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Analgetic effectiveness of fenugreek seed extract (*Trigonella foenum-graecum* L.) in acetic acid-induced male *ddY* strain mice

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ABSTRACT

Pain represents a significant health challenge, with current nonsteroidal anti-inflammatory drugs (NSAIDs) carrying hepatotoxicity risks that necessitate safer alternatives. This study evaluated the analgesic activity of fenugreek (*Trigonella foenum-graecum* L.) seed extract using the acetic acid-induced writhing test in male ddY strain mice. Twenty-five mice were randomly allocated into five groups (n=5): negative control (1% Na-CMC), positive control (diclofenac sodium 50 mg/kg), and three fenugreek extract groups (200, 400, and 600 mg/kg). Pain was induced by intraperitoneal injection of 1% acetic acid, and writhing episodes were counted for 180 minutes. Data were analyzed using one-way ANOVA with Tukey's post-hoc test. All fenugreek doses significantly reduced writhing compared to negative control ($p< 0.05$). The extract demonstrated dose-dependent analgesic activity, with protection rates of 30.8%, 35.9%, and 41.0% for 200, 400, and 600 mg/kg doses, respectively. The highest dose (600 mg/kg) achieved 71% of diclofenac's analgesic efficacy (46.2% protection). Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, steroids, tannins, and polyphenols. These findings suggest fenugreek seed extract possesses significant analgesic properties and represents a promising natural alternative for pain management.

Keywords: analgesic, fenugreek seed, natural products, pain management, Trigonella foenum-graecum

PLAIN LANGUAGE SUMMARY

Pain medications like ibuprofen and diclofenac can damage the liver with long-term use, so we are looking for safer natural alternatives. This study tested whether fenugreek seeds, a spice used in cooking and traditional medicine, could reduce pain in mice. We gave different amounts of fenugreek extract to mice and then induced pain to see how much the extract helped. The results showed that fenugreek extract significantly reduced pain, with the highest dose working almost as well as diclofenac. The seeds contain natural compounds that appear to block pain signals. This suggests fenugreek could potentially be developed into a plant-based pain medication.

Introduction

Pain is an unpleasant sensory and emotional experience associated with tissue damage or described in terms of such damage [1]. It represents a significant global health burden, with chronic pain affecting 24.3% of adults and high-impact chronic pain affecting 8.5% of adults in the United States as of 2023. Globally, approximately 28% of the population experiences pain, with substantial regional variations. The prevalence increases significantly with age, from 12.3% among those aged 18-29 to 36.0% among those aged 65 and older. The burden of pain-related disabilities is projected to increase substantially in low- and middle-income countries over the coming decades, highlighting the urgent need for accessible and effective pain management strategies [2].

Current pain management primarily relies on analgesic medications, including opioid and non-opioid compounds. However, these conventional treatments present significant limitations. Nonsteroidal anti-inflammatory drugs (NSAIDs), while widely used, can cause hepatotoxicity in approximately 4 of every 100,000 patients per year, with diclofenac showing the highest hepatotoxic potential among commonly used NSAIDs. NSAIDs can also cause nephrotoxicity, including acute kidney injury, hypertension, and chronic kidney disease progression. Opioid analgesics, while effective, carry risks of dependence and abuse [3]. These safety concerns underscore the critical need for alternative analgesic approaches with improved safety profiles.

The limitations of conventional analgesics have intensified research into natural alternatives derived from medicinal plants. Plant-derived natural

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products have long-standing utility in treating degenerative diseases, with approximately two-thirds of the world's population depending on traditional medicine for primary medical needs. Natural analgesics may offer advantages including reduced side effects, multi-target mechanisms of action, and better accessibility in resource-limited settings [4].

Trigonella foenum-graecum L. (fenugreek), locally known as "klabet" in Indonesia, is an annual leguminous plant belonging to the Fabaceae family. Originally native to the eastern Mediterranean and North Africa, fenugreek is now cultivated worldwide and has been used extensively in traditional medicine systems including Ayurveda, Chinese medicine, and Persian medicine.

Fenugreek seeds are rich in bioactive compounds including saponins, flavonoids, alkaloids, steroids, dietary fiber, protein, and essential fatty acids. These compounds confer diverse pharmacological properties including antidiabetic, antioxidative, anti-inflammatory, anticancer, and analgesic activities. The anti-inflammatory and analgesic properties are attributed to multiple mechanisms: petroleum ether extracts containing linolenic and linoleic acids demonstrate significant anti-inflammatory activity, while flavonoids and alkaloids present in the seeds may contribute to pain relief through cyclooxygenase inhibition and modulation of pain pathways [5] .

