



Evaluation of the Anti-Joint Pain Activity of Genjer Leaf Extract in AgNO₃-Induced Rats

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Article Info	ABSTRACT
Received: 15-06-2026 Revised: 17-07-2026 Accepted: 29-07-2026 *Corresponding author: Disa Andriani Email: disa.andriani@stikesnas.ac.id	Background: Joint pain is a common musculoskeletal condition that can significantly reduce mobility, physical function, and quality of life. Despite the availability of conventional analgesics, their long-term use is often associated with adverse effects, highlighting the need for safer therapeutic alternatives. Genjer (<i>Limnocharis flava</i>), a medicinal plant rich in flavonoids, phenolic compounds, and other bioactive constituents, has shown potential analgesic and anti-inflammatory activities, making it a promising natural candidate for the management of joint pain. Therefore, investigating the therapeutic potential of <i>L. flava</i> is important for the development of safer and more effective treatments for joint pain. Objective: This study aimed to determine the anti-joint pain activity of genjer extract in AgNO₃-induced rats and evaluate its effectiveness at different dose levels. Method: The experiment was conducted using four treatment groups: negative control (CMC-Na), positive control (diclofenac sodium), genjer extract at a dose of 100 mg, and genjer extract at a dose of 200 mg. The observed parameters included limping score, paw usage, mechanical hyperalgesia, and joint swelling at 30, 60, 90, and 120 minutes after treatment. Results: The positive control group showed the greatest reduction in pain scores among all groups. Both doses of genjer extract also reduced pain compared with the negative control, and the 200 mg dose produced a stronger effect than the 100 mg dose. These findings suggest that genjer extract has antinociceptive activity by reducing sensitivity to mechanical pain. However, all treatment groups showed only slight reductions in joint swelling. Conclusion: Genjer extract exhibited anti-joint pain activity in AgNO₃-induced rats. The most effective dose was 200 mg, which showed greater pain-relieving activity compared to the 100 mg dose. Keywords: genjer, joint pain, mechanical hyperalgesia, AgNO₃

INTRODUCTION

Joint pain (arthralgia) is one of the most common musculoskeletal health problems. The prevalence of joint pain increases with age and lifestyle factors (Berenbaum, 2013). Conventional treatments generally involve the use of nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, or opioid analgesics; however, their long-term use may cause gastrointestinal, cardiovascular, and renal side effects (Hunter et al., 2020). One

approach that has gained increasing attention is the use of topical formulations derived from natural products, as they provide direct local effects at the site of pain with minimal systemic side effects (Graham et al., 2024). Topical formulations such as gels containing plant extracts have the potential to serve as effective and safe alternative therapies for joint pain (Guy, 2024).

Limnocharis flava has traditionally been used to reduce fever and treat mild inflammation. Phytochemical analysis has shown that genjer leaves contain high levels of flavonoids, saponins, alkaloids, tannins, and phenolic compounds (Baehaki et al., 2019). These compounds are known to play important roles in anti-inflammatory and analgesic activities through inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) pathways (Ekor, 2014). Its ethanol extract has been reported to exhibit strong antioxidant activity based on DPPH and FRAP assays (Baehaki et al., 2019), as well as protective effects against oxidative stress in mammalian cell models (Rury Trisa Utami, 2023).

Anti-inflammatory activity has also been demonstrated through the reduction of inflammatory mediators such as NO and TNF- α in in vitro models (Wijesekara et al., 2024). In this study, AgNO₃ (silver nitrate) was used as a local induction agent because it can induce inflammatory reactions and hyperalgesia in subcutaneous tissue, resembling local inflammatory processes (Zheng et al., 2023). Although these pharmacological activities have been reported, the development of *L. flava* as a topical gel for joint pain management has not been adequately investigated, and evidence of its efficacy in a localized inflammatory joint pain model remains limited. Therefore, the novelty of this study lies in the development and evaluation of a topical gel containing *L. flava* ethanol extract as a potential natural therapy for joint pain using an AgNO₃ induced local inflammatory model. This approach provides new evidence regarding the analgesic potential of *L. flava* in topical dosage form and supports its future development as a safer alternative for the management of joint pain.

