# Nano-Kratom Leaf Suspension Versus Tramadol for Burn Pain: Effects on Mechanical Withdrawal Threshold and Glutamate Levels in Rats

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**Received:** 26 January 2026; **Revised:** 31 January 2026; **Accepted:** 4 February 2026; **Published:** 25 February 2026

**Abstract**

**Background**: Burn pain involves nociceptive, inflammatory, and central sensitization mechanisms, including increased glutamatergic activity. Although tramadol is commonly used as an analgesic, its adverse effects and dependence potential support the development of alternative therapies. Nano-formulated kratom leaf (Mitragyna speciosa) may improve the delivery and bioavailability of its active alkaloids. This study compared the effects of nano-kratom leaf suspension and tramadol on mechanical pain threshold and glutamate levels in a rat model of burn pain.

**Methods**: This randomized post-test-only controlled experimental study included 15 male Wistar rats aged 4–6 weeks and weighing 140–180 g. The animals were randomly allocated into control, tramadol, and nano-kratom leaf suspension groups. A second-degree burn was induced on the right hind paw by immersion in water at 65°C for 3 seconds. Treatments were administered orally. Mechanical pain threshold was evaluated using the von Frey test, and glutamate levels were measured using enzyme-linked immunosorbent assay. Data were analyzed using one-way analysis of variance, followed by Tukey's honestly significant difference post hoc test.

**Results**: Baseline von Frey values were comparable among the groups (p=0.383). At 24 hours after treatment, von Frey values differed significantly among the control, tramadol, and nano-kratom groups, with values of 3.60±0.96, 9.59±1.29, and 13.78±2.70, respectively (p=0.001). Glutamate levels were also significantly different, measuring 6.22±0.82 ng/mL in the control group, 3.84±0.45 ng/mL in the tramadol group, and 1.78±0.51 ng/mL in the nano-kratom group (p=0.001). Glutamate levels were strongly and negatively correlated with 24-hour von Frey values (r=−0.911; p=0.001).

**Conclusion**: Nano-kratom leaf suspension produced a higher mechanical pain threshold and lower glutamate levels than tramadol in a rat model of burn pain. These findings support further preclinical investigation of nano-formulated kratom as a potential analgesic intervention for burn pain.

***Keywords****: burn pain, glutamate, Mitragyna speciosa, nano-kratom, tramadol, von Frey*

**How to cite this article:** de Lima FVI, Airlangga PS, Veterini AS, Santoso KH, Susila D. Nano-Kratom Leaf Suspension Versus Tramadol for Burn Pain: Effects on Mechanical Withdrawal Threshold and Glutamate Levels in Rats. Int J Drug Deliv Technol. 2026;16(70s): 780-788. DOI: 10.25258/ijddt.16.70s.80

## Introduction

Burn injuries are significant physical traumas caused by thermal exposure, radiation, chemicals, electricity, or friction, with most cases resulting from contact with hot liquids, solid objects, or flames. These injuries cause tissue damage through energy transfer and trigger complex physiological and pathophysiological responses (Jeschke et al., 2020). Disruption of the skin barrier increases the risk of fluid loss, impaired thermoregulation, and infection. Burn injuries also induce excessive inflammatory responses and oxidative stress, which may delay tissue regeneration and increase the risk of clinical complications (Treesh et al., 2020; Żwierełło et al., 2023).

Despite advances in burn management, including wound excision and skin grafting, pain control remains a major clinical challenge (WHO, 2023). Burn pain is multidimensional and may involve thermal, mechanical, and chemical nociceptive stimuli (Chinchilla et al., 2022). Tissue injury activates nociceptors and promotes the release of excitatory neurotransmitters, including glutamate and substance P, which transmit nociceptive signals to the central nervous system. The release of inflammatory mediators, such as prostaglandins and bradykinin, further increases nociceptor sensitivity and intensifies pain responses (Hettiaratchy et al., 2024). Persistent inflammation, infection, delayed wound healing, and hypertrophic scar formation may further aggravate pain during recovery (Kaddoura et al., 2017; Wang et al., 2017). Moreover, simultaneous peripheral sensory nerve injury and inflammation may contribute to the transition from acute nociceptive pain to chronic pain with neuropathic characteristics. Burn-related procedures, including dressing changes, wound excision, skin grafting, and physical therapy, may also produce pain that is more severe than the pain caused by the initial injury (Morgan et al., 2018). Inadequately controlled pain can impair wound healing, disrupt sleep, reduce quality of life, and increase the risk of anxiety and post-traumatic stress, emphasizing the importance of effective pain management in burn care (Griggs et al., 2017; Romanowski et al., 2020).