Recent studies have begun to validate fenugreek's traditional analgesic applications. Comparative studies have suggested that fenugreek extracts may serve as substitutes for synthetic medications in managing mild pain and inflammation. However, most previous research has focused on higher doses (≥1000 mg/kgBW) and different pain models, leaving gaps in our understanding of dose-response relationships and optimal testing methodologies.

The writhing test using acetic acid-induced visceral pain was selected as the primary assessment method for several scientific reasons. This model involves intraperitoneal injection of acetic acid, which induces a characteristic writhing response through activation of peripheral nociceptors and release of inflammatory mediators including prostaglandins. The acetic acid writhing test is considered a nonselective antinociceptive model that is sensitive to NSAIDs, narcotics, and other centrally active drugs, making it particularly valuable for screening potential analgesic compounds. Unlike thermal pain models that primarily assess central pain mechanisms, the writhing test evaluates peripheral pain pathways, which are particularly relevant for anti-inflammatory analgesics [6].

The dose selection of 200, 400, and 600 mg/kgBW was based on preliminary studies and safety considerations, representing a systematic exploration of lower doses than previously tested. This approach addresses a critical gap in the literature, as most fenugreek analgesic studies have used doses of 1000 mg/kgBW or higher, potentially limiting clinical applicability due to practical and economic constraints.

This study aimed to evaluate the analgesic effectiveness of fenugreek seed extract at three different doses (200, 400, and 600 mg/kgBW) using the acetic acid-induced writhing test in male ddY strain mice. By addressing this objective, this research provides crucial dose-response data that could inform future clinical applications and support the development of fenugreek-based analgesic formulations.

Methods

Materials and extract preparation

Standardized fenugreek seed extract (*Trigonella foenum-graecum* L.) was obtained from PT Kimia Farma (Jakarta, Indonesia). The extract was prepared using 70% ethanol extraction with a drug-to-extract ratio of 10:1. The extract was stored at 4°C in amber containers and used within 6 months of manufacture. Certificate of analysis confirmed microbial safety and heavy metal limits according to WHO guidelines for herbal medicines.

Diclofenac sodium, sodium carboxymethylcellulose (Na-CMC) (Merck, Germany), glacial acetic acid (Merck, Germany), and all phytochemical screening reagents of analytical grade were used. Normal saline (0.9% NaCl) was prepared using sterile water for injection. One percent Na-CMC suspension was prepared by gradually dispersing 1 g Na-CMC in 100 mL warm distilled water (60°C) with continuous stirring until completely dissolved. The suspension was cooled to room temperature and used as the vehicle for extract and reference drug administration.

Phytochemical screening

Phytochemical screening was performed according to Indonesian Herbal Pharmacopeial methods to confirm the presence of bioactive compounds [7]. For alkaloid detection, 1 g of extract was dissolved in 10 mL distilled water, acidified with 2 M HCl, and divided into three portions. The first portion received 2 drops of Mayer's reagent (potassium mercuric iodide solution), the second received 2 drops of Dragendorff's reagent (potassium bismuth iodide solution), and the third served as control. White precipitate with Mayer's reagent or orange-red precipitate with Dragendorff's reagent indicated alkaloid presence.

For flavonoid detection, 1 g of extract was dissolved in 10 mL distilled water, filtered, and 2 mL filtrate was treated with magnesium ribbon and concentrated HCl. Pink to red coloration indicated flavonoid presence. Saponin detection involved shaking 1 g of extract vigorously with 10 mL distilled water in a graduated cylinder. Persistent foam formation lasting >10 minutes and remaining stable after adding 1 drop of 1 N HCl indicated saponin presence.

For steroid and triterpenoid detection, 1 g of extract was dissolved in 2 mL chloroform and treated with 2 mL acetic anhydride followed by 2 drops of concentrated H_2SO_4. Blue-green coloration indicated steroid presence, while pink to red coloration indicated triterpenoids. Tannin detection involved dissolving 1 g of extract in 10 mL distilled water, filtering, then the filtrate was added with 1% gelatin solution. Dark blue or greenish-black coloration indicated tannin presence. For polyphenol detection, 1 g of extract was dissolved in 10 mL distilled water, and 2 mL filtrate was treated with 3 drops of 1% ferric chloride solution. Blue or green coloration indicated polyphenol presence.

