RESEARCH PAPER

Synergistic Analgesic and Anti-Inflammatory Effects of Kratom (Mitragyna speciosa) Leaf Extract as an Adjuvant to Paracetamol in a Rat Neuropathic Pain Model

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Received: 7th Feb, 2026; Revised: 17th Feb 2026; Accepted: 20th Feb, 2026; Available Online: 11th Mar, 2026

ABSTRACT

Neuropathic pain is a chronic condition with a substantial impact on quality of life. An Indonesian multicentre survey reported a neuropathic pain prevalence of 21.8%, exceeding global estimates.[1] Paracetamol is widely used for pain management, but high doses are associated with hepatotoxicity.[2] Kratom (Mitragyna speciosa) contains opioid like and anti inflammatory alkaloids and may serve as an adjuvant to reduce paracetamol dose.[3] C X C motif chemokine ligand 1 (CXCL1) is a pro inflammatory chemokine implicated in neuropathic and inflammatory pain.[4–6] This study evaluated the effect of kratom leaf extract as an adjuvant to paracetamol on mechanical pain threshold and serum CXCL1 levels in a chronic constriction injury (CCI) rat neuropathic pain model.

Twenty eight male Wistar rats (Rattus norvegicus; 2–3 months; 150–250 g) underwent sciatic nerve CCI.[7] Animals were randomised into four groups (n=7): negative control (placebo), paracetamol 10 mg/kg body weight (BW), paracetamol 5 mg/kgBW + kratom extract 30 mg/kgBW, and paracetamol 5 mg/kgBW + kratom extract 60 mg/kgBW. Treatments were administered orally once daily for 7 days. Mechanical pain threshold was assessed with the von Frey test at baseline, 7 days post CCI (pre treatment), and after treatment.[8] Serum CXCL1 was measured by ELISA on day 14. Two animals (one in the paracetamol 10 mg/kgBW group and one in the paracetamol 5 mg/kgBW + kratom 30 mg/kgBW group) died during the experiment and were excluded from post treatment analysis.

CCI significantly reduced von Frey thresholds compared with baseline (mean difference 6.25 ± 1.89 g; $p<0.001$), confirming neuropathic pain. Baseline and post CCI thresholds did not differ between groups. After treatment, thresholds differed significantly among groups ($p<0.001$), increasing in a dose dependent manner from control to the high dose kratom combination, and all pairwise comparisons were significant ($p<0.01$). Serum CXCL1 levels also differed significantly ($p=0.007$), and pairwise analysis showed a significant difference between the two kratom doses ($p=0.001$), indicating dose dependent modulation of CXCL1. Experimental mortality was most likely related to anaesthesia and surgical stress, although idiosyncratic drug reactions cannot be excluded.

In conclusion, kratom leaf extract significantly enhanced the analgesic effect of paracetamol in CCI induced neuropathic rats, with a clear dose–response on mechanical pain threshold and associated changes in CXCL1 levels. The combination of low dose paracetamol with high dose kratom provided superior analgesia compared with full dose paracetamol alone, suggesting potential for paracetamol dose sparing in neuropathic pain management...

Keywords: Neuropathic pain; Mitragyna speciosa; kratom; CXCL1; paracetamol; chronic constriction injury; rat..

How to cite this article: Anindito GPA, Waloejo CS, Airlangga PS, Veterini AS, Susila D, Synergistic Analgesic and Anti-Inflammatory Effects of Kratom (Mitragyna speciosa) Leaf Extract as an Adjuvant to Paracetamol in a Rat Neuropathic Pain Model..Int J Drug Deliv Technol. 2026;16 (5s): 540-547; DOI: 10.25258/ijddt.16.5s.71.

