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Adjunctive Nano-Formulated Kratom (Mytragina Speciosa) with Paracetamol for Neuropathic Pain in Rats: Mechanical Allodynia and Serum Interleukin-6 Outcomes

Rahmat Kukuh Noviandomo[1], Anna Surgean Veterini[1,2]*, Kohar Hari Santoso[1,2], Prananda Surya Airlangga[1,2], Christrijogo Sumartono Waloejo[1,2]

[1]*Department of Anesthesiology and Reanimation, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.*

[2]*Department of Anesthesiology and Reanimation, Dr. Soetomo General Academic Hospital, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.*

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ABSTRACT

Background: Neuropathic pain is a refractory chronic pain condition driven in part by neuroinflammation. Interleukin-6 (IL-6) is a key pro-inflammatory mediator, and paracetamol monotherapy is often insufficient. Alkaloids of Mitragyna speciosa (kratom) have μ-opioid receptor agonist and anti-inflammatory activity, while nanoformulation may improve oral bioavailability. The objective is to evaluate the analgesic and anti-inflammatory effects of oral nano-kratom as an adjuvant to paracetamol on mechanical withdrawal threshold and serum IL-6 in a rat chronic constriction injury (CCI) model.

Methods: A post-test-only randomized controlled animal study was conducted in 21 male Wistar rats assigned to vehicle control (K−), paracetamol 10 mg/kg (K1), or nano-kratom 30 mg/kg plus paracetamol 5 mg/kg (K2) (n = 7/group). After acclimatization, unilateral CCI of the left sciatic nerve was induced. Treatments were given orally once daily from day 7 to day 13, and endpoints were assessed on day 14. Mechanical withdrawal threshold was measured using von Frey filaments, and serum IL-6 was quantified by ELISA. One K− rat died before endpoint assessment; the final analyzed n = 6, 7, and 7.

Results: Baseline von Frey thresholds were comparable across groups. CCI significantly reduced withdrawal thresholds in all groups. On day 14, thresholds differed significantly, with K2 showing the highest values, followed by K1 and K− (all pairwise $p < 0.001$). Serum IL-6 was lowest in K2; the difference was significant versus K− ($p = 0.037$) and approached significance versus K1 ($p = 0.053$).

Conclusion: Nano-kratom combined with half-dose paracetamol produced greater antinociception than paracetamol alone and was associated with the lowest serum IL-6 levels. These findings support further evaluation of nano-formulated kratom as a multimodal adjuvant for neuropathic pain.

Introduction

Neuropathic pain is a chronic pain state arising from a lesion or disease of the somatosensory nervous system [1-6]. It is characterized by spontaneous pain, hyperalgesia, and allodynia and frequently resists conventional analgesic therapy [2,7-13]. Global prevalence estimates range from 3% to 17% of the general population [14–17], whereas hospital-based Indonesian data report a substantially higher prevalence of approximately 21.8%, reflecting a

The authors declare no conflicts of interest.
*Corresponding author.
E-mail address: annasurgean@gmail.com
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considerable public health burden [18]. Pharmacological options remain limited: first-line agents such as gabapentinoids, tricyclic antidepressants, and serotonin–norepinephrine reuptake inhibitors provide meaningful relief in only a minority of patients [13,19–21], while opioids carry risks of tolerance, dependence, and respiratory depression [20–22]. Paracetamol, though widely used and generally well tolerated, has limited efficacy because its central cyclo-oxygenase inhibition and endocannabinoid-related actions confer only weak anti-inflammatory effects at sites of nerve injury [23–27]. There is, therefore, an unmet need for adjuvant therapies that specifically target the neuroinflammatory component of neuropathic pain [1,28].

Interleukin-6 (IL-6) is a pleiotropic pro-inflammatory cytokine induced after nerve injury that promotes peripheral and central sensitization, and elevated IL-6 correlates with mechanical allodynia and hyperalgesia [1,3–5,29-33]. Mitragyna speciosa (kratom) contains alkaloids with μ-opioid receptor agonism and anti-inflammatory effects [6–8,34-37], and nanoformulation may enhance their oral bioavailability [9–11,28]. This study evaluated whether an oral nano-kratom suspension as an adjuvant to paracetamol could improve mechanical withdrawal threshold and reduce serum IL-6 levels in a rat chronic constriction injury model [38-40].

