 mg/kg/day. Intracerebral hemorrhage was induced by stereotactic injection of 0.16 mL autologous whole blood into the right basal ganglia. Thymoquinone (≥ 98 % purity; dissolved in olive oil) was administered orally once daily for three consecutive days following ICH induction.

Tissue processing and histological examination

On day 3 post-ICH induction, animals were re-anesthetized and euthanized and brains were rapidly harvested. Brain tissues were immersion-fixed in 10 % neutral buffered formalin for 48 h at 4 °C, followed by dehydration through graded ethanol, clearing in xylene and paraffin embedding. Sagittal sections (5 µm thickness) encompassing the perihematomal region were obtained, as this area represents the primary site of secondary injury following ICH. Hematoxylin and eosin (H & E) staining was performed for evaluation of general histological features, neuronal integrity and inflammatory cell infiltration.

Immunohistochemistry

Paraffin-embedded sections were subjected to heat-induced antigen retrieval using citrate buffer (pH 6.0). Endogenous peroxidase activity and non-specific protein binding were blocked prior to antibody incubation. Sections were incubated overnight at 4 °C with the following primary antibodies:

(a) Tumor necrosis factor-alpha (TNF-α): rabbit polyclonal antibody (Bio-Rad AAR33; 1:200), (b) Ki-67: mouse monoclonal antibody (1:100), used as a marker of cellular proliferation, (c) Peroxisome proliferator-activated receptor gamma (PPARγ): rabbit polyclonal antibody (1:150), (d) NeuN: mouse monoclonal antibody (1:500) used to identify mature neurons.

Immunoreactivity was detected using biotinylated secondary antibodies and a streptavidin-horseradish peroxidase (HRP) system, with 3.3′-diaminobenzidine (DAB) as the chromogen. Sections were counterstained with hematoxylin.

Image acquisition and quantitative analysis

Images were captured using a digital light microscope at magnifications of 100×, 400× and 1000×. Quantitative analyses were performed by investigators blinded to experimental group allocation to minimize observer bias. Image analysis was conducted using ImageJ software. NeuN-positive neurons were quantified as the number of positive cells per mm² in the perihematomal region. TNF-α and PPARγ expression levels were quantified as integrated optical density of DAB-positive staining. Ki-67 was expressed as the percentage of positive nuclei relative to the total number of cells. For each animal, 3 – 5 sections were analyzed, with 5 – 8 randomly selected fields per section evaluated to ensure representative sampling of the perihematomal area.

Statistical analysis

The data were analyzed using SPSS version 26.0 with descriptive statistics presented as means and standard deviations (SD). Prior to hypothesis testing, data normality was verified using the Kolmogorov–Smirnov and Shapiro–Wilk tests ($p > 0.05$), while homogeneity of variances was assessed using Levene's test. Differences between groups were determined using one-way ANOVA followed by Tukey's HSD post-hoc test and path analysis was conducted to examine relationships among parameters. To ensure data integrity and avoid area selection bias, all immunohistochemical quantification was performed consistently using a blinded method by researchers unaware of the treatment group identities, through evaluating 5 – 8 randomly selected fields of view per specimen in the perihematomal region.

RESULTS

Before conducting hypothesis testing, data were first examined to ensure they met the assumptions of normality and homogeneity, which are fundamental for the validity of parametric statistical analysis. The normality test was performed using the Kolmogorov–Smirnov and Shapiro–Wilk methods. Results showed that all parameters in the three treatment groups, namely ICH control group, thymoquinone (150 mg/kg) group and thymoquinone (250 mg/kg body weight per day obtained *p*-values greater than 0.05. These findings indicate that data in each group were normally distributed and fulfilled

the normality assumption required for further statistical testing.

Furthermore, a homogeneity test was conducted to determine whether the variances among the groups were equal. The results showed that all parameters produced *p*-values above 0.05, which means that the data were homogeneous and had similar variance across the groups.