Source of support:. None

Conflict of interest: None

INTRODUCTION

Neuropathic pain is defined as pain arising directly from a lesion or disease affecting the somatosensory nervous system.[9] It is characterised by spontaneous pain, allodynia, hyperalgesia, and various sensory disturbances, and is often accompanied by sleep disturbance, anxiety, depression, and disability.[10] Population-based studies have reported prevalence rates between 3% and 17% worldwide,[11] but a multicentre hospital-based survey in Indonesia reported a prevalence of 21.8%, indicating a substantial burden.[1]

Neuroinflammation is critical in the development and maintenance of neuropathic pain. Chemokines, including CXCL1, are key mediators linking immune responses to nociceptive processing. CXCL1 is a C-X-C motif chemokine that signals mainly via CXCR2 to promote neutrophil recruitment and activation.[4,12] In models of inflammatory and neuropathic pain, CXCL1 is upregulated in peripheral tissues, dorsal root ganglia (DRG), and spinal cord.[4,5,13] In the spinal cord, CXCL1 enhances NMDA receptor activity and induces COX-2 expression via ERK signalling, contributing to central sensitisation and

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persistent pain.[4,13] Targeting the CXCL1/CXCR2 axis has thus been proposed as a potential strategy to attenuate chronic pain.[4–6]

Paracetamol (acetaminophen) is one of the most commonly used analgesics and antipyretic drugs. Its mechanisms include inhibition of central cyclo-oxygenase and the actions of its metabolite, AM404, on TRPV1 and CB1 receptors.[14,15] Although paracetamol is relatively safe at recommended doses, overdose leads to excessive formation of the hepatotoxic metabolite N-acetyl-p-benzoquinone imine (NAPQI) and is a leading cause of acute liver failure.[2,16] High doses or prolonged use further increase hepatotoxicity risk, especially in susceptible individuals.[2] There is therefore a clinical need for strategies that maintain analgesic efficacy while reducing paracetamol exposure.

Kratom (*Mitragyna speciosa* Korth.), a member of the Rubiaceae family, is native to Southeast Asia, including Indonesia, and has been used traditionally for its stimulant and analgesic effects.[3,17] Kratom leaves contain over 40 alkaloids, dominated by the indole alkaloids mitragynine and 7-hydroxymitragynine.[3, 18] Mitragynine acts as a partial agonist at μ-opioid receptors with G-protein–biased signalling and limited β-arrestin-2 recruitment, which may reduce the risk of respiratory depression compared with classical opioids.[19,20] Preclinical studies have demonstrated that kratom extracts and mitragynine increase nociceptive thresholds and reduce allodynia in neuropathic pain models.[21,22] Kratom also exhibits anti-inflammatory and antioxidant effects, including inhibition of pro-inflammatory cytokines and dual COX-2/5-lipoxygenase inhibition.[3,23]

Given this pharmacological profile, kratom leaf extract could be a promising adjuvant to paracetamol, enhancing analgesic effects and allowing dose reduction in neuropathic pain. However, data on kratom–paracetamol combinations and their effects on neuroinflammatory markers such as CXCL1 remain limited.

The present study was designed to evaluate the effect of kratom leaf extract as an adjuvant to paracetamol on mechanical pain threshold and serum CXCL1 levels in a rat model of chronic constriction injury (CCI)–induced neuropathic pain.

MATERIALS AND METHODS

Study design and ethics

This was a true experimental laboratory study with a randomised post-test–only control group design. The study was conducted at the Institute of Tropical Disease and the Faculty of Veterinary Medicine, Universitas Airlangga. The protocol was approved by the Animal Ethics Committee, Faculty of Veterinary Medicine, Universitas Airlangga (Ethical Clearance No. 2.KEH.142.09.2025), and followed institutional guidelines for animal care and use.

Animals

Twenty-eight male Wistar rats (Rattus norvegicus, Wistar strain), aged 2–3 months and weighing 150–250 g, were obtained from the local government animal facility with a veterinary health certificate. Rats were acclimatised for 7 days in pathogen-free cages under controlled environmental conditions (12 h light/12 h dark cycle; lights on 06:00–18:00), with ad libitum access to standard chow and water. Rats were considered healthy based on clinical examination (clear eyes, smooth coat, stable weight).

Inclusion criteria were: male Wistar rats; 2–3 months; 150–250 g; clinically healthy. Exclusion criteria were: death during the protocol; signs of illness before treatment; or failure to develop neuropathic pain (no reduction in von Frey threshold after CCI).