Opioids remain effective for the management of burn pain; however, their use is limited by adverse effects, including tolerance, physical dependence, respiratory depression, and gastrointestinal disturbances. These limitations have increased the need for safer analgesic strategies that reduce opioid exposure without compromising pain control (Ciornei B et al., 2024). Tramadol is a synthetic opioid analgesic that reduces central pain perception through a dual mechanism involving opioid receptor agonism and inhibition of serotonin and norepinephrine reuptake. Although tramadol provides effective analgesia, prolonged or high-dose use may cause nausea, vomiting, vertigo, and dependence, supporting the investigation of alternative analgesic agents (Cannon B et al., 2020).

Kratom (Mitragyna speciosa) is a medicinal plant native to Southeast Asia, including Indonesia, Malaysia, and Thailand (Hassan Z et al., 2013). Its leaves have traditionally been used for wound treatment, fever, muscle pain, appetite suppression, and diarrhea. Kratom has also demonstrated antinociceptive and antidepressant effects (Cheaha et al., 2015). At higher doses, it produces opioid-like analgesic and anti-inflammatory effects and has also been used to reduce symptoms of opioid withdrawal (Parthasarathy et al., 2015). However, the therapeutic application of herbal compounds may be limited by poor water solubility, low bioavailability, inefficient absorption, and limited stability (Parthasarathy et al., 2015).

Nanotechnology may address these limitations by reducing particle size, increasing surface area, improving solubility and dissolution, and enhancing the absorption and bioavailability of active compounds (Lie Y et al., 2024). Nano-based delivery systems may also improve compound stability and facilitate controlled or sustained drug release, potentially allowing therapeutic effects to be achieved at lower doses (Kathole KS., 2025). Nevertheless, the analgesic effects of nano-formulated kratom and its relationship with glutamatergic activity in burn pain have not been adequately established. Therefore, this study aimed to compare the effects of nano-kratom leaf suspension and tramadol on mechanical pain threshold and glutamate levels in a rat model of burn pain.

## Methods

### *Study Design and Ethical Approval*

This study was a true experimental laboratory investigation using a randomized post-test-only control group design. The study was conducted at the Institute of Tropical Disease Laboratory, Universitas Airlangga, Surabaya, Indonesia. Ethical approval for the use of experimental animals was obtained from the relevant ethics committee at the Faculty of Veterinary Medicine, Universitas Airlangga, before the study was conducted.

### *Experimental Animals*

Fifteen healthy male Wistar rats (Rattus norvegicus), aged 4–6 weeks and weighing 140–180 g, were included. The animals were obtained from the Faculty of Veterinary Medicine, Universitas Airlangga. Rats were considered eligible when they were male Wistar rats, were within the predefined age and weight ranges, and were declared physically healthy by a veterinarian. Animals that became ill or died during the study were excluded.

Before the experiment, the rats were acclimatized for 14 days in clean cages with adequate access to food. Their health status was confirmed before initiation of the experimental procedures. The animals were then randomly allocated into three groups of five rats each: a control group, a tramadol group, and a nano-kratom leaf suspension group.

***Preparation of Tramadol Suspension***

Tramadol tablets were ground into a fine powder and suspended in carboxymethylcellulose sodium solution. The suspension was prepared so that 1 mL contained 10 mg of tramadol. Tramadol was administered orally at a dose of 50 mg/kg body weight. Based on the body weight range of the animals, each rat received approximately 7–9 mg of tramadol.

***Preparation of Nano-Kratom Leaf Suspension***

Kratom leaves were collected from trees aged at least two years. The leaves were harvested in the morning, dried until their moisture content was below 10%, ground, and stored in a closed container protected from direct light.

Mitragynine was separated from the crude kratom leaf extract using chromatographic methods and characterized using gas chromatography–mass spectrometry. The kratom preparation was combined with a solid lipid nanoparticle delivery system produced using a microemulsion technique. The mixture, consisting of a low-melting-point fatty acid, polysorbate 20, butanol, and water, was stirred at 65–70°C. The resulting microemulsion was subsequently dispersed into cold water at 2–3°C while being stirred to facilitate lipid crystallization and minimize particle aggregation. The nano-kratom leaf suspension was administered orally at a dose of 15 mg/kg body weight.