Table 1: Descriptive statistics of immunohistochemical markers (Mean, SD, Median, and Range) in rats with intracerebral hemorrhage (ICH) and TQ treatment

Parameter	Group	Minimum	Maximum	Median	Mean	SD
Neuron	ICH	1	8	5	4.20	2.098
	ICH+TQ 150 mg	5	11	9	8.09	1.758
	ICH+TQ 250 mg	8	15	12	11.36	2.111
Ki67	ICH	1	5	4.5	3.10	1.370
	ICH+TQ 150 mg	4	9	9	6.55	1.864
	ICH+TQ 250 mg	8	15	10	10.64	2.378
PPARgamma	ICH	1	6	4	3.20	1.549
	ICH+TQ 150 mg	5	10	9	7.36	1.629
	ICH+TQ 250 mg	6	14	14	10.82	2.272
TNFalpha	ICH	7	13	11.5	10.20	1.874
	ICH+TQ 150 mg	5	12	8	8.45	2.115
	ICH+TQ 250 mg	1	6	1	3.73	1.794

This outcome confirms that the dataset satisfied both the normality and homogeneity assumptions, allowing the subsequent hypothesis testing to be carried out using parametric statistical methods with reliable and valid results.

The descriptive analysis revealed distinct variations among the three experimental groups across all parameters. The ICH control group exhibited the lowest mean neuron count (4.20), which was significantly higher in the groups treated with thymoquinone (TQ) at 150 and 250 mg/kg body weight/day (8.09 and 11.36, respectively). It is important to note that this observed increase in NeuN-positive cells does not imply the proliferation of post-mitotic neurons or rapid neurogenesis within the 3-day treatment window. Instead, these findings suggest a dose-dependent neuroprotective effect, where TQ enhances neuronal survival by mitigating the secondary injury cascade.

Similar improvements were observed in Ki67 and PPARγ expression, reflecting enhanced cell proliferation (likely of glial and progenitor origin) and neuronal regeneration pathways. In contrast, TNF-α levels decreased from 10.20 in control group to 8.45 and 3.73 in TQ-treated groups, indicating a significant reduction in inflammatory activity. Overall, thymoquinone administration demonstrated significant potential in promoting neuronal recovery and preserving brain tissue integrity following intracerebral hemorrhage.

The results of the hypothesis testing using One-Way ANOVA followed by the Tukey HSD post hoc analysis showed significant differences among all experimental groups. Thymoquinone administration at both 150 and 250 mg/kg per day significantly increased neuron counts, Ki67 expression and PPARγ levels, while markedly reducing TNF-α levels compared to the ICH control group ($p < 0.05$). The highest dose of thymoquinone (250 mg/kg) produced the most substantial improvement across all parameters, indicating a dose-dependent effect. These findings demonstrate that thymoquinone effectively inhibits inflammatory responses and promotes neuronal regeneration in Wistar rats with intracerebral hemorrhage. The image was made by searching for the most damaged part of ICH and then it was cut sagittally. The image shows immunohistochemical staining of PPARγ expression in brain tissue at different magnifications (100x, 400x and 1000x). The top row shows the ICH control group, with weak or limited brown staining, indicating low PPARγ expression. The middle row shows ICH + TQ 150 mg/kg group, with moderate brown staining intensity, suggesting partial activation of PPARγ. The bottom row shows the ICH + TQ 250 mg/kg group exhibiting strong, widespread brown staining in the cytoplasm and nuclei, indicating markedly increased PPARγ expression. This pattern demonstrates that thymoquinone administration enhances PPARγ activation in a dose-dependent manner, contributing to neuroprotective and anti-inflammatory effects.