Two animals died during the experimental period: one in the paracetamol 10 mg/kgBW group (K1) and one in the paracetamol 5 mg/kgBW + kratom 30 mg/kgBW group (K2). These animals were excluded from post-treatment analyses.

2.3 Sample size and group allocation

Sample size was calculated using the Lemeshow formula for comparison of means, based on variability in mechanical thresholds and tumour necrosis factor-alpha (TNF-α) reported in a previous rat neuropathic pain study.[24] With α=0.05, power 95% (β=0.05), an expected mean difference of 5 pg/mL and standard deviation 3.5 pg/mL, the minimum required sample was seven rats per group.

Rats were randomly allocated to four groups (n=7):

K− (negative control): CCI + placebo (1% carboxymethyl cellulose sodium, CMC-Na)

K1: CCI + paracetamol 10 mg/kgBW

K2: CCI + paracetamol 5 mg/kgBW + kratom extract 30 mg/kgBW

K3: CCI + paracetamol 5 mg/kgBW + kratom extract 60 mg/kgBW

Randomisation was performed using simple random allocation. At the end of the study, usable sample sizes for post-treatment analyses were: K− (n=7), K1 (n=6), K2 (n=6), K3 (n=7).

2.4 Preparation of kratom extract

Fresh kratom leaves were collected from trees aged ≥2 years in Kalimantan, washed and shade-dried to <10% moisture, then milled into powder. Thirty grams of powder were macerated in 150 mL of 70% ethanol at room temperature for 24 hours with intermittent stirring, then filtered. The filtrate was concentrated under reduced pressure using a rotary evaporator to yield a crude ethanolic extract (EK). LC–high-resolution mass spectrometry confirmed the presence of mitragynine and related indole alkaloids consistent with previous reports.[23,25]

For oral administration, the extract was freshly suspended in 1% CMC-Na to achieve doses of 30 or 60 mg/kgBW.

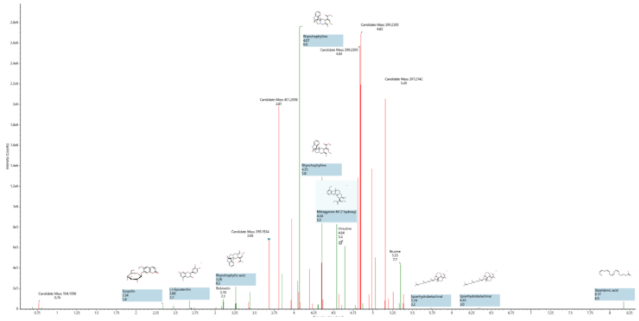

Fig 1. Total Ion Chromatogram (TIC) showing separation of alkaloids and other phytochemicals.

Metabolic profiling of the kratom leaf extract was conducted using liquid chromatography–tandem mass spectrometry (LC–MS/MS). To prepare the sample, the extract was dissolved in methanol, filtered, and then analyzed with a high-resolution mass spectrometer in both positive and negative electrospray ionization modes. Data were collected over an m/z range of 100–1,000 and compared to a reference library of kratom alkaloids (figure 1). This analysis confirmed the presence of major indole alkaloids, including mitragynine and 7-hydroxymitragynine, as well as minor constituents such as speciociliatine, paynantheine, and speciogynine. Overall, these results demonstrate that the extract contained the typical alkaloid profile of Mitragyna speciosa leaves.

2.5 Preparation of paracetamol suspension

Paracetamol tablets were finely powdered. An amount corresponding to 100 mg of paracetamol was triturated with a small volume of 1% CMC-Na and brought to 10 mL with 1% CMC-Na, producing a 10 mg/mL suspension. Doses of 5 or 10 mg/kgBW were calculated from body weight and administered orally.

2.6 Induction of neuropathic pain (chronic constriction injury)

Neuropathic pain was induced using the chronic constriction injury (CCI) model of the sciatic nerve.[7] Under anaesthesia with ketamine/xylazine/acepromazine (90/10/1 mg/kg, intraperitoneal), the left sciatic nerve was exposed at the mid-thigh level via a small incision. Three to four loose ligatures of 5-0 silk were placed around the sciatic nerve at 1 mm intervals, tight enough to mildly constrict the nerve without occluding epineurial blood flow. The muscle and skin were sutured in layers. Rats were placed in warmed cages and monitored until recovery.

