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Safinamide Attenuates Systemic Inflammatory Hyperalgesia Through Modulation Of Neuroimmune Signaling And Nav1.7 Expression

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Article

Keywords: hyperalgesia, NaV1.7, TNF-α, IL-1β, neuroimmunomodulation, safinamide, TLR4, NF-κB

Posted Date: July 29th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10327455/v1

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on September 29th, 2026. See the published version at https://doi.org/10.1038/s41598-026-73225-z.

Abstract

Systemic inflammatory states are associated with neuroimmune activation and peripheral sensitization mediated by dysregulation of ion channels and inflammatory mediators. Safinamide, a multi-target drug approved for Parkinson's disease, exhibits sodium channel–blocking and anti-inflammatory properties, suggesting potential relevance in inflammatory pain. This study investigated the analgesic and neuroimmune modulatory effects of safinamide in a systemic lipopolysaccharide (LPS)-induced inflammatory hyperalgesia model in rats. Thirty-two male Wistar rats were divided into control, LPS, safinamide-treated, and indomethacin-treated groups. Thermal nociception was assessed using Tail-Flick and Hot Plate tests, and motor coordination was evaluated using the Rotarod assay. Expression of NaV1.7, TLR4, TNF-α, and IL-1β was evaluated by qRT-PCR and ELISA, whereas NF-κB p65 protein expression was assessed by Western blot. LPS administration induced significant thermal hyperalgesia and increased expression of NaV1.7, TLR4, NF-κB p65, TNF-α, and IL-1β in DRG tissue. Safinamide significantly attenuated thermal hyperalgesia while reducing NaV1.7 expression and these neuroinflammatory markers, with effects comparable to or greater than indomethacin, without impairing motor coordination. These findings suggest that safinamide alleviates inflammatory pain through coordinated modulation of neuroimmune signaling and NaV1.7 expression, supporting its further evaluation as a candidate therapy for inflammatory pain.

1. Introduction

Pain is a complex physiological and pathological phenomenon defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [1]. Inflammatory pain, in particular, arises from tissue damage and the subsequent release of a "biochemical soup" containing cytokines, bradykinin, and growth factors. These mediators sensitize peripheral nociceptors, leading to the clinical hallmarks of hyperalgesia and allodynia [2].

Current pharmacological gold standards, primarily Non-steroidal Anti-inflammatory Drugs (NSAIDs) and opioids, are fraught with significant clinical limitations. Long-term NSAID use is associated with gastrointestinal erosion and renal toxicity [3]. Conversely, the opioid crisis—driven by high rates of addiction, tolerance, and respiratory depression—underscores the urgent need for non-opioid alternatives that target specific molecular pathways involved in pain transduction [4].

Recent advances have identified voltage-gated sodium channels (VGSCs) as pivotal players in the signaling of inflammatory pain [5]. Specifically, the NaV1.7 subtype, highly expressed in the dorsal root ganglia (DRG), acts as a "threshold setter" for neuronal firing [6]. During inflammation, the upregulation and post-translational modification of these channels are associated with increased neuronal excitability [7]. Consequently, molecules capable of modulating NaV1.7 activity represent a promising frontier in analgesic research [8, 9].

Neuroimmune signaling also plays a central role in inflammatory pain. Activation of Toll-like receptor 4 (TLR4) stimulates nuclear factor-kappa B (NF-κB), leading to increased production of pro-inflammatory

cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β). These mediators amplify peripheral sensitization and enhance neuronal excitability, partly through regulation of ion channels, including NaV1.7 [10, 11]. Consequently, therapies capable of simultaneously modulating neuroimmune signaling and neuronal excitability may provide greater analgesic efficacy than approaches targeting a single pathway.

Safinamide is a multimodal drug currently approved as an adjunct treatment for Parkinson's disease. In addition to its highly selective and reversible monoamine oxidase-B (MAO-B) inhibition, safinamide exhibits state- and use-dependent blockade of voltage-gated sodium channels and modulates glutamate release [12]. Emerging evidence also suggests that safinamide exerts anti-inflammatory effects by suppressing TLR4/NF-κB signaling and reducing the production of pro-inflammatory cytokines. These pharmacological properties suggest that safinamide may influence both neuronal and inflammatory mechanisms involved in pain processing [13, 14].

