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Research Article

Evaluation of the Analgesic Activity of Earthworm Gel (*Lumbricus rubellus*) in Male Mice with Formalin-induced Pain Model

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ABSTRACT

Inflammatory pain is one of the body's defense mechanisms, characterized by redness, swelling, and pain due to the release of inflammatory mediators. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as diclofenac sodium, are commonly used topically to relieve pain but may cause local side effects with long-term use. Accordingly, a safer, natural-based alternative is needed. Earthworms (*Lumbricus rubellus*) are known to contain polyphenols and lumbrokinase, which have anti-inflammatory and analgesic properties. This study aimed to evaluate the effectiveness of earthworm gel as a topical analgesic in male mice (*Mus musculus*) induced with formalin. A total of 24 mice were divided into four groups: untreated, gel base, diclofenac sodium gel, and 5% earthworm gel. Pain response was assessed by measuring the duration of licking behavior at two key phases: acute pain (0–5 minutes) and late inflammatory pain (25–30 minutes). Statistical analysis using the Kruskal-Wallis and Mann–Whitney U tests showed that earthworm gel significantly reduced licking duration in the inflammatory phase, with effectiveness comparable to diclofenac sodium gel. These findings support the potential of earthworm gel as a safe and effective natural alternative for managing inflammatory pain topically.

INTRODUCTION

Pain is a subjective sensation that causes discomfort and is often associated with tissue damage. It functions as a protective mechanism that sends warning signals in response to potential threats such as inflammation, bacterial infections, or muscle spasms (Faisol, 2022). The International Association for the Study of Pain (IASP) defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Raja *et al*., 2020). According to the 2020 Global Pain Index Report by GlaxoSmithKlein (GSK), pain is a highly prevalent condition, with 93% of respondents across 19 countries reporting having experienced pain in the past year, and 34% of them experiencing pain daily. Chronic pain is also present among younger populations, with 20% of sufferers under the age of 30, indicating that pain affects all age groups, not just the elderly (GSK, 2020).

Inflammatory pain is one of the most common types of pain, occurring as a natural response of the body to injury or infection. Typical symptoms include heat, pain, redness, swelling, and loss of function (Calder, 2006). To manage this condition, non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, mefenamic acid, and diclofenac sodium are commonly used (Kesehatan *et al*., 2019). Topical NSAIDs, like diclofenac sodium gel, are generally considered safer than oral formulations due to lower systemic absorption (Arfania *et al*., 2023). However, in some cases, topical use may still result in local irritation, including contact dermatitis and skin sensitization. Furthermore, the limited availability of natural-based topical analgesics highlights the need to explore

alternative, more natural, and sustainable options.

Earthworms (*Lumbricus rubellus*) are one of the natural ingredients long used in traditional medicine. Research indicates that earthworms contain bioactive compounds such as polyphenols and lumbrokinase with antioxidant, anti-inflammatory, and fibrinolytic activities (Asminah, 2023), (Syamsuri & Rimbun, 2024). These compounds potentially reduce pain by suppressing inflammatory mediators and accelerating tissue repair. Although the analgesic effects of earthworms have been widely studied in systemic formulations, their topical application, particularly in gel form, has not been extensively explored (Luo *et al*., 2018). The extract of earthworms, particularly the species *Lumbricus rubellus*, contains various bioactive compounds such as proteins, peptides (including the enzyme lumbrokinase), amino acids, as well as small amounts of minerals, lipids, and polysaccharides. These main hydrophilic components have good solubility in water-based solvents, such as distilled water or phosphate buffer solution with a neutral pH (Stephani *et al*., 2023). Therefore, in terms of solubility, earthworm extract is considered easily soluble in suitable media, especially when formulated at a pH of 6–7, which closely resembles the physiological conditions of the skin. Based on these characteristics, earthworm extract is highly suitable for formulation into a gel, particularly for topical use. In this context, the use of gelling agents such as Carbopol 940, Hydroxypropyl Methylcellulose (HPMC), or Carboxymethylcellulose sodium (CMC-Na) can accommodate the solubility and stability of the extract.

