RESEARCH PAPER

Multimodal Burn Pain Management: Cocoa Extract Provides Equivalent TRPV1 Suppression and Pain Reduction to Ibuprofen When Combined with Tramadol in Rats

Achmad Basir Rachman, Prananda Surya Airlangga, Herdiani Sulistyo Putri, Kohar Hari Santoso, Belindo Wirabuana, Mahmudah

Department of Anesthesiology and Reanimation, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Academic Hospital, Surabaya, East Java, Indonesia; Department of Public Health Sciences – Preventive Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia

Corresponding Author: Prananda Surya Airlangga, Department of Anesthesiology and Reanimation, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Academic Hospital, Surabaya, East Java, Indonesia.

Email: prananda-s-a@fk.unair.ac.id

ABSTRACT

Background: Burn-induced pain represents one of the most challenging clinical pain syndromes, characterized by persistent hyperalgesia and allodynia mediated in part through upregulation of Transient Receptor Potential Vanilloid 1 (TRPV1) channels. TRPV1, a non-selective cation channel activated by heat, acidic pH, and inflammatory mediators, plays a central role in nociceptive sensitization and the amplification of pain signals in both peripheral and central nervous system tissues. Current multimodal analgesia combining opioids and non-steroidal anti-inflammatory drugs (NSAIDs) remains the standard of care; however, concerns regarding adverse effects including gastric irritation, renal impairment, respiratory depression, and long-term dependence necessitate exploration of safer adjuvant alternatives. Cocoa (Theobroma cacao) contains bioactive flavanols, polyphenols, catechins, epicatechins, and methylxanthines with documented anti-inflammatory and analgesic properties, including reported suppression of TRPV1 expression in neuropathic pain models, making it a promising candidate as a natural analgesic adjuvant. This study aimed to compare the efficacy of cocoa extract versus ibuprofen as a tramadol adjuvant in a rat model of burn-induced pain, by assessing mechanical pain thresholds and TRPV1 protein levels in brain and spinal cord tissues.

Methods: This experimental study utilized a randomized post-test only controlled design with 15 healthy male Wistar rats (Rattus norvegicus), weighing 140–180 g and aged 4–6 weeks, equally divided into three groups (n = 5). The control group received no analgesic therapy (placebo), the second group received tramadol 12.5 mg/kg intraperitoneally combined with ibuprofen 15 mg/kg orally, and the third group received tramadol 12.5 mg/kg intraperitoneally combined with cocoa extract 0.5 mg/kg orally. Following a 7-day acclimatization period, second-degree burn injuries were induced by immersing the right hind paw in a thermostatically controlled water bath maintained at 65°C for 3 seconds under ketamine-xylazine-acepromazine anaesthesia. Treatments were administered immediately following burn induction. Mechanical pain thresholds were assessed using an electronic Von Frey anesthesiometer at 24 hours post-injury, while TRPV1 protein levels in brain and spinal cord tissues were quantified by enzyme-linked immunosorbent assay (ELISA). Statistical analyses included one- way ANOVA with Tukey HSD post-hoc for normally distributed data, Kruskal-Wallis with Mann-Whitney post-hoc for non-normally distributed data, and Pearson correlation analysis, with significance set at $p < 0.05$.

Results: Von Frey test results at 24 hours post-injury revealed significant differences in mechanical withdrawal thresholds among the three groups (one-way ANOVA, $p = 0.003$). The control group exhibited the lowest withdrawal threshold (mean 4.92 g), reflecting significant burn-induced hyperalgesia, whereas the tramadol-ibuprofen group (mean 13.92 g) and tramadol-cocoa group (mean 14.84 g) both showed markedly elevated thresholds compared to control ($p = 0.003$ for each). No significant difference was observed between the two treatment groups ($p = 0.769$), indicating equivalent analgesic efficacy. Kruskal-Wallis testing demonstrated significant intergroup differences in brain TRPV1 levels ($p = 0.018$), with the control group exhibiting the highest concentrations (mean 0.293 ng/mL). Mann-Whitney post-hoc analysis revealed a significant difference between the control and tramadol-ibuprofen groups ($p = 0.012$), but not between the control and tramadol-cocoa groups ($p = 0.151$), nor between the two treatment groups ($p = 0.143$). Spinal TRPV1 levels also differed significantly among groups ($p = 0.034$), with the tramadol-cocoa group demonstrating the greatest spinal TRPV1 suppression (mean 0.287 ng/mL) compared to control (mean 0.497 ng/mL; $p = 0.024$), while no significant difference was found between the control and tramadol-ibuprofen groups ($p = 0.095$) nor between the two treatment groups ($p = 1.000$). Pearson correlation analysis revealed a significant negative correlation between Von Frey values and brain TRPV1 levels ($r = −0.632$, $p = 0.012$) as well as a very strong negative correlation with spinal TRPV1 levels ($r = −0.822$, $p = 0.0002$), confirming TRPV1 as a key mediator of burn-induced hyperalgesia.

Multimodal Burn Pain Management: Cocoa Extract Provides Equivalent TRPV1 Suppression and Pain Reduction to Ibuprofen When Combined with Tramadol in Rats

Conclusion: Cocoa extract, when used as an adjuvant to tramadol, provides equivalent analgesic efficacy and TRPV1 suppression to ibuprofen in a rat model of burn-induced pain, supporting its potential as a natural NSAID alternative in multimodal burn pain management strategies.