Methods

This true experimental laboratory study used a randomized, post test only control group design in a chronic constriction injury (CCI) model of neuropathic pain in male Wistar rats. All procedures were performed at Universitas Airlangga, Surabaya, Indonesia, and reported in accordance with ARRIVE 2.0 guidelines. Ethical approval was obtained from the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga (No. 2.KEH.202.12.2025); animal care complied with institutional and international standards and the 3Rs (replacement, reduction, and refinement).

Twenty one healthy 4 month old male Wistar rats (150–200 g) were obtained from the Faculty of Veterinary Medicine and housed in pathogen free polycarbonate cages with ad libitum access to standard chow and water at 22 ± 2 °C on a 12 h light/dark cycle. After 7 days of acclimatization, baseline mechanical thresholds were recorded and CCI of the left sciatic nerve was induced under ketamine/xylazine/acepromazine anesthesia, using four loose 4 0 silk ligatures placed around the nerve.

Sample size was calculated using $\alpha = 0.05$ and 80% power based on previous CCI data, yielding a minimum of six rats per group; allowing for drop out, seven rats were enrolled per arm (total n = 21). Animals were randomly allocated to three groups (n = 7): vehicle control (K−, 1% CMC Na), paracetamol 10 mg/kg (K1), and nano kratom 30 mg/kg plus paracetamol 5 mg/kg (K2), administered orally once daily for 7 days. One K− rat died before the endpoint, giving a final analyzed n = 6, 7, and 7.

The primary outcome was mechanical withdrawal threshold measured with von Frey filaments at baseline, post CCI, and day 14. The secondary outcome was serum IL 6 concentration measured by ELISA at the day 14 endpoint. Statistical analyses used Shapiro–Wilk and Levene tests, a paired t test, and a one way ANOVA with Scheffé/Tukey post hoc tests (two sided $p < 0.05$).

Results

Animal and Disposition

Twenty-one male Wistar rats were enrolled and underwent CCI. One rat in the K− group died during the treatment period before endpoint assessment and was excluded because no necropsy was performed, and the cause of death remained undetermined. Thus, 20 rats were included in the final analyses: K−, n = 6; K1, n = 7; and K2, n = 7. No additional animals were excluded for protocol deviations, and predefined humane endpoints were not triggered in the surviving animals.

Baseline Mechanical Withdrawal Threshold

At baseline, von Frey thresholds were normally distributed in each group, and variances were homogeneous. Mean thresholds were similar between groups (K− 11.42 ± 1.92 g; K1 11.32 ± 1.72 g; K2 11.34 ± 1.34 g), and one-way ANOVA confirmed no significant difference ($F(2,18) = 0.007$, $p = 0.993$), indicating successful randomization.

Validation of the CCI Model

Seven days after surgery, mechanical thresholds decreased uniformly in all groups, with a mean within-subject reduction of 5.13 ± 1.10 g (95% CI 4.63–5.63; $t(20) = 21.33$; $p < 0.001$). Post-CCI thresholds did not differ significantly between groups ($F(2,18) = 0.748$, $p = 0.534$), confirming consistent establishment of neuropathic pain.

Treatments' Effect on Mechanical Withdrawal Threshold

On day 14, after seven daily oral doses, mechanical thresholds differed markedly between groups. Mean (± SD) thresholds were 6.25 ± 0.68 g (K−), 9.50 ± 1.27 g (K1), and 17.04 ± 1.25 g (K2). One-way ANOVA showed a highly significant group effect ($F(2,17) = 79.34$, $p < 0.001$), and post hoc testing demonstrated significant pairwise differences among all groups (K− vs. K1, K− vs. K2, K1 vs. K2; all $p < 0.001$), indicating the greatest antinociceptive effect in the nano kratom plus half dose paracetamol group.