Topical analgesics offer several advantages, including site-specific application, reduced systemic side effects, and greater convenience for patients. Based on this background, this study aims to evaluate the *in vivo* analgesic effectiveness of earthworm (*Lumbricus rubellus*) gel in male mice (*Mus musculus)* using a formalin-induced pain model. The formalin model is a standard method for assessing analgesic activity in inflammatory pain. Formalin injection generates two distinct phases of pain: an acute phase caused by direct nociceptor activation and a subsequent inflammatory phase involving the release of inflammatory mediators, providing a comprehensive framework for evaluating topical analgesic effectiveness.

This study measures pain responses based on the duration of paw-licking behavior observed in mice induced with formalin. The licking behavior is recorded at five-minute intervals from 0 to 30 minutes post-injection. Data used for hypothesis testing are taken from two distinct phases: the acute phase (0–5 minutes) and the late inflammatory phase (25–30 minutes), reflecting the mechanisms of nociceptive and inflammatory pain respectively. The transition phase (5–25 minutes) is excluded from analysis due to its limited validity in representing consistent pain responses. The novelty of this research lies in the evaluation of the topical analgesic potential of *Lumbricus rubellus* gel using the formalin-induced pain model. While prior studies have focused primarily on oral preparations of earthworm extract, this study provides new insights into its topical application. The findings are expected to contribute scientific evidence supporting the use of earthworm extract as a natural active ingredient in topical analgesics.

METHODS

This study employed a quasi-experimental design to evaluate the topical analgesic effect of earthworm (*Lumbricus rubellus*) gel in formalin-induced male mice. The design used was post-test only control group. The test was conducted at the Pharmacology Laboratory, Universitas Santo Borromeus. Ethical approval for the use of animals in this study was obtained from the Animal Ethics Committee, Faculty of Medicine, Universitas Kristen Maranatha, under approval number 094/KEP/V/2025.

Materials and Chemicals

The materials used in this study included diclofenac sodium gel 1% (Megatic®, BPOM Reg. No. DTL1009220628A1), gel base composed of Carbopol 940 0.8% (Brataco, analytical grade), earthworm extract powder (*Lumbricus rubellus*) 5% w/w, from Vermint® (BPOM Reg. No. TR023317301), and formalin solution 5% (Merck Millipore). Vermint is an herbal supplement product made from dried earthworm powder, specifically from the species *Lumbricus rubellus*. Its production process does not involve chemical extraction, but rather a series of physical steps such as washing, boiling to inactivate microorganisms, drying using low-temperature ovens or freeze-drying methods, and grinding into a fine powder. This process aims to preserve the natural bioactive compounds, including proteins, peptides (such as lumbrokinase), amino acids, and essential

minerals like zinc and iron. The resulting powder is then encapsulated for ease of consumption and has been widely used as a traditional supplement, claimed to help support the immune system, treat mild infections, and promote healthy blood circulation. The instruments used in this research were digital analytical balance (Ohaus PA214), stopwatch (Casio HS-80TW), 1 mL syringes with 26G needles (Terumo), and Elizabethan collars (custom-made from soft plastic). All instruments were used according to the manufacturer's instructions. No modifications were made to the equipment.

The earthworm gel was formulated by incorporating 5 g of dried earthworm extract into 100 g of gel base. The gel base itself was prepared using Carbopol 940 dissolved in distilled water, neutralized with triethanolamine until a homogeneous gel was formed. The use of 5% earthworm (*Lumbricus rubellus*) extract in the gel formulation was based on prior research demonstrating optimal efficacy at this concentration. This study used an experimental method by comparing five groups of mice. Each group received different treatments: no treatment, 10% Kalmicetin® gel, and earthworm (*Lumbricus rubellus*) gel at concentrations of 3%, 4%, and 5%. A cut wound was made on the back of each mouse, and the treatment was applied three times a day. Wound healing time and wound size were observed daily. A study showed that gel containing 5% earthworm extract accelerated incised wound healing in mice more effectively than 3% and 4% concentrations, as well as a 10% Kalmicetin® positive control. This indicates that 5% concentration exhibits significant biological activity in promoting wound recovery (Deng *et al*., 2018).