Most experimental studies of inflammatory pain employ localized inflammatory models that do not fully reproduce the systemic immune activation observed in conditions such as sepsis or systemic inflammatory response syndrome. Therefore, the present study used a subacute systemic lipopolysaccharide (LPS)-induced inflammatory model to investigate the effects of safinamide on thermal hyperalgesia and neuroimmune responses. Specifically, we evaluated the expression of NaV1.7, TLR4, NF-κB p65, TNF-α, and IL-1β in the L4–L6 DRG 72 hours after LPS administration to determine whether modulation of these pathways contributes to the antinociceptive effects of safinamide.

2. Materials and Methods

2.1. Ethical Statement and Animal Housing

The present study was conducted in accordance with the ethical guidelines for the care and use of laboratory animals and was approved by the Ethics Committee of Alborz University of Medical Sciences (Ethics Code: IR.ABZUMS.AEC.1403.004) and was conducted and reported in accordance with the ARRIVE 2.0 guidelines. Thirty-two male Wistar rats (weighing 200–300 g) were obtained from the Animal House of the Faculty of Pharmacy, Alborz University of Medical Sciences. The animals were housed in standard cages at a controlled temperature (25 °C) and humidity (40%–60%) under a 12-hour light/dark cycle, with ad libitum access to food and water.

2.2. Experimental Design and Groups

The rats were randomly assigned to four experimental groups (n = 8 per group):

1. **Negative Control**: Healthy rats receiving an equal volume of sterile saline (i.p.) (vehicle)
2. **LPS Group (Model)**: Rats subjected to LPS-induced inflammatory pain

3. **Treatment Group (Safinamide)**: Rats receiving Safinamide (30 mg/kg, p.o.) 1 hour post-LPS induction, followed by daily dosing at 24-hour intervals until tissue collection
4. **Positive Control (Indomethacin)**: Rats receiving Indomethacin (10 mg/kg, p.o.) 1 hour post-LPS induction, followed by daily dosing at 24-hour intervals until tissue collection

2.3. Induction of Inflammatory Pain and Drug Administration

Inflammatory pain was induced by a single intraperitoneal (i.p.) injection of LPS (1 mg/kg) [15]. The Safinamide dose (30 mg/kg/day, p.o.) was selected based on clinical translational data indicating that oral doses between 15–45 mg/kg in rats produce maximum plasma concentrations consistent with the therapeutic range observed in humans (50–100 mg/day) [16]. This dose has been previously validated to provide significant antinociceptive effects in rodent models without inducing sedation or compromising locomotor activity.

2.4. Behavioral Assessments

2.4.1. Hot Plate Test

To assess thermal hyperalgesia, rats were placed on a heated surface (52 °C). The latency to the first sign of a pain response (hind paw licking or jumping) was recorded, with a 30-second cutoff to prevent tissue damage.

2.4.2. Tail-Flick Test

Thermal nociceptive thresholds were measured by exposing the rat's tail to a focused radiant heat source. The time taken for the animal to flick its tail (reaction latency) was recorded as an indicator of spinal nociceptive processing.

2.4.3. Rotarod Test (Motor Coordination)

To evaluate potential motor impairment or sedative effects, motor coordination was assessed using an accelerating Rotarod apparatus. After two days of habituation, rats were placed on the rod, which accelerated from 4 to 40 rpm over 300 s. The latency to fall (s) was recorded, with a 300 s cut-off time.

Behavioral assessments and molecular analyses were performed by investigators blinded to group allocation

2.5. Tissue Collection and Preparation

On day 3, following behavioral testing, rats were anesthetized with an i.p. injection of Ketamine (60 mg/kg) and Xylazine (10 mg/kg). Terminal blood was collected via cardiac puncture, and the animals were euthanized. The spinal column was dissected, and DRGs were isolated and immediately stored at -80 °C for molecular analysis (qRT-PCR and ELISA).