METHODS

Materials

The equipment used in this study included a rotary evaporator (IKA RV 10), analytical balance (OHAUS), oven (Mettler), water bath (Mettler), blender, digital caliper, oral gavage, 1 mL syringe, and laboratory glassware such as measuring cylinders, beakers, Erlenmeyer flasks, funnels, test tubes, dropper pipettes, and stirring rods. The materials used in this study included fresh genjer leaves *Limnocharis flava* obtained from Mojolaban, Sukoharjo Regency, Central Java, which had been taxonomically identified. Other materials used were 96% ethanol, diclofenac sodium, CMC-Na, AgNO₃ solution, distilled water, FeCl₃, NaOH, concentrated HCl, magnesium powder, Dragendorff reagent, Wagner reagent, Mayer reagent, and Liebermann–Burchard reagent. The experimental animals used were 24 male Wistar rats (*Rattus norvegicus*), aged 2–3 months and weighing between 120–211 g.

Methods

Preparation of Simplisia and Ethanolic Extract of Genjer Leaves

Fresh genjer leaves were collected and sorted. The dried simplisia was then ground using a blender until a uniformly fine powder was obtained and stored in a tightly closed container. Extraction was carried out using the maceration method with 70% ethanol as the solvent. The powdered simplisia was mixed with 70% ethanol at a ratio of 1:10 (w/v). The mixture was macerated for 3 × 24 hours at room temperature with occasional stirring. The filtrate was evaporated using a rotary evaporator at a temperature below 50°C. The extract was further concentrated using a water bath until a thick extract with minimal water content was obtained. The extraction yield was then calculated

Phytochemical Screening of Genjer Leaf Extract

Phytochemical screening was conducted to identify the secondary metabolite compounds present in the ethanolic extract of genjer leaves. Qualitative analysis was performed following the standard methods of Harborne (1998) and modifications of Trease & Evans (2002), including tests for alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids.

Joint Pain Activity Test Using AgNO₃ Induction

1. Joint Pain Test

This study used four treatment groups, each consisting of five rats:

Negative control K(-): 0.5% CMC-Na

Positive control K(+): diclofenac sodium 25 mg/kg BW

P1: ethanolic extract of genjer leaves at 100 mg/kg BW

P2: ethanolic extract of genjer leaves at 200 mg/kg BW

The joint pain model was induced by intra-articular injection of 0.05 mL of 0.1% AgNO₃. After injection, the animals were left for 15–30 minutes to observe pain responses, including limping, reduced paw usage, and joint swelling. Once pain symptoms appeared, treatments were administered according to each group. Observations were conducted at 30, 60, 90, and 120 minutes after treatment administration.

2. Measurement of Joint Pain Response

The observed parameters included:

a. Limping Score

Observation method: Rats were placed on a flat surface and allowed to move freely for approximately 3–5 minutes. Hind limb walking patterns were visually observed. Scoring system:

0 : Normal walking, paw fully used

1 : Mild limping, paw still used

2 : Obvious limping, paw rarely used

3 : Paw not used at all

Interpretation: Lower scores indicate milder joint pain and better analgesic effects.

b. Hind Paw Use Score

Observation method: Animals were observed while standing and walking for approximately 3 minutes. Scoring system:

0 : Normal paw usage

1 : Slightly reduced paw usage

2 : Paw rarely used

3 : Paw not used

Interpretation: A lower score indicates improved joint function.

c. Mechanical Hyperalgesia

Observation method: The area around the knee joint or paw was stimulated using a mild mechanical stimulus (e.g., von Frey filament or blunt tip). Scoring system:

0 : No response

1 : Mild response

2 : Strong response (rapid paw withdrawal/avoidance)

Interpretation: Lower scores indicate an increased pain threshold.

d. Joint Swelling (Edema)

Measurement method: The diameter of the knee joint was measured using a digital caliper before induction (baseline), after induction, and after treatment.

Recorded parameters: Joint diameter (mm) and Change in diameter from baseline

Interpretation: A decrease in joint diameter indicates an anti-inflammatory effect.