A total of 24 male mice (*Mus musculus*), aged 2–3 months, weighing 20–30 g, were obtained from a certified research animal breeder. Mice were housed under standard laboratory conditions with 12-hour light/dark cycles, and given standard feed and water ad libitum. The sample size was determined using Federer's formula (n-1)(t-1) > 15, where t = 4 groups and n = 6 replicates.

Procedure

The mice were randomly divided into four groups (n = 6 per group): negative control: no treatment, only formalin induction; gel base group: gel base application, then formalin induction; positive control: diclofenac sodium gel application, then formalin induction; test group: earthworm gel application, then formalin induction.

Mice were acclimatized for 7 days. A 100 mg sample of each respective gel was applied to the plantar surface of the right hind paw. Elizabethan collars were fitted immediately after application to prevent ingestion or removal of the gel. After 30 minutes, 0.02 mL of 5% formalin solution was injected subcutaneously into the same paw (Tjølsen *et al*., 1992). Pain behavior was assessed based on the cumulative duration of licking the injected paw, measured with a stopwatch across six 5-minute intervals: 0–5, 5–10, 10–15, 15–20, 20–25, and 25–30 minutes, as shown in Figure 1.

Formalin was chosen as the pain-inducing agent due to its ability to produce a consistent and standardized pain model in experimental animals. A subcutaneous injection of 5% formalin into the plantar surface of the mouse's paw induces two distinct phases of pain: the early (acute) phase and the late (inflammatory) phase. The acute phase occurs within the first 0–5 minutes following injection and reflects direct nociceptor activation caused by chemical irritation. The inflammatory phase, which appears between 15–30 minutes, is associated with the release of inflammatory mediators such as prostaglandins, histamine, and serotonin. Therefore, this model is considered reliable for evaluating the analgesic effects of test substances on both acute and inflammatory pain.

Moreover, the formalin-induced pain model is particularly suitable for assessing the efficacy of topical formulations. Since the injection and pain response are localized to the paw, it allows for direct evaluation of how well a topically applied agent can reduce pain at the application site. This makes it highly relevant for studies involving topical analgesics, because it mimics the intended route of administration and provides targeted data on local pain relief.

This study included four treatment groups for comparison. The negative control group (no treatment) was used to observe the natural pain response without any intervention. The gel base group received topical application of the formulation base without active ingredients to assess any potential effect of the vehicle itself. As a positive control, a group received diclofenac gel, a topical NSAID (non-steroidal anti-inflammatory drug) with clinically established analgesic properties. Lastly, the test group was treated with a gel containing *Lumbricus rubellus* extract, and its analgesic effect was compared to the other groups.