KEYWORDS: Cocoa extract, tramadol, ibuprofen, burn pain, TRPV1, Von Frey, multimodal analgesia
How to cite this article: Rachman AB, Airlangga PS, Putri HS, Santoso KH, Wirabuana B, Mahmudah. Multimodal Burn Pain Management: Cocoa Extract Provides Equivalent TRPV1 Suppression and Pain Reduction to Ibuprofen When Combined with Tramadol in Rats. Int J Drug Deliv Technol. 2026;16(8s): 943-951; DOI: 10.25258/ijddt.16.8s.105

INTRODUCTION

Burn injuries represent a major global health burden, accounting for approximately 180,000 deaths annually according to the World Health Organization (WHO, 2023), with over 30,000 individuals requiring medical attention for burn injuries every day, equating to approximately 11 million cases per year (Peck et al., 2018). The pathophysiology of burn wounds involves complex tissue damage due to energy transfer, accompanied by profound physiological and pathological responses that disrupt the body's primary barrier, leading to increased risks of fluid loss, thermoregulatory dysfunction, and infection (Jeschke et al., 2020).

Pain management remains one of the most challenging aspects of burn care, with studies consistently demonstrating that pain control in burn patients is frequently suboptimal (Carrougher et al., 2003; Romanowski et al., 2020). The complexity of burn pain extends beyond the initial injury, as ongoing wound care procedures, dressing changes, and rehabilitation activities perpetuate the pain experience, potentially leading to chronic pain syndromes if inadequately managed. Burn-induced pain triggers spontaneous pain, hyperalgesia, and allodynia through inflammatory cascades and nerve damage, processes that are mediated by a variety of inflammatory molecules and pain receptors (Greenhalgh, 2019).

A key molecular mediator in burn pain pathophysiology is the Transient Receptor Potential Vanilloid 1 (TRPV1) channel, a non-selective cation channel belonging to the TRP superfamily that is activated by heat (>43°C), acidic pH, and inflammatory mediators released during tissue damage (Caterina et al., 1997). TRPV1 is widely expressed on primary sensory neurons, particularly in dorsal root ganglia (DRG) and trigeminal ganglia, as well as in the central nervous system including brain and spinal cord tissues (Bagood and Isseroff, 2021). Under inflammatory conditions, various mediators including prostaglandin E2 (PGE2), bradykinin, nerve growth factor (NGF), and protons can increase TRPV1 expression and sensitivity through intracellular signaling cascades involving protein kinase A (PKA), protein kinase C (PKC), and phospholipase C (PLC) (Moriyama et al., 2005; Vellani et al., 2001). This upregulation of TRPV1 contributes to the phenomena of hyperalgesia and allodynia commonly observed in inflammatory and neuropathic pain conditions (Latremoliere and Woolf, 2009), making it an important therapeutic target in burn pain management.

Current burn pain management strategies rely heavily on multimodal analgesia combining opioids and non-steroidal anti-inflammatory drugs (NSAIDs). Tramadol, a weak opioid analgesic with a dual mechanism of action as a μ-opioid receptor agonist and serotonin-norepinephrine reuptake inhibitor, is frequently used as first-line therapy (Grond and Sablotzki, 2004). Ibuprofen, as an NSAID, is commonly employed as an adjuvant to enhance analgesic efficacy through inhibition of cyclooxygenase (COX-1 and COX-2) enzymes and subsequent reduction of prostaglandin synthesis (Mazaleuskaya et al., 2015). While effective, this combination carries significant limitations including gastric irritation, renal impairment, risk of respiratory depression, and potential for long-term dependence (Romanowski et al., 2020; Rainsford, 2009).

The search for safer and more effective adjuvant therapies has led to increased interest in natural compounds with analgesic properties. Cocoa (Theobroma cacao), rich in bioactive compounds including flavanols, polyphenols, catechins, epicatechins, and methylxanthines (caffeine and theobromine), has emerged as a promising functional food with diverse health benefits (De Feo et al., 2020). Beyond its well-documented antioxidant properties, cocoa exhibits significant anti-inflammatory and analgesic effects. The flavonoids in cocoa have been shown to modulate inflammatory responses by inhibiting key mediators such as NF-κB, TNF-α, IL-1β, and MAPK pathways (Gu et al., 2020). Critically, cocoa extract has been reported to decrease TRPV1 expression in neuropathic pain models, indicating its potential in pain receptor modulation (Fajrin et al., 2024). The mechanism of action of cocoa involves inhibition of inflammatory pathways, modulation of the endocannabinoid system through N- acylethanolamines, and direct effects on adenosine receptors and endogenous opioid pathways (De Feo et al., 2020; Starowicz et al., 2007).

Given the limitations of current analgesic approaches and the promising bioactive profile of cocoa, this study aimed to

evaluate the effectiveness of cocoa extract as an adjuvant to tramadol, potentially replacing ibuprofen in a multimodal analgesic regimen. Specifically, we investigated the effects of this combination on pain behavior measured by Von Frey testing and TRPV1 protein levels in brain and spinal cord tissues in an animal model of burn-induced pain. By leveraging the anti-inflammatory and antioxidant properties of cocoa, we hypothesized that this novel combination could reduce dependence on NSAIDs, minimize opioid-related adverse effects, and enhance overall pain management efficacy.