Table 2: Hypothesis test results

Parameter	Group	Sub-group	Mean difference	*P*-value
Neuron	ICH	ICH+TQ 150 mg	-3.891*	0.000
		ICH+TQ 250 mg	-7.164*	0.000
	ICH+TQ 150 mg	ICH	3.891*	0.000
		ICH+TQ 250 mg	-3.273*	0.002
	ICH+TQ 250 mg	ICH	7.164*	0.000
		IC H+TQ 150 mg	3.273*	0.002
Ki67	ICH	ICH+TQ 150 mg	-3.445*	0.001
		ICH+TQ 250 mg	-7.536*	0.000
	ICH+TQ 150 mg	ICH	3.445*	0.001
		ICH+TQ 250 mg	-4.091*	0.000
	ICH+TQ 250 mg	ICH	7.536*	0.000
		IC H+TQ 150 mg	4.091*	0.000
PPARgamma	ICH	ICH+TQ 150 mg	-4.164*	0.000
		ICH+TQ 250 mg	-7.618*	0.000
	ICH+TQ 150 mg	ICH	4.164*	0.000
		ICH+TQ 250 mg	-3.455*	0.000
	ICH+TQ 250 mg	ICH	7.618*	0.000
		IC H+TQ 150 mg	3.455*	0.000
TNFalpha	ICH	ICH+TQ 150 mg	1.745	0.115
		ICH+TQ 250 mg	6.473*	0.000
	ICH+TQ 150 mg	ICH	-1.745	0.115
		ICH+TQ 250 mg	4.727*	0.000
	ICH+TQ 250 mg	ICH	-6.473*	0.000
		IC H+TQ 150 mg	-4.727*	0.000

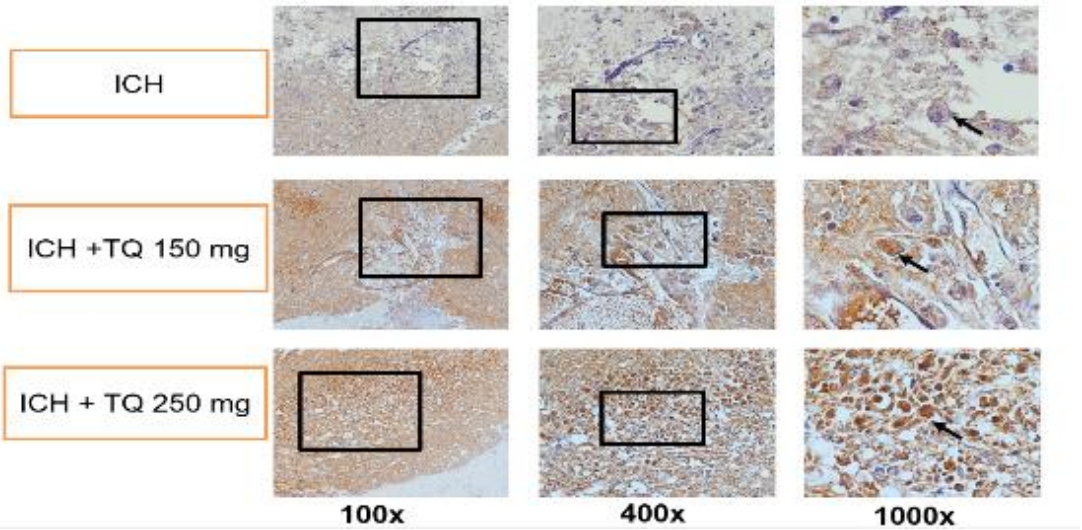


Figure 1: Immunohistochemical Expression of PPARγ in Brain Tissue of ICH Rats after Thymoquinone Treatment (100x, 400x and 1000x)