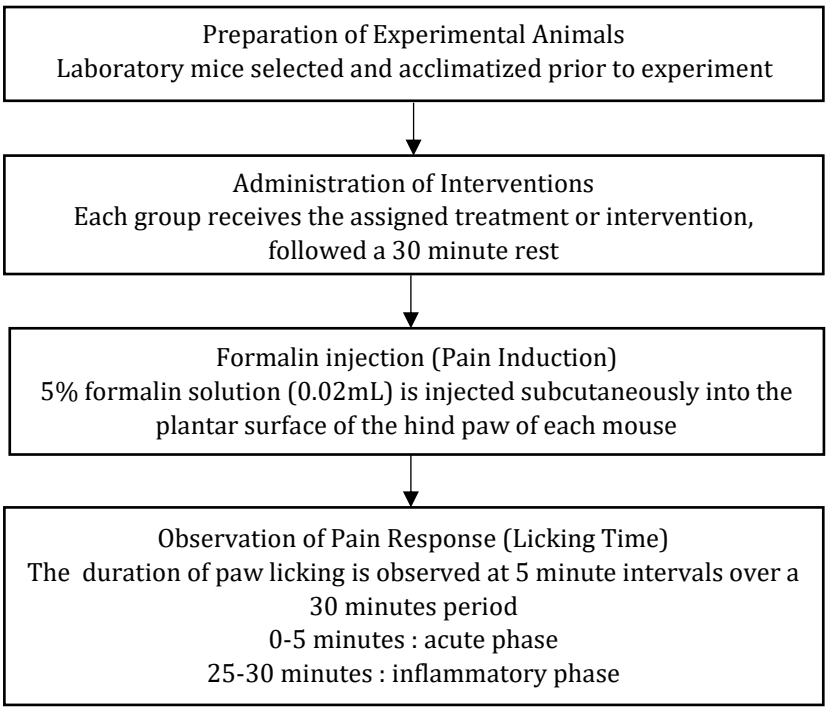

Figure 1. Flowchart of the Formalin-Induced Pain Test Procedure in Mice.

Table 1. Mean Licking Duration (Seconds) in Mice During the Activity Test

Treatment Group	Licking Duration (Mean ± SD) n=6					
	0-5 min	5-10 min	10-15 min	15-20 min	20-25 min	25-30 min
Gel Base	47.67±34.49	7.50±6.22	5.67±2.16	27.17±2.32	17.17±2.48	52.00±17.23
Diclofenac Gel	44.17±18.06	5.83±7.52	28.33±17.55	30.33±11.47	24.67±22.84	7.67±7.23
Earthworm Gel	42.83±9.33	7.33±13.74	1.83±2.64	14.50±9.81	10.33±6.12	3.50±2.43
No Treatment	127.17±17.93	24.33±8.89	20.50±8.48	39.00±8.07	85.33±26.26	61.17±12.66

SD, standard deviation.

Statistical analysis was conducted using SPSS v25.0 (IBM Corp., Chicago, USA). Because the number of samples was fewer than 30, non-parametric tests were applied. The Kruskal–Wallis test was used to detect differences across groups. If significant, pairwise comparisons were conducted using the Mann–Whitney U test. A *p*-value < 0.05 was considered statistically significant. Hypothesis testing was conducted using the Kruskal–Wallis test to assess whether there were significant differences in pain responses (licking duration) among the four treatment groups (gel base, diclofenac sodium gel, earthworm gel, and untreated) during the 0–5 minute and 25–30 minute intervals post-formalin induction. If the result was statistically significant, a post hoc Mann–Whitney U test was performed to determine which group exhibited the greatest reduction in licking time, based on the mean rank values.

RESULTS AND DISCUSSION

Activity Test

This study was conducted to evaluate the analgesic effect of four treatment groups: diclofenac sodium gel (positive control), earthworm gel (*Lumbricus rubellus*), gel base, and no treatment (negative control). The untreated group served to verify the success of pain induction through the intraplantar injection of 5% formalin at a volume of 0.02 mL into the

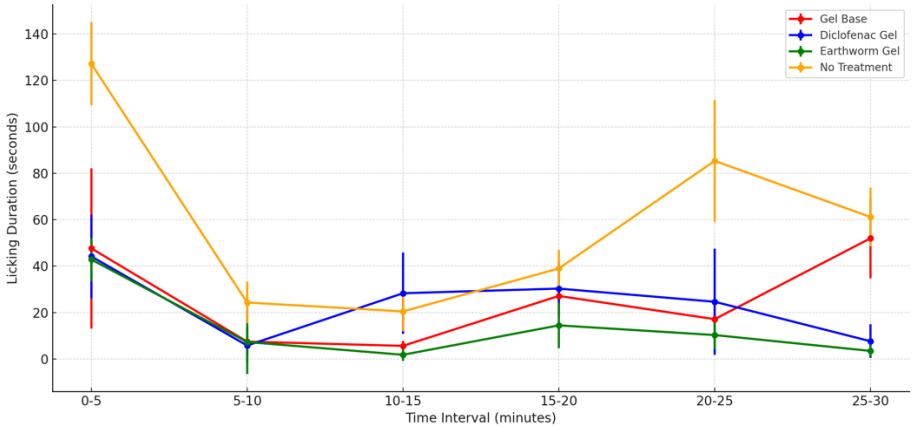

Figure 2. Graph of licking duration across all treatment groups (Mean±SD)

mice's paw, and as a comparison against the treated groups. Each group consisted of six mice. Observations were conducted every 5 minutes over a 30-minute period after formalin induction. Measurements were done using a stopwatch when mice exhibited licking behavior on their paw. If a mouse did not lick, the stopwatch was paused and resumed when licking resumed, until data were collected in intervals of 0–5 minutes (acute phase) and 25–30 minutes (inflammatory phase). The average licking duration (in seconds) for each group is shown in Table 1 and Figure 2.