(Table 1), Post hoc pairwise comparisons of the day-14 mechanical withdrawal threshold and serum IL-6 levels between treatment groups. Negative mean differences indicate that the first-listed group had a lower mean value than the second-listed group. Von Frey thresholds were analyzed with Tukey HSD and serum IL-6 with Scheffé post hoc tests ($\alpha = 0.05$).

(Figure 1), Mechanical withdrawal threshold measured by the von Frey test at baseline, after chronic constriction injury (post-CCI), and after treatment in the three groups: vehicle control (K−), paracetamol 10 mg/kg (K1), and nano-kratom 30 mg/kg plus paracetamol 5 mg/kg (K2). Bars represent mean ± standard deviation. CCI reduced withdrawal thresholds in all groups, confirming successful induction of neuropathic pain, whereas post-treatment thresholds were highest in K2, followed by K1 and K−. Asterisks indicate significant between-group differences at day 14 (***$p < 0.001$).

(Figure 2), Serum interleukin-6 (IL-6) concentrations at the day-14 endpoint in the vehicle control (K−, n = 6), paracetamol 10 mg/kg (K1, n = 7), and nano-kratom 30 mg/kg plus paracetamol 5 mg/kg (K2, n = 7) groups. Bars represent median IL-6 values (pg/mL), and error bars indicate the observed range. Serum IL-6 tended to be lower in K2 compared with K− and K1; the difference between K− and K2 was statistically significant ($p = 0.037$), whereas K1 versus K2 approached but did not reach significance ($p = 0.053$).

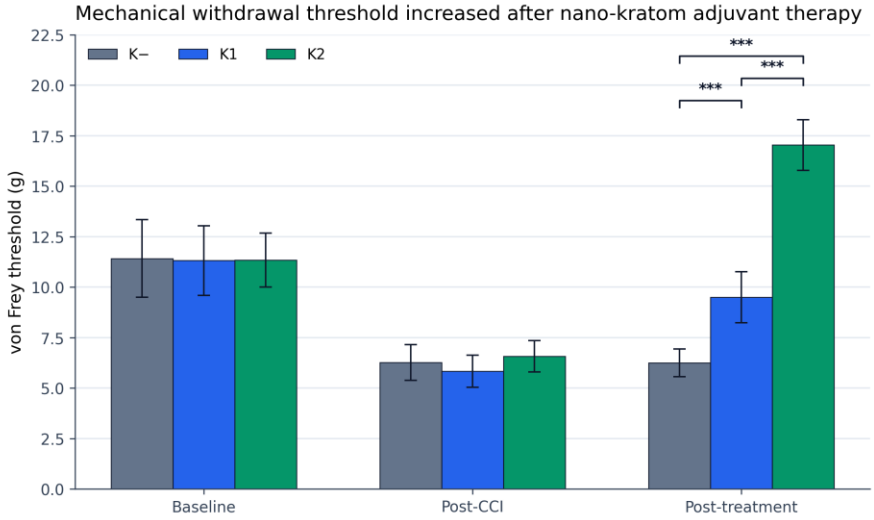


Figure 1- Mechanical withdrawal thresholds at baseline, after chronic constriction injury, and after treatment in male Wistar rats

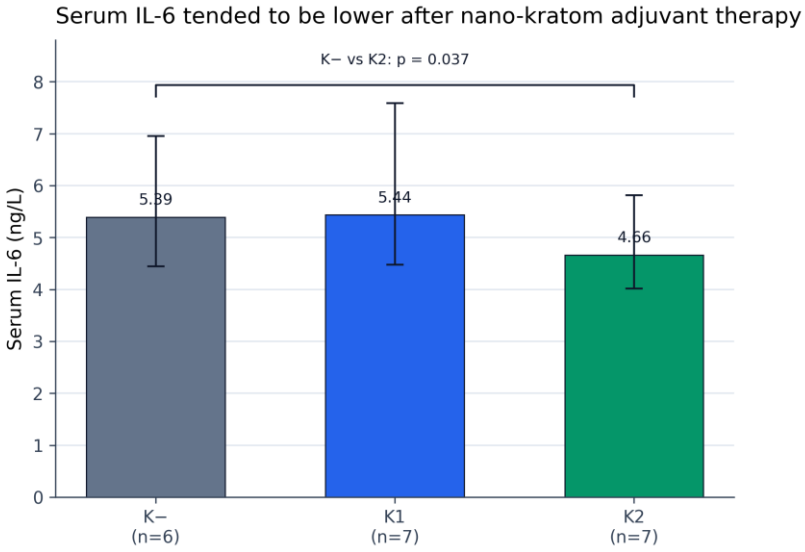


Figure 2- Serum interleukin-6 concentrations on day 14 in male Wistar rats with chronic constriction injury-induced neuropathic pain