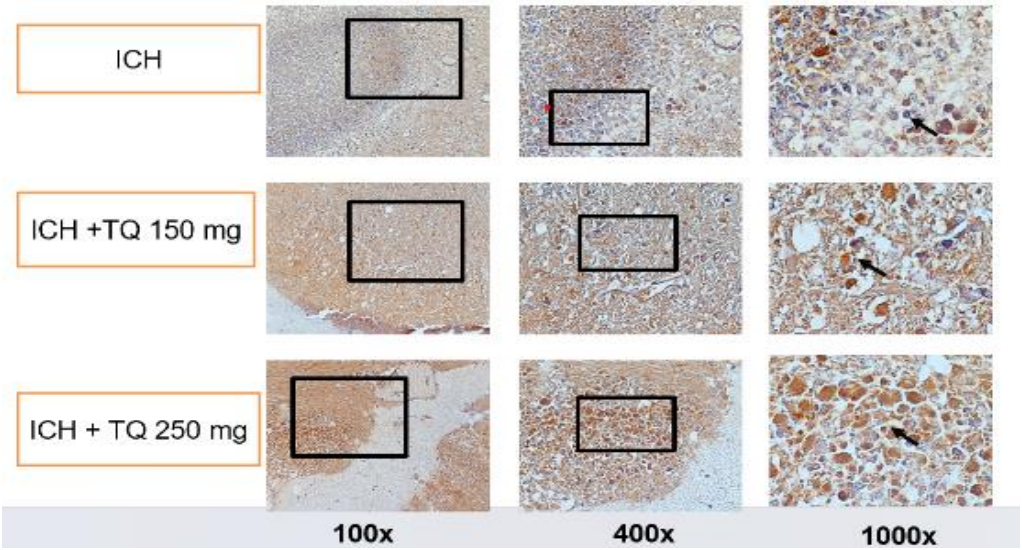


Figure 2: Immunohistochemical Expression of Ki67 in Brain Tissue of ICH Rats after Thymoquinone Treatment (100x, 400x and 1000x)

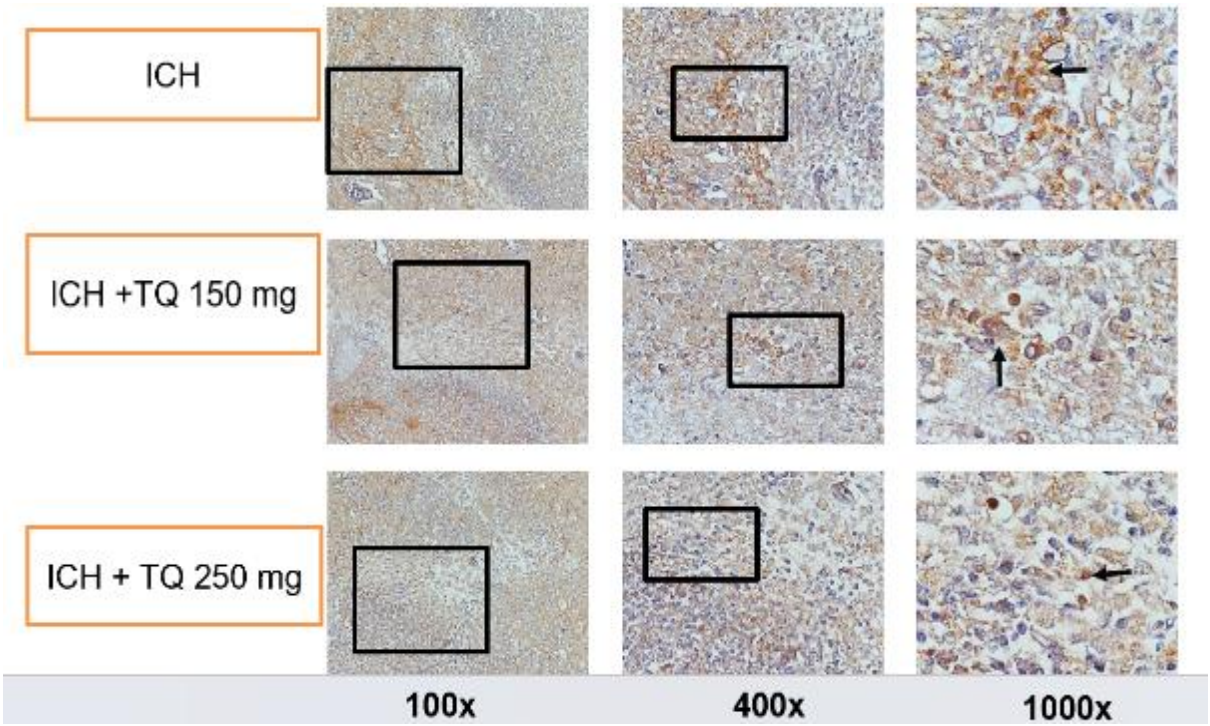


Figure 3: Immunohistochemical Expression of TNF-α in Brain Tissue of ICH Rats after Thymoquinone Treatment (100x, 400x and 1000x)

