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Potential of Cacao Extract as a Natural Adjuvant Analgesic in Burn Injury: Integrating Behavioral and Molecular Findings

Rizqy Tafinna Lazuardi[1], Prananda Surya Airlangga[1], Herdiani Sulistyo Putri[1*], Kohar Hari Santoso[1], Christrijogo Sumartono Waloejo[1], and Mahmudah[2]

[1]Department of Anesthesiology and Reanimation, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
herdiani-s-p@fk.unair.ac.id
[2]Department of Public Health and Preventive Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

Background: Burn injuries cause extensive tissue damage and trigger systemic inflammatory responses characterized by elevated inducible nitric oxide synthase (iNOS) and interleukin-1β (IL-1β) levels. These mediators sensitize nociceptors and activate multiple pain transduction pathways, leading to hyperalgesia. Conventional multimodal analgesia combining opioids and NSAIDs is effective but limited by gastrointestinal, cardiovascular, and dependence-related adverse effects. Cocoa (Theobroma cacao), rich in flavanols and polyphenols, exhibits anti-inflammatory and analgesic properties, offering potential as a natural adjuvant analgesic.

Methods: This experimental study used a randomized post-test only control group design with fifteen male Wistar rats (6–8 weeks, 160–180 g) divided into three groups (n=5): Group I (control) received placebo, Group II received tramadol 12.5 mg/kgBW + ibuprofen 15 mg/kgBW, and Group III received tramadol 12.5 mg/kgBW + cocoa extract 0.5 g/kgBW. A second-degree burn injury was induced. After 24 hours, mechanical pain threshold was assessed using the Von Frey test, and serum levels of iNOS and IL-1β were measured using ELISA.

Results: There is significant differences in Von Frey scores and serum inflammatory markers among groups, revealed that both tramadol–ibuprofen and tramadol–cocoa groups showed significantly higher pain thresholds and lower serum iNOS and IL-1β levels compared to control with no significant difference between cocoa and ibuprofen groups.

Conclusion: Cocoa extract, when used as an adjuvant, exhibits analgesic and anti-inflammatory properties that are comparable to those of ibuprofen in experimental models of burn-induced pain.

Major Findings: Cocoa extract as adjuvant provides analgesic and anti-inflammatory effects comparable to ibuprofen in burn-induced pain models. These findings suggest cocoa may serve as a promising natural alternative in multimodal analgesia for burn-related pain management.

KEYWORDS: analgesia, burn injury, cocoa , IL-1β, iNOS

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INTRODUCTION

Cacao (Theobroma cacao) is a natural source rich in flavonoids, methylxanthines, and polyphenolic compounds known for their antioxidant, anti-inflammatory, and neuroprotective effects.1 Recent research has highlighted cacao's potential as an adjuvant therapy in pain management, particularly due to its ability to modulate both peripheral and central pain pathways.2–5 The bioactive constituents of cacao are suggested to influence neurotransmitter systems, including the opioidergic pathway, thereby enhancing analgesic efficacy while potentially reducing drug-related side effects. Evidence supports that cacao polyphenols can indirectly interact with μ-opioid receptors, regulate nitric oxide signaling, and suppress pro-inflammatory cytokine release, making cacao a promising opioid adjuvant capable of augmenting analgesia and minimizing inflammation.1

Pain resulting from burn injury is multifactorial, comprising both nociceptive and neuropathic components. The neuropathic aspect is primarily due to peripheral nerve damage, neuronal hyperexcitability, and sustained inflammatory responses that drive central sensitization. Over time, these processes manifest clinically as persistent hyperalgesia and allodynia, often extending beyond the wound site and long after tissue healing.6

Pro-inflammatory mediators implicated in neuropathic pain, including the neuropathic components of burn injury, are inducible nitric oxide synthase (iNOS) and interleukin-1β (IL-1β). iNOS catalyzes the excessive production of nitric oxide during inflammation, leading to oxidative tissue damage and neuronal sensitization, whereas IL-1β contributes to peripheral and central inflammatory signaling, including glial activation and modulation of synaptic transmission. Elevated expression of iNOS and IL-1β has been consistently associated with increased pain hypersensitivity in various acute pain models, suggesting their central role in sustaining inflammatory nociception.7,8 Therefore, interventions capable of reducing these markers may represent promising strategies for attenuating neuroinflammation and chronic pain.