Data Analysis

Pain score data were analyzed using the Kruskal–Wallis and Mann–Whitney tests, while joint swelling data were analyzed using one-way ANOVA. The treatment groups showed a significant reduction in pain compared to the negative control group and positive control group.

RESULTS AND DISCUSSION

Extraction of Genjer Leaf Extract

The extraction process of genjer leaf simplisia was carried out using the maceration method with 96% ethanol as the solvent. The maceration method was selected because it is effective in extracting polar and semi-polar compounds such as flavonoids, saponins, and tannins, which are abundant in plant tissues (Pawarti et al., 2023). The maceration process produced a thick extract with a yield value of 15.42%. This value falls within the normal range for ethanolic extracts of herbal leaves, where yields generally range from 5–30% depending on the plant species, plant part used, solvent concentration, maceration duration, and solvent-to-material ratio (Pawarti et al., 2023; Syamsul et al., 2020). Previous studies have shown that the yield value of ethanolic genjer leaf extract varies depending on the extraction conditions used. A higher yield indicates that more compounds are extracted, although it is not always directly proportional to the concentration of active compounds responsible for pharmacological activity (Ferdinan & Audiah, 2021).

Phytochemical Screening of Genjer Leaf Extract

Qualitative identification was performed using specific reagents that produce color changes or precipitate formation as indicators of the presence of

particular classes of compounds (Pongpasan et al., 2022). The phytochemical screening results of the ethanolic extract of genjer leaves are presented in Table 1.

The presence of phenolic compounds in genjer leaves has been confirmed by Ooh et al., (2015), who identified various phenolic acids using HPLC analysis of genjer extract. Phenolic compounds play important roles as antioxidants and anti-inflammatory agents through free radical scavenging mechanisms and lipoxigenase enzyme inhibition. The positive flavonoid result obtained through the NaOH test is supported by the findings of (Ferdinan & Audiah, 2021), who successfully isolated flavonoid compounds from ethanolic genjer leaf extract. The presence of flavonoids, particularly quercetin and kaempferol, is highly relevant to the joint pain activity observed in this study, as these compounds are known to inhibit COX-2 and suppress the NF-κB pathway (Wang et al., 2026).

The presence of saponins in genjer leaves has been reported in several previous studies. Saponins are thought to contribute to anti-inflammatory activity through inhibition of the NF-κB pathway and modulation of macrophage activity, which supports the analgesic effects observed in this study (Wang et al., 2026; Wijesekara et al., 2024). The alkaloid test results also indicated that the ethanolic extract of genjer leaves positively contained alkaloids. The tannin test using 1% FeCl₃ reagent showed positive results.

Table 1. Phytochemical Screening Results of Ethanolic Genjer Leaf Extract

Compound Class	Observation Result	Result
Phenolics	Solution turned dark greenish-black	(+) Positive
Flavonoids	Solution turned reddish-brown	(+) Positive
Alkaloids	Orange-red precipitate formed	(+) Positive
Saponins	Stable foam formed	(+) Positive
Tannins	Solution turned greenish-black	(+) Positive
Terpenoids	Solution turned dark red	(+) Positive

Tannins are known to possess anti-inflammatory properties through their ability to inhibit lipid oxidation, suppress pro-inflammatory cytokine production, and interact with immune cell proteins. The presence of tannins in genjer is consistent with phytochemical screening reports from several previous studies on this plant. The positive terpenoid result is important in the context of joint pain activity because several triterpenoid compounds are known to possess anti-inflammatory and analgesic activities through inhibition of COX enzymes and suppression of inflammatory mediator production (Jamila et al., 2021).

Evaluation of Anti-Joint Pain Activity

Anti-joint pain activity was evaluated using an AgNO₃-induced inflammatory pain model. AgNO₃ induces localized inflammation and increases peripheral nociceptor sensitivity, making it suitable for evaluating analgesic activity in experimental animals (Al-Khayri et al., 2022). Four indicators were used to assess the analgesic effect of the treatments: limping score, paw usage score, mechanical hyperalgesia, and joint swelling. The limping score reflects the severity of pain during walking, with lower scores indicating reduced pain and improved locomotion. The paw usage score evaluates the animal's ability to bear weight on the affected limb, where lower scores indicate better functional recovery. Mechanical hyperalgesia measures sensitivity to mechanical stimuli and represents peripheral nociceptor sensitization; lower scores indicate reduced pain sensitivity. Joint swelling reflects the degree of local inflammation around the affected joint, with smaller swelling indicating reduced inflammatory response.