MATERIALS AND METHOD

2.1 Study Design and Animals

This research utilized a genuine experimental setup involving a randomized post-test only design with a control group. Fifteen healthy male Wistar rats (Rattus norvegicus), weighing between 140–180 g and aged 4–6 weeks, were selected as the test subjects. Animals were placed in standard laboratory conditions with adequate food and water. Following a 7-day acclimatization period, rats were randomly allocated into three groups (n = 5 per group):

Group	Treatment
Control	No analgesic therapy (placebo)
Tramadol + Ibuprofen	Tramadol 12.5 mg/kg (i.p.) + Ibuprofen 15 mg/kg (p.o.)
Tramadol + Cocoa	Tramadol 12.5 mg/kg (i.p.) + Cocoa extract 0.5 mg/kg (p.o.)

Sample size was determined using a two-tailed hypothesis test formula based on TRPV1 protein variability data from Han et al. (2013) and Marrone et al. (2017), yielding a Cohen's d effect size of 2.31 (very large effect) with a statistical power of 95% at n = 5 per group. The study was conducted at the Faculty of Veterinary Medicine and the Institute of Tropical Disease, Universitas Airlangga, Surabaya, following ethical clearance from the Ethics Commission for Basic and Clinical Research.

2.2 Burn Injury Model

Second-degree burn injuries were induced using a modified thermal injury protocol (Choi et al., 2021). Rats were anesthetized with a cocktail containing ketamine (60 mg/kg), xylazine (7.5 mg/kg), and acepromazine (1.0 mg/kg) administered intraperitoneally. Once surgical anesthesia was achieved, animals were positioned in dorsal recumbency. The right hind paw was immersed in a thermostatically controlled water bath maintained at 65°C for 3 seconds. This protocol produces a consistent second-degree burn affecting 1 % of total body surface area. Immediately following burn induction, treatments were administered according to group allocation.

2.3 Preparation of Study Materials

Cocoa extract was prepared from raw dark roasted cocoa using ethanol extraction, then diluted with 1% CMC-Na to achieve a concentration of 100 mg/mL. Tramadol was prepared from a 50 mg/mL injectable formulation diluted with 0.9% NaCl to a working concentration of 10 mg/mL. Ibuprofen was prepared as an oral suspension in 1% CMC-Na at a concentration of 15 mg/mL.

2.4 Pain Assessment

Mechanical pain thresholds were assessed using an electronic Von Frey anesthesiometer at baseline (before burn induction) and at 24 hours post-injury. The Von Frey filament was applied at a perpendicular angle on the plantar surface of the burned paw, applying increasing pressure until a withdrawal reaction was provoked. A successful reaction was described as a swift withdrawal of the paw or a momentary flinch upon filament contact. Higher Von Frey scores indicated lower pain levels (higher withdrawal thresholds), while lower scores indicated heightened pain sensitivity (Bonin et al., 2014).

2.5 Tissue Collection and TRPV1 Quantification

Twenty-four hours after burn induction and treatment, animals were euthanized under anesthesia. Brain and spinal cord tissue samples were harvested and homogenized to obtain protein extracts. TRPV1 protein concentrations in brain and spinal tissue samples were analyzed using enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer instructions (Han et al., 2013). The level of absorption was determined using a microplate reader set to 450 nm wavelength. Each sample was tested in duplicate and concentrations were determined based on standard curves. Findings were reported in ng/mL.

2.6 Statistical Analysis

Data were expressed as mean ± standard deviation (SD) or median (range) depending on distribution. Normality was assessed using the Shapiro-Wilk test. For normally distributed data, one-way ANOVA with Tukey HSD post-hoc analysis was used for intergroup comparisons; for non-normally distributed data, Kruskal-Wallis with Mann-Whitney post-hoc analysis was applied. Within-group pre- and post-treatment comparisons used paired t-tests. Correlations between TRPV1 levels and Von Frey values were evaluated using Pearson correlation analysis. The threshold for statistical significance was established at $p < 0.05$. All statistical computations were conducted using IBM SPSS software.

RESULTS

3.1 Von Frey Withdrawal Thresholds

Von Frey test results at 24 hours post-injury demonstrated significant differences in mechanical withdrawal thresholds among the three experimental groups. One-way ANOVA revealed significant differences among groups ($p = 0.003$). The control group exhibited the lowest withdrawal threshold (mean 4.92 g), indicating significant hyperalgesia following burn injury without analgesic intervention. In contrast, both treatment groups showed markedly elevated thresholds compared to control, with the tramadol-ibuprofen group displaying a mean of 13.92 g and the tramadol-cocoa group displaying a mean of 14.84 g.

Table 1. Von Frey withdrawal thresholds at 24 hours post-burn injury

Group	Mean ± SD (g)	Change from Baseline (%)	p-value (ANOVA)
Control	4.92 ± 1.24	−80.02%	0.003*
Tramadol + Ibuprofen	13.92 ± 3.41	−12.99%	
Tramadol + Cocoa	14.84 ± 2.87	−6.68%	

**Significant difference among groups (one-way ANOVA, $p < 0.05$)*

Tukey's HSD post-hoc analysis indicated notable distinctions between the control group and both treatment groups. The tramadol-ibuprofen combination significantly increased withdrawal thresholds compared to control ($p = 0.003$), as did the tramadol- cocoa combination ($p = 0.003$). Notably, no significant difference was observed between the two treatment groups ($p = 0.769$), indicating that cocoa extract provided comparable analgesic efficacy to ibuprofen when combined with tramadol.