Table 1- Post hoc pairwise comparisons of day-14 mechanical withdrawal thresholds and serum interleukin-6 concentrations between treatment groups

Outcome	Comparison	Mean difference ± SE	95% CI	P value
Von Frey, day 14	K− vs. K1	−3.25 ± 0.62	−4.90 to −1.50	< 0.001
	K− vs. K2	−10.79 ± 0.62	−12.47 to −9.10	< 0.001
	K1 vs. K2	−7.54 ± 0.60	−9.15 to −5.96	< 0.001
Serum IL-6 (pg/mL)	K− vs. K1	0.13 ± 0.46	−1.10 to 1.37	0.950
	K− vs. K2	1.16 ± 0.46	0.07 to 2.25	0.037
	K1 vs. K2	1.17 ± 0.44	−0.01 to 2.36	0.053

Negative mean differences indicate that the first listed group had a lower mean value than the second listed group. Von Frey thresholds were analyzed with Tukey HSD and serum IL 6 with Scheffé post hoc tests ($\alpha = 0.05$).

Discussion

In this preclinical study, an oral nano-formulated suspension of *Mitragyna speciosa* administered as an adjuvant to half-dose paracetamol produced a marked and statistically significant improvement in mechanical withdrawal threshold compared with paracetamol monotherapy in male Wistar rats with CCI-induced neuropathic pain [27,39-40]. Serum IL-6 levels followed the predicted direction, with the nano-kratom combination group showing the lowest IL-6 and a statistically significant reduction relative to the negative control [3,5]. The robustness of the behavioral observation is supported by three internal validity checks. First, baseline thresholds were homogeneous across the three arms, indicating that randomization was effective. Second, the CCI insult produced a uniform and large reduction in withdrawal threshold across all groups, confirming consistent establishment of mechanical allodynia [39-40]. Third, post-CCI thresholds did not differ between groups, implying that any subsequent differences in day-14 thresholds can be attributed to the intervention rather than to differential injury severity. The magnitude of the effect (a 79% increase in mean threshold for K2 over K1) is unlikely to be a chance finding and is consistent with reports that kratom alkaloids produce dose-dependent antinociception in rodent models of inflammatory and neuropathic pain [6-8,33,41-42]. Mechanistically, the additive effect of nano-kratom on top of paracetamol is biologically plausible. Mitragynine and 7-hydroxymitragynine are partial μ-opioid receptor agonists biased toward G-protein signaling rather than β-arrestin recruitment, a profile associated with analgesia and a potentially reduced incidence of opioid-typical adverse effects [6-8]. In addition, kratom alkaloids have been shown to inhibit COX-2 and NF-κB and to attenuate pro-inflammatory cytokine production [34-36,43], providing an anti-neuroinflammatory action that conventional opioids lack. Paracetamol acts predominantly through central COX inhibition and possibly through endocannabinoid- and TRPV1-mediated mechanisms, with a limited peripheral anti-inflammatory effect [23,25-26,44]. The two agents therefore engage complementary pathways at the levels of nociceptive transmission, modulation, and inflammation. Comparable preclinical evidence from other plant-derived extracts has also shown concurrent reductions in inflammatory cytokines and nociceptive responses in rat models [45], supporting the broader rationale for botanical adjuvants in inflammatory and neuropathic pain. Our IL-6 findings should be interpreted cautiously. The overall ANOVA indicated a significant between-group effect, and the direction of change was consistent with the behavioral data: the group with the highest withdrawal threshold (K2) also had the lowest IL-6 [1,3-5]. Post hoc comparisons demonstrated a significant difference between the nano-kratom combination group (K2) and the negative control (K−) ($p = 0.037$), while the contrast between K2 and paracetamol monotherapy (K1) approached statistical significance ($p = 0.053$), suggesting a numerically larger but not yet conclusively distinct cytokine response. With small group sizes ($n = 6–7$), serum IL-6 measured at a single late time point captures only a partial picture of the dynamic neuroinflammatory response, and the present analyses are therefore best interpreted as supportive of, rather than fully confirmatory for, an anti-neuroinflammatory contribution from nano-kratom [3,29-30]. The partial dissociation between behavioral and IL-6 results highlights the multifactorial nature of neuropathic pain. K1 (paracetamol alone) produced a significant analgesic effect without a clearly detectable change in serum IL-6, indicating that mechanical antinociception in this model can be achieved through non-inflammatory pathways such as central COX inhibition [25-27]. Conversely, the additional benefit conferred by nano-kratom in K2 may reflect a combined contribution of opioid-receptor–mediated analgesia and anti-neuroinflammatory action [8,33,36,42]. This pattern is consistent with the contemporary view that effective neuropathic pain management often requires multimodal targeting of nociceptive, descending modulatory, and neuroinflammatory mechanisms [2,13,20-22].