The untreated mice group confirmed that 5% formalin at a 0.02 mL dosage consistently triggered acute and inflammatory pain responses, as evidenced by increased licking behavior. The licking duration varied over time, affirming the dose and method of induction as valid for the activity test. The longest licking duration occurred during the 0–5 minutes interval, representing the acute pain phase caused by the direct activation of nociceptors by formalin. A significant reduction in licking time between minutes 5 to 15 was observed, indicating a transition into the quiescent phase, which is physiologically associated with the activation of the body's endogenous analgesic system.

During this phase, the body releases endogenous opioids such as endorphins and enkephalins that bind to opioid receptors in the central and peripheral nervous systems. These receptor activations inhibit the release of pain neurotransmitters and block pain signal transmission to the brain, creating a temporary analgesic effect. This process reflects the body's homeostatic mechanism in regulating pain perception after the acute stimulation phase. This phenomenon aligns with the formalin test model, which comprises two phases: the first phase (0–5 minutes) as acute pain, and the second phase (15–30 minutes) as inflammatory pain, with the quiescent phase between them reflecting the work of the endogenous opioid system (Tjølsen *et al*., 1992).

Following the quiescent phase (5–15 minutes), most mice exhibited increased licking duration between 15 and 30 minutes. This phase represents the second or inflammatory phase of the formalin test, physiologically related to peripheral inflammatory responses and central sensitization. In this phase, formalin no longer directly stimulates nociceptors but causes local tissue damage that stimulates the release of pro-inflammatory mediators (e.g., prostaglandins, bradykinin, histamine, substance P, tumor necrosis factor-alpha [TNF-α], and interleukin [IL]-6) (Young, 2021).

In the treated groups, all three gels reduced the licking duration (in seconds) in male mice after formalin induction. During the 0–5 minute interval, all groups showed peak licking activity, indicating acute nociceptor activation by formalin injection (Puspitasari *et al*., 2021). Table 1 shows that the gel base group (containing Carbopol 940) experienced a reduction in licking duration starting at minute 0. This may be attributed to the gel base's water-absorbing properties, resulting in a stable, elastic consistency. When applied to the skin, water in the gel may evaporate due to the temperature difference between the skin and the air. This evaporation absorbs heat energy from the skin, creating a temporary cooling sensation that provides comfort, reduces inflammation, and relieves pain. The cooling effect also causes vasoconstriction, reducing blood flow to the inflamed area and inhibiting the release of inflammatory mediators such as prostaglandins, bradykinin, and histamine. Additionally, this

cooling effect can stimulate Transient Receptor Potential Melastatin 8 (TRPM8) receptors on the skin and peripheral nerve endings. TRPM8 activation diverts pain perception toward temperature sensation, thereby reducing inflammatory responses and pain perception (De Caro *et al*., 2019). However, this effect is temporary, as increased licking behavior returned at the 25–30 minute interval, likely due to loss of skin moisture and the fading cooling effect of the gel (Dahiya *et al*., 2018).

Diclofenac sodium gel significantly reduced pain, as indicated by decreased licking duration, starting from minute 0 to minute 30. As an NSAID, diclofenac works by inhibiting cyclooxygenase (COX-1 and COX-2), which converts arachidonic acid into prostaglandins. Prostaglandins are key mediators in inflammation, increasing nociceptor sensitivity and causing pain, heat, and swelling. By inhibiting COX, diclofenac reduces prostaglandin synthesis, lowering inflammation and pain, and promoting tissue healing (Hida, 2025; Ercan, 2013).