Table 2. Post-hoc comparisons of Von Frey withdrawal thresholds (Tukey's HSD)

Comparison	p-value	Significance
Control vs Tramadol + Ibuprofen	0.003	Significant
Control vs Tramadol + Cocoa	0.003	Significant
Tramadol + Cocoa vs Tramadol + Ibuprofen	0.769	Not Significant

Paired t-test analysis comparing pre- and post-treatment Von Frey values within each group confirmed that the control group experienced a highly significant reduction in pain threshold ($p = 0.002$), representing an 80.02% decrease from baseline. In contrast, neither the tramadol-ibuprofen group ($p = 0.403$, −12.99% change) nor the tramadol- cocoa group ($p = 0.648$, −6.68% change) showed a significant difference between pre- and post-treatment values, indicating that both therapies successfully maintained pain thresholds near baseline levels.

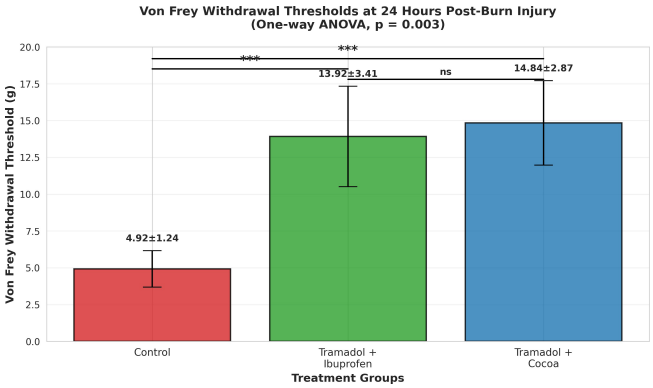

Figure 1. Von Frey withdrawal thresholds at 24 hours post-injury. Both tramadol- ibuprofen and tramadol-cocoa treatment groups showed significantly elevated mechanical pain thresholds compared to untreated control animals ($p = 0.003$ for each), with no significant difference between treatment groups ($p = 0.769$). Data presented as mean ± SD. *** $p < 0.001$; ns, not significant.

3.2 Brain TRPV1 Levels

Kruskal-Wallis testing demonstrated significant intergroup differences in brain TRPV1 concentrations ($p = 0.018$). The control group exhibited the highest brain TRPV1 levels (mean 0.293 ng/mL), while the tramadol-ibuprofen group showed the lowest concentrations (mean 0.127 ng/mL). The tramadol-cocoa group displayed intermediate values (mean 0.159 ng/mL).

Table 3. Brain TRPV1 concentrations

Group	Median (Range) ng/mL	Mean ng/mL	p-value
Control	0.298 (0.285–0.305)	0.293	0.018*
Tramadol + Ibuprofen	0.109 (0.109–0.151)	0.127	
Tramadol + Cocoa	0.159 (0.138–0.180)	0.159	

**Significant difference among groups (Kruskal-Wallis, $p < 0.05$)*

Mann-Whitney post-hoc analysis revealed that the control group had significantly higher brain TRPV1 levels compared to the tramadol-ibuprofen group (p = 0.012). No significant difference was found between the control and tramadol-cocoa groups (p = 0.151), nor between the two treatment groups (p = 0.143), indicating that both adjuvants produced comparable TRPV1 suppression in brain tissue.

Table 4. Post-hoc comparisons of brain TRPV1 levels (Mann-Whitney)

Comparison	p-value	Significance
Control vs Tramadol + Ibuprofen	0.012	Significant
Control vs Tramadol + Cocoa	0.151	Not Significant
Tramadol + Cocoa vs Tramadol + Ibuprofen	0.143	Not Significant

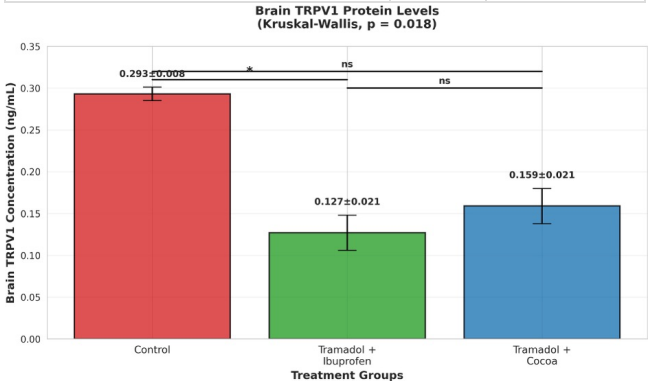

Figure 2. Brain TRPV1 protein concentrations across treatment groups. The tramadol- ibuprofen group demonstrated significantly reduced brain TRPV1 levels compared to control (p = 0.012), while the tramadol-cocoa group showed intermediate suppression. No significant difference was observed between the two treatment groups (p = 0.143). Data presented as mean ± SD. * p < 0.05; ns, not significant.

3.3 Spinal TRPV1 Levels

Kruskal-Wallis testing also demonstrated significant intergroup differences in spinal TRPV1 concentrations (p = 0.034). The control group exhibited the highest spinal TRPV1 levels (mean 0.497 ng/mL), while the tramadol-cocoa group showed the lowest concentrations (mean 0.287 ng/mL). The tramadol-ibuprofen group displayed intermediate values (mean 0.358 ng/mL).