***Burn Injury Model***

The animals were anesthetized using a rat anesthetic cocktail consisting of ketamine, xylazine, and acepromazine at doses of 60, 7.5, and 1 mg/kg body weight, respectively. Each rat was placed in the supine position, and the right hind paw was immersed in water maintained at 65°C for 3 seconds to induce a second-degree burn injury. This procedure produced a burn involving less than 1% of the total body surface area.

***Experimental Intervention***

Following induction of the burn injury, the animals received the intervention according to their assigned group. The control group received no active analgesic treatment, the tramadol group received oral tramadol at 50 mg/kg body weight, and the nano-kratom group received oral nano-kratom leaf suspension at 15 mg/kg body weight. Outcomes were evaluated 24 hours after treatment.

***Mechanical Pain Threshold Assessment***

Mechanical pain sensitivity was evaluated using an electronic von Frey apparatus. A blunt von Frey filament was applied to the plantar surface of the affected hind paw with gradually increasing force. A positive response was defined as rapid withdrawal of the paw or an immediate flinching movement during or immediately after filament application.

The force at which paw withdrawal occurred was automatically recorded by the device and expressed as the von Frey value. A higher von Frey value indicated a higher mechanical withdrawal threshold and lower mechanical pain sensitivity, whereas a lower value indicated greater pain sensitivity. Measurements were performed at baseline, after burn induction, and 24 hours after treatment.

***Glutamate Measurement***

At 24 hours after treatment, the rats were anesthetized and euthanized. Brain and spinal cord tissues were collected, homogenized, and processed to obtain tissue supernatants for protein extraction. Glutamate concentrations were measured using an ELISA kit, 1775×Mark™ with catalog number RE10060, Reed Biotech, according to the manufacturer's instructions.

Absorbance was measured using a microplate reader at a wavelength of 450 nm. Glutamate concentrations were reported in ng/mL.

***Statistical Analysis***

Data were initially entered into Microsoft Excel and subsequently analyzed using SPSS Statistics. Continuous variables were summarized using the mean, standard deviation, median, and range, as appropriate.

Data distribution was assessed using the Shapiro–Wilk test. Normally distributed data were compared among the three groups using one-way analysis of variance. Tukey's honestly significant difference test was used for post hoc pairwise comparisons when the overall analysis showed a significant difference. Non-normally distributed data were planned to be analyzed using the Kruskal–Wallis test.

The association between glutamate concentrations and 24-hour von Frey values was assessed using Pearson's correlation analysis for normally distributed data or Spearman's correlation analysis for non-normally distributed data. Statistical significance was defined as a two-sided p-value of <0.05.

**Results**

A total of 15 male Wistar rats were included in the final analysis and randomly allocated into three groups, with five animals in each group: control, tramadol, and nano-kratom leaf suspension.

***Mechanical Withdrawal Threshold***

Baseline von Frey values were comparable among the three groups. The mean baseline values were 12.48 ± 1.23 in the control group, 12.44 ± 1.23 in the tramadol group, and 13.32 ± 0.85 in the nano-kratom group. There was no significant difference among the groups at baseline (p = 0.383), indicating similar mechanical sensitivity before burn induction (Table 1).

| Outcome | Control group (n = 5) | Tramadol group (n = 5) | Nano-kratom group (n = 5) | p-value |
|---|---|---|---|---|
| **Baseline von Frey value** | 12.48 ± 1.23 | 12.44 ± 1.23 | 13.32 ± 0.85 | 0.383 |
| **Post-burn von Frey value** | 4.24 ± 0.85 | 4.00 ± 0.79 | 4.17 ± 1.38 | 0.916 |
| **Von Frey value at 24 hours after treatment** | 3.60 ± 0.96 | 9.59 ± 1.29 | 13.78 ± 2.70 | 0.001 |
| **Glutamate level, ng/mL** | 6.22 ± 0.82 | 3.84 ± 0.45 | 1.78 ± 0.51 | <0.001 |

Table 1. Mechanical withdrawal threshold and glutamate levels across the study groups

Following induction of the second-degree burn and before treatment administration, the mean von Frey values decreased to 4.24 ± 0.85 in the control group, 4.00 ± 0.79 in the tramadol group, and 4.17 ± 1.38 in the nano-kratom group. No significant difference was observed among the groups at this time point (p = 0.916) (Table 1). Paired comparisons between baseline and post-burn measurements showed significant reductions in von Frey values in all groups, with mean differences of 8.24 ± 1.90 in the control group, 8.48 ± 0.34 in the tramadol group, and 9.32 ± 1.33 in the nano-kratom group (all p < 0.001), confirming successful induction of mechanical hypersensitivity.