Our findings extend the preclinical literature on kratom in several ways. First, most published rodent studies have used crude ethanolic or methanolic extracts at doses of 100–300 mg/kg [8,33,42]; the use here of a 30 mg/kg nano-suspension achieving substantial antinociceptive effects is consistent with the proposition that

nanoformulation can meaningfully increase the pharmacological efficiency of mitragynine, presumably through improvements in oral bioavailability and tissue penetration [9-11,37-38]. Second, the present design isolates the adjuvant effect of nano-kratom on top of a reduced paracetamol dose, which is therapeutically relevant: it speaks directly to a paracetamol-sparing strategy in which a herbal adjuvant could permit lower doses of conventional analgesics, potentially reducing cumulative gastrointestinal, renal, and hepatic risk over time [22,25-26]. Translation to humans requires caution. Allometric scaling and species differences in alkaloid metabolism (notably CYP-mediated inhibition by mitragynine) imply that the human dose equivalent will be considerably lower than 30 mg/kg, and drug–drug interactions are plausible [8,37-38]. The safety profile of long-term kratom use in humans remains debated, with case reports of hepatotoxicity, dependence, and withdrawal at high or chronic doses [8,31-32,42]. Carefully designed phase-I pharmacokinetic and safety studies of standardized nano-kratom formulations will be needed before any clinical recommendation can be made.

Limitations

Several limitations of this study should be acknowledged. First, the sample size (n = 6–7 per group) was based on a standard formula for rodent experiments and is adequate to detect large effects on the mechanical withdrawal threshold but is likely underpowered for the more variable IL-6 outcome, where the K1 versus K2 contrast approached but did not reach statistical significance. Second, IL-6 was measured at only one time point on day 14, so the temporal dynamics of neuroinflammation through the acute and subacute phases of CCI cannot be inferred. Third, only a single cytokine was assayed; a broader panel including TNF-α, IL-1β, IL-10, and chemokines, together with tissue-level measurements at the injured nerve and spinal dorsal horn, would have provided a more complete picture of the inflammatory milieu. Fourth, behavioral assessment was restricted to mechanical thresholds with von Frey filaments; thermal hyperalgesia, cold allodynia, and spontaneous pain behaviors were not evaluated. Fifth, histopathology and immunohistochemistry (e.g., microglial and astrocytic markers) were not performed, so the cellular substrate of the observed effects cannot be directly confirmed. Sixth, detailed physicochemical characterization of the nano-kratom formulation (particle size, polydispersity index, zeta potential, encapsulation efficiency, and in vitro release) was outside the scope of the present biological study and should accompany future translational work.