2.6. RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from DRG tissues using the TRIzol method (Qiazol, Kiazist, Iran). Tissue samples (50–100 mg) were homogenized in 1 mL of TRIzol, followed by chloroform phase separation and isopropanol precipitation. RNA concentration and purity were verified using a NanoDrop spectrophotometer (OD 260/280).

Complementary DNA (cDNA) was synthesized using the Easy cDNA Synthesis Kit (Parstous, Iran) according to the manufacturer's instructions. The expression of NaV1.7 mRNA was quantified using SYBR Green (Addbio, Korea) on an ABI StepOne system. The primer sequences used were:

GAPDH: F: 5'-ACTCCACTCACGGCAAATTC-3', R: 5'-TCTCCATGGTGGTGAAGACA-3'

NaV1.7: F: 5'-GTGTGCTGTGTGGGGAGTGGA-3', R: 5'-TGGAGGTTGTTTGCGTCGGTGT-3'

Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, with GAPDH as the internal control.

2.7. Enzyme-Linked Immunosorbent Assay (ELISA)

The protein expression levels of TLR-4, NaV1.7, and IL-1β and TNF-α in the L4–L6 DRG were quantified using commercially available ELISA kits (Biochem MBS026966, Antibodies A303548, Antibodies A78305, Antibodies A78898) according to the manufacturer’s instructions. Briefly, DRG tissues were homogenized in PBS containing protease inhibitors. After centrifugation, the supernatant was collected, and the total protein concentration was determined using the BCA assay. Absorbance was measured at 450 nm using a microplate reader.

2.8. Western blot analysis

DRG tissues were homogenized in RIPA lysis buffer containing protease inhibitors, and total protein was quantified using a BCA protein assay. Equal amounts of protein were separated by SDS-PAGE and transferred onto PVDF membranes. After blocking, the membranes were incubated overnight at 4°C with rabbit anti-NF-κB p65 primary antibody (Abcam, Cambridge, UK; ab32536; 1:1000), followed by HRP-conjugated goat anti-rabbit IgG secondary antibody (1:5000) for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence detection system. β-Actin was used as the loading control, and band intensities were quantified by densitometric analysis using ImageJ software.

2.9. Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). All data are presented as Mean ± standard error of the mean (SEM). Data normality and homogeneity of variances were confirmed using the Shapiro-Wilk and Brown-Forsythe tests, respectively, verifying that all datasets satisfied the assumptions required for parametric analysis. To compare the differences between the four experimental groups, a one-way analysis of variance (ANOVA) was utilized.

Following ANOVA, Tukey's post hoc test was conducted to determine specific intergroup differences. A p-value < 0.05 was considered statistically significant. A sample size of n = 8 per group was determined based on a power analysis to achieve 80% power at a 0.05 significance level.

3. Results

3.1. Behavioral Assessment of Nociception and Motor Safety

Tailflick

On Day 1, no statistically significant differences in tailflick latency were observed among the experimental groups (P > 0.05), indicating a baseline uniformity across cohorts shortly after induction. However, by Day 3, the LPS-treated model group exhibited a significant decrease in tailflick response latency compared to the control group (P < 0.05), confirming the successful development of persistent spinal thermal hyperalgesia. Daily treatment with Safinamide (30 mg/kg, p.o.) robustly reversed this inflammatory hyperalgesia by Day 3, significantly extending reaction latencies compared to the untreated LPS model group (P < 0.05). Inter-treatment comparisons on Day 3 revealed that Safinamide produced a significantly greater antinociceptive effect than the positive control, Indomethacin, demonstrating an enhanced effect on elevating spinal pain thresholds (P < 0.05) (Fig. 1a).