Therefore, this study aims to evaluate the analgesic and anti-inflammatory effects of cacao extract as an adjuvant to tramadol in a rat model of second-degree burn injury, using interleukin-1β (IL-1β) and inducible nitric oxide synthase (iNOS) as inflammatory

biomarkers and the Von Frey test as a behavioral measure of pain threshold. The findings are expected to contribute to the development of natural adjuvant therapies for more effective and safer burn pain management.

MATERIALS AND METHODS

2.1 Animal Subjects

Fifteen healthy male Wistar rats (Rattus norvegicus) were obtained from the Experimental Animal Laboratory, UNAIR Stem Cell Research and Development Center. Animals were acclimatized for 7 days in cages under controlled conditions with adequate daily feeding. Prior to testing, the animals were confirmed to be healthy, showing active movements, clear eyes, and clean fur. Ethical Committee approval was obtained from Faculty of Veterinary Airlangga University Surabaya number 2.KEH.28.02.2025.

2.2 Study Designs

This study was true experimental research with randomized post-test control group design. A total of 15 rats were randomly assigned to three groups (n=5):

- K0: suspension of 1% CMC-Na orally and saline intraperitoneally
- K1: suspension of cacao extract (0.5 mg/kg BW orally) and tramadol (12.5 mg/kg BW, orally)
- K2: ibuprofen (15 mg/kg BW orally) and tramadol (12.5 mg/kg BW, intraperitoneally).

The treatment in each group was administered every 8 hours.

2.3 Treatment preparations

Cacao beans were obtained from PUSLITKOKA (Center of Coffee and Cacao Research Indonesia) Jember. The roasted and ground beans were extracted using 70% ethanol, and the extract was resuspended in 1% CMC-Na to concentration 100 mg/mL. Ibuprofen 200 mg tablets crushed, suspended in 1% CMC-Na to 100 mg/mL concentration. Oral suspension was administered at dose 10 mg/kg BW to each rat (2-2.25 mg). Tramadol 50 mg/mL injection was diluted into 0.9% NaCl to concentration of 10 mg/mL and administered intraperitoneally at dose of 12.5 mg/kg BW.10

2.4 Burn-injury Pain Animal Model

Second-degree injury was induced by immersing the right hind paw of rats in 65o±0.5o C water for 3 seconds.11 This procedure produced stratum corneum damage, weakened the epidermis, and disruption of collagen in less than 1% of total body surface area. Before induction, the animals were anesthetized with mixture of ketamine (60 mg/kg BW), xylazine (7.5 mg/kg BW), and acepromazine (1 mg/kg BW), administered intraperitoneally.

2.5 Pain Assessment

Mechanical pain thresholds were measured using an electronic von Frey apparatus (Ugo Basile Co., Varese, Italy), producing measurements within 0-5 gram-force (gf) range. Rats were acclimatized for one hour in a wired-mesh cage before testing. A filament force of 5.9098 mn was applied to the plantar surface of the left hind paw for 2-5 seconds. The withdrawal thresholds were recorded and stored in the computer system. Measurements were taken at baseline and 24 hours after treatment.

2.6 IL-1β serum level measurement

Blood sample was drawn by cardiac puncture under anesthesia 24 hours after treatment. Serum IL-1β concentrations were measured by ELISA (Bioassay Technology Laboratory). Absorbance was read at 450 nm using a microplate reader. Results were expressed in ng/mL.

2.7 iNOS measurement

Blood sample was drawn from experimental rats 24 hours after burn injury pain induction using scalding water. The rats were anesthetized, then blood was drawn by cardiac puncture. Concentration of iNOS in the serum was analyzed using an ELISA device according to manufacturer's instructions. The results were analyzed using an ELISA (Bioassay Technology Laboratory) microplate reader system at a wavelength of 450 nm with a unit of measurement ng/mL.

RESULT

3.1 Body Weight

There was no statistically significant difference in the mean body weight among the control, tramadol–cacao, and tramadol–ibuprofen groups ($p = 0.889$) (Table 1). This finding indicates that the administered treatments did not induce significant alterations in weight, confirming that the interventions were well-tolerated and did not affect general health or metabolism during the study period.