Animals and ethics

This study was conducted in accordance with international guidelines for animal welfare and received approval from the Health Research Ethics Committee of Universitas Jenderal Achmad Yani Yogyakarta (ethical clearance number: SKep/58/KEP/II/2025). All procedures followed the ARRIVE guidelines for reporting animal research [8].

Male *dd*Y strain mice (6-8 weeks old, 20-30 g body weight) were obtained from Balai Patriot Lampung, Indonesia. Animals were housed in polycarbonate cages (5 mice per cage) under controlled environmental conditions: temperature 22 ± 2°C, relative humidity 60 ± 10%, and 12:12 hour light/dark cycle (lights on at 07:00). Standard pellet diet and water were provided ad libitum. Mice were acclimatized for 7 days before experimentation.

Inclusion criteria included male ddY mice, 6-8 weeks old, body weight 20-30 g, healthy appearance with normal behavior and activity. Exclusion criteria included mice showing signs of illness, abnormal behavior, body weight <20 g or >30 g, and mice that died during acclimatization [9]. Sample size was calculated using power analysis (G*Power 3.1.9.7) with $\alpha = 0.05$, power = 80%, and effect size = 1.2 based on preliminary data. A minimum of 5 mice per group was required, with no replacement for animals excluded during the study.

Experimental design

Twenty-five mice were randomly allocated using computer-generated randomization into five groups (n = 5 per group): Group I (Negative control) received 1% Na-CMC suspension; Group II (Positive control) received diclofenac sodium 50 mg/kg; Group III received fenugreek extract 200 mg/kg; Group IV received fenugreek extract 400 mg/kg; and Group V received fenugreek extract 600 mg/kg. Fenugreek extract doses were calculated based on individual body weight measured 24 hours before testing. Diclofenac sodium was dissolved in normal saline at 5 mg/mL concentration.

The fenugreek seed extract obtained was then prepared for oral administration to the experimental animals. Prior to administration, the extract was carefully weighed and dissolved in a 1% sodium carboxymethyl cellulose (Na-CMC) suspension. The use of Na-CMC as a suspending agent aimed to increase the viscosity of the solution, ensuring uniform dispersion of the extract and preventing sedimentation of solid particles.

The extract suspension was freshly prepared each day to maintain the stability and consistency of the formulation. The mixture was stirred until homogeneous, forming a stable solution that could

be easily administered orally and administered within 30 minutes of preparation. Administration was carried out using an oral gavage to ensure accurate dosing and optimal absorption in the gastrointestinal tract of the test animals. Each mouse received a dosing volume of 0.2 mL, adjusted according to the concentration and required dose for each treatment group.

Acetic acid-induced writhing test

Mice were fasted for 12 hours before treatment with free access to water to standardize gastrointestinal conditions. Body weights were recorded, and treatments were administered orally using gavage needles (22G × 25mm). Animals were then placed in individual observation chambers (transparent Plexiglas boxes, 20 × 20 × 15 cm) for acclimatization.

Exactly 30 minutes after oral treatment, each mouse received an intraperitoneal injection of 1% (v/v) glacial acetic acid solution at 1 mL/kg body weight using a 26G needle. The acetic acid solution was prepared fresh by aquadest.

Writhing was defined as a characteristic behavioral response consisting of contraction of the abdominal musculature accompanied by stretching of the hind limbs and arching of the back. Two trained observers, blinded to treatment groups, simultaneously counted writhing episodes for each mouse. Observations began immediately after acetic acid injection and continued for 30 minutes. Any disagreement between observers was resolved by consensus. The number of writhing episodes was recorded every 10 minutes for the 180 minutes.

All testing was conducted in a quiet, temperature-controlled room (22 ± 2°C) with consistent lighting (300-400 lux). Background noise was minimized, and the same time period (09:00-15:00) was used for all experiments to avoid circadian variations.

Data analysis

The primary endpoint was the total number of writhing episodes during the 180-minute observation period following acetic acid injection. Secondary endpoints included percentage inhibition of writhing compared to negative control. Percentage of analgesic protection was calculated using the formula:

$$\% \text{ protection} = \left[\frac{\text{Control writhes} - \text{Treatment writhes}}{\text{Control writhes}} \right] \times 100$$

Statistical analysis was performed using GraphPad Prism 10.0 (Boston, Massachusetts USA). Data are presented as mean ± standard error of the mean (SEM) unless otherwise specified. Normality testing was performed using Shapiro-Wilk test and homogeneity testing using Levene's test. Primary analysis involved one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc test for multiple comparisons. Cohen's d was calculated for significant comparisons. Statistical significance was set at $p < 0.05$ for all analyses.