The image shows immunohistochemical staining of Ki67 expression in brain tissue at three magnifications (100x, 400x and 1000x). The top row represents the ICH control group, which displays weak brown staining, indicating low cellular proliferation. The middle row, corresponding to the ICH + TQ 150 mg/kg group, shows moderate brown staining intensity, suggesting increased proliferative activity. In the bottom row, representing ICH + TQ 250 mg/kg group, the nuclei exhibit strong, dense brown staining, indicating significantly elevated Ki67 expression. These findings suggest that thymoquinone enhances neuronal proliferation in a dose-dependent manner following intracerebral hemorrhage.

The image presents immunohistochemical staining of TNF-α expression in brain tissue at 100x, 400x and 1000x magnifications. The top row (ICH group) shows strong brown cytoplasmic staining, indicating high TNF-α expression and marked inflammatory response following intracerebral hemorrhage. The middle row (ICH +

TQ 150 mg/kg) demonstrates moderate staining intensity, suggesting a reduction in inflammatory activity. The bottom row (ICH + TQ 250 mg/kg) exhibits weak brown staining, indicating the lowest TNF-α expression among the groups. These findings suggest that thymoquinone administration reduces TNF-α–mediated neuroinflammation in a dose-dependent manner.

DISCUSSION

This evidence highlights the neuroprotective effects of thymoquinone in ICH models, which are driven by interconnected anti-inflammatory and pro-regenerative processes. The data presented not only show the repair functional and histological, but also reveals modulation underlying the molecular effect of TQ therapy.

One of the findings central to the current study is the significant decline of TNF-α levels in the groups treated with TQ. The ICH control group shows high TNF-α levels, which is consistent with literature stating that ICH triggers an acute inflammation cascade dominated by pro-inflammatory cytokines such as TNF-α and IL-1β [6]. Administration of TQ at doses of 150 and 250 mg/kg successfully lowered TNF-α levels to 8.45 and 3.73, respectively. The remarkable decrease is especially pronounced at the 250 mg/kg dose, indicating TQ's strong anti-inflammatory potential. This observation is consistent with a previous study, which reported that TQ represses pro-inflammatory gene expression by inhibiting NF-κB signaling, a master regulator of cytokine transcription involved in inflammation [18]. Furthermore, TQ capabilities in increasing glutathione (GSH) levels and reducing the excitotoxicity of glutamate in the central nervous system contribute to the creation of an antioxidant environment that does not support excessive production of TNF-α [15]. Immunohistochemical data show a gradual weakening of TNF-α staining intensity with increasing TQ dose. This observation is supported by the quantitative findings and indicates the resolution of inflammation at the network level.

By suppressing NF-κB activation, TQ breaks the inflammatory cascade signal at a very early stage, leading to a measurable reduction in TNF-α protein level. In addition, the ability of TQ to increase glutathione (GSH) levels, the main endogenous antioxidant, also contributes to creating a microenvironment less conducive to TNF-α production and neutralizing oxidative stress, which also triggers inflammation [16]. Thus, a significant decrease in TNF-α may be attributed to two TQ harmonious mechanisms: direct inhibition of cytokine transcription and repair of the antioxidant defense system. The dose-dependent improvement in Ki-67 and PPARγ expression represents a significant finding of this study. Ki-67, a marker of cellular proliferation, showed an increase from 3.10 ± 1.37 in control group to 10.64 ± 2.38 in 250 mg/kg TQ group. This upregulation within the 3-day post-ICH window is well-documented in rodent models and predominantly reflects the early proliferation of glial cells (microglia and astrocytes) as well as neural progenitor cells in the perihematomal area as part of the reparative response.

