VER
Vascular & Endovascular Review

Cocoa vs Ibuprofen as Paracetamol Adjuncts for Postoperative Pain and Inflammation in Femoral Fracture Rats

Albert Simon1, Herdiani Sulistyo Putri[1]*, Prananda Surya Airlangga[1], Christrijogo Soemartono Waloejo[1], Ira Sari Yudaniayanti[2], Pudji Lestari[3]

[1]Department of Anesthesiology and Reanimation, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Clinical Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia
[3]Department of Public Health and Preventive Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Correspondence:
Herdiani Sulistyo Putri, Universitas Airlangga, Surabaya, Indonesia. Email: herdiani-s-p@fk.unair.ac.id

ABSTRACT

Background: The management of acute postoperative pain commonly relies on multimodal regimens combining paracetamol with nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen. These approaches may pose adverse effects, highlighting the need for safer adjuvant alternatives. Cocoa, rich in polyphenols and flavonoids, shows potential as an anti-inflammatory and analgesic agent. Objectives: To compare the efficacy of cocoa and ibuprofen as adjuvants to paracetamol in reducing acute pain and plasma interleukin-1 beta (IL-1β) levels in a rat model of postoperative femoral fracture. Methods: Eighteen healthy male Wistar rats (150–300g, 16–24 weeks) were randomized into three groups (n=6): control (placebo), cocoa-paracetamol, and ibuprofen-paracetamol. Standardised femoral fracture surgery was performed under anaesthesia, followed by assigned treatments. Pain evaluation was performed 24 hours postoperatively using the Von Frey filament test; plasma IL-1β was measured by ELISA. Data were analyzed using ANOVA and post-hoc Tukey test ($p<0.05$). Results: IL-1β levels were significantly lower in the cocoa-paracetamol (2.50±0.89ng/ml) and ibuprofen-paracetamol (4.32±0.52ng/ml) groups compared to control (7.41±0.65ng/ml; $p=0.000$). Cocoa-paracetamol showed significantly lower IL-1β than ibuprofen-paracetamol ($p=0.001$). Von Frey scores were significantly higher (indicating better analgesia) in the cocoa-paracetamol (19.38±4.15gf) and ibuprofen-paracetamol (26.19±8.01gf) groups than control (9.52±1.63gf; $p<0.001$); there was no statistically significant difference between cocoa-paracetamol and ibuprofen-paracetamol ($p=0.098$). IL-1β levels and Von Frey scores were moderately negatively correlated ($r=-0.529$, $p=0.024$). Conclusion: Cocoa as an adjuvant to paracetamol provided analgesic efficacy comparable to ibuprofen and superior anti-inflammatory effect as assessed by IL-1β. Cocoa is a promising, safer adjuvant alternative to NSAIDs for postoperative pain management.

KEYWORDS: Cocoa, Ibuprofen, Paracetamol, Postoperative Pain, Von Frey, IL-1β, Multimodal Analgesia, Rat Model

How to Cite: Albert Simon, Herdiani Sulistyo Putri, Prananda Surya Airlangga, Christrijogo Soemartono Waloejo, Ira Sari Yudaniayanti, Pudji Lestari, (2025) Cocoa vs Ibuprofen as Paracetamol Adjuncts for Postoperative Pain and Inflammation in Femoral Fracture Rats, Vascular and Endovascular Review, Vol.8, No.2, 1-6.