1. Limping Score (A)

The limping score was used to evaluate pain-related impairment during locomotion.

Higher scores indicate more severe limping caused by joint pain, whereas lower scores indicate improved mobility (Figure 1). The negative control group maintained high limping scores throughout the observation period, indicating that AgNO₃ successfully induced inflammatory joint pain. In contrast, the positive control group treated with diclofenac sodium showed a rapid reduction in limping scores, consistent with the analgesic mechanism of NSAIDs through inhibition of prostaglandin synthesis (Shamsudin et al., 2022). Both genjer extract-treated groups also demonstrated progressive reductions in limping scores, with the 200 mg dose producing greater improvement than the 100 mg dose.

2. Paw Usage Score (B)

The paw usage score assesses functional use of the affected limb during movement. Lower scores indicate that the animals were able to use the injured paw more normally, suggesting pain relief and functional recovery (Figure 2). Similar improvements were observed in the paw usage score. Animals receiving genjer extract showed better use of the affected limb than those in the negative control group. The improvement became more evident after 60 minutes, and the 200 mg dose produced greater functional recovery than the 100 mg dose, suggesting a dose-dependent analgesic effect (Shamsudin et al., 2022).

3. Hyperalgesia

Mechanical hyperalgesia measures increased sensitivity to mechanical stimulation, which is a hallmark of peripheral inflammatory pain. Lower scores indicate reduced nociceptor sensitization and improved pain control (Figure 3). The genjer extract groups at doses of 100 mg and 200 mg showed reduced pain scores compared to the negative control group. The reduction became evident at 60 minutes and more pronounced at 90 and 120 minutes. The 200

mg dose demonstrated better anti-joint pain activity than the 100 mg dose and approached the effect of the positive control, indicating a dose-response relationship (Ysrafil et al., 2023). The anti-joint pain activity of genjer extract is thought to be associated with the presence of flavonoids

and phenolic compounds, which are capable of inhibiting inflammatory mediators and reducing peripheral nociceptor sensitivity (Ferraz et al., 2020). The reduction in mechanical hyperalgesia scores also indicates antinociceptive activity against peripheral pain (Zhou et al., 2024).

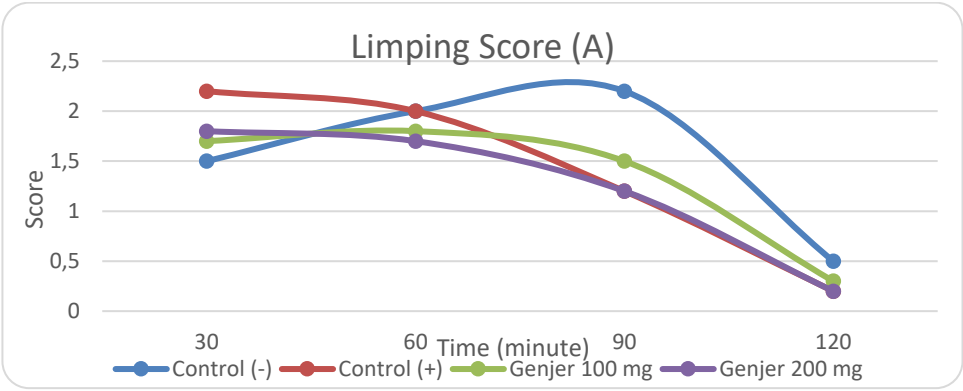


Figure 1. Limping Score Graph in AgNO₃-Induced Rats: Negative Control Group, Positive Control Group, Genjer Extract 100 mg, and Genjer Extract 200 mg