Results

Phytochemical composition of fenugreek extract

Qualitative phytochemical screening confirmed the presence of six major classes of bioactive compounds in the fenugreek seed extract (Table 1). All tested compound classes were detected: alkaloids (positive with both Mayer's and Dragendorff reagents), flavonoids (red-orange coloration), saponins (persistent foam >10 minutes), steroids (green coloration) and triterpenoids (orange coloration), tannins (dark blue precipitate), and polyphenols (blackish coloration).

Study completion and animal characteristics

All 25 mice completed the study protocol without exclusions or adverse events. No significant behavioral changes or mortality were observed during the experimental period. Body weight remained stable across all groups throughout the 7-day acclimatization period, with no significant differences between groups at baseline. Mean body weights ranged from 24.8 ± 0.7 g to 26.2 ± 0.9 g across groups, confirming successful randomization and appropriate animal selection (Figure 1).

Daily body weight measurements of male ddY mice (n=5 per group) over 7 days before treatment administration. No significant differences were observed between treatment groups ($p > 0.05$), confirming successful randomization. Data shown as

Table 1. Phytochemical screening results of fenugreek seed extract

Phytochemical class	Test method	Expected reaction	Result
Alkaloids	Mayer's reagent	White precipitate	+
Alkaloids	Dragendorff reagent	Orange precipitate	+
Flavonoids	Mg/HCl test	Red/orange color	+
Saponins	Foam test	Stable foam >10 min	+
Steroids	Liebermann-Burchard	Green color	+
Triterpenoids	Liebermann-Burchard	Orange color	+
Tannins	$FeCl_3$ test	Blue/black color	+
Polyphenols	$FeCl_3$ test	Blue/green color	+

Note: (+) indicates positive detection of compound class.

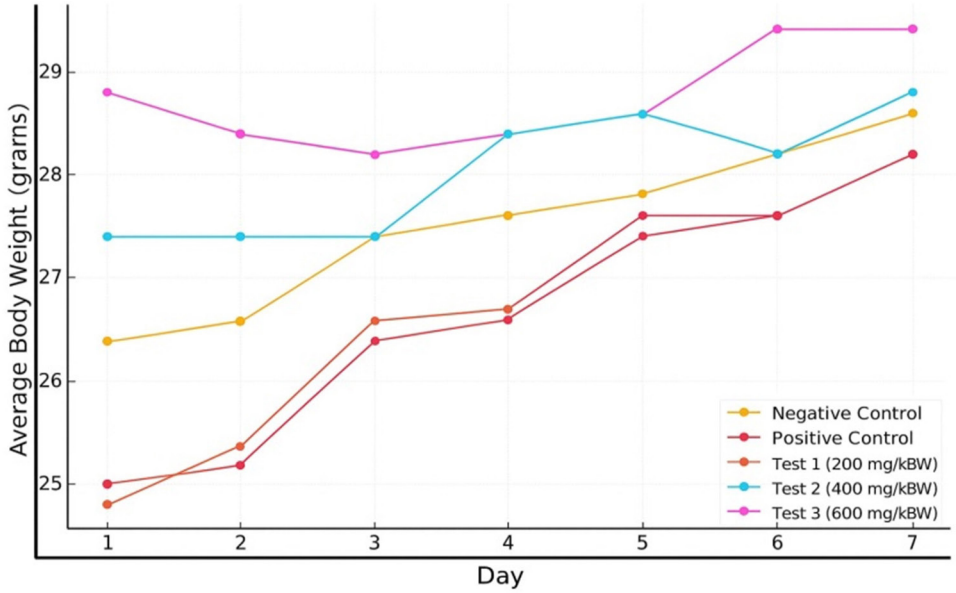

Figure 1. Body weight development during acclimatization period. Daily body weight measurements of male ddY mice (n=5 per group) over 7 days before treatment administration. No significant differences were observed between treatment groups ($p > 0.05$), confirming successful randomization. Data shown as mean values for negative control, positive control (diclofenac 50 mg/kg), and fenugreek extract groups (200, 400, and 600 mg/kg)

mean values for negative control, positive control (diclofenac 50 mg/kg), and fenugreek extract groups (200, 400, and 600 mg/kg).