INTRODUCTION

Effective management of acute postoperative pain is essential for patient comfort, recovery, and prevention of chronic pain.1–3 Multimodal analgesia, typically combining paracetamol and NSAIDs such as ibuprofen, enhances pain control through distinct mechanisms.4,5 However, long-term or high-dose NSAID use increases risks of gastrointestinal, renal, and cardiovascular adverse effects.6,7 Therefore, the search for natural products with analgesic and anti-inflammatory properties8 and a better safety profile is warranted.9

Cocoa (Theobroma cacao) is a rich source of polyphenols, particularly flavonoids such as epicatechin and proanthocyanidins, which have demonstrated anti-inflammatory, neuroprotective, and analgesic effects in preclinical models.10,11 Animal studies suggest cocoa extracts can reduce central and peripheral inflammation and modulate pain pathways by inhibiting nuclear factor kappa-B (NF-κB), cytokine production, and oxidative stress.12,13

Interleukin-1 beta (IL-1β) is a key pro-inflammatory cytokine involved in nociception and central sensitization in acute and chronic pain states.14,15

Most research on the analgesic and anti-inflammatory effects of chocolate/cocoa has been conducted on general inflammatory pain models (such as orofacial pain, neuropathic, soft tissue inflammation), not on femur fracture or bone fracture models. Therefore, it remains unclear whether the cocoa + paracetamol effect would be equally effective in bone trauma conditions involving hard tissue damage, cytokine release, and bone healing mechanisms. Research by De Feo et al. shows effects on various inflammation and oxidative stress models, but not specifically on bone fractures.9

Several studies compare chocolate-rich diets with controls, but there is very rarely research that directly compares the effects of chocolate + paracetamol combination with NSAID + paracetamol combination. Ammar et al. shows the analgesic effect of chocolate as a paracetamol adjuvant, but only on neuropathic pain models, not on bone fracture models.16 Research by Fathani et al. shows that low doses of cocoa are already effective in several models (for example, a dose of 0.5 mg/gBW) for reducing TNF-α and increasing pain threshold.17 However, it is still unclear what the optimal cocoa dose is in combination with other analgesics for fracture models, as well as whether its effects are dose-dependent and safe in the short/long term.

This study aims to compare the effects of cocoa and ibuprofen as adjuvants to paracetamol on acute pain behaviour and plasma IL-1β in a rat model of complete femoral fracture.

METHODS

Experimental Design and Animals

This laboratory-based true experimental study employed a randomized post-test only control group design. Eighteen healthy male Wistar rats (Rattus norvegicus), 150–300g, aged 16–24 weeks, were housed under standard conditions with 7-day acclimatization, and randomized into three groups (n=6/group):

1. K0: Control (placebo)
2. K1: Cocoa extract 1g/kg body weight + Paracetamol 15mg/kg BW
3. K2: Ibuprofen 10mg/kg BW + Paracetamol 15mg/kg BW

The study received ethical clearance from the Animal Ethics Committee, Airlangga University, Surabaya.

Femoral Fracture Model

Rats underwent anaesthesia with rat cocktail (ketamine/xylazine/acepromazine, dosed as per standard protocols). A complete right mid-femur fracture was surgically induced, stabilized with intramedullary pinning, and followed by closure with layered sutures.

Treatments

Treatments were administered orally via gavage every 8 hours post-surgery for 24 hours, adjusted for individual body weights. Control rats received placebos in the same vehicle (CMC-Na 1%).

Cocoa Extract Preparation

Raw, roasted cocoa beans from Puslitkoka Jember (Vicco brand) were dried, ground, subjected to 70% ethanol extraction, filtration, evaporation, and resuspension in 1% CMC-Na to yield 100mg/ml cocoa suspension.

Measurement of Outcomes

Pain Behaviour

Mechanical pain thresholds were assessed using the electronic Von Frey test 24 hours postoperatively. The pressure (gram-force) required to elicit paw withdrawal on the operated limb was recorded.

Plasma IL-1β

After pain testing, animals were euthanized, and blood was collected via cardiac puncture. Plasma was separated and IL-1β levels quantified using ELISA (Bioassay Technology Laboratory, Shanghai).

Statistical Analysis

Data were tested for normality (Shapiro-Wilk), followed by ANOVA with Tukey's post-hoc test for intergroup comparisons. Correlation between IL-1β and Von Frey thresholds was analyzed by Pearson's method. Significance was set at $p<0.05$.