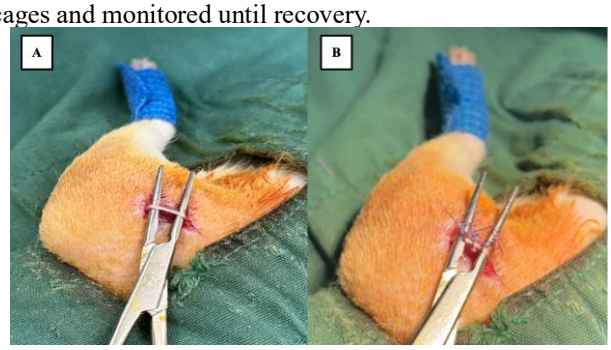

Fig 2. Sciatic Nerve on Rat (A), Nerve ligation to make Chronic Constriction Injury Models (B)

2.7 Treatment schedule

The protocol was:

Day 0: acclimatisation completed; baseline von Frey test; CCI surgery.

Day 7: von Frey test to confirm neuropathic pain (post-CCI, pre-treatment).

Days 7–13: once-daily oral administration of assigned treatments.

Day 14: von Frey test after treatment; deep anaesthesia and sacrifice; blood collection for CXCL1.

Treatments were administered between 08:00 and 16:00.

2.8 Mechanical pain assessment (von Frey test)

Mechanical pain threshold was assessed using von Frey filaments as described by Deuis et al.[8] Rats were placed individually in transparent cages on an elevated wire mesh platform and acclimatised for 60 minutes.

The plantar surface of the ipsilateral hind paw (CCI side) was stimulated:

A 0.6 g filament was applied perpendicularly until it just bent, for 1–2 seconds.

If no withdrawal, licking or shaking occurred, the next higher filament was tested; if a positive response occurred, the next lower filament was applied.

Each paw was tested five times with ≥2-minute intervals.

Mechanical threshold (g) was determined using the up-down method, with higher values indicating higher nociceptive threshold (lower mechanical hypersensitivity). Measurements were taken at baseline (day 0), post-CCI (day 7) and post-treatment (day 14).

2.9 Measurement of serum CXCL1

On day 14, rats were deeply anaesthetised and sacrificed by cardiac puncture. Blood was collected into plain tubes, allowed to clot and centrifuged at 6,000 g for 15 minutes at 4°C. Serum was stored at −20°C until assay.

Serum CXCL1 levels were measured using a rat CXCL1 ELISA kit (E2350Ra, BT Lab, USA) following the manufacturer's protocol. Absorbance was measured at 450 nm using a microplate reader (Mark™ 1775, BT Laboratories, Inc.), and CXCL1 concentrations were derived from a standard curve.

2.10 Statistical analysis

Statistical analysis was performed using SPSS version 23. Data are presented as mean ± standard deviation (SD) or median (range) as appropriate. Normality was assessed with the Shapiro–Wilk test and homogeneity of variance with Levene's test.

Baseline and post-CCI von Frey thresholds: one-way ANOVA for intergroup differences; paired t-test for baseline vs post-CCI.

Post-treatment von Frey thresholds: one-way ANOVA with Scheffe post hoc tests.

CXCL1: Kruskal–Wallis test for intergroup differences; Mann–Whitney U test for pairwise comparisons.

A p-value <0.05 was considered statistically significant.

3. RESULTS

3.1 Result

The **Von Frey test** was performed to evaluate the mechanical pain threshold in experimental animals before treatment (baseline), after induction of neuropathic pain through *chronic constriction injury* (CCI), and after intervention with paracetamol and kratom (*Mitragyna speciosa*) leaf extract in various combination doses. At the **baseline phase,** the mean mechanical pain thresholds were relatively homogeneous among the four groups: control group 12.73 ± 1.17, K1 12.43 ± 1.19, K2 12.66 ± 1.77, and K3 12.89 ± 1.67. One-way ANOVA analysis showed no significant differences between groups (p = 0.95). After **CCI induction** (Von Frey post-CCI), a decrease in the mean Von Frey value was observed across all groups, indicating successful establishment of the neuropathic pain model.