3.2 Pain Threshold (Von Frey Test)

The mechanical pain threshold showed a significant difference among groups ($p = 0.001$). Post hoc analysis revealed that both the tramadol–cacao and tramadol–ibuprofen groups had significantly higher pain thresholds compared to the control group ($p = 0.001$ for both). However, no significant difference was observed between the tramadol–cacao and tramadol–ibuprofen groups ($p = 0.955$). The mean Von Frey score in the control group was 4.36 (3.5–5.1) g, while tramadol–cacao and tramadol–ibuprofen groups reached 14.66 (12.8–16.5) g and 14.44 (13.0–15.8) g, respectively.

3.3 Inflammatory Marker IL-1β

Serum IL-1β levels were significantly reduced in both treatment groups compared with the control group ($p = 0.005$ for tramadol–

cacao and p = 0.013 for tramadol–ibuprofen). There was no significant difference between the tramadol and cacao and tramadol and ibuprofen groups (p = 0.846). The mean IL-1β concentrations were 8.68 (7.2–9.9) ng/mL in the control group, 6.98 (6.3–7.5) ng/mL in the tramadol–cacao group, and 6.70 (6.0–7.5) ng/mL in the tramadol–ibuprofen group

Table 1. Descriptive Statistics among Experimental Groups

Variable	N	Mean ±SD	p-value*
Weight			
Control	5	171.8 (163-180.6)	
Tramadol and Cacao	5	169.4 (163.3-175.5)	0.889
Tramadol and Ibuprofen	5	170.3 (162.5-178)	
Von Frey			
Control	5	4.36 (3.5-5.1)	
Tramadol and Cacao	5	14.66 (12.8-16.5)	0.001
Tramadol and Ibuprofen	5	14.44 (13.0-15.8)	
IL-1β			
Control	5	8.68 (7.2-9.9)	
Tramadol and Cacao	5	6.98 (6.3-7.5)	0.003
Tramadol and Ibuprofen	5	6.70 (6.0-7.5)	
iNOS			
Control	5	21.53 (20.4-23.1)	
Tramadol and Cacao	5	19.90 (18.8-20.6)	0.01
Tramadol and Ibuprofen	5	19.74 (19.0-20.6)	

3.4 Inducible Nitric Oxide Synthase (iNOS)

Analysis of iNOS levels also revealed significant intergroup differences (p = 0.01). Post hoc comparison showed that both tramadol–cacao and tramadol–ibuprofen significantly reduced iNOS levels compared to control. No significant difference was observed between the two treatment groups (p = 0.963). The mean serum iNOS levels were 21.53 (20.4–23.1) ng/mL in the control group, 19.90 (18.8–20.6) ng/mL in the tramadol–cacao group, and 19.74 (19.0–20.6) ng/mL in the tramadol–ibuprofen group (Table 2).

Table 2. Post-hoc Comparison of Pain Threshold (Von Frey Test), IL-1β, and iNOS Expression among Experimental Groups

Variable		p-value
Von Frey		
Control	**Tramadol and Ibuprofen**	0.001
	Tramadol and Cacao	0.001
Tramadol and Cacao		0.955
IL-1β		0.001
Control	**Tramadol and Ibuprofen**	0.013
	Tramadol and Cacao	0.005
Tramadol and Cacao		0.846
iNOS		
Control	**Tramadol and Ibuprofen**	0.048
	Tramadol and Cacao	0.030
Tramadol and Cacao		0.963

DISCUSSION

This study demonstrates that both cacao extract and ibuprofen, when used as adjuvants to tramadol, effectively reduced acute pain and inflammation following burn injury, as evidenced by increased mechanical pain thresholds and decreased serum IL-1β and iNOS concentrations. The burn injury model induced by scalding water successfully produced hyperalgesia and allodynia, as pain thresholds were assessed on the contralateral paw to evaluate central sensitization. After 24 hours of treatment, the combination of cacao extract and tramadol significantly increased the pain threshold compared to the control group. Interestingly, the analgesic effect of cacao extract was comparable to that of the tramadol–ibuprofen combination, suggesting that cacao may serve as a potential natural adjuvant analgesic in burn injury pain management.