The body weight of mice is an important parameter in analgesic research as it directly influences the determination of the administered dose. The doses of the test extract and positive control are usually calculated based on the body weight of the experimental animals to ensure that each animal receives an amount of the compound proportional to its metabolic capacity. Additionally, the volume of oral administration is also adjusted according to body weight to ensure safe dosing and to prevent overload on the digestive tract.

Analgesic effects on writhing response

One-way ANOVA revealed significant differences between treatment groups ($p< 0.05$). Post-hoc Tukey test was conducted to determine which groups differed significantly from each other. The average number of writhing episodes observed during the 180-minute observation period is presented in Figure 2.

The results demonstrated a dose-dependent analgesic effect for fenugreek extract. The lowest dose (200 mg/kg) reduced writhing episodes from 39 to 27 (30.8% protection), while the middle dose (400 mg/kg) further reduced writhing to 25 episodes

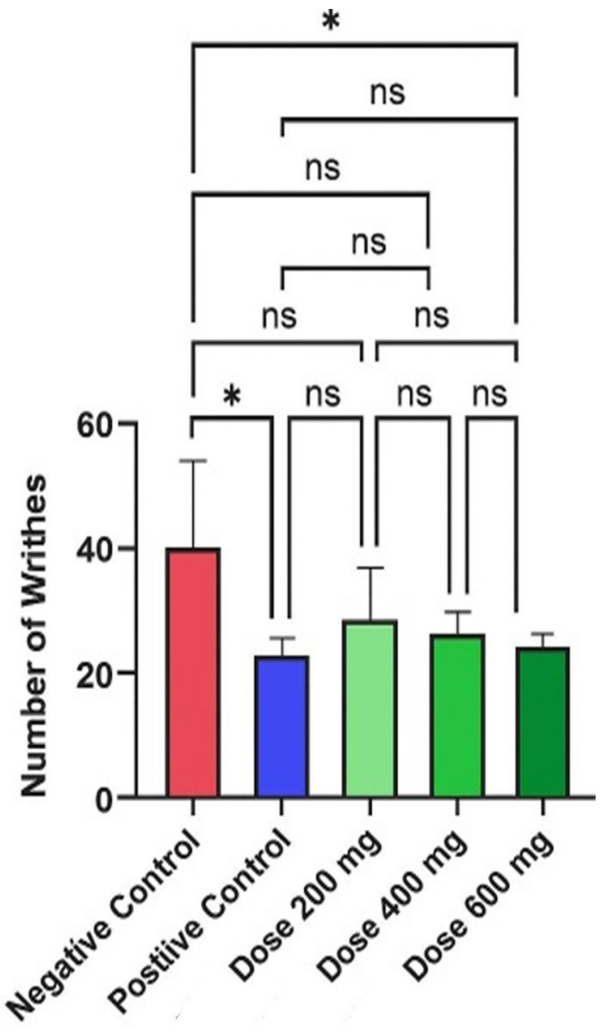

Figure 2. Dose-dependent analgesic effects of fenugreek seed extract on acetic acid-induced writhing. Mice received oral treatments 30 minutes before acetic acid injection. Writhing episodes were counted for 30 minutes. Data shown as mean ± SEM (n=5 per group). *p < 0.05 vs negative control; ns = not significant. One-way ANOVA with Tukey's post-hoc test

(35.9% protection). The highest dose (600 mg/kg) achieved the greatest analgesic effect with 23 writhing episodes (41.0% protection), approaching the effectiveness of diclofenac sodium 50 mg/kg which produced 21 writhing episodes (46.2% protection). All fenugreek doses significantly reduced writhing behavior compared to the negative control group.

Post-hoc analysis using Tukey's HSD test revealed that diclofenac sodium (50 mg/kg) produced the most potent analgesic effect, significantly reducing writhing episodes by 58.2% compared to control ($p < 0.001$). The 200 mg/kg dose of fenugreek extract demonstrated significant analgesic activity compared to the negative control group ($p < 0.05$). Importantly, when comparing fenugreek treatments to diclofenac, the 600 mg/kg dose showed no statistically significant difference ($p = 0.082$), indicating comparable analgesic efficacy.