Evidence suggests that while microglia may exhibit initial cell death at day 1, they undergo rapid proliferation (indicated by Ki-67+ co-localization) starting early post-ICH, a process often driven by factors such as thrombin. Furthermore, glial proliferation is detectable within 1 to 3 days after brain injury in ICH models, supporting the timeline of the observed changes. To ensure the validity of these Ki-67 values and to confirm they are not artifactual due to focal sampling, all immunohistochemical quantifications were performed in a strictly consistent and fully blinded manner. This protocol involved evaluating multiple random fields across the perihematomal region rather than selective positioning, thereby ensuring the data reflect the overall tissue response.

The concurrent increase in Ki-67 and PPARγ expression, alongside decreased TNF-α levels, suggests that TQ may assist in redirecting proliferative activity toward a reparative M2 phenotype rather than a destructive one. PPARγ activation is known to promote microglial polarization towards the anti-inflammatory M2 phenotype, which supports resolution of inflammation and network repair [13,14]. It is important to note that microglial proliferation in phase I can be damaging in nature. However, in the context of TQ administration, the increase in Ki-67 correlated positively with improved PPARγ expression and decreased TNF-α level. This indicates that TQ directs proliferative activity in the reparative direction rather than a destructive phenotype. Though the findings must be interpreted within a broader, holistic context. The increase in Ki-67 occurred simultaneously with a decrease in TNF-α, and a marked increase in PPARγ, leading to a compelling hypothesis: TQ not only suppresses immune cell proliferation, but may reprogram proliferating cells towards a reparative phenotype. The concept of microglial polarization from a pro-inflammatory M1 phenotype to an anti-inflammatory, reparative M2 phenotype (M2) is increasingly recognized as a

key mechanism in neurological recovery [5]. Proliferative M2 cells play a role in phagocytosis of hematoma debris and secretion of neurotrophic factors. Therefore, increased Ki-67 observed in the groups treated with TQ possibly reflects the growth of beneficial M2 cells, a proposition supported by PPARγ data.

PPARγ expression increased drastically from 3.20 in control group to 10.82 in the 250 mg/kg TQ group. PPARγ is a transcriptional regulator with dual roles in neuroprotection as an anti-inflammatory regulator. PPARγ forms transcriptional complexes with NF-κB, thereby hindering its activity and ultimately reducing the production of pro-inflammatory cytokines such as TNF-α [12]. This reciprocal relationship is clearly observed in data from this study: increased PPARγ expression was accompanied by a decrease in TNF-α. In addition, PPARγ activation promotes microglia polarization towards an anti-inflammatory M2 phenotype, which supports inflammation resolution and tissue repair network [12]. Through these mechanisms, TQ-mediated activation of PPARγ not only suppresses inflammation-induced damage but also activates a regenerative program.

Upregulation of PPARγ connects the anti-inflammatory and pro-regenerative effects of TQ. PPARγ is a transcriptional regulator that plays a crucial role in post-injury neural homeostasis [6]. These data signify that TQ activates PPARγ in a dose-dependent manner, providing an elegant mechanistic explanation for its dual effects. Firstly, PPARγ exerts trans-repression on NF-κB. preventing NF-κB from binding to the regulatory elements of its target genes. This obstruction suppresses transcription of pro-inflammatory genes such as TNF-α [7]. This explains the inverse relationship observed between PPARγ activation and TNF-α levels. Secondly, on regeneration, PPARγ functions as a "master regulator" of genes involved in tissue repair. Activation of PPARγ drives microglial polarization towards M2 phenotype, which is in line with the interpretation of Ki-67 data [8]. Furthermore, PPARγ promotes neurogenesis and angiogenesis by upregulating growth factors such as Brain-Derived Neurotrophic Factor (BDNF) and Vascular Endothelial Growth Factor (VEGF) [17]. Basically, activation of PPARγ by TQ acts as a switch that redirects the brain from a destructive state to a reparative one. This shift allows the proliferation of cells (Ki-67) that fosters meaningful recovery, which is ultimately demonstrated as significant increase in the number of neurons.