Table 1. Von Frey Descriptive and Analysis

Variable	N	Von Frey Baseline Mean ± SD	N	Von Frey post CCI Mean ± SD	N	Von Frey post Intervention Mean ± SD
Control	7	12.73 ± 1.17	7	5.64 ± 1.54	7	6.09 ± 1.88
K1	7	12.43 ± 1.19	7	6.89 ± 1.70	6	11.90 ± 1.24
K2	7	12.66 ± 1.77	7	6.53 ± 1.80	7	19.10 ± 3.59
K3	7	12.89 ± 1.67	7	6.64 ± 1.44	6	25.52 ± 2.11
*P value**		0.95		0.52		<0.001

K1 : Group receiving paracetamol 10 mg/kgBW; K2 : Group receiving kratom leaf extract 30 mg/kgBW and paracetamol 5 mg/kgBW; K3 : Group receiving kratom leaf extract 60 mg/kgBW and paracetamol 5 mg/kgBW.
*One-way ANOVA *Significant if p < 0.05.*

The mean Von Frey scores were 5.64 ± 1.54 in the control group, 6.89 ± 1.70 in K1, 6.53 ± 1.80 in K2, and 6.64 ± 1.44 in K3. No significant difference was found among groups at this stage (p = 0.52). Following **treatment intervention,** there was a marked increase in the mechanical pain threshold, particularly in groups receiving the combination of kratom leaf extract and paracetamol. The mean Von Frey scores increased to 6.09 ± 1.88 in the control group, 11.90 ± 1.24 in K1, 19.10 ± 3.59 in K2, and reached the highest level in K3 (25.52 ± 2.11). One-way ANOVA analysis demonstrated a highly significant difference among groups (p < 0.001).

Measurement of **CXC Motif Chemokine Ligand 1 (CXCL1)** levels was performed to assess the inflammatory response in rats with neuropathic pain after administration of the combination of kratom leaf extract and paracetamol. The mean CXCL1 levels in the control group were 121.36 ± 73.29 pg/mL, while the treatment groups showed distinct variations: 109.63 ± 35.50 pg/mL in K1 (paracetamol 10 mg/kgBW), 66.80 ± 25.42 pg/mL in K2 (kratom leaf extract 30 mg/kgBW + paracetamol 5 mg/kgBW), and 259.81 ± 143.55 pg/mL in K3 (kratom leaf extract 60 mg/kgBW + paracetamol 5 mg/kgBW).

Table 2. CXCL1 Levels Descriptive and Analysis

Variabel	N	Mean ± SD	*P value**	Comparison	*P value*#*
Control	7	121.36 ± 73.29	0.007	K1	0.945
				K2	0.073
				K3	0.073
K1	6	109.63 ± 35.50		K2	0.035
				K3	0.041
K2	7	66.80 ± 25.42		K3	0.001
K3	6	259.81 ± 143.55			
Total	26	108.65 ± 77.83			

K1 : Group receiving paracetamol 10 mg/kgBW; K2 : Group receiving kratom leaf extract 30 mg/kgBW and paracetamol 5 mg/kgBW; K3 : Group receiving kratom leaf extract 60 mg/kgBW and paracetamol 5 mg/kgBW.
**Kruskal Wallis #Mann-whitney, Exact Sig, Significant if p < 0.05*

Statistical analysis using the **Kruskal–Wallis test** showed a significant difference among groups (p = 0.007), indicating that administration of kratom–paracetamol combinations significantly affected serum CXCL1 levels. Further analysis with the **Mann–Whitney test** revealed that the CXCL1 level in K2 was significantly different from K1 (p = 0.035) and K3 (p = 0.041). Moreover, a highly significant difference was also found between K2 and K3 (p < 0.001).