These results are consistent with Erchan *et al*. (2013), who evaluated the anti-inflammatory effect of topical diclofenac in a carrageenan-induced paw edema model in rats. The study showed that topical diclofenac significantly reduced paw edema volume and weight, with maximum effect at 3 hours post-application. Edema volume reduction became significant at 2 hours, with peak anti-inflammatory effect at 3 hours (Mufaiduddin *et al*., 2024). This supports the notion that topical diclofenac requires time for skin penetration and tissue targeting. In this study, the significant reduction in licking time at 25–30 minutes reflects a consistent pharmacodynamic response.

Earthworm gel reduced licking duration as a pain response post-formalin induction. Its analgesic activity may be attributed to bioactive compounds like polyphenols, which exhibit antioxidant properties and counter oxidative stress and inflammation (Asminah, 2023). Additionally, the enzyme lumbrokinase in earthworms is known for its fibrinolytic activity that aids recovery and pain reduction during inflammation (Luo *et al*., 2018).

Lumbrokinase acts as an anti-inflammatory agent by inhibiting the Toll-like receptor 4 (TLR4) and nuclear factor-kappa B (NF-κB) pathways, crucial in triggering inflammation. TLR4 detects bacterial molecules or cell damage, activating NF-κB to produce inflammatory mediators such as TNF-α and IL-6. Lumbrokinase suppresses TLR4 activity and prevents NF-κB nuclear translocation, reducing mediator production and alleviating symptoms like pain and swelling. Polyphenols also lower the production of cytokines like TNF-α and IL-6 via inhibition of inflammatory signaling pathways like NF-κB (Shofiatiningsih, 2014; Hamdi *et al*., 2023).

Hypothesis Test: Differences in Licking Duration Between Groups

The hypothesis was analyzed using the Kruskal–Wallis test to determine whether significant differences existed in licking duration among groups: gel base, diclofenac gel, earthworm gel, and no treatment. Analysis focused on two main intervals: 0–5 minutes (acute pain phase) and 25–30 minutes (late inflammatory phase).

In the 0–5 minute interval, Kruskal–Wallis test returned a significance value of $p = 0.0062$ ($p < 0.05$), rejecting H_0 and accepting H_1. Thus, significant differences in acute pain response (licking duration) existed among the group. For the 25–30 minute interval, $p = 0.0004$ ($p < 0.05$), also rejecting H_0 and accepting H_1, indicating significant differences in late inflammatory pain response across groups. Post hoc Mann–Whitney U tests were used to determine specific group differences (Table 2).

Table 2. Post Hoc Mann–Whitney Test Results in Acute and Inflammatory Phases

Group Pair	*p*-value (0–5 min) & Interpretation	*p*-value (25–30 min) & Interpretation
Gel Base vs Diclofenac Gel	0.522 (ns)	0.0039 (significant)
Gel Base vs Earthworm Gel	0.378 (ns)	0.0039 (significant)
Gel Base vs No Treatment	0.010 (significant)	0.336 (ns)
Diclofenac vs Earthworm Gel	0.810 (ns)	0.198 (ns)
Diclofenac vs No Treatment	0.004 (significant)	0.0039 (significant)
Earthworm Gel vs No Treatment	0.004 (significant)	0.0038 (significant)

During the acute pain phase (0–5 minutes post-formalin), post hoc Mann–Whitney U results revealed significant differences between the untreated group and all treated groups (gel base, diclofenac, and earthworm), with the untreated group showing the highest mean rank (21.17), indicating the longest licking duration. No significant differences were found among the treated groups themselves, suggesting that all three interventions provided relatively equal reduction in acute pain. Despite active compounds in diclofenac and earthworm gels, the gel base group also showed similar reduction, possibly due to the temporary cooling sensation of the gel.

During the late inflammatory phase (25–30 minutes post-formalin), significant differences were observed among some group pairs. No significant difference was found between the gel base and untreated groups, indicating the gel base had no substantial effect on reducing inflammatory pain. This finding may be due to the temporary nature of the cooling effect of Carbopol 940 without any active ingredients.