Conclusion

In a male Wistar rat CCI model of neuropathic pain, oral administration of a nano-formulated *Mitragyna speciosa* suspension at 30 mg/kg as an adjuvant to half-dose paracetamol produced substantially greater mechanical antinociception than paracetamol monotherapy and was associated with the lowest median serum IL-6 levels among the three groups. These exploratory preclinical findings are consistent with a multimodal mechanism that combines opioid-receptor–mediated analgesia with anti-neuroinflammatory activity and justify further preclinical work—including dose–response characterization, multi-cytokine and tissue-level analyses, head-to-head comparison with first-line neuropathic-pain agents, and rigorous toxicology—before any clinical translation can be considered. The findings should not be over-interpreted as evidence of clinical efficacy in humans.

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Ethics approval and consent to participate

All animal procedures were reviewed and approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga (approval no. 2.KEH.202.12.2025). Experiments complied with applicable national regulations on the use of laboratory animals and were conducted in accordance with the ARRIVE 2.0 guidelines.

Data availability

The de-identified individual-animal data underlying the analyses reported in this article are available from the corresponding author on reasonable request. Raw individual-animal von Frey and IL-6 values will also be provided as Supplementary Table S1.

References

[1] Clark AK, Old EA, Malcangio M. Neuropathic pain and cytokines: current perspectives. J Pain Res. 2013; 6:803-14.

[2] Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D, et al. Neuropathic pain.