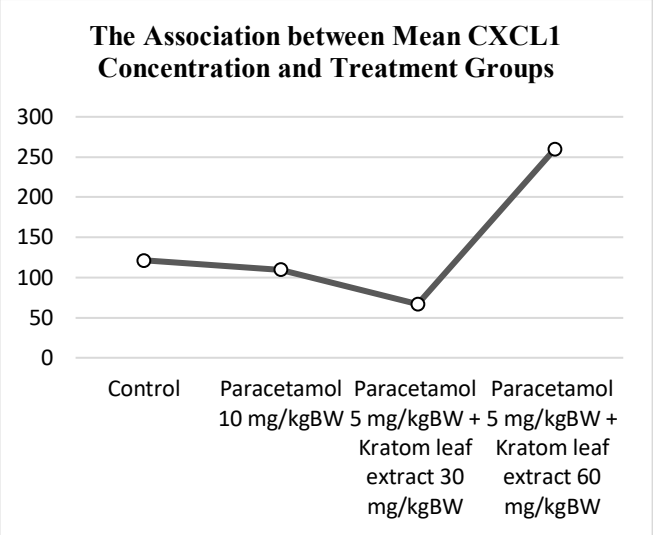

Fig 3. Graph Association between Mean CXCL1 and Treatment Groups

The graph in Figure 3 illustrates the comparison of mean serum CXCL1 levels among the treatment groups. Descriptively, the highest mean CXCL1 level was observed in the group receiving kratom leaf extract at a dose of 60 mg combined with 5 mg of paracetamol, with a mean value of 259.81 pg/mL. Conversely, the lowest CXCL1 level was found in the group treated with kratom leaf extract at a dose of 30 mg combined with 5 mg of paracetamol, with a mean value of 66.80 pg/mL.

Experimental mortality

One rat in K1 (paracetamol 10 mg/kgBW) and one rat in K2 (paracetamol 5 mg/kgBW + kratom 30 mg/kgBW) died during the treatment period and were excluded from post-treatment analyses. Both animals had successfully undergone CCI surgery and survived to the treatment phase. No gross signs of severe respiratory distress, convulsions or profuse bleeding were observed before death; necropsy and histopathology were not performed.

DISCUSSION

This study demonstrated that kratom leaf extract significantly enhanced the analgesic effect of paracetamol in a rat model of chronic constriction injury (CCI)–induced neuropathic pain. The combination of low-dose paracetamol (5 mg/kgBW) with high-dose kratom (60 mg/kgBW; K3) produced the greatest increase in mechanical pain threshold, outperforming full-dose paracetamol (10 mg/kgBW; K1), and thus suggests a paracetamol-sparing effect. At the same time, kratom modulated serum CXCL1 levels in a dose-dependent but non-linear manner, with the highest CXCL1 levels observed in K3 despite the best behavioural analgesia.

4.1 Analgesic effects of kratom–paracetamol combinations

The dose-dependent improvement in von Frey thresholds is consistent with the known pharmacology of kratom alkaloids. Mitragynine and 7-hydroxymitragynine are indole alkaloids that act as μ-opioid receptor agonists with G-protein–biased signalling, producing strong antinociception with reduced β-arrestin-2 recruitment and potentially lower risk of respiratory depression compared with classical opioids.[3, 18–20] Preclinical work has shown that kratom extracts and mitragynine increase pain thresholds and reduce mechanical allodynia in several models, including neuropathic pain.[21,22] In addition to opioid mechanisms, kratom exhibits anti-inflammatory and antioxidant effects, including suppression of TNF-α, IL-6, and COX-2/5-LOX pathways, which may contribute to attenuation of peripheral and central sensitisation.[3, 23]

Paracetamol acts centrally through multiple mechanisms, including inhibition of cyclo-oxygenase and actions of its metabolite AM404 at TRPV1 and CB1 receptors in pain-modulating regions such as the spinal cord and brainstem.[14,15] The combination of kratom and paracetamol therefore provides multimodal analgesia, engaging opioid, monoaminergic, endocannabinoid, and anti-inflammatory mechanisms. This likely underlies the clear dose–response pattern observed in von Frey thresholds, with the kratom–paracetamol combinations (K2 and K3) outperforming paracetamol alone (K1).