RESULTS

IL-1β Levels

Mean IL-1β plasma concentrations (ng/ml):

Table 1: IL-1β distribution between groups

Group	Mean ±SD	p value Shapiro w	Information
K0 (control)	7,407 ± 0,648 ng/ml	0,242*	Normal
K1 (cocoa 1 mg/gBW and oral paracetamol 15 mg/kgBW)	2,500 ± 0,889 ng/ml	0,624*	Normal
K2 (oral ibuprofen 10 mg/kgBW and oral paracetamol mg/kgBW)	4,318 ± 0,523 ng/ml	0,771*	Normal

Based on Table 1, plasma IL-1β levels in the control group (K0) showed the highest mean value of 7.407 ± 0.648 ng/ml. This indicates a relatively high inflammatory response without any treatment. Meanwhile, the group given a combination of cocoa 1 mg/gBW and paracetamol 15 mg/kgBW (K1) experienced the greatest decrease in IL-1β levels, reaching 2.500 ± 0.889 ng/ml. This reduction demonstrates that the combination has a quite strong anti-inflammatory effect. In the group with ibuprofen 10 mg/kgBW and paracetamol 15 mg/kgBW combination (K2), IL-1β levels also decreased to 4.318 ± 0.523 ng/ml, but not as effectively as group K1. The Shapiro-Wilk normality test showed that all groups had normal data distribution ($p > 0.05$), allowing further analysis with parametric tests. Overall, these results indicate that cocoa combined with paracetamol is capable of

suppressing pro-inflammatory cytokine IL-1β levels better than the ibuprofen-paracetamol combination, pointing to the potential role of bioactive compounds in cocoa as anti-inflammatory agents.

Table 2: IL-1β Anova test

IL-1β	Mean ±SD	P value Anova	Information
K0 (control)	7,407 ± 0,648 ng/ml		
K1 (cocoa 1 mg/gBW and oral paracetamol 15 mg/kgBW)	2,500 ± 0,889 ng/ml	0,000*	Significant
K2 (oral ibuprofen dose 10 mg/kgBW and oral paracetamol 15 mg/kgBW	4,318 ± 0,523 ng/ml		

The ANOVA test results for IL-1β levels showed significant differences between treatment groups ($p = 0.000$; $p < 0.05$). This confirms that the treatments significantly affected IL-1β level reduction. In the control group (K0), IL-1β levels remained high at 7.407 ± 0.648 ng/ml, while in the treatment groups, these pro-inflammatory cytokine levels decreased. Group K1 (cocoa 1 mg/gBW and paracetamol 15 mg/kgBW) had the lowest mean IL-1β level at 2.500 ± 0.889 ng/ml, showing the strongest effectiveness in suppressing inflammatory response. Meanwhile, group K2 (ibuprofen 10 mg/kgBW and paracetamol 15 mg/kgBW) also reduced IL-1β levels to 4.318 ± 0.523 ng/ml, but the reduction was not as significant as group K1. Thus, these results strengthen the finding that cocoa combined with paracetamol provides more effective anti-inflammatory effects compared to ibuprofen combined with paracetamol, indicating an active role of bioactive compounds in cocoa that can significantly modulate IL-1β levels.

Table 3: IL-1β Post Hoc

Group		P value Anova	Information
K0 (control) 7,407 ± 0,648 ng/ml	K1 (cocoa and paracetamol) 2,500 ± 0,889 ng/ml*	<,001	Significant
K0 (control) 7,407 ± 0,648 ng/ml	K2 (ibuprofen and paracetamol) 4,318 ± 0,523 ng/ml*	<.001	Significant
K1 (cocoa dan paracetamol) 2,500 ± 0,889 ng/ml	K2 (ibuprofen and paracetamol) 4,318 ± 0,523 ng/ml*	0,001	Significant