The earthworm and diclofenac gel groups showed no significant difference, suggesting comparable effectiveness in reducing late-phase inflammatory pain. Diclofenac works via prostaglandin inhibition, while earthworm extract contains lumbrokinase and polyphenols that suppress TLR4/NF-κB pathways and reduce pain mediators like TNF-α, IL-6, and nitric oxide (NO) (Mufaiduddin *et al*., 2024).

Significant differences were found between several other group pairs: no treatment vs. earthworm gel, no treatment vs. diclofenac gel, gel base vs. earthworm gel, and gel base vs. diclofenac gel. These results indicate that earthworm and diclofenac gels are significantly more effective than no treatment or gel base in reducing inflammatory pain. Therefore, earthworm gel can be considered a topically effective analgesic comparable to diclofenac and holds promise as a safe and effective natural alternative for inflammatory pain management.

CONCLUSIONS

This study demonstrates that there were significant differences in licking duration, representing pain response, among male mice treated with gel base, diclofenac gel, earthworm (*Lumbricus rubellus*) gel, and the untreated control group during both the acute pain phase (0–5 minutes) and the late inflammatory phase (25–30 minutes) following formalin induction. The findings suggest that earthworm gel exhibited topical analgesic effects comparable to those of diclofenac gel and was significantly more effective than the gel base and untreated groups in reducing pain responses. Therefore, earthworm gel has the potential to be developed as a natural and effective alternative for topical pain management.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. The internal funding from the Institute for Research and Community Service (LPPM) of Universitas Santo Borromeus did not influence the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors had full access to all data and independently conducted the research without any sponsor intervention.

REFERENCES

Abdulbaqi, I. M., Darwis, Y., Khan, N. A. K., Assi, R. A., & Khan, A. A. (2016). Ethosomal nanocarriers: The impact of constituents and formulation techniques on ethosomal properties, *in vivo* studies, and clinical trials. *International Journal of Nanomedicine, 11*, 2279–304. doi: 10.2147/IJN.S105016

Arfania, M., Friyanto, D., Musfiroh, E. N., Sathi'ah, F. A., Irawan, L., Yuliani, N. D., & Herawati, S. H. (2023). Efek samping terhadap pemakaian analgetik golongan NSAID (ibuprofen). *Journal of Social Science Research, 3*(2), 8065–75. https://j-innovative.org/index.php/Innovative/article/view/1342

Asminah, M. (2023). Efektivitas kapsul kombinasi cacing tanah *Lumbricus rubellus* Hoffmeister dan kayu manis *Cinnamomum burmannii* (Nees & T. Nees) Blume sebagai sediaan herbal

antidiabetes [Skripsi, Universitas Hasanuddin].

Calder, P. C. (2006). n–3 Polyunsaturated fatty acids, inflammation, and inflammatory. *The American Journal of Clinical Nutrition, 83*(6), 1505S–1519S. https://doi.org/10.1093/ajcn/83.6.1505S

Dahiya, R. S., Farooq, S. A., & Saini, V. (2018). Evaluation of novel cooling gel sheets for antipyretic effect. *International Journal of Current Pharmaceutical Review and Research, 9*(1), 12–15.

De Caro, C., Cristiano, C., Avagliano, C., Bertamino, A., Ostacolo, C., Campiglia, P., Gomez-Monterrey, I., La Rana, G., Gualillo, O., Calignano, A., & Rossi, F. (2019). Characterization of new TRPM8 modulators in pain perception. *International Journal of Molecular Sciences, 20*(22), 5544. https://doi.org/10.3390/ijms20225544

Ercan, N., et al. (2013). The anti-inflammatory effect of diclofenac is considerably augmented by topical capsaicinoids-containing patch in carrageenan-induced paw oedema of rat. *Inflammopharmacology, 21*(6), 413–419. https://doi.org/10.1007/s10787-013-0175-7

Faisol, S. K. M. (2022). Manajemen nyeri. *Kementerian Kesehatan Republik Indonesia*. https://yankes.kemkes.go.id/view_artikel/1052/manajemen-nyeri

GSK. (2020). *Global Pain Index Report 4th edition – 2020* (pp. 1–110).