The significant increase in NeuN-positive cell counts per field, from 4.20 ± 0.85 in control group to 11.36 ± 1.24 at the highest dose of 250 mg/kg, serves as definitive evidence of the neuroprotective efficacy of thymoquinone (TQ). This observed elevation in the number of mature neurons reflects enhanced neuronal survival rather than the proliferation of post-mitotic cells within the short treatment window. To ensure the validity of these findings and to confirm that the results are not artifactual or due to selective positioning during image acquisition, all immunohistochemical quantifications were performed in a strictly consistent and fully blinded manner by researchers unaware of the treatment assignments. Furthermore, analysis was conducted across multiple random fields within the perihematomal region to ensure a representative assessment of the entire affected area [11]. The findings suggest that TQ facilitates this process through convergence of several mechanisms: creating a supportive environment by suppressing inflammation (decreased TNF-α), activating the reparative master regulator (increased PPARγ) and stimulating progenitor cell proliferation (increased Ki-67). PPARγ, in particular, has been reported not only as an anti-inflammatory mediator but also as a trigger for the expression of neurotrophic factors and angiogenesis promoters, both of which are important for the survival and integration of newly generated neurons [18]. Thus, TQ acts as multi-target therapeutic agent, intervening in several pathological pathways of ICH simultaneously.

The consistent dose-dependent pattern observed across all measured parameters (TNF-α, Ki-67, PPARγ, and neuronal counts) gives credence to the validity of these findings and reveals that the therapeutic benefits of TQ are optimized through dose adjustment. The effect of TQ 250 mg/kg was consistently superior to that of TQ 150 mg/kg, suggesting the existence of a therapeutic "sweet spot" that warrants further exploration. These findings pave the way for the development of TQ as an adjunctive therapy for ICH. Its potential advantages lie in its ability to address the dual sides of ICH pathology: primary damage due to hematoma and secondary damage resulting from inflammation. TQ simultaneously promotes reparative processes by remediating injury and enhancing recovery [19].

CONCLUSION

This study demonstrates that thymoquinone (TQ) confers significant neuroprotection in a rat model of intracerebral hemorrhage by effectively counteracting the secondary injury cascade and actively promoting repair. Its efficacy is

evidenced by a dose-dependent attenuation of the pro-inflammatory response, as shown by the marked reduction of TNF-α levels, thereby mitigating inflammation-driven neuronal damage. Concurrently, thymoquinone stimulates a robust regenerative response characterized by enhanced cellular proliferation, indicated by elevated Ki-67 expression and a substantial upregulation of the master regulatory protein PPARγ, which orchestrates repair mechanisms. This favorable modulation of the post-hemorrhagic milieu culminates in a significant increase in neuronal survival and regeneration. Critically, 250 mg/kg body weight dose proved to be consistently more effective than 150 mg/kg dose across all parameters, underscoring a clear dose-response relationship and highlighting its superior therapeutic potential for fostering neurological recovery after ICH**.**

Based on the findings of this study, it is recommended that thymoquinone, particularly at a dose of 250 mg/kg, be further investigated as a promising neuroprotective adjuvant therapy for intracerebral hemorrhage. Future studies should prioritize elucidating the precise molecular pathways through which thymoquinone activates PPARγ and its long-term effects on functional neurological recovery in advanced animal models. to expedite its translational potential into clinical settings.

DECLARATIONS

Acknowledgement/Funding

The authors are grateful to all parties who assisted in the implementation of this study.

Conflict of interest

No conflict of interest is associated with this work.

Ethical approval

The animal study protocol was approved by the Animal Care and Use Committee (ACUC) of the Universitas Airlangga (no. 2.KEH.107.07.2024).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Khamim Thohari: Conceptualization, Methodology, Software, Data curation, Writing-Original draft preparation. Asra Al Fauzi, Djoko Agus Purwanto: Supervision, Validation, Writing-Reviewing and Editing. All authors have read and approved the manuscript for publication.