Hot Plate

The Hot Plate assay corroborated the hyperalgesic trends observed at the spinal level (Fig. 1b). On Day 1, baseline thermal latencies remained statistically comparable across all cohorts (P > 0.05). By Day 3, systemic LPS administration induced a profound reduction in thermal pain thresholds, characterized by a sharp decrease in response latency relative to control animals (P < 0.001). Therapeutic intervention with Safinamide significantly mitigated this supraspinal hyperalgesia by Day 3, resulting in a marked increase in withdrawal latencies compared to the LPS model group (P < 0.05). While the positive control group treated with Indomethacin trended toward an increased latency on Day 3, it failed to reach statistical significance against the LPS group (P = 0.86), highlighting Safinamide's distinct translational advantage in this subacute inflammatory window.

Rotarod

To ensure these results were not confounded by sedation, the Rotarod test was performed (Fig. 1c). Neither the LPS-induced SIRS model nor Safinamide treatment significantly affected locomotor activity (P > 0.05). Safinamide-treated rats exhibited a mean latency of 285.0 ± 7.0 s, statistically indistinguishable from the Control group (287.5 ± 9.3 s), suggesting that the observed antinociceptive effects were unlikely to be secondary to motor impairment or sedation.

3.2. Effects of Safinamide on DRG Neuroimmune and Nociception-Related Markers

3.2.1. Safinamide attenuates LPS-induced activation of the TLR4/NF-κB signaling pathway in DRG

LPS administration significantly increased the expression of TLR4 in dorsal root ganglion (DRG) compared with the control group ($p < 0.001$). Safinamide treatment markedly reduced TLR4 levels relative to the LPS group, although values remained higher than control animals. Indomethacin also significantly decreased TLR4 expression, confirming the anti-inflammatory effect of the treatment (Fig. 2a).

Similarly, NF-κB p65 protein expression was significantly increased following LPS exposure ($p < 0.001$). Both safinamide and indomethacin significantly reduced NF-κB p65 protein expression compared with the LPS group (Fig. 2b-c).

3.2.2. Safinamide reduces pro-inflammatory cytokine production in DRG

LPS markedly increased TNF-α and IL-1β levels in DRG tissue compared with control animals ($p < 0.001$). Treatment with safinamide significantly reduced both TNF-α and IL-1β concentrations, although levels remained elevated compared with control. Indomethacin showed a comparable inhibitory effect on cytokine production (Fig. 2d–e).

3.2.3. Safinamide downregulates NaV1.7 expression in DRG

NaV1.7 mRNA expression was significantly upregulated following LPS administration ($p < 0.001$). Both safinamide and indomethacin significantly reduced NaV1.7 mRNA levels compared with the LPS group. At the protein level, both treatments also decreased NaV1.7 expression; however, safinamide produced a significantly greater reduction than indomethacin (Fig. 2f–g), suggesting a stronger inhibitory effect on NaV1.7 expression and potentially reduced neuronal excitability in sensory neurons.

4. Discussion

The present study demonstrates that safinamide attenuates LPS-induced inflammatory hyperalgesia and suppresses neuroimmune activation in the dorsal root ganglion (DRG). Behavioral improvements were accompanied by reductions in TLR4, NF-κB p65, TNF-α, IL-1β, and NaV1.7 expression, suggesting that safinamide exerts antinociceptive effects through coordinated modulation of inflammatory signaling and neuronal excitability. Although safinamide is clinically approved for Parkinson’s disease, these findings extend its pharmacological profile to include potential relevance in inflammatory pain conditions.

4.1. Persistent neuroimmune activation in DRG following systemic LPS exposure

Systemic LPS administration induced sustained neuroimmune alterations within the DRG that persisted 72 hours after exposure. Despite clearance of circulating endotoxin, elevated expression of TLR4, NF-κB p65, TNF-α, IL-1β, and NaV1.7 indicates prolonged activation of peripheral nociceptive signaling pathways.

This sustained response is likely maintained by interactions between sensory neurons, satellite glial cells, and infiltrating immune cells within the ganglion microenvironment. These non-neuronal elements contribute to a feed-forward inflammatory loop that amplifies nociceptive signaling and maintains peripheral sensitization even after the initial systemic insult resolves. [17, 18]. These findings support the DRG as a key integrative site linking systemic inflammation to persistent pain states.