4.2 CXCL1 modulation and neuroinflammation

CXCL1 is a C-X-C chemokine that signals primarily via CXCR2 on neutrophils and other cells.[4,12] Elevated CXCL1 has been reported in models of inflammatory and neuropathic pain, where it contributes to pain via recruitment of immune cells, sensitisation of primary afferent neurons, and facilitation of spinal central sensitisation through NMDA receptor activation and COX-2 upregulation.[4,5,12,13] In this study, CXCL1 levels differed significantly between groups, and the lower-dose kratom combination (K2) tended to reduce CXCL1 relative to paracetamol alone, consistent with an anti-inflammatory effect.[3,23]

However, the highest kratom dose (K3) showed the highest median CXCL1 levels, despite producing the greatest behavioural analgesia. At first glance, this appears paradoxical. Several factors may help reconcile these findings.

First, CXCL1 is only one element within a complex chemokine network, and its systemic concentration may not linearly correlate with pain behaviour when strong central analgesia is present. In K3, kratom's opioid and non-opioid mechanisms may effectively suppress nociceptive signalling and central sensitisation, overshadowing any pronociceptive contribution of CXCL1. Second, CXCL1 is closely linked to neutrophil biology and neutrophil extracellular trap (NET) formation, thereby introducing important positive feedback loops.

4.3 CXCL1–CXCR2 signalling, neutrophils and NETs

Neutrophils are the most abundant circulating leukocytes and play a critical role in innate immunity. Upon activation, they can release neutrophil extracellular traps (NETs), which are web-like structures composed of decondensed chromatin decorated with histones and granular proteins.[67] NET formation (NETosis) can be induced by a variety of stimuli, including chemokines that signal through CXCR1

and CXCR2, such as CXCL1 (murine homolog of human GRO-α and IL-8), CXCL2, and CXCL8. [24,29,31]

Activation of CXCR1/2 on neutrophils triggers intracellular pathways involving calcium mobilisation, PKC and MAPKs, leading to NADPH oxidase activation, reactive oxygen species (ROS) production and, in some settings, PAD4-dependent NETosis.[24,29,31] In models of sepsis and acute lung injury, high levels of CXCR1/2 ligands (e.g., IL-8, MIP-2, CXCL1) strongly drive NETosis, and pharmacological inhibition of CXCR1/2 reduces NET formation and improves outcomes.[19,26] Similarly, tumour-derived CXCR1/2 agonists can promote NETosis, and NETs in turn reinforce a pro-inflammatory microenvironment and interfere with antitumour immunity.[25,27]

Importantly, NETs do not merely reflect downstream neutrophil activation; they can themselves amplify inflammation by stimulating surrounding cells (e.g., endothelial cells, epithelial cells, macrophages) to produce additional chemokines, including CXCL1, CXCL2, and CXCL8, thereby attracting a second wave of neutrophils.[20,21,24] In vitro, NETs have been shown to induce airway epithelial cells to secrete CXCL1, CXCL2, and CXCL8, creating a self-perpetuating chemokine loop.[21] This bidirectional relationship suggests that high CXCL1 may both drive and be driven by NET formation

.

4.4 Possible NET-linked explanation for higher CXCL1 in K3

In our study, the K3 group displayed the best behavioural analgesia but also the highest median CXCL1 level. One plausible mechanistic hypothesis is that higher kratom doses modulate immune responses in a way that favours increased NETosis and chemokine feedback without necessarily enhancing nociceptive signalling. Kratom's dual COX-2/5-LOX inhibitory and antioxidant effects may blunt nociceptor sensitisation and spinal plasticity,[28,29] while at the same time systemic immune cells (including neutrophils) remain responsive to chemokine cues, potentially producing more NETs under conditions of tissue injury and low-grade inflammation.

In this scenario, elevated CXCL1 in K3 could reflect an active CXCL1–CXCR2–NET axis: CXCL1 recruits and activates neutrophils via CXCR2; activated neutrophils form NETs; NETs then stimulate resident cells to release additional CXCL1 and other chemokines; and the resulting chemokine milieu is detectable in serum.[30-34] While NETs can exacerbate tissue damage and amplify inflammation in many diseases, the net effect on pain behaviour depends on the balance between peripheral inflammatory signalling and central analgesic mechanisms. In K3, kratom-induced central and peripheral analgesia may dominate, so that increased CXCL1 and possible NET activity do not translate into higher mechanical hypersensitivity.