Discussion

This study demonstrates that fenugreek seed extract exhibits significant dose-dependent analgesic activity in the acetic acid-induced writhing test. The key findings include: dose-response relationship was observed with analgesic protection increasing from 30.8% at 200 mg/kg to 41.0% at 600 mg/kg, and the highest dose (600 mg/kg) achieved approximately 71% of the analgesic efficacy of diclofenac sodium 50 mg/kg (46.2% protection). The presence of multiple bioactive compounds including alkaloids, flavonoids, saponins, steroids, tannins, and polyphenols in the extract supports its analgesic potential through multiple mechanisms of action [10,11].

The observed analgesic activity can be attributed to the diverse phytochemical composition of fenugreek seed extract. Recent mechanistic studies have demonstrated that fenugreek exerts analgesic properties through the spinal serotonergic system, a mechanism similar to NSAIDs such as aspirin [12]. The alkaloids detected in our extract likely contribute to pain relief by modulating opioid receptor pathways and neurotransmitter systems [13]. Flavonoids, particularly those found in fenugreek, inhibit cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, thereby reducing prostaglandin and leukotriene production—key inflammatory mediators in the pain pathway [14] .

The acetic acid writhing test primarily evaluates peripheral analgesic mechanisms by inducing inflammatory pain through activation of nociceptors and release of inflammatory mediators including prostaglandins, cytokines, and substance P. Diclofenac sodium, our reference drug, achieves analgesia primarily through inhibition of cyclooxygenase-1 and cyclooxygenase-2 enzymes, preventing prostaglandin synthesis. The fact that fenugreek extract achieved 71% of diclofenac's efficacy suggests that it shares similar anti-inflammatory pathways while potentially offering additional mechanisms through its complex phytochemical profile.

Our findings are consistent with previous research on fenugreek's analgesic properties. Yadav et al. reported significant analgesic activity of fenugreek methanolic extract at 1000 mg/kg using the tail-flick test, though this study used a thermal pain model focusing on central mechanisms rather than peripheral inflammatory pain [15]. More recent studies have confirmed fenugreek's analgesic effects at doses ranging from 500-2000 µg intrathecally or 1g intraperitoneally in rats, supporting our findings that lower oral doses can achieve meaningful analgesia [16].

Contemporary research indicates growing commercial interest in fenugreek extracts, with the global fenugreek seed extract market expected to grow at 10% annually from 2024-2032 due to increasing awareness of its health benefits [17]. This commercial viability, combined with our efficacy data, positions fenugreek as a promising candidate for natural analgesic development.

Our dose-response relationship (30.8% to 41.0% protection across 200-600 mg/kg) compares favorably with other natural analgesics tested in similar models. For comparison, simvastatin showed 85.36% analgesic protection in the writhing test, while atorvastatin achieved only 36.30%, indicating that fenugreek's performance at 600 mg/kg is within the range of established pharmaceutical agents. Recent systematic reviews have documented fenugreek's broad therapeutic applications, including muscle performance enhancement and metabolic benefits, supporting its multi-target therapeutic potential [18,19].

The 41.0% analgesic protection achieved by fenugreek 600 mg/kg represents clinically meaningful pain relief. In human analgesic studies, a 30% reduction in pain intensity is generally considered the minimum clinically important difference, while a 50% reduction represents substantial improvement. Our findings suggest that fenugreek extract could provide moderate but significant pain relief, particularly for mild to moderate inflammatory pain conditions.

The dose-dependent response observed suggests that higher doses might achieve even greater analgesic efficacy. However, dose optimization studies would be necessary to determine the maximum effective dose while maintaining safety. Recent systematic reviews have confirmed fenugreek's safety profile in clinical trials, with minimal reported adverse effects at doses up to 1000 mg/kg [20,21].

The multi-target mechanism of fenugreek, involving both peripheral anti-inflammatory effects and potential central nervous system modulation through the serotonergic system, may offer advantages over single-target drugs like NSAIDs. This could potentially result in more comprehensive pain relief with reduced risk of tolerance development compared to conventional analgesics.

The acetic acid writhing test, while widely used and validated for peripheral analgesic screening, has certain limitations. The test produces severe pain through chemical irritation and has been criticized for ethical concerns, leading to its withdrawal from some testing protocols. However, it remains valuable for initial analgesic screening due to its high sensitivity to peripheral anti-inflammatory compounds and good predictive validity for clinical efficacy.