*P value <0,05 significant difference

ANOVA indicated a significant effect of treatment on IL-1β levels ($p < 0.01$). Tukey's post-hoc test confirmed all pairwise differences were significant (K0 vs K1, K0 vs K2, K1 vs K2; all $p<0.05$). This confirms that both cocoa-paracetamol and ibuprofen-paracetamol combinations are equally effective in reducing pro-inflammatory cytokine levels compared to no treatment. Additionally, the comparison between K1 and K2 also showed significant differences ($p = 0.001$), where group K1 had lower IL-1β levels than K2. This finding strengthens evidence that cocoa combined with paracetamol can provide stronger anti-inflammatory effects compared to ibuprofen combined with paracetamol. Therefore, it can be concluded that all treatments affect IL-1β levels, but the cocoa-paracetamol combination shows the highest effectiveness in suppressing inflammation.

Pain Thresholds (Von Frey Test)

Mean Von Frey scores (gram-force):

Table 4: Von Frey distribution between groups

Von Frey	Mean ±SD	p value Shapiro wilk	Information
K0 (control)	9,517 ± 1,625 gf	0,846	Normal
K1 (cocoa dosage 1 mg/gBW and oral paracetamol mg/kgBW)	19,380 ± 4,146 gf	0,126	Normal
K2 (ibuprofen dosage 10 mg/kgBW and oral paracetam 15 mg/kgBW)	26,190 ± 8,007 gf	0,993	Normal

*P value > 0,05 normal distribution

Pain threshold test results using the Von Frey method showed that all groups had normal data distribution ($p > 0.05$, Shapiro-Wilk test). The mean Von Frey score in the control group (K0) was 9.517 ± 1.625 gf, indicating the lowest pain threshold. Increased pain thresholds were seen in the treatment groups. Group K1 (cocoa 1 mg/gBW + paracetamol 15 mg/kgBW) showed a mean pain threshold of 19.380 ± 4.146 gf, almost twice as high as the control group. Meanwhile, group K2 (ibuprofen 10 mg/kgBW + paracetamol 15 mg/kgBW) had the highest mean value of 26.190 ± 8.007 gf, meaning the strongest pain threshold increase effect. Overall, these results show that combination therapy administration can increase pain thresholds compared to control, with effectiveness order: K2 > K1 > K0. This indicates that both cocoa and ibuprofen combined with paracetamol have analgesic effects, but the ibuprofen-paracetamol combination provides greater pain threshold increases compared to the cocoa-paracetamol combination.

Table 5: Von Frey Anova test

Von Frey	Mean ±SD	P value Anova	Information
K0 (control) K1 (cocoa dosage 1 mg/gBW and oral paracetamol 15 mg/kgBW)	9,517 ± 1,625 gf 19,380 ± 4,146 gf	<,001	Significant

K2 (ibuprofen dosage 10 mg/kgBW and oral paracetamol mg/kgBW)	26,190 ± 8,007 gf

*P value <0,05 significant difference

The ANOVA test on Von Frey data showed $p < 0.001$ ($p < 0.05$), meaning there were significant pain threshold differences between groups. The control group (K0) had the lowest pain threshold at 9.517 ± 1.625 gf, indicating that conditions without treatment resulted in high pain sensitivity. Administration of cocoa 1 mg/gBW combined with paracetamol 15 mg/kgBW (K1) increased the pain threshold to 19.380 ± 4.146 gf, while ibuprofen 10 mg/kgBW combined with paracetamol 15 mg/kgBW (K2) produced the highest pain threshold at 26.190 ± 8.007 gf. These results confirm that all treatments significantly affected pain threshold increases. Thus, both K1 and K2 combination therapies are effective in reducing pain sensitivity, but the strongest effect was obtained in group K2, indicating ibuprofen's superiority in increasing pain thresholds compared to cocoa when combined with paracetamol.