At 24 hours after treatment, the mean von Frey value was 3.60 ± 0.96 in the control group, 9.59 ± 1.29 in the tramadol group, and 13.78 ± 2.70 in the nano-kratom group. One-way ANOVA demonstrated a significant difference among the three groups (p = 0.001) (Table 1). Tukey's post hoc analysis showed significant differences between the control and tramadol groups (p < 0.001), between the control and nano-kratom groups (p < 0.001), and between the tramadol and nano-kratom groups (p = 0.010) (Table 2).

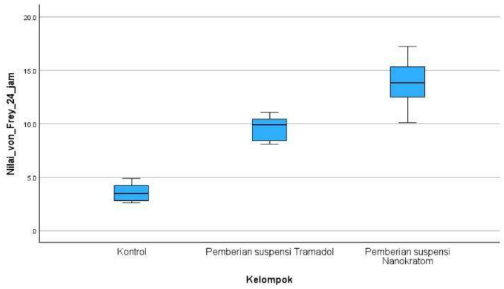
| Outcome | Pairwise comparison | p-value |
|---|---|---|
| **Von Frey value at 24 hours** | Control vs tramadol | <0.001 |
| | Control vs nano-kratom | <0.001 |
| | Tramadol vs nano-kratom | 0.010 |
| **Glutamate level** | Control vs tramadol | <0.001 |
| | Control vs nano-kratom | <0.001 |
| | Tramadol vs nano-kratom | <0.001 |

Table 2. Pairwise comparisons of 24-hour von Frey values and glutamate levels

The pattern of change across the three assessment points is presented in Figure 1. Von Frey values decreased after burn induction in all groups. At 24 hours, the control group showed a further reduction, whereas the tramadol and nano-kratom groups showed increased mechanical withdrawal thresholds, with the highest mean value observed in the nano-kratom group.

Von Frey values at 24 hours after treatment across the experimental groups.

Within-group analysis showed that the control group had a mean post-burn minus 24-hour difference of 0.64 ± 0.40, with a 95% confidence interval of 0.13




to 1.14 (p = 0.013). The corresponding mean differences were −5.66 ± 1.40 in the tramadol group, with a 95% confidence interval of −7.46 to −3.85 (p < 0.001), and −9.70 ± 2.66 in the nano-kratom group, with a 95% confidence interval of −13.00 to −6.46 (p < 0.001).

***Glutamate Levels***

The mean glutamate concentration was 6.22 ± 0.82 ng/mL in the control group, 3.84 ± 0.45 ng/mL in the tramadol group, and 1.78 ± 0.51 ng/mL in the nano-kratom group. One-way ANOVA showed a significant difference among the three groups (p < 0.001) (Table 1).

Post hoc analysis demonstrated significant differences between the control and tramadol groups, between the control and nano-kratom groups, and between the tramadol and nano-kratom groups (all p < 0.001) (Table 2). The lowest glutamate concentration was observed in the nano-kratom group.

***Correlation Between Glutamate Levels and Mechanical Withdrawal Threshold***

Pearson's correlation analysis demonstrated a significant negative correlation between glutamate concentrations and 24-hour von Frey values (r = −0.911; p = 0.001). Higher glutamate concentrations were associated with lower mechanical withdrawal thresholds, whereas lower glutamate concentrations were associated with higher mechanical withdrawal thresholds.

**Discussion**

This study demonstrated that nano-kratom leaf suspension and tramadol significantly affected the mechanical withdrawal threshold and glutamate levels in a rat model of second-degree burn pain. The principal finding was that nano-kratom produced a greater increase in the 24-hour von Frey value and a greater reduction in glutamate levels than tramadol. Thermal injury induces the release of inflammatory mediators and neuromodulators, including tumor necrosis factor-α, interleukin-6, nerve growth factor, and glutamate, which activate peripheral nociceptors and amplify nociceptive transmission (Colloca et al., 2017). Kratom has long been used empirically as a complementary therapy in Southeast Asia, while nanoformulation may improve the delivery and bioavailability of its active compounds (Azhar, 2019).