4.2. Safinamide attenuates inflammatory hyperalgesia without motor impairement

LPS significantly decreased thermal withdrawal latencies in both Tail-Flick and Hot Plate tests, confirming the development of robust inflammatory hyperalgesia at spinal and supraspinal levels [19]. Safinamide treatment significantly reversed these behavioral changes without affecting Rotarod performance, indicating that its antinociceptive effects are not attributable to sedation or motor impairment.

These findings are consistent with previous reports showing analgesic and anti-hyperexcitability effects of safinamide in preclinical pain models [16, 20, 21]. The preservation of motor coordination strengthens the interpretation that safinamide modulates nociceptive processing rather than producing nonspecific suppression of behavior [22, 23].

4.3. Modulation of TLR4/NF-κB signaling and neuroimmune response

LPS exposure significantly increased TLR4 expression and NF-κB p65 protein levels in the DRG. Safinamide reduced both markers, suggesting attenuation of LPS-driven neuroimmune signaling.

Importantly, NF-κB p65 measurement in this study reflects total protein expression rather than nuclear translocation or phosphorylation status. Therefore, the observed changes should be interpreted as reduced NF-κB pathway-associated expression, rather than direct evidence of altered transcriptional activity.Similarly, TLR4 was quantified by ELISA on whole DRG tissue lysate, which reflects total receptor expression rather than receptor activation, ligand engagement, or downstream signaling flux; consequently, the proposed TLR4→NF-κB causal link remains inferential rather than directly demonstrated in this study.

Given that TLR4/NF-κB signaling regulates transcription of multiple inflammatory mediators, suppression of this pathway likely contributes to reduced cytokine production and downstream nociceptive sensitization [24]. In addition to its anti-inflammatory actions, safinamide may indirectly reduce NF-κB activation by reducing sodium-dependent neuronal hyperexcitability and calcium-dependent intracellular signaling [25]. Previous studies have similarly demonstrated inhibition of TLR4/NF-κB signaling by safinamide in macrophages and experimental models of spinal cord injury and cerebral ischemia, supporting a broader immunomodulatory profile [26, 27].

4.4. Cytokine-driven regulation of NaV1.7 and neuronal excitability

Marked increases in TNF-α and IL-1β following LPS administration confirm sustained activation of neuroimmune signaling within the DRG. These cytokines are key mediators of inflammatory pain and contribute to peripheral sensitization by enhancing nociceptor excitability and amplifying inflammatory cascades [11, 28].

Importantly, accumulating evidence indicates that TNF-α and IL-1β can upregulate voltage-gated sodium channels, including NaV1.7, thereby lowering action potential thresholds and promoting hyperexcitability. In this context, cytokine elevation may represent an upstream mechanism linking TLR4/NF-κB activation to altered ion channel expression [10].

Safinamide significantly reduced TNF-α and IL-1β levels, suggesting attenuation of this inflammatory cascade. This reduction may indirectly contribute to decreased NaV1.7 expression by limiting cytokine-mediated channel regulation.

Consistent with this mechanism, NaV1.7 was significantly upregulated at both mRNA and protein levels following LPS exposure, reinforcing its role in inflammatory nociceptive sensitization. Safinamide reduced NaV1.7 expression more effectively than indomethacin at the protein level, despite comparable effects at the mRNA level, suggesting additional post-transcriptional or post-translational regulation [29, 30].

Together, these findings support a model in which safinamide attenuates inflammatory pain through dual actions: suppression of neuroimmune signaling (TLR4/NF-κB/cytokines) and direct or indirect modulation of NaV1.7-mediated neuronal excitability.

4.5. Therapeutic implications

Safinamide is a multimodal compound with MAO-B inhibitory, sodium channel–blocking, and glutamate-modulating properties. The present findings suggest that these pharmacological features may converge to produce antinociceptive effects in inflammatory pain by targeting both immune signaling and neuronal excitability.