Table 6: Von Frey Post Hoc

Group		P value Anova	Information
K0 (control) 9,517 ± 1,625 gf	K1 (cocoa and paracetamol) 19,380 ± 4,146 gf*	0,015	Significant
K0 (control) 9,517 ± 1,625 gf	K2 (ibuprofen and paracetamol) 26,190 ± 8,007 gf*	<,001	Significant
K1 (cocoa and paracetamol) 19,380 ± 4,146 gf	K2 (ibuprofen and paracetamol) 26,190 ± 8,007 gf	0,098	Not significant

*P value <0,05 significant difference

ANOVA indicated significant differences among groups ($p<0.001$). Both treatment groups had higher pain thresholds than control (K0 vs K1: $p=0.015$; K0 vs K2: $p<0.001$). The difference between K1 and K2 did not reach statistical significance ($p=0.098$). This shows that although K2 had higher average pain thresholds compared to K1, the difference was not statistically strong enough. Therefore, it can be concluded that both combination treatments effectively increase pain thresholds, but there is no significant difference between the analgesic effects of cocoa-paracetamol combination compared to ibuprofen-paracetamol combination.

Correlation Between IL-1β and Von Frey

Table 7: Correlation IL-1β with Von Frey

	N	r	P value	Information
IL-1β with Von Frey	18	-0,529	0,024	Correlate

*significant correlation if p value < 0,05

DISCUSSION

This study demonstrates that acute pain and inflammation following surgical femoral fracture can be effectively mitigated by both cocoa and ibuprofen when used as adjuvants to paracetamol, as judged by IL-1β levels and mechanical pain thresholds. Cocoa-paracetamol was similar in analgesic efficacy to ibuprofen-paracetamol (Von Frey test), but cocoa exerted significantly stronger anti-inflammatory action (lower IL-1β). This may be attributed to the multi-modal anti-inflammatory actions of cocoa polyphenols, which suppress NF-κB, cytokine production, and oxidative stress.11–13 The superiority of cocoa in lowering IL-1β levels highlights its potential to reduce postoperative inflammation more safely than NSAIDs, whose gastrointestinal, cardiovascular, and renal risks are well known.6,7

The results of this research show that there is no significant difference in analgesic effects (pain threshold) between the cocoa-paracetamol combination and the ibuprofen-paracetamol combination. This finding supports previous clinical data showing that several natural products, including flavonoids and polyphenols, can function as adjuvants or alternatives in pain management without reducing the effectiveness of standard analgesics.18 In other words, cocoa is capable of providing analgesic effects equivalent to NSAIDs, but with potentially lower risk of side effects, considering that cocoa is natural and has a good safety profile.

This finding also extends previous animal studies that have demonstrated the analgesic and anti-inflammatory effects of cocoa in various pain models, including neuropathic and inflammatory pain.16,17,19 Cocoa contains polyphenols such as epicatechin and proanthocyanidins that are known to suppress the NF-κB inflammatory pathway, reduce COX-2 expression, and decrease oxidative stress, thereby reducing the production of pro-inflammatory cytokines such as IL-1β. This effect explains why cocoa can significantly reduce inflammation, even in post-fracture pain models, and supports the idea that inflammation modulation can contribute to analgesic effects indirectly.

Furthermore, the moderate negative correlation between plasma IL-1β levels and pain threshold confirms the role of inflammatory cytokines as biomarkers and mediators of hyperalgesia.14 Increased IL-1β is associated with increased pain sensitivity through activation of sensory neurons in the spinal cord and induction of prostaglandins, which causes post-trauma hyperalgesia. Thus, the reduction of IL-1β by cocoa not only demonstrates anti-inflammatory effects but also indicates an indirect analgesic mechanism through modulation of inflammatory pathways.

Overall, these results emphasize that cocoa can function as an effective analgesic adjuvant, providing analgesic effects equivalent to NSAIDs while reducing inflammatory mediators such as IL-1β. This has important clinical translational implications, especially for postoperative patients who require short to medium-term pain management, where reducing NSAID use can lower the risk of gastrointestinal, cardiovascular, and renal side effects. Further studies are needed to explore optimal dosing, long-term duration effects, as well as clinical trials in humans to confirm the effectiveness and safety of cocoa as an analgesic adjuvant.