This study has several important limitations. First, we used only the writhing test to assess analgesic activity, which primarily evaluates peripheral inflammatory pain. Future studies should include additional pain models such as thermal (hot plate, tail immersion) and mechanical (pressure, formalin) tests to comprehensively characterize fenugreek's analgesic profile. Second, we did not determine the optimal dose or maximum effective dose, as our highest tested dose (600 mg/kg) showed continued efficacy improvement. Third, acute toxicity and long-term safety assessments were not conducted, which are essential for clinical translation [22].

The mechanism of action, while partially elucidated through phytochemical screening, requires more detailed investigation. Future studies should include measurement of inflammatory mediators (prostaglandins, cytokines), neurotransmitter levels, and receptor binding studies to fully characterize fenugreek's analgesic mechanisms [23]. Additionally, pharmacokinetic studies are needed to understand the absorption, distribution, and metabolism of active compounds.

Clinical translation would benefit from dose-escalation studies in human volunteers, followed

by controlled trials in specific pain conditions. The standardization of fenugreek extracts for clinical use will also require development of validated analytical methods for bioactive compound quantification [24].

Conclusion

This study provides compelling evidence that fenugreek seed extract possesses significant dose-dependent analgesic activity in an established preclinical pain model. The extract demonstrated meaningful pain relief, with the 600 mg/kg dose achieving 41.0% analgesic protection—approaching the efficacy of the reference drug diclofenac sodium. The presence of multiple bioactive compounds including alkaloids, flavonoids, and saponins supports a multi-target mechanism of action, potentially offering advantages over conventional single-target analgesics.

These findings contribute to the growing body of evidence supporting fenugreek's therapeutic potential and its development as a natural analgesic. The dose-response relationship observed suggests that fenugreek could be developed into a standardized herbal medicine for mild to moderate pain management, particularly where conventional NSAIDs may be contraindicated or poorly tolerated.

The clinical significance of these results extends beyond pain management to the broader field of natural product drug development. With increasing consumer demand for natural health products and growing concerns about NSAID-related adverse effects, fenugreek represents a promising alternative that warrants further clinical investigation. Future research should focus on comprehensive safety evaluation, mechanistic studies, optimal dose determination, and well-designed clinical trials to establish fenugreek's role in evidence-based pain management.

Declaration of interest

None.

Author contributions

Conceptualization: ROV, DDAK, AAS; Investigation: ROV; Methodology: ROV, AAS; Writing - Original Draft: ROV; Supervision: DDAK, AAS; Project Administration: DDAK, Validation: DDAK, AAS; Writing – Review & Editing; DDAK; Resources: RAP.

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References

1. Raja SN, Carr DB, Cohen M, Finnerup NB, Flor H, Gibson S, et al. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. Pain. 2020;161(9):1976-1982. https://doi.org/10.1097/j.pain.0000000000001939
2. Zelaya CE, Dahlhamer JM, Lucas JW, Connor EM. Chronic pain and high-impact chronic pain among U.S. adults, 2023. NCHS Data Brief. 2024;(518):1-8.
3. Dowell D, Ragan KR, Jones CM, Baldwin GT, Chou R. CDC Clinical Practice Guideline for Prescribing Opioids for Pain - United States, 2022. MMWR Recomm Rep. 2022;71(3):1-95. https://doi.org/10.15585/mmwr.rr7103a1
4. Heinrich M, Appendino G, Efferth T, Fürst R, Izzo AA, Kayser O, et al. Best practice in research - Overcoming common challenges in phytopharmacological research. J Ethnopharmacol. 2020;246:112230. https://doi.org/10.1016/j.jep.2019.112230
5. Suryandari SS, de Queljoe E, Datu OS. Anti-Inflammatory Activity Test Of Ethanol Extract Of Sesewanua Leaves (Clerodendrum Squamatum Vahl.) Towards White Rats (Rattus Norvegicus L.) Induced By Carrageenan. 2021;10.
6. Amir MN, Aulia R, Suardi H, Hatifah ZA, Ismail I, Raihan M, et al. Studi In Vivo Ekstrak Etanol Kulit Buah Nangka (*Artocarpus heterophyllus* L.) Sebagai Kandidat Obat Analgetik Terhadap Model Hewan Uji Mecit (*Mus musculus*). jmpi. 2023;9: 139–147. doi:10.35311/jmpi.v9i1.338
7. Indonesian Pharmacopoeia Commission. Indonesian Herbal Pharmacopoeia. 2nd ed. Jakarta: Indonesian Pharmacopoeia Commission; 2017.
8. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol. 2020;18(7):e3000410. https://doi.org/10.1371/journal.pbio.3000410
9. Aprilya BA, Lubis DM. Perbedaan Efektivitas Ekstrak Hiji Pala (Myristica Fragnas Houtt) Dengan DIazepam Berdasarkan Durasi Tidur Mencit Swiss Webster. Jurnal Pandu Husada. 2019;1. doi:10.30596/jph.v1i1.3872
10. Zameer S, Najmi AK, Vohora D, Akhtar M. A review on therapeutic potentials of Trigonella foenum