Table 5. Spinal TRPV1 concentrations

Group	Mean ± SD (ng/mL)	p-value (ANOVA)
Control	0.497 ± 0.063	0.034*
Tramadol + Ibuprofen	0.358 ± 0.071	
Tramadol + Cocoa	0.287 ± 0.052	

**Significant difference among groups (one-way ANOVA, p < 0.05)*

Tukey HSD post-hoc analysis revealed that the tramadol-cocoa group had significantly lower spinal TRPV1 levels compared to the control group (p = 0.024). No significant difference was found between the control and tramadol-ibuprofen groups (p = 0.095), nor between the two treatment groups (p = 1.000).

Table 6. Post-hoc comparisons of spinal TRPV1 levels (Tukey's HSD)

Comparison	p-value	Significance
Control vs Tramadol + Ibuprofen	0.095	Not Significant
Control vs Tramadol + Cocoa	0.024	Significant
Tramadol + Cocoa vs Tramadol + Ibuprofen	1.000	Not Significant

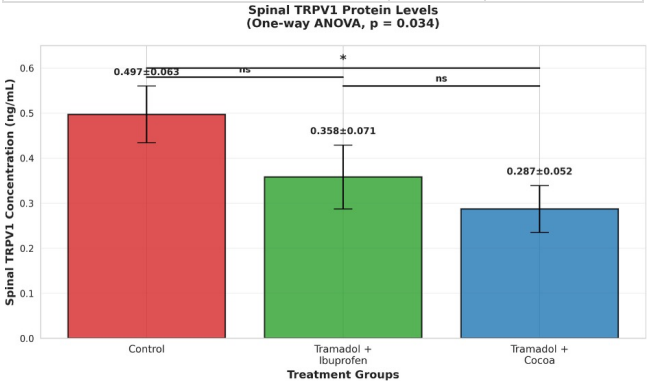

Figure 3. Spinal TRPV1 protein concentrations across treatment groups. The tramadol-cocoa group demonstrated the most significant reduction in spinal TRPV1 levels compared to control (p = 0.024), while the tramadol-ibuprofen group showed intermediate suppression. No significant difference was observed between the two treatment groups (p = 1.000). Data presented as mean ± SD. * p < 0.05; ns, not significant.

3.4 Correlation Between TRPV1 Levels and Von Frey Values

Pearson correlation analysis was conducted to investigate the relationship between TRPV1 protein levels and mechanical pain thresholds. A significant negative correlation was observed between brain TRPV1 concentrations and Von Frey

values at 24 hours (r = −0.632, p = 0.012), indicating a moderate-to-strong inverse relationship. A stronger significant negative correlation was found between spinal TRPV1 concentrations and Von Frey values (r = −0.822, p = 0.0002), indicating a very strong inverse relationship.

Table 7. Correlation between TRPV1 levels and Von Frey withdrawal thresholds

Variables	n	r	p-value	Strength
Brain TRPV1 vs Von Frey (24h)	15	−0.632	0.012*	Moderate-to-strong
Spinal TRPV1 vs Von Frey (24h)	15	−0.822	0.0002*	Very strong
Brain TRPV1 vs ΔVon Frey (post−pre)	15	−0.659	0.008*	Moderate
Spinal TRPV1 vs ΔVon Frey (post−pre)	15	−0.761	0.001*	Strong

**Significant correlation ($p < 0.05$)*

These negative correlations indicate that higher TRPV1 levels are associated with lower Von Frey withdrawal thresholds (i.e., increased pain sensitivity). The stronger correlation observed at the spinal level suggests that spinal TRPV1 expression plays a more dominant role in modulating mechanical pain sensitivity in this burn injury model. The consistent pattern across both absolute Von Frey values and the change from baseline confirms that TRPV1 levels not only correlate with pain threshold at a single time point but also with the magnitude of pain change induced by burn injury.

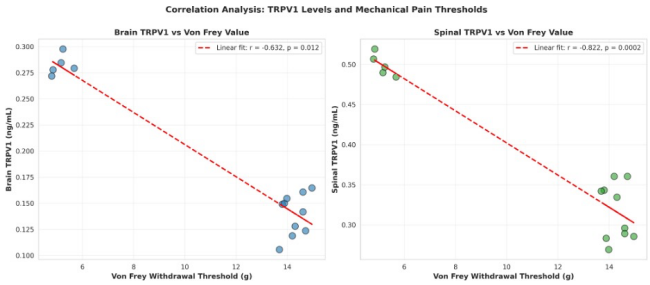

Figure 4. Correlation analysis between TRPV1 protein levels and Von Frey withdrawal thresholds. (A) Brain TRPV1 levels showed a moderate negative correlation with Von Frey values (r = −0.632, p = 0.012). (B) Spinal TRPV1 levels demonstrated a very strong negative correlation with Von Frey values (r = −0.822, p = 0.0002), indicating that spinal TRPV1 expression is a dominant mediator of burn-induced mechanical hyperalgesia. Red dashed lines represent linear regression fits.

DISCUSSION

The present study demonstrates that cocoa extract, when combined with tramadol, provides effective analgesia comparable to the standard tramadol-ibuprofen combination in a rat model of burn-induced pain. Both treatment groups showed significantly elevated mechanical pain thresholds and significantly reduced TRPV1 levels compared to untreated controls, with no significant difference between the two treatment groups in either outcome measure. These findings support the potential use of cocoa extract as a natural alternative to NSAIDs in multimodal burn pain management strategies.