Although this study is limited to a single acute inflammatory model, the observed effects provide a rationale for further investigation of safinamide in chronic and systemic pain conditions. Future studies should clarify whether NaV1.7 modulation is a direct pharmacological effect or secondary to neuroimmune suppression.

Study limitations and future directions

Several limitations should be acknowledged. Only one dose (as noted in Methods, this dose corresponds to human-equivalent exposure in the approved clinical range) and time point were evaluated, preventing assessment of dose–response relationships and temporal dynamics. NaV1.7 functional activity was not directly measured using electrophysiological approaches. NF-κB signaling was assessed at the protein expression level without evaluation of nuclear translocation or phosphorylation, limiting mechanistic resolution.

Additionally, analyses were restricted to DRG tissue, without assessment of spinal cord or peripheral immune compartments. Finally, only male animals and an acute LPS model were used, limiting generalizability to chronic pain states and sex-dependent effects.

Future studies should incorporate electrophysiological validation of NaV1.7 function, mechanistic pathway inhibition, and chronic pain models to strengthen translational relevance.

5. Conclusion

In this acute, single-dose model of systemic LPS-induced hyperalgesia in male rats, safinamide attenuated inflammatory hyperalgesia through coordinated modulation of neuroimmune signaling and NaV1.7 expression in the dorsal root ganglion. These findings suggest that its antinociceptive effects involve suppression of TLR4/NF-κB–mediated inflammatory pathways and regulation of sodium channel–dependent neuronal excitability. Further studies incorporating dose-response, longer-term, and sex-inclusive designs are required to clarify underlying mechanisms and evaluate the translational potential of safinamide as a candidate therapy for chronic inflammatory pain.

Declarations

Conflict of Interest

The authors declare no competing interests.

Ethical Approval

The present study was conducted in accordance with the ethical guidelines for the care and use of laboratory animals and was approved by the Ethics Committee of Alborz University of Medical Sciences (Ethics Code: IR.ABZUMS.AEC.1403.004).

Funding

This study was supported by Alborz University of Medical Sciences under grant number [0-0-94-7061].

Author Contribution

PG, SS, and MAA designed the study. PG performed the experiments, collected the data, and conducted the behavioral and molecular analyses. PG drafted the manuscript. SS supervised the study, contributed to the study design, interpreted the results, critically revised the manuscript, and acquired funding. MAA contributed to the methodology, data interpretation, and critical revision of the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Acknowledgement

The authors would like to acknowledge the Student Research Committee of Alborz University of Medical Sciences for their support and assistance in conducting this study