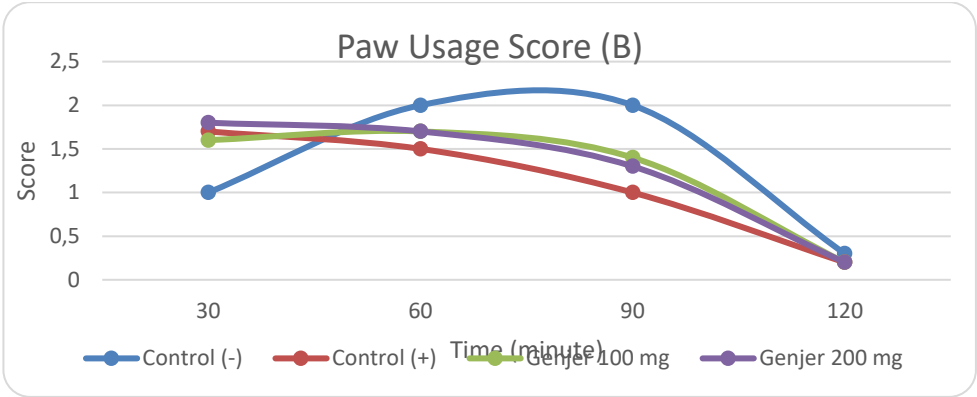


Figure 2. Paw Usage Score Graph in AgNO₃-Induced Rats: Negative Control Group, Positive Control Group, Genjer Extract 100 mg, and Genjer Extract 200 mg

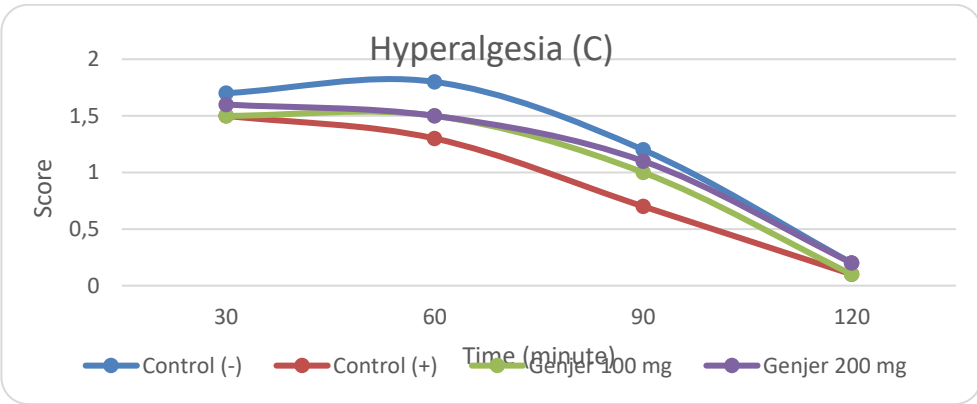


Figure 3. Hyperalgesia Score Graph in AgNO₃-Induced Rats: Negative Control Group, Positive Control Group, Genjer Extract 100 mg, and Genjer Extract 200 mg

For the edema parameter, the observed changes were relatively minor in all groups, although the 200 mg genjer extract group showed a tendency toward reduced

swelling. This suggests that the effect of genjer extract in this study was more dominant as an anti-joint pain agent than as an anti-inflammatory agent. Flavonoid

compounds are known to exert mild anti-inflammatory effects through inhibition of pro-inflammatory cytokines (Fatmawati et al., 2024). Overall, genjer extract demonstrated anti-joint pain activity characterized by reduced pain scores in experimental animals, with the best effectiveness observed at the 200 mg dose.

CONCLUSION

Genjer (*Limnocharis flava*) extract demonstrated moderate anti-joint pain activity in AgNO₃-induced rats, as evidenced by reductions in limping score, paw usage score, and mechanical hyperalgesia compared with the negative control group. The 200 mg dose produced greater analgesic effects than the 100 mg dose and approached the activity of the positive control (diclofenac sodium), although its effect on joint swelling was relatively limited. These findings suggest that *L. flava* extract has promising potential as a natural agent for the management of joint pain.

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CONFLICT OF INTEREST

The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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