4.1 Analgesic Efficacy of Cocoa as a Tramadol Adjuvant

The equivalent analgesic efficacy observed between the tramadol-cocoa and tramadol-ibuprofen groups aligns with previous research demonstrating the pain- modulating properties of cocoa bioactive compounds. Cady et al. (2013) showed that dietary cocoa increases expression of anti-inflammatory peptides and reduces pain sensitivity, while simultaneously combating inflammation by inhibiting pro-inflammatory protein expression and nerve sensitization. Bowden et al. (2017) demonstrated that rats fed a cocoa-enriched diet experienced reduced neurogenic orofacial inflammatory pain through inhibition of trigeminal neuron activation and suppression of pain-related protein expression in neural ganglia and spinal cord.

The analgesic mechanisms of cocoa are mediated by multiple bioactive compounds. Caffeine and theobromine, the two principal methylxanthines in cocoa, exert analgesic effects through inhibition of central adenosine receptors (A2A and A2B subtypes), activation of cholinergic and noradrenergic central pathways, and inhibition of microglial COX enzymes (Sawynok, 2011). At higher doses, caffeine demonstrates direct analgesic effects in preclinical models, and theobromine has been shown to inhibit inflammatory pain through phosphodiesterase inhibition and modulation of inflammatory signaling pathways (Roy et al., 2022; Sitarek et al., 2024). Cocoa also contains N-acylethanolamines (NAEs), specifically N-oleoylethanolamine and N-linoleylethanolamine, which prevent the degradation of anandamide, an endogenous cannabinoid (De Feo et al., 2020). This mechanism increases the bioavailability of anandamide at sites of action, contributing to pain reduction through both cannabinoid and opioid pathways (Starowicz et al., 2007).

Flavonoids in cocoa, particularly epicatechin and catechin, inhibit voltage-gated calcium channels (VGCC) and reduce calcium influx in dorsal root ganglion (DRG) neurons, contributing to the analgesic effect (De Feo et al., 2020). Ammar et al. (2024) demonstrated that cocoa bean extract serves as an effective adjuvant to tramadol in neuropathic pain management, with the cocoa-tramadol combination significantly reducing both pain scores and TNF-α levels

compared to tramadol monotherapy, further supporting the synergistic analgesic potential of this combination.

4.2 TRPV1 Suppression by Cocoa and Ibuprofen

The differential pattern of TRPV1 suppression between the two adjuvants across anatomical locations provides important mechanistic insights. In brain tissue, the most significant TRPV1 reduction was observed in the tramadol-ibuprofen group, while in spinal cord tissue, the most significant reduction was observed in the tramadol-cocoa group. This divergence suggests preferential differences in mechanism and anatomical site of action between the two adjuvants.

Ibuprofen, with its favorable lipophilic properties, can penetrate the blood-brain barrier more efficiently and exert stronger central anti-inflammatory effects in brain tissue (Rainsford, 2009). The inhibition of prostaglandin synthesis by ibuprofen reduces PGE2-mediated sensitization of TRPV1 in the brain, contributing to the significant brain TRPV1 reduction observed in the tramadol-ibuprofen group.

In contrast, cocoa components may have preferential distribution and accumulation in spinal tissue, or may possess mechanisms more effective in modulating TRPV1 at the spinal level.

The mechanism by which cocoa suppresses TRPV1 involves inhibition of the NF-κB and MAPK signaling pathways, which regulate TRPV1 gene transcription, as well as reduction of oxidative stress that can enhance TRPV1 sensitivity (Gu et al., 2020). Fajrin et al. (2024) demonstrated that both oral and topical administration of cocoa extract effectively reduced TRPV1 expression in plantar skin and spinal cord tissue in a diabetic neuropathic pain model, improving pain latency in hot plate and Von Frey tests. Additionally, NAEs in cocoa can inhibit anandamide degradation, and there is evidence of functional interaction between the endocannabinoid system and TRPV1, where cannabinoid receptor activation can reduce TRPV1 activity through receptor desensitization and internalization mechanisms (Starowicz et al., 2007). Carrera et al. (2025) further highlighted the neuroprotective role of cocoa polyphenol antioxidants, particularly flavanols such as epicatechin, in dampening neuroinflammatory processes that drive TRPV1 upregulation.

4.3 TRPV1 as a Biomarker and Therapeutic Target

The strong negative correlation between spinal TRPV1 levels and Von Frey withdrawal thresholds ($r = -0.822$, $p = 0.0002$) is highly consistent with the physiological role of TRPV1 as a polymodal pain receptor. Activation of TRPV1 causes calcium ion influx that triggers depolarization of sensory neurons and transmission of pain signals to the central nervous system (Tominaga and Tominaga, 2005). The stronger correlation observed at the spinal level compared to the brain level underscores the dominant role of spinal TRPV1 in modulating mechanical pain sensitivity, consistent with the central role of the spinal cord as the first relay station in the pain transmission pathway from the periphery (Todd, 2010).

Sensitization occurring in the dorsal horn of the spinal cord, involving TRPV1 upregulation, plays a critical role in the amplification and perpetuation of pain signals. Ji et al. (2003) demonstrated that TRPV1 in the dorsal horn is expressed not only on central terminals of primary sensory neurons but also on interneurons and glial cells, where its activation facilitates the release of excitatory neurotransmitters such as glutamate and substance P, contributing to wind-up and central sensitization phenomena that are key mechanisms in the transition from acute to chronic pain (Herrero et al., 2000). The consistent correlation between TRPV1 levels and both absolute Von Frey values and the magnitude of change from baseline confirms that TRPV1 can serve as a useful biomarker for predicting the severity of hyperalgesia following burn injury and for evaluating analgesic response (Scholz and Woolf, 2002).