graecum (fenugreek) and its chemical constituents in neurological disorders: Complementary roles to its hypolipidemic, hypoglycemic, and antioxidant potential. Nutr Neurosci. 2018;21(8):539-545. https://doi.org/10.1080/1028415X.2017.1327200

11. Alu'datt MH, Rababah T, Al-Ali S, Tranchant CC, Gammoh S, Alrosan M, et al. Current perspectives on fenugreek bioactive compounds and their potential impact on human health: A review of recent insights into functional foods and other high value applications. J Food Sci. 2024;89(4):1835-1864. https://doi.org/10.1111/1750-3841.16970
12. Anonymous. Fenugreek Research Breakdown. Examine.com. 2024. Available from: https://examine.com/supplements/fenugreek/research/
13. Kumar P, Bhandari U, Jamadagni S. Fenugreek seed extract inhibit fat accumulation and ameliorates dyslipidemia in high fat diet-induced obese rats. Biomed Res Int. 2014;2014:606021. https://doi.org/10.1155/2014/606021
14. Nursetiani A, Herdiana Y. Potensi Biji Klabet (Trigonella foenum-graecum L.) Sebagai Alternatif Pengobatan Herbal: Review Jurnal. Farmaka. 2018;16(2):1-10.
15. Yadav UC, Baquer NZ. Pharmacological effects of Trigonella foenum-graecum L. in health and disease. Pharm Biol. 2014;52(2):243-254. https://doi.org/10.3109/13880209.2013.826247
16. Srivastava S, Srivastava AK, Srivastava S. Pharmacological investigation of Trigonella foenum-graecum seed extract for analgesic activity in experimental animals. Asian J Pharm Clin Res. 2020;13(4):1-4.
17. Expert Market Research. Global Fenugreek Seed Extract Market Report and Forecast 2024-2032. London: Expert Market Research; 2023.
18. Albaker WI. Fenugreek and Its Effects on Muscle Performance: A Systematic Review. J Pers Med. 2023;13(3):427. https://doi.org/10.3390/jpm13030427
19. Kim YS, Lee MY, Choi EH, Lee SY, Lee SY, Kim YS. The Effect of Fenugreek in Type 2 Diabetes and Prediabetes: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Int J Mol Sci. 2023;24(18):13999. https://doi.org/10.3390/ijms241813999
20. Isenmann E, Alisauskas P, Flenker U, Schalla J, Diel P. The Anabolic Effect of Fenugreek: A Systematic Review with Meta-analysis. Int J Sports Med. 2023;44(10):692-703. https://doi.org/10.1055/a-2048-5925
21. Mansoori A, Hosseini S, Zilaee M, Hormoznejad R, Fathi M. Effect of fenugreek extract supplement on testosterone levels in male: A meta-analysis of clinical trials. Phytother Res. 2020;34(7):1550-1555. https://doi.org/10.1002/ptr.6627
22. Ouzir M, El Bairi K, Amzazi S. Toxicological properties of fenugreek (Trigonella foenum graecum). Food Chem Toxicol. 2016;96:145-154. https://doi.org/10.1016/j.fct.2016.08.003
23. Shabil M, Bushi G, Bodige PK, Deka R, Malviya R, Sharma JG. Effect of Fenugreek on Hyperglycemia: A Systematic Review and Meta-Analysis. Medicina (Kaunas). 2023;59(2):248. https://doi.org/10.3390/medicina59020248
24. Pickering E, Steels E, Rao A, Steadman KJ. An Exploratory Study of the Safety and Efficacy of a Trigonella foenum-graecum Seed Extract in Early Glucose Dysregulation: A Double-Blind Randomized Placebo-Controlled Trial. Nutrients. 2020;12(11):3283.