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

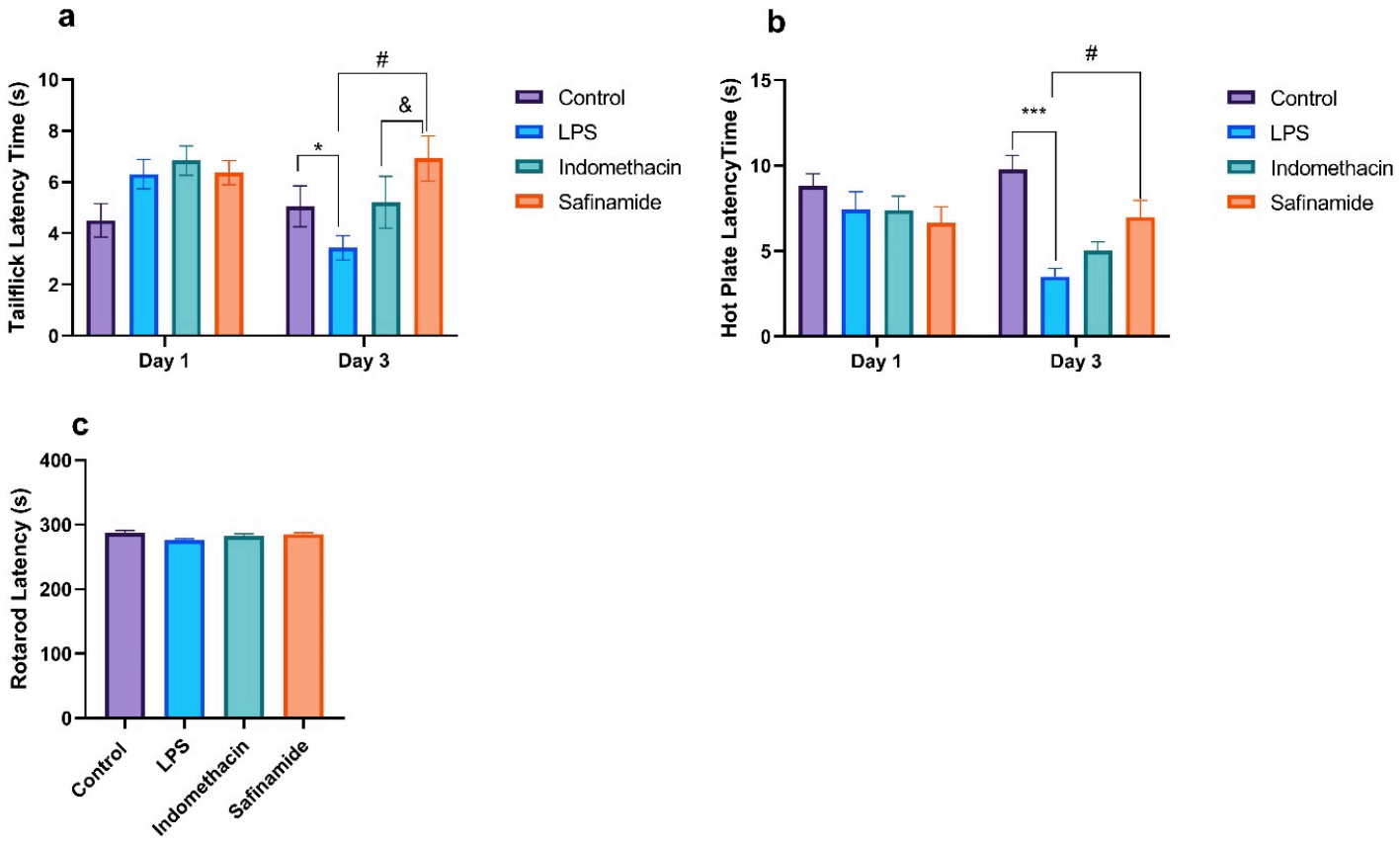


Figure 1

Effects of Safinamide on nociceptive thresholds and motor coordination. Reaction latencies were assessed on Days 1 and 3 post-induction using the (a) Tail-Flick test and (b) Hot Plate test. (c) Rotarod latency time (s) was evaluated on Day 3 to assess motor coordination and potential sedative effects. Data are presented as mean ± SEM (n = 8 per group). *P < 0.05 and ***P < 0.001 vs. Control; #P < 0.05 vs. LPS; &P < 0.05 vs. Indomethacin