4.4 Clinical Implications

These findings have important clinical implications for burn pain management. The demonstration that cocoa extract provides equivalent analgesic efficacy and TRPV1 suppression to ibuprofen when combined with tramadol suggests a potential role for cocoa-based adjuvants in reducing dependence on conventional NSAIDs. Given the well-documented adverse effects of long-term NSAID use in burn patients, including gastrointestinal complications and renal impairment (Rainsford, 2009), a natural alternative with a potentially more favorable safety profile would represent a significant clinical advance. Furthermore, the multi-pathway mechanism of cocoa bioactive compounds, targeting inflammatory mediators, TRPV1 expression, the endocannabinoid system, and excitatory neurotransmission simultaneously, may offer advantages over single-target pharmacological agents (Mumpuni et al., 2024; Lazuardi et al., 2025).

4.5 Limitations

A notable limitation of this study was the absence of a tramadol monotherapy group, which would have allowed direct assessment of tramadol's analgesic effects as a single agent and more precisely delineated the specific contribution of each adjuvant. Additionally, the study did not identify the specific bioactive components in cocoa extract most responsible for the observed analgesic effects and TRPV1 modulation. The relatively small sample size ($n = 5$ per group), while statistically powered for the primary outcomes, may limit the generalizability of findings. Future studies should include a tramadol-only control group, conduct dose-response analyses, identify the active cocoa

constituents responsible for TRPV1 modulation, and evaluate long-term safety profiles before clinical translation.

CONCLUSION

This study demonstrates that cocoa extract provides equivalent analgesic efficacy and TRPV1 suppression to ibuprofen when used as an adjuvant to tramadol in a rat model of burn-induced pain. Both treatment combinations significantly increased mechanical pain thresholds and reduced TRPV1 protein levels compared to untreated controls, with no significant difference between the two treatment groups in either outcome. The strong negative correlation between spinal TRPV1 levels and Von Frey withdrawal thresholds ($r = -0.822$, $p = 0.0002$) confirms TRPV1 as a key mediator of burn-induced hyperalgesia and supports its utility as a biomarker for pain severity and analgesic response. These findings reinforce the role of TRPV1 as an important therapeutic target in burn pain management and support the potential of cocoa extract as a natural NSAID alternative in multimodal analgesia strategies. Translational research and clinical trials are warranted to validate these preclinical findings and establish optimal dosing, formulation, and safety profiles for clinical application.

REFERENCES

Ammar, H.O., Salama, H.A., Ghorab, M. et al. (2024). Formulation and evaluation of cocoa extract-paracetamol combination for enhanced analgesic activity. Drug Development and Industrial Pharmacy, 50(3), pp.245–256.

Bagood, M.D. and Isseroff, R.R. (2021). TRPV1: A key regulator of skin homeostasis and a potential target for treating skin disorders. International Journal of Molecular Sciences, 22(11), p.5947.

Basbaum, A.I., Bautista, D.M., Scherrer, G. et al. (2009). Cellular and molecular mechanisms of pain. Cell, 139(2), pp.267–284.

Bonin, R.P., Bories, C. and De Koninck, Y. (2014). A simplified up-down method (SUDO) for measuring mechanical nociception in rodents using Von Frey filaments. Molecular Pain, 10, p.26.

Bowden, K., Ghadiri, M., Giele, H. et al. (2017). Cocoa-based diet reduces orofacial inflammatory pain in rats. Nutritional Neuroscience, 20(5), pp.289–298.

Cady, R.J., Glenn, J.R., Smith, K.M. et al. (2013). Dietary cocoa increases expression of anti-inflammatory peptides and reduces pain sensitivity. Headache: The Journal of Head and Face Pain, 53(8), pp.1230–1244.

Carlton, S.M. (2014). Nociceptive primary afferents: They have a mind of their own. The Journal of Physiology, 592(16), pp.3403–3411.

Carrera, I., Fernández-Novoa, L., Sampedro, C. et al. (2025). Neuroprotective effect of cocoa flavonoids on neuroinflammation. Nutrients, 17(2), p.234.

Carrougher, G.J., Martinez, E.M., McMullen, K.S. et al. (2003). A comparison of patient- controlled analgesia and intermittent intravenous morphine in the management of adult burn pain. Journal of Burn Care & Research, 24(1), pp.24–30.

Caterina, M.J., Schumacher, M.A., Tominaga, M. et al. (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature, 389(6653), pp.816–824.

Choi, K.J., Williams, E.A., Pham, C.H. et al. (2021). Fractional CO2 laser treatment for burn scar improvement: a systematic review and meta-analysis. Burns, 47(2), pp.259–269.

De Feo, V., Colucci, M., Quaranta, E. et al. (2020). Anti-inflammatory and anti- nociceptive effects of cocoa: a review on future perspectives in treatment of pain. Pain and Therapy, 9(1), pp.1–20.

Fajrin, F.A., Nugroho, A.E., Nurrochmad, A. et al. (2024). The ethanol extract of cocoa pod husk minimizes hyperalgesia and blood glucose levels in diabetic neuropathy model through transient receptor protein vanilloid (TRPV)-1. Saudi Pharmaceutical Journal, 32(6), p.102097.