IL-1β is a major mediator of hyperalgesia that increases the excitability of sensory neurons in the spinal cord and increases prostaglandin production, thereby increasing pain sensitivity. Polyphenols in cocoa, including epicatechin and proanthocyanidins, are capable of inhibiting the NF-κB, COX-2, and MAPK pathways, thereby reducing pro-inflammatory cytokine production and oxidative stress. Interestingly, although cocoa reduces IL-1β more significantly compared to ibuprofen, its analgesic effect is equivalent, indicating that cocoa targets inflammatory processes more than direct pain pathways, and possibly works synergistically with paracetamol. From a clinical perspective, this finding shows the potential of cocoa as a safe analgesic adjuvant to reduce postoperative NSAID use, thereby reducing the risk of gastrointestinal, cardiovascular, and renal side effects.

This study's limitations include lack of single-drug intervention groups (cocoa or ibuprofen alone), relatively short follow-up, and use of an animal model. Future research should include longer-term, dose-finding, and human trials.

CONCLUSION

Cocoa as a paracetamol adjuvant provides analgesic effects equivalent to the ibuprofen-paracetamol combination, as evidenced by mechanical pain thresholds, while simultaneously demonstrating stronger anti-inflammatory effects, shown by the significant reduction in IL-1β levels. This finding supports the role of cocoa as a natural product with multimodal mechanisms, which can reduce inflammation through inhibition of NF-κB, COX-2, and MAPK pathways, as well as reduce oxidative stress. These results confirm that cocoa has potential as a safe analgesic adjuvant, particularly in postoperative pain management, with the possibility of reducing NSAID use and the risk of gastrointestinal, cardiovascular, and renal side effects. Further studies are needed to explore optimal dosing, long-term effects, as well as translational trials in humans so that the effectiveness and safety of cocoa as an analgesic adjuvant can be clinically confirmed.