Hida, I., & Fajrin, R. (2025). Gambaran penggunaan obat antiinflamasi nonsteroid pada pasien penderita osteoarthritis di instalasi rawat jalan RSUD Kajen periode Januari–Oktober 2024. *Jurnal Ilmu Farmasi dan Kesehatan, 3*(1). https://doi.org/10.59841/an-najat.v3i1.2239

Kesehatan, J. I., Husada, S., Wardoyo, A. V., & Oktarlina, R. Z. (2019). Tingkat pengetahuan masyarakat terhadap obat analgesik pada swamedikasi untuk mengatasi nyeri akut. *Jurnal Ilmu Sosial dan Kesehatan, 10*(2), 156–60. https://doi.org/10.35816/jiskh.v10i2.138

Luo, W., Deng, Z. H., Li, R., Cheng, G., Kotian, R. N., Li, Y. S., & Li, W. P. (2018). Study of analgesic effect of earthworm extract. *Bioscience Reports, 38*(1). https://doi.org/10.1042/BSR20171554

Hamdi, M., Azmi, N. A., Harun, I., Fahmi, A., & Haris, M. S. (2023). An insight into the use and advantages of Carbopol in topical mucoadhesive drug delivery system: A systematic review. *Journal of Pharmacy, 3*(1). https://doi.org/10.31436/jop.v3i1.156

Mufaiduddin, M., Innelya, & Rahmatika, A. (2024). A review: Oral lumbrokinase as potential agent prevents myocardial reinfarction post-reperfusion in ST-elevation myocardial infarction (STEMI) patients. *SCRIPTA SCORE Scientific Medical Journal, 5*(2), 121–31. https://doi.org/10.32734/scripta.v5i2.15156

Syamsuri, M. I., & Rimbun, N. H. (2024). Studi pustaka efikasi ekstrak cacing tanah sebagai pengobatan herbal bagi penderita diabetes mellitus tipe 2. *Unairnews*. https://unair.ac.id/studi-pustaka-efikasi-ekstrak-cacing-tanah-sebagai-pengobatan-herbal-bagi-penderita-diabetes-mellitus-tipe-2/

Puspitasari, V., Wahjuni, R. S., Saputro, A. L., Hamid, I. S., & Wibawati, P. A. (2021). Effectiveness of clove flower extract (*Syzygium aromaticum* L.) as analgesic on licking time reaction in male mice with formalin induction. *Jurnal Medik Veteriner, 4*(2), 226–30. https://doi.org/10.20473/jmv.vol4.iss2.2021.226-230

Raja, S. N., Carr, D. B., Cohen, M., Finnerup, N. B., Flor, H., Gibson, S., Keefe, F. J., Mogil, J. S., Ringkamp, M., Sluka, K. A., Song, X. J., Stevens, B., Sullivan, M. D., Tutelman, P. R., Ushida, T., & Vader, K. (2020). The revised International Association for the Study of Pain definition of pain: Concepts, challenges, and compromises. *Pain, 161*(9), 1976–82. https://doi.org/10.1097/j.pain.0000000000001939

Shofiatiningsih, A. R. H. (2014). Uji daya antiinflamasi serbuk cacing tanah (*Lumbricus rubellus*) dengan metode udema terinduksi karagenin pada

telapak kaki tikus [Skripsi, Universitas Gadjah Mada].

Tjølsen, A., Berge, O. G., Hunskaar, S., Rosland, J. H., & Hole, K. (1992). The formalin test: An evaluation of the method. *Pain, 51*(1), 5–17. https://doi.org/10.1016/0304-3959(92)90003-T

Young, S. Y. P., Kim, S. R. C., Kang, J. H., Lee, S. C., Jung, S. Y., & Jung, J. H. (2021). Antinociceptive effect of BPC-157 in the formalin-induced pain model. *Kosin Medical Journal*, *36*(1), 1-13. doi: 10.7180/kmj.2021.36.1.1