REFERENCES

1. *Montaño A, Hanley DF, Hemphill III JC. Hemorrhagic stroke. Handb Clin Neurol 2021; 176: 229-248*
2. *Lindsay MP, Norrving B, Sacco RL, Brainin M, Hacke W, Martins S, et al. World Stroke Organization (WSO): Global Stroke Fact Sheet 2019. Int J Stroke 2019; 14(8): 806–817*
3. *Wang S, Zou XL, Wu LX, Zhou HF, Xiao L, Yao T, Zhang Y, Ma J, Zeng Y, Zhang L. Epidemiology of intracerebral hemorrhage: a systematic review and meta-analysis. Front Neurol 2022; 13: 915813*
4. *GBD 2019 Stroke Collaborators. Global, regional, and national burden of stroke and its risk factors, 1990–2019: A systematic analysis for the Global Burden of Disease Study 2019. Lancet Neurol 2021; 20(10): 795–820.*
5. *Aronowski J, Zhao X. Molecular pathophysiology of cerebral hemorrhage: Secondary brain injury. Stroke 2011; 42(6): 1781–1786*
6. *Yang G, Fan X, Mazhar M, Guo W, Zou Y, Dechsupa N, Wang L. Neuroinflammation of microglia polarization in intracerebral hemorrhage and its potential targets for intervention. Front Mol Neurosci 2022; 15: 1013706*
7. *Zhang Y, Khan S, Liu Y, Zhang R, Li H, Wu G, et al. Modes of brain cell death following intracerebral hemorrhage. Front Cell Neurosci 2022; 16: 1-20*
8. *Zhang Z, Zhang Z, Lu H, Yang Q, Wu H, Wang J. Microglial Polarization and inflammatory mediators after intracerebral hemorrhage. Mol Neurobiol 2017; 54(3): 1874-1886*
9. *Chen X, Jiang Y, Zhao W, Zhang Z. Microglial proliferation and phenotypic transformation in the injured brain: Implications for neural repair. Glia 2024; 72(3): 512-526*
10. *Wang J, Doré S. Neuroinflammation after intracerebral hemorrhage: mechanisms and therapeutic targets. J Neuroinflam 2023; 20(1): 45*
11. *Miller I, Min M, Yang C, Tian CV, Gookin S, Charter D, Sabrina L Spence. Ki67 is a graded rather than a binary marker of proliferation versus quiescence. Cell Rep 2018; 24: 1105–1112*
12. *Fatima Shad K, Soubra W, Cordato DJ. The role of thymoquinone, a major constituent of Nigella sativa, in the treatment of inflammatory and infectious diseases. Clin Exp Pharmacol Physiol 2021; 48(11): 1445-1453*
13. *Lan X, Han X, Liu J, Wang J. PPARγ activation promotes microglia/macrophage M2 polarization and functional recovery after experimental intracerebral hemorrhage. Brain Behav Immun 2024; 115: 450-463*

14. Cai W, Yang T, Liu H, Han L, Zhang K, Hu X, Zhang X, Yin K, Gao Y, Michael Bennett K, et al. Peroxisome proliferator-activated receptor γ (PPARγ): A master gatekeeper in CNS injury and repair. Prog Neurobiol 2018; 163–164: 27-58
15. Al-Majed AA, Al-Omar FA, Nagi MN, Al-Shabanah OA. Thymoquinone modulates glutathione and glutamate levels in the brain: implications for neuroprotection. Neurosci Lett 2023; 802: 137175
16. Li Y, Zhang K, Wang P, Liu F, Xu D. Thymoquinone attenuates neuroinflammation via suppressing TLR4/MyD88/NF-κB pathway in a rat model of intracerebral hemorrhage. Int Immunopharmacol 2024; 128: 111489
17. Behairy A, Elkomy A, Elsayed F, Gaballa MMS, Soliman A, Aboubakr M. Antioxidant and anti-inflammatory potential of spirulina and thymoquinone mitigate the methotrexate-induced neurotoxicity. Naunyn Schmiedebergs Arch Pharmacol 2023; 1-14
18. Zhao H, Li M, Zhang L, Chen S. PPARγ agonist promotes angiogenesis and neurogenesis after experimental intracerebral hemorrhage. CNS Neurosci Ther 2024; 30(1): e14565
19. Kvistad CE, Kråkenes T, Gavasso S, Bø L. Neural regeneration in the human central nervous system-from understanding the underlying mechanisms to developing treatments. Front Neurol 2024; 15: 1398089