The absence of significant between-group differences at baseline and immediately after burn induction indicates that the groups had comparable initial mechanical sensitivity and developed a relatively homogeneous nociceptive response before treatment. The marked reduction in von Frey values following burn induction confirmed the development of acute mechanical hypersensitivity. Second-degree burns cause tissue injury and peripheral nociceptor activation through the release of prostaglandins, bradykinin, histamine, proinflammatory cytokines, adenosine triphosphate, substance P, and calcitonin gene-related peptide. These mediators lower nociceptor activation thresholds through transient receptor potential channels, purinergic receptors, and voltage-gated sodium channels. Consequently, normally innocuous or mildly noxious mechanical stimuli can produce an exaggerated withdrawal response (You et al., 2025; Sosanya et al., 2025). Activation of microglia and increased spinal N-methyl-D-aspartate receptor activity may further maintain hyperalgesia and central sensitization after thermal injury (You et al., 2025; Sosanya et al., 2025; Stanton et al., 2024).

Tramadol increased the mechanical withdrawal threshold compared with the control intervention. This finding is consistent with its dual central analgesic mechanism. Tramadol acts as a weak μ-opioid receptor agonist and inhibits norepinephrine and serotonin reuptake, thereby enhancing descending inhibitory pathways and reducing nociceptive transmission in the spinal dorsal horn. Its active metabolite, O-desmethyltramadol, has a higher affinity for μ-opioid receptors than the parent compound and contributes to its analgesic activity (Miotto, 2017; You et al., 2025; Andriyanto et al., 2025).

Nano-kratom produced a higher 24-hour von Frey value than tramadol. The analgesic properties of kratom are primarily attributed to mitragynine and 7-hydroxymitragynine, which interact with opioid receptors, particularly the μ-opioid receptor. These compounds have been described as partial agonists

with preferential G-protein signaling relative to β-arrestin-2 recruitment (Kruegel et al., 2016; Kamble et al., 2021; He et al., 2021). G-protein-mediated signaling may reduce nociceptive neuronal excitability by decreasing cyclic adenosine monophosphate, inhibiting presynaptic calcium channels, activating postsynaptic potassium channels, and reducing the release of excitatory neurotransmitters such as glutamate. Nevertheless, the clinical implications of biased agonism and its potential relationship with opioid-related adverse effects remain incompletely established and should not be inferred directly from the present findings.

Kratom may also exert non-opioid and anti-inflammatory effects. Preclinical evidence suggests that Mitragyna speciosa has antinociceptive and anti-inflammatory activity and may modulate pathways involving inflammatory mediators and transient receptor potential vanilloid 1 (Mat et al., 2023; Waloejo et al., 2026; Alford et al., 2025). Suppression of peripheral inflammation could reduce nociceptor sensitization at the burn site, thereby increasing the mechanical withdrawal threshold. Experimental work has also suggested that kratom may enhance mechanical pain thresholds when used as an analgesic adjuvant, supporting its possible role in multimodal analgesia (Anindito et al., 2026).

The greater effect observed in the nano-kratom group may also be related to the pharmaceutical formulation. Herbal alkaloids may have limited water solubility, gastrointestinal stability, and oral bioavailability. Nanoencapsulation can increase particle surface area, improve dispersion and dissolution, protect active compounds against premature degradation, and enhance intestinal absorption (Chelly et al., 2024). Thus, the higher von Frey value in the nano-kratom group may reflect a combination of improved exposure to active alkaloids, opioid receptor modulation, attenuation of spinal nociceptive transmission, and reduced peripheral inflammation. However, the present study did not assess particle pharmacokinetics, tissue distribution, alkaloid concentrations, or receptor activity; therefore, these mechanisms remain plausible interpretations rather than directly demonstrated effects.

The glutamate findings were consistent with the behavioral results. The control group had the highest glutamate concentration, while the nano-kratom group had the lowest concentration. Glutamate is the principal excitatory neurotransmitter involved in nociceptive transmission between primary afferent fibers and second-order neurons in the spinal dorsal horn. Following burn injury, increased glutamate release activates α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-D-aspartate receptors, producing postsynaptic depolarization, calcium influx, and intracellular signaling that strengthens nociceptive transmission. Excessive or sustained glutamatergic activity contributes to central sensitization, hyperalgesia, and allodynia (Temmermand et al., 2022; Sosanya et al., 2025). This pathway represents an important link between peripheral burn inflammation and central nervous system plasticity (You et al., 2025; Stanton et al., 2024).