Nat Rev Dis Primers. 2017; 3:17002.
[3] Zhou YQ, Liu Z, Liu ZH, Chen SP, Li M, Shahveranov A, et al. Interleukin-6: an emerging regulator of pathological pain. J Neuroinflammation. 2016; 13(1):141.
[4] Lee KM, Jeon SM, Cho HJ. Interleukin-6 induces microglial CX3CR1 expression in the spinal cord after peripheral nerve injury through the activation of p38 MAPK. Eur J Pain. 2010; 14(7):682.e1-12.
[5] Scheller J, Garbers C, Rose-John S. Interleukin-6: from basic biology to selective blockade of pro-inflammatory activities. Semin Immunol. 2014; 26(1):2-12.
[6] Karunakaran T, Ngew KZ, Zailan AAD, Mian Jong VY, Abu Bakar MH. The chemical and pharmacological properties of mitragynine and its diastereomers: an insight review. Front Pharmacol. 2022; 13:805986.
[7] Kruegel AC, Gassaway MM, Kapoor A, Váradi A, Majumdar S, Filizola M, et al. Synthetic and receptor signaling explorations of the Mitragyna alkaloids: mitragynine as an atypical molecular framework for opioid receptor modulators. J Am Chem Soc. 2016; 138(21):6754-64.
[8] Prozialeck WC, Jivan JK, Andurkar SV. Pharmacology of kratom: an emerging botanical agent with stimulant, analgesic and opioid-like effects. J Am Osteopath Assoc. 2012; 112(12):792-9.
[9] Azhar S, Ashari S. Strategies for the development of Mitragyna speciosa (kratom) leaves extract loaded with solid lipid nanoparticles. JMEST. 2019; 6(12):100-4.
[10] Azhar SN, Ashari SE, Tan JK, Kassim NK, Hassan M, Zainuddin N, et al. Screening and selection of formulation components of nanostructured lipid carriers system for Mitragyna speciosa (Korth). Havil drug delivery. Ind Crops Prod. 2023; 198:116668.
[11] Peng Y, Chen L, Ye S, Kang Y, Liu J, Zeng S, et al. Research and development of drug delivery systems based on drug transporter and nano-formulation. Asian J Pharm Sci. 2020; 15(2):220-36.
[12] Dubin AE, Patapoutian A. Nociceptors: the sensors of the pain pathway. J Clin Invest. 2010; 120(11):3760-72.
[13] Cavalli E, Mammana S, Nicoletti F, Bramanti P, Mazzon E. The neuropathic pain: an overview of the current treatment and future therapeutic approaches. Int J Immunopathol Pharmacol. 2019; 33:2058738419838383.
[14] Bouhassira D, Lantéri-Minet M, Attal N, Laurent B, Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. Pain. 2008; 136(3):380-7.
[15] van Hecke O, Austin SK, Khan RA, Smith BH, Torrance N. Neuropathic pain in the general population: a systematic review of epidemiological studies. Pain. 2014; 155(9):1907.
[16] Torrance N, Smith BH, Bennett MI, Lee AJ. The epidemiology of chronic pain of predominantly neuropathic origin. Results from a general population survey. J Pain. 2006; 7(4):281-9.
[17] Zimmer Z, Fraser K, Grol-Prokopczyk H, Zajacova A. A global study of pain prevalence across 52 countries: examining the role of country-level contextual factors. Pain. 2022; 163(9):1740-50.
[18] Purwata TE, Sadeli HA, Amir D, Asnawi C, Partoatmodjo L, Aulina S, et al. Characteristics of neuropathic pain in Indonesia: a hospital based national clinical survey. Neurol Asia. 2015; 20(4).
[19] Dureja GP, Iyer RN, Das G, Ahdal J, Narang P. Evidence and consensus recommendations for the pharmacological management of pain in India. J Pain Res. 2017; 10:709-36.
[20] Gilron I, Baron R, Jensen T. Neuropathic pain: principles of diagnosis and treatment. Mayo Clin Proc. 2015; 90(4):532-45.
[21] Wright ME, Rizzolo D. An update on the pharmacologic management and treatment of neuropathic pain. JAAPA. 2017; 30(3):13-7.
[22] Vargas-Schaffer G. Is the WHO analgesic ladder still valid? Twenty-four years of experience. Can Fam Physician. 2010; 56(6):514-7.
[23] Dani M, Guindon J, Lambert C, Beaulieu P. The local antinociceptive effects of paracetamol in neuropathic pain are mediated by cannabinoid receptors. Eur J Pharmacol. 2007; 573(1-3):214-5.
[24] Gulcu N, Karaaslan K, Ozturan KE, Kocoglu H. Intravenous paracetamol for pain relief in a patient with neuropathic pain. Acta Anaesthesiol Scand. 2007; 51(7):956-7.
[25] Mazaleuskaya LL, Sangkuhl K, Thorn CF, FitzGerald GA, Altman RB, Klein TE. PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses. Pharmacogenet Genomics. 2015; 25(8):416-26.
[26] Ohashi N, Kohno T. Analgesic effect of acetaminophen: a review of known and novel mechanisms of action. Front Pharmacol. 2020; 11:580289.
[27] Im KS, Jung HJ, Kim JB, Lee JM, Park HJ, Joo CH, et al. The antinociceptive effect of acetaminophen in a rat model of neuropathic pain. Kaohsiung J Med Sci. 2012; 28(5):251-8.
[28] Beiranvand S, Sorori MM. Pain management using nanotechnology approaches. Artif Cells Nanomed Biotechnol. 2019; 47(1):462-8.
[29] Gunn J, Hill MM, Cotten BM, Deer TR. An analysis of biomarkers in patients with chronic pain. Pain Physician. 2020; 23(2):235.
[30] Persson AK, Gebauer M, Jordan S, Metz-Weidmann C, Schulte AM, Schneider HC, et al. Correlational analysis for identifying genes whose regulation contributes to chronic neuropathic pain. Mol Pain. 2009; 5:7.
[31] Assanangkornchai S, Muekthong A, Sam-Angsri N, Pattanasattayawong U. The use of Mitragyna speciosa ("krathom"), an addictive plant, in