The absence of a significant difference in body weight among the control, tramadol–cacao, and tramadol–ibuprofen groups (p = 0.889) suggests that the administered treatments were well tolerated and did not interfere with metabolic or nutritional balance throughout the study period. Body weight is an indirect but important indicator of systemic toxicity and overall health status in animal studies. The stability in weight across groups confirms that the combination of tramadol with cacao extract or ibuprofen did not induce appetite suppression, catabolic stress, or metabolic alterations, thereby ensuring that observed analgesic and anti-inflammatory effects were pharmacological rather than secondary to systemic toxicity.1

The mechanical pain threshold results demonstrated a significant increase in nociceptive tolerance in both tramadol–cacao and tramadol–ibuprofen groups compared to control (p = 0.001). This indicates that both adjuvants potentiated tramadol's analgesic

activity.

The cacao extract showed a comparable analgesic effect to ibuprofen when combined with tramadol ($p = 0.955$), suggesting that the bioactive compounds in cacao particularly flavonoids such as epicatechin and procyanidins may modulate pain perception through central and peripheral mechanisms involving opioid and nitric oxide (NO) pathways.2

Mechanistically, cacao polyphenols have been shown to inhibit cyclooxygenase (COX) activity and reduce prostaglandin synthesis similar to nonsteroidal anti-inflammatory drugs (NSAIDs) 2,5,8. Furthermore, their interaction with μ-opioid receptors enhances the efficacy of opioid analgesics by modulating endogenous β-endorphin and enkephalin release. This synergy between tramadol and cacao aligns with findings that certain polyphenols can act as mild opioid receptor agonists or potentiators, thereby enhancing central analgesia without increasing opioid dosage requirements.2,4,5

Serum IL-1β levels significantly decreased in both treatment groups relative to control ($p = 0.005$ and $p = 0.013$), supporting the hypothesis that cacao extract exerts anti-inflammatory effects comparable to ibuprofen. IL-1β is a key pro-inflammatory cytokine released by activated macrophages and microglia during tissue injury and plays a critical role in the sensitization of peripheral nociceptors.12 Cacao polyphenols have demonstrated inhibitory effects on NF-κB signaling and subsequent IL-1β transcription, reducing neuroinflammation and peripheral sensitization.2,4,5,13 The reduction in IL-1β parallels the observed elevation in pain threshold, indicating that modulation of inflammatory cytokines may underlie the analgesic synergy observed with tramadol–cacao treatment.

A significant reduction in iNOS levels was also observed in both treatment groups compared to control ($p = 0.01$), with similar magnitude of suppression between tramadol–cacao and tramadol–ibuprofen groups ($p = 0.963$). Overexpression of iNOS contributes to excessive NO production, which amplifies inflammatory pain via peripheral vasodilation, oxidative stress, and central sensitization.8,14 The downregulation of iNOS following cacao administration likely reflects the antioxidant and radical-scavenging capacity of its flavanols, which suppress peroxynitrite formation and attenuate NF-κB-mediated transcription of pro-inflammatory enzymes.15 This aligns with previous findings demonstrating that dietary cocoa attenuates iNOS and TNF-α expression in experimental inflammation models.7,1

CONCLUSION

These findings suggest that cacao extract acts as an effective opioid adjuvant by enhancing tramadol's analgesic efficacy through multiple mechanisms, suppression of inflammatory mediators (IL-1β), inhibition of NO synthesis via downregulation of iNOS, and potential modulation of endogenous opioid signaling. The comparable outcomes between cacao and ibuprofen combinations highlight cacao's promise as a natural, low-toxicity alternative for pain management in burn injury models, where inflammation-driven hyperalgesia is predominant.

Future studies should focus on elucidating cacao's specific bioactive compounds responsible for this synergistic effect, as well as dose–response relationships and pharmacokinetic interactions with tramadol to support translational potential in clinical analgesia.

ETHIC APPROVAL

The ethical guidelines of the Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, Universitas Airlangga, were followed for all experimental procedures. Ethical approval for this study was obtained from the Animal Ethics Committee, Faculty of Veterinary Medicine, Universitas Airlangga (Ethical Clearance Number: 2.KEH.26.02.2025)

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AUTHOR CONTRIBUTION

All authors, Rizqy Tafinna Lazuardi, Prananda Surya Airlangga, Herdiani Sulistyo Putri, Kohar Hari Santoso, Christrijogo Sumartono Waloejo, and Mahmudah, contributed equally to the conception and design of the study, data collection and analysis, interpretation of results, and preparation of the manuscript. All authors have read and approved the final version of the manuscript.

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