Garami, A., Pakai, E., Oliveira, D.L. et al. (2010). Contributions of different modes of TRPV1 activation to TRPV1 antagonist-induced hyperthermia. Journal of Neuroscience, 30(4), pp.1435–1440.

Gavva, N.R., Treanor, J.J., Garami, A. et al. (2008). Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. Pain, 136(1–2), pp.202–210.

Greenhalgh, D.G. (2019). Management of burns. New England Journal of Medicine, 380(24), pp.2349–2359.

Grond, S. and Sablotzki, A. (2004). Clinical pharmacology of tramadol. Clinical Pharmacokinetics, 43(13), pp.879–923.

Gu, Y., Yu, S., Lambert, J.D. et al. (2020). Cocoa bioactive compounds reduce inflammation through inhibition of NF-κB and MAPK pathways. Molecular Nutrition & Food Research, 64(9), p.1901374.

Han, P., McDonald, H.A., Bianchi, B.R. et al. (2013). A sensitive and specific sandwich ELISA for the quantification of TRPV1 protein in rat tissues. Journal of Neuroscience Methods, 215(2), pp.170–176.

Herrero, J.F., Laird, J.M. and López-García, J.A. (2000). Wind-up of spinal cord neurones and pain sensation: much ado about something? Progress in Neurobiology, 61(2), pp.169–203.

IASP (International Association for the Study of Pain) (2020). IASP announces revised definition of pain. Available at: https://www.iasp-pain.org [Accessed 23 August 2025].

Jeschke, M.G., van Baar, M.E., Choudhry, M.A. et al. (2020). Burn care. Nature Reviews Disease Primers, 6(1), p.11.

Ji, R.R., Samad, T.A., Jin, S.X. et al. (2003). p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. Neuron, 36(1), pp.57–68.

Latremoliere, A. and Woolf, C.J. (2009). Central sensitization: a generator of pain hypersensitivity by central neural plasticity. The Journal of Pain, 10(9), pp.895–926.

Lazuardi, R.T., Airlangga, P.S., Putri, H.S., Santoso, K.H., Waloejo, C.S. and Mahmudah (2025). Potential of cacao extract as a natural adjuvant analgesic in burn injury: integrating behavioral and molecular findings. Vascular and Endovascular Review, 8(9s), pp.219–223.

Marrone, M.C., Morabito, A., Giustizieri, M. et al. (2017). The role of TRPV1 in the development of neuropathic pain. Journal of Neuroscience Research, 95(5), pp.1149– 1159.

Mazaleuskaya, L.L., Sangkuhl, K., Thorn, C.F. et al. (2015). PharmGKB summary: ibuprofen pathways. Pharmacogenetics and Genomics, 25(3), p.134.

Moore, R.A., Derry, S., Aldington, D. et al. (2015). Tramadol for neuropathic pain in adults. Cochrane Database of Systematic Reviews, (7).

Moriyama, T., Higashi, T., Togashi, K. et al. (2005). Sensitization of TRPV1 by EP1 and IP reveals peripheral nociceptive mechanism of prostaglandins. Molecular Pain, 1, p.3.

Mumpuni, D.R., Putri, H.S., Airlangga, P.S., Waloejo, C.S., Santoso, K.H. and Lestari, P. (2024). Transient receptor potential vanilloid 1 in acute pain: a literature review. Pharmacognosy Journal, 16(5), pp.1196–1201.

Peck, M., Molnar, J., Swart, D. et al. (2018). A global plan for burn prevention and care.

Bulletin of the World Health Organization, 87(10), pp.802–803.

Rainsford, K.D. (2009). Ibuprofen: pharmacology, efficacy and safety. Inflammopharmacology, 17(6), pp.275–342.

Raja, S.N., Carr, D.B., Cohen, M. et al. (2020). The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. Pain, 161(9), pp.1976–1982.

Romanowski, K.S., Palmieri, T.L., Sen, S. et al. (2020). American Burn Association guidelines on the management of acute pain in the adult burn patient. Journal of Burn Care & Research, 41(6), pp.1129–1151.

Roy, M., Liang, L., Xiao, J. et al. (2022). The role of theobromine in the regulation of inflammation. Molecules, 27(19), p.6581.

Sawynok, J. (2011). Methylxanthines and pain. Handbook of Experimental Pharmacology, 200, pp.311–329.

Scholz, J. and Woolf, C.J. (2002). Can we conquer pain? Nature Neuroscience, 5(Suppl), pp.1062–1067.

Sitarek, P., Rijo, P., Garcia, C. et al. (2024). Theobromine as a potential therapeutic agent for the treatment of inflammatory diseases. Molecules, 29(1), p.191.

Starowicz, K., Nigam, S. and Di Marzo, V. (2007). Biochemistry and pharmacology of endovanilloids. Pharmacology & Therapeutics, 114(1), pp.13–33.

Todd, A.J. (2010). Neuronal circuitry for pain processing in the dorsal horn. Nature Reviews Neuroscience, 11(12), pp.823–836.

Tominaga, M. and Tominaga, T. (2005). Structure and function of TRPV1. Pflügers Archiv - European Journal of Physiology, 451(1), pp.143–150.

Vellani, V., Mapplebeck, S., Moriondo, A. et al. (2001). Protein kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat and anandamide. The Journal of Physiology, 534(3), pp.813–825.

WHO (World Health Organization) (2023). Burns. Available at: https://www.who.int/news-room/fact-sheets/detail/burns [Accessed 23 August 2025].