REFERENCES

1. Gan TJ. Poorly controlled postoperative pain: prevalence, consequences, and prevention. J Pain Res. 2017;10:2287.
2. Raja SN, Carr DB, Cohen M, Finnerup NB, Flor H, Gibson S, et al. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. Pain. 2020;161(9):1976-1982. doi:10.1097/j.pain.0000000000001939
3. Putri HS, Hanindito E, Sulistyono H. Ketamine Versus Tramadol Effectiveness as Postoperative Oral Analgesics on Pediatric Patients Age 5-10 Years in Elective Surgery at Dr. Soetomo Hospital Surabaya. Indones J Anesthesiol Reanim. 2020;2(2):38. doi:10.20473/ijar.V2I22020.38-46
4. Chou R, Gordon DB, de Leon-Casasola OA, Rosenberg JM, Bickler S, Brennan T, et al. Management of Postoperative Pain: A Clinical Practice Guideline From the American Pain Society, the American Society of Regional Anesthesia and Pain Medicine, and the American Society of Anesthesiologists' Committee on Regional Anesthesia, Executive Commi. J Pain. 2016;17(2):131-157. doi:10.1016/j.jpain.2015.12.008
5. Mumpuni DR, Putri HS, Airlangga PS, Waloejo CS, Santoso KH, Lestari P. Transient Receptor Potential Vanilloid 1 in Acute Pain: A Literature Review. Pharmacogn J. 2024;16(5):1196-1201. doi:10.5530/pj.2024.16.196
6. Castellsague J, Riera-Guardia N, Calingaert B, Varas-Lorenzo C, Fourrier-Reglat A, Nicotra F, et al. Individual NSAIDs and Upper Gastrointestinal Complications. Drug Saf. 2012;35(12):1127-1146. doi:10.2165/11633470-000000000-00000
7. McGettigan P, Henry D. Use of Non-Steroidal Anti-Inflammatory Drugs That Elevate Cardiovascular Risk: An Examination of Sales and Essential Medicines Lists in Low-, Middle-, and High-Income Countries. Turnbull FM, ed. PLoS Med. 2013;10(2):e1001388. doi:10.1371/journal.pmed.1001388
8. Saristiana Y, Salmasfattah N, Prasetyawan F, Savitri L, Kadir MBA. Identification of Active Compounds from Sambung Nyawa Leaves (Gynura procumbens (Lour.) Merr) as Potential Natural Antioxidant and Anti-inflammatory Agents. Pharmacol Med REPORTS, Orthop Illn DETAILS. 2025;4(3):110-126. doi:10.55047/comorbid.v4i3.1865
9. De Feo M, Paladini A, Ferri C, Carducci A, Del Pinto R, Varrassi G, et al. Anti-Inflammatory and Anti-Nociceptive Effects of Cocoa: A Review on Future Perspectives in Treatment of Pain. Pain Ther. 2020;9(1). doi:10.1007/s40122-020-00165-5
10. Katz DL, Doughty K, Ali A. Cocoa and Chocolate in Human Health and Disease. Antioxid Redox Signal. 2011;15(10):2779-2811. doi:10.1089/ars.2010.3697
11. Pérez-Cano FJ, Massot-Cladera M, Franch À, Castellote C, Castell M. The effects of cocoa on the immune system. Front Pharmacol. 2013;4. doi:10.3389/fphar.2013.00071
12. Ramiro E, Franch À, Castellote C, Pérez-Cano F, Permanyer J, Izquierdo-Pulido M, et al. Flavonoids from Theobroma cacao Down-Regulate Inflammatory Mediators. J Agric Food Chem. 2005;53(22):8506-8511. doi:10.1021/jf0511042
13. Cady RJ, Denson JE, Durham PL. Inclusion of cocoa as a dietary supplement represses expression of inflammatory proteins in spinal trigeminal nucleus in response to chronic trigeminal nerve stimulation. Mol Nutr Food Res. 2013;57(6):996-1006. doi:10.1002/mnfr.201200630
14. Ren K, Torres R. Role of interleukin-1β during pain and inflammation. Brain Res Rev. 2009;60(1):57-64. doi:10.1016/j.brainresrev.2008.12.020

15. Gonçalves dos Santos G, Delay L, Yaksh TL, Corr M. Neuraxial Cytokines in Pain States. Front Immunol. 2020;10. doi:10.3389/fimmu.2019.03061
16. Ammar FM, Waloejo CS, Putri HS, Santoso KH, Airlangga PS, Utomo B. Effect of Cacao Bean Extract as a Paracetamol Adjuvant on Pain Scale and Tumor Necrosis Factor-Alpha in Neuropathic Pain: An Animal Model Study. Pharmacogn Journal,. 2025;16(6):1336-1341. doi:10.5530/pj.2024.16.215
17. Fathani HC, Sulistyo Putri H, Airlangga PS, Waloejo CS, Yudaniayanti IS, Lestari P. Effective Dose of Cocoa as a Preemptive Analgesic and Anti- Inflammatory Agent Assessed through Pain Scale and Tumor Necrosis Factor Alpha (TNF-α) in an Acute Pain Animal Model. Pharmacogn J. 2024;16(5):1134-1137. doi:10.5530/pj.2024.16.185
18. Nuha K, Rusmil K, Ganiem AR, Permadi W, Diah Herawati DM. Single-Blind Randomized Controlled Trial: Comparative Efficacy of Dark Chocolate, Coconut Water, and Ibuprofen in Managing Primary Dysmenorrhea. Int J Environ Res Public Health. 2023;20(16):6619. doi:10.3390/ijerph20166619
19. Fikri A, Sulistiyowati H, Sumartono C, Santoso KH, Airlangga PS, Lestari P. Phytochemicals as Modulators of TRPV1 in Pain and Inflammation a Review. African J Biol Sci. 2024;6(9). doi:10.48047/AFJBS.6.9.2024.5231-5244