The reduction in glutamate levels after tramadol treatment may reflect the combined effects of opioid receptor activation and enhancement of descending monoaminergic inhibition, which can suppress excitatory neurotransmitter release within nociceptive pathways (Miotto, 2017; Temmermand et al., 2022). However, glutamate levels remained higher in the tramadol group than in the nano-kratom group. The greater reduction observed with nano-kratom may indicate broader antinociceptive, anti-inflammatory, and neuromodulatory activity. Current preclinical reviews suggest that kratom acts primarily through μ-opioid receptors but may also influence non-opioid pathways involved in pain modulation (Waloejo et al., 2026; Alford et al., 2025; Garza-Garcia et al., 2024).

A very strong negative correlation was identified between glutamate concentration and the 24-hour von Frey value. Animals with higher glutamate levels tended to have lower mechanical withdrawal thresholds, whereas animals with lower glutamate levels had higher thresholds. This relationship is biologically consistent with the role of glutamate in spinal nociceptive transmission and central sensitization. A lower von Frey value indicates that a relatively weak mechanical stimulus is sufficient to evoke paw withdrawal, while a higher value reflects reduced mechanical hypersensitivity. The correlation therefore supports an association between reduced glutamatergic activity and improved behavioral pain responses. However, correlation does not establish that the reduction in glutamate directly caused the increase in the mechanical withdrawal threshold.

The direction of these results is consistent with previous experimental studies reporting that natural-product adjuvants may modify glutamate and inflammatory mediators in burn pain models and that kratom supplementation may increase mechanical pain thresholds in neuropathic pain models (Andriyanto et al., 2025; Anindito et al., 2026). Nevertheless, these findings remain preclinical. Evidence regarding kratom use in humans is still limited and heterogeneous, and its efficacy, optimal dosing, long-term safety, dependence potential, and

drug interactions have not been adequately established (Waloejo et al., 2026). Mitragynine may inhibit cytochrome P450 3A activity and alter the exposure to drugs such as midazolam, creating a potential risk of enhanced or prolonged sedation when combined with central nervous system depressants (Tanna et al., 2023; Hanapi et al., 2021; Lim et al., 2012; Kamble et al., 2019). Therefore, the present results should not be interpreted as supporting the immediate clinical use of nano-kratom for burn pain.

This study had several limitations. First, only one principal observation time at 24 hours was evaluated, preventing assessment of the onset, peak, duration, and persistence of the analgesic and neuromodulatory effects. Second, glutamate was measured in combined brain and spinal cord tissue supernatants without separating specific neuroanatomical regions involved in pain processing. Third, the study did not measure pharmacokinetic parameters, circulating or tissue alkaloid concentrations, or additional mechanistic biomarkers such as inflammatory cytokines, N-methyl-D-aspartate or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor expression, transient receptor potential vanilloid 1, or oxidative stress markers. Fourth, only one nano-kratom dose was assessed, and detailed safety outcomes, including hepatic and renal function, sedation, motor behavior, and other signs of toxicity, were not evaluated. Consequently, the proposed mechanisms and comparative safety of nano-kratom remain inferential.

Overall, nano-kratom leaf suspension was associated with a higher mechanical withdrawal threshold and lower glutamate concentration than tramadol in this acute burn pain model. These findings provide a preclinical basis for further investigation of nano-formulated kratom as a potential analgesic or analgesic adjuvant. Future studies should evaluate multiple doses, serial observation periods, pharmacokinetics, tissue-specific mechanisms, toxicity, and clinically relevant safety outcomes before considering translation to human studies.

## Conclusion

Nano-kratom leaf suspension at a dose of 15 mg/kg body weight produced a higher mechanical withdrawal threshold and lower glutamate levels than tramadol in a rat model of second-degree burn pain. The strong negative correlation between glutamate concentrations and 24-hour von Frey values indicates that lower glutamatergic activity was associated with reduced mechanical hypersensitivity. These findings support the potential antinociceptive and neuromodulatory effects of nano-formulated kratom. However, because this study was preclinical, used a single dose and assessment time, and did not evaluate pharmacokinetics or detailed safety outcomes, the findings cannot yet be directly translated into clinical practice. Further studies are required to evaluate dose–response relationships, duration of effect, tissue-specific mechanisms, pharmacokinetics, toxicity, and safety before clinical investigation.

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