Thailand. Subst Use Misuse. 2007; 42(14):2145-57.
[32] Wahyono S, Widowati L, Handayani L, Sampurno OD, Haryanti S, Fauzi F, et al. Kratom, prospek kesehatan dan sosial ekonomi. Jakarta: Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan; 2019.
[33] Stolt AC, Schröder H, Neurath H, Grecksch G, Höllt V, Meyer MR, et al. Behavioral and neurochemical characterization of kratom (Mitragyna speciosa) extract. Psychopharmacology (Berl). 2014; 231(1):13-25.
[34] Anantaworasakul P, Preedalikit W, Anantaworasakul P, Singh S, Intharuksa A, Arunotayanun W, et al. Phytochemical characterization, bioactivities, and nanoparticle-based topical gel formulation development from four Mitragyna speciosa varieties. Gels. 2025; 11(7):494.
[35] Haspi NFZ, Mat So'ad SZ, Arzmi MH, Khatib A. Sequential extraction and phytochemical study of the extracts of Mitragyna speciosa. Malays J Chem. 2025; 27(1):84-91.
[36] Kafo AS, Elsalami RM, Hassan M. Mitragyna speciosa Korth. downregulates macrophage inflammatory responses by inhibiting TLR-4 and increasing IL-10 production. İstanbul J Pharm. 2024; 54(3):350-8.
[37] Berthold EC, Kamble SH, Raju KS, King TI, Popa R, Sharma A, et al. Preclinical pharmacokinetic study of speciociliatine, a kratom alkaloid, in rats using an UPLC-MS/MS method. J Pharm Biomed Anal. 2021; 194:113778.
[38] Ramanathan S, Parthasarathy S, Murugaiyah V, Magosso E, Tan SC, Mansor SM. Understanding the physicochemical properties of mitragynine, a principal alkaloid of Mitragyna speciosa, for preclinical evaluation. Molecules. 2015; 20(3):4915-27.
[39] Decosterd I, Woolf CJ. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain. 2000; 87(2):149-58.
[40] Savastano LE, Laurito SR, Fitt MR, Rasmussen JA, Gonzalez Polo V, Patterson SI. Sciatic nerve injury: a simple and subtle model for investigating many aspects of nervous system damage and recovery. J Neurosci Methods. 2014; 227:166-80.
[41] Elsa L, Yuwono M, Prawita A. Pengembangan metode isolasi dan identifikasi mitragynine dalam daun kratom (Mitragyna speciosa). J Biosains Pascasarjana. 2016; 18(3):191.
[42] Indriani DW, Rahmawati SI, Bayu A, Ahmadi P, Sari AN, Zuraida Z, et al. Serotonin release mediates analgesia via opioidergic system and withdrawal symptoms in chronic kratom extract-treated mice. BMC Complement Med Ther. 2025; 25(1):205.
[43] Palit P, Mandal SC, Bhunia B. Total steroid and terpenoid enriched fraction from Euphorbia neriifolia Linn offers protection against nociceptive-pain, inflammation, and in vitro arthritis model: an insight of mechanistic study. Int Immunopharmacol. 2016; 41:106-15.
[44] McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M, et al. TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci U S A. 2007; 104(33):13525-30.
[45] Razieh Amini Zadeh, Mojtaba Emam Bakhsh, Fariba Sharififar, Rehman NU, Arif M, Esmaeilpour K. Anti-inflammatory and analgesic activity of Hertia intermedia leaf extract in rats. Int J Pharm Integr Health Sci. 2025; 6(1).

Supplementary Table S1

	Sample	VONFREY			IL6
		BASAL	POST CCI	POST KRATOM	
Group K-	1	13,2	6,7	7,3	5,303
	2	9,6	4,8	5,3	6,958
	3	11,5	5,9	6,1	5,047
	4	12,3	7,1	6,6	4,446
	5	10,5	6,3	5,8	5,496
	6	8,8	5,7	6,4	6,544
	7	14,1	7,4	Excluded	
Group K1	1	12,9	7,2	11,1	6,943
	2	8,8	5,4	9,6	5,472
	3	9,6	5,5	8,2	7,586
	4	13,4	6,2	10,3	5,441
	5	12,5	6,4	7,4	5,074
	6	11,3	4,9	10,1	4,476
	7	10,8	5,3	9,8	4,645
Group K2	1	11,7	6,8	16,8	4,645
	2	9,6	5,3	17,9	4,66
	3	12,2	7,2	15,3	4,737
	4	11,4	6,6	19,1	4,019
	5	9,6	5,9	17,6	4,134
	6	13,3	7,6	16,4	5,824
	7	11,6	6,7	16,2	5,38

Group K3	1	13,4	7,9	26,3	3,342
	2	11,1	6,2	23,8	3,327
	3	12,3	5,4	27,3	5,84
	4	9,1	4,3	24,9	5,288
	5	10,9	7,1	25,6	3,728
	6	12,8	7,8	27,2	3,925
	7	13,6	6,1	24,6	3,096