This interpretation remains hypothetical, as NET markers (e.g., citrullinated histone H3, MPO-DNA complexes) were not measured in this study. Nonetheless, it is consistent with the broader literature showing that CXCL1/CXCR2 signalling is a major driver of NETosis in various inflammatory and neoplastic conditions and that NETs can form chemokine-rich, self-amplifying inflammatory niches.[34,35,36] Future studies should directly assess NET formation and related pathways to clarify the relationship between kratom dosage, CXCL1 levels, NETosis, and neuropathic pain.

4.5 Experimental mortality and safety considerations

Two rats (one in K1 and one in K2) died during the experiment and were excluded from post-treatment analyses. Experimental mortality in rodent studies involving major surgery and general anaesthesia is not uncommon and may be related to anaesthetic complications, cardiovascular or respiratory depression, hypothermia, inadequate analgesia, dehydration, infection, or underlying vulnerability.21,23,25,28 Combinations of ketamine and xylazine, especially with additional sedatives, can cause bradycardia, hypotension, and respiratory depression and have been associated with increased mortality in rodents, particularly in aged or stressed animals.[21,23,26]

In the present study, no gross signs of severe kratom toxicity such as seizures, prolonged coma, or profuse diarrhoea were observed prior to death, and the kratom doses used fall within ranges reported to be analgesic in rats without overt acute toxicity.[23,27,30] Kratom-related fatalities in humans are typically associated with high doses, polydrug use, and comorbidities.[24, 27-29] Although idiosyncratic drug reactions cannot be excluded, anaesthetic and surgical factors are the most plausible contributors to the observed mortality. To minimise such events, future studies should incorporate stricter perioperative monitoring and supportive care protocols, including temperature maintenance, fluid support, and postoperative analgesia.[34,35,36]

Strengths and limitations

Strengths of this work include the use of a well-validated neuropathic model (CCI), objective behavioural assessment of mechanical allodynia, and measurement of a mechanistically relevant chemokine biomarker (CXCL1). The randomised design and inclusion of two kratom doses allow assessment of dose–response relationships for both analgesic and inflammatory outcomes.

Limitations include the relatively small sample sizes per group, the use of only male rats and a single neuropathic model, and the measurement of only one chemokine. NET markers and other key cytokines (e.g. TNF-α, IL-1β, IL-6, CCL2) were not evaluated, precluding a more detailed understanding of the immunological effects of kratom. Histopathological evaluation of tissues from deceased animals was not performed. Finally, direct extrapolation of doses and safety from rats to humans is not appropriate without further pharmacokinetic and toxicological studies.

CONCLUSION

Kratom leaf extract significantly enhanced the analgesic effect of paracetamol in a rat model of CCI-induced neuropathic pain. The combination of low-dose paracetamol (5 mg/kgBW) with high-dose kratom (60 mg/kgBW) provided superior analgesia compared with full-dose paracetamol alone, indicating a paracetamol-sparing effect.

Kratom also modulated serum CXCL1 levels in a dose-dependent but non-linear manner, suggesting complex effects on neuroinflammatory pathways. Two deaths occurred (one in the paracetamol-only group and one in the lower-dose kratom group), most likely related to anaesthetic and surgical factors.

Kratom leaf extract appears to be a promising adjuvant candidate in neuropathic pain management, but translation to clinical practice must await further mechanistic and safety studies.

ACKNOWLEDGEMENTS

The author thanks the staff of the Institute of Tropical Disease and the Veterinary Hospital, Universitas Airlangga, and the Department of Anesthesiology and Intensive Therapy, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Hospital, for their technical assistance and institutional support.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this article.

FUNDING

This research was funded by the Directorate of Research and Community Service, Ministry of Higher Education, Science, and Technology (No. SP DIPA-139.04.1.693320/2025), through Universitas Airlangga (No. 2308/B/UN3.LPPM/PT.01.03/2025) by the Regular Fundamental Research Scheme 2025.

ETHICAL APPROVED

Ethical clearance was obtained from the Ethics Committee for Basic and Clinical Research, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, prior to the initiation of the study (Ethic number : 2.KEH.142.09.2025)

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