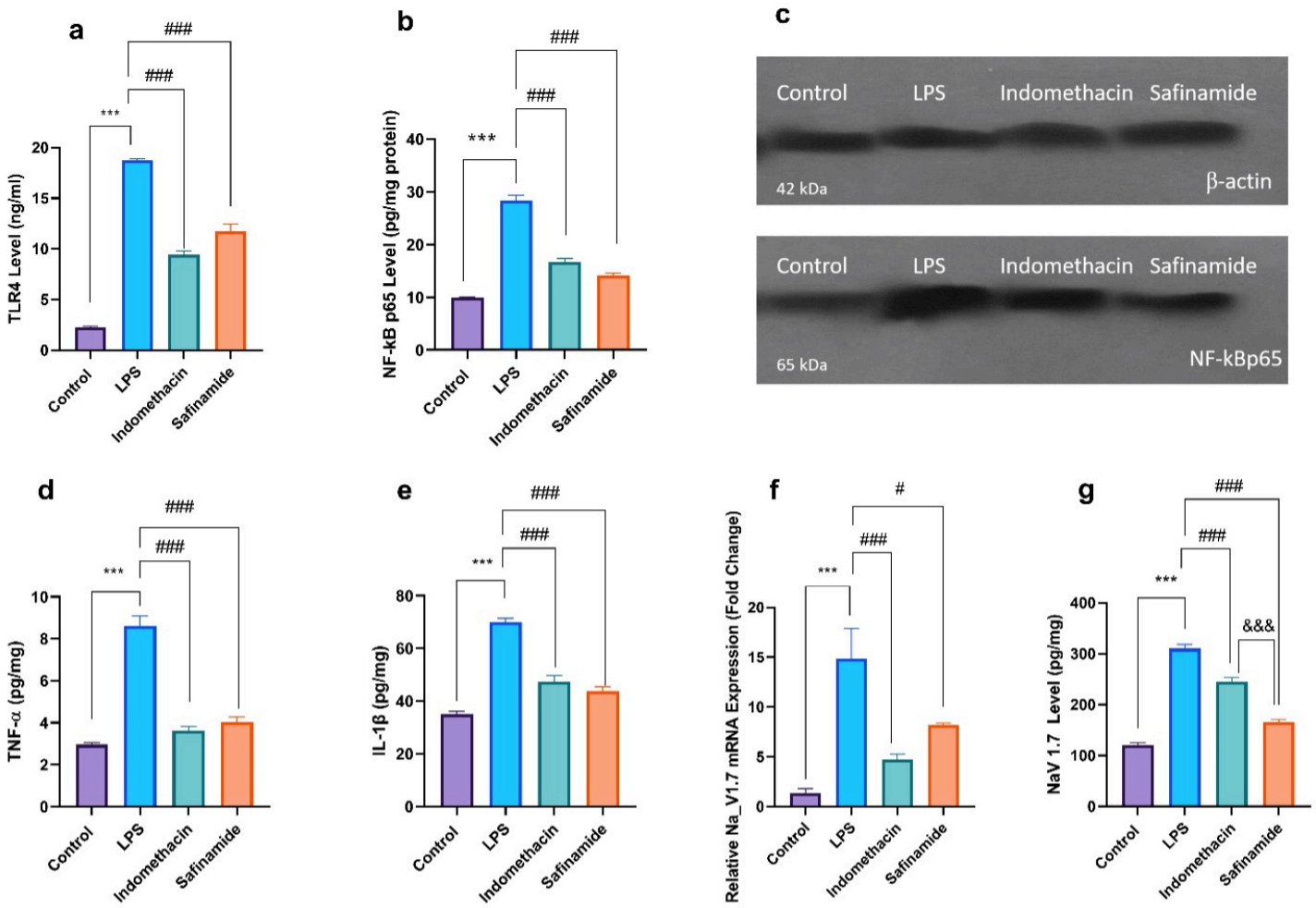


Figure 2

Effects of safinamide on the TLR4/NF-κB signaling pathway, pro-inflammatory cytokines, and NaV1.7 expression in DRGs (a) TLR4 protein expression measured by ELISA. (b) Representative Western blot showing NF-κB p65 and β-actin expression. Full-length uncropped Western blot images corresponding to NF-κB p65 and β-actin are provided in Supplementary Figures S1 and S2. The areas used for quantification and presentation in Figure 2c are indicated by boxes. (c) Densitometric analysis of NF-κB p65 normalized to β-actin. (d) TNF-α levels measured by ELISA. (e) IL-1β levels measured by ELISA. (f) Relative NaV1.7 mRNA expression measured by qRT-PCR. (g) NaV1.7 protein expression measured by ELISA. Data are presented as mean ± SEM (n = 8 per group). ***P < 0.001 vs. Control; #P < 0.05, ##P < 0.01, and ###P < 0.001 vs. LPS; &&&P < 0.001 vs. Indomethacin

Supplementary Files

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- SupplementaryFile.pdf