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Pharmacological Evaluation of the Analgesic, Antipyretic, and Anti-Inflammatory Effects of Ethanol Extract from *Gardenia stenophylla* Merr. Fruits in Animal Models

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ABSTRACT

This study investigates the therapeutic effects of *Gardenia stenophylla* Merr. Using experimental animal models, fruit extract (GSME) on pain, inflammation, and fever. The primary objective was to evaluate the analgesic, anti-inflammatory, and antipyretic activities of GSME at varying dosages. Mice were administered GSME at 100, 150, and 200 mg/kg doses to assess their analgesic potential through writhing and hot plate tests. The antipyretic effect was evaluated using a fever-inducing model, while the anti-inflammatory potential was examined via the carrageenan-induced paw edema model at doses of 100, 200, and 300 mg/kg. Key parameters measured included reaction time, pain inhibition, rectal temperature, fever suppression, paw diameter, edema inhibition, and serum cytokine levels (IL-1β, IL-6, TNF-α). The results indicated that GSME significantly enhanced pain tolerance in the hot plate test and reduced writhing behavior, particularly at the dose of 200 mg/kg, resulting in a reduction of 54.01% ($p < 0.05$). In the fever model, GSME demonstrated a substantial reduction of 60.19% in rectal temperature after 4 hours ($p < 0.05$). Additionally, GSME effectively diminished paw edema and lowered serum concentrations of pro-inflammatory cytokines. These findings support the traditional use of *Gardenia stenophylla* for alleviating pain, reducing fever, and managing inflammation, highlighting its potential as a natural remedy for inflammatory conditions.

***Keywords*:** Analgesic effects, Antipyretic properties, Anti-inflammatory activity, *Gardenia stenophylla* Merr., Mice model.

Introduction

Gardenia, a genus within the Rubiaceae family, encompasses approximately 60 species primarily distributed across tropical and subtropical regions. Many of these species, originating from East Asia, Africa, and tropical islands, are characterized as evergreen shrubs or small trees with dark green foliage and fragrant, pale yellow or white flowers.[1] Certain Gardenia species are known to contain pharmacologically active compounds such as iridoids and flavonoids, which are recognized for their antioxidant and anti-inflammatory properties. For example, *Gardenia sootepensis* has demonstrated a range of biological activities including cytotoxicity, anti-implantation, anti-ulcer, antibacterial, diuretic, analgesic, antihypertensive, and anthelmintic effects. Similarly, compounds found in *Gardenia coronaria* and *Gardenia sootepensis* exhibit broad-spectrum cytotoxicity against human cancer cell lines.[2] *Gardenia jasminoides*, containing active compounds such as geniposide, genipin, crocin, and crocetin, has been utilized for its analgesic, diuretic, antihypertensive, antibacterial, anxiolytic, antispasmodic, antipyretic, headache relief, anti-arthritic, anticancer, and neuroprotective effects.[3]

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Gardenia stenophylla Merr. has long been recognized in traditional medicine for its therapeutic properties. The fruit of *G. stenophylla* is notably acknowledged in folk medicine for its potential benefits in treating various conditions.[4] Traditionally, the fruit has been employed for its anti-inflammatory, hepatoprotective, nephroprotective, and antidiabetic effects, highlighting its historical significance and widespread use in traditional remedies.[5]

Investigating the managing pain, fever, and inflammation effects of *G. stenophylla* fruit extract (GSME) is important for several reasons. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage pain, fever, and inflammation by inhibiting cyclooxygenase (COX) enzymes, which play a critical role in the synthesis of prostaglandins (PGE) involved in inflammatory, febrile, and pain responses. However, NSAIDs are associated with potential risks including hepatotoxicity, nephrotoxicity, gastrointestinal issues, and cardiovascular complications. As a result, there is growing interest in exploring alternative treatments with a lower incidence of adverse effects.[6] Despite its traditional use for managing pain, fever, and inflammation, there remains a significant gap in scientifically validated evidence supporting these claims for *Gardenia stenophylla*. This study evaluated the analgesic, antipyretic, and anti-inflammatory properties of GSME, thereby providing a scientific basis for its traditional uses and potentially revealing new therapeutic applications. The findings could enhance the credibility of traditional medicine practices and lead to the identification of novel therapeutic agents derived from *Gardenia stenophylla*.

Materials and Methods

Collection of material and preparation of the extract

In April 2024, fruits of *Gardenia stenophylla* were collected from Buon Don District, Dak Lak Province, Vietnam. A voucher specimen, labeled VST-GSM260424, was deposited at the Laboratory of Plant Biotechnology, Institute of Biotechnology and Food Technology, Ho Chi Minh City University of Industry, for future reference. The fruits

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were screened to eliminate any damaged or diseased samples, then thoroughly washed with clean water, followed by a rinse with distilled water to ensure the removal of surface impurities. The selected fruits were air-dried in the shade for 48 hours before being further dehydrated in a Memmert drying cabinet (Germany) at 40°C until their moisture content dropped below 12%. The dried fruits were then finely ground into powder and stored in moisture-proof containers, ready for extraction.

The extraction process of *Gardenia stenophylla* fruits was conducted according to the method described by Falodun *et al*. with some modifications.[7] Fifty grams of fully mature *G. stenophylla* fruit pulp were immersed in 50 mL of 70% ethanol and extracted using a Soxhlet apparatus for 10 hours. The resulting extract was then filtered through a vacuum Buchner funnel (Sigma-Aldrich, USA), collecting the upper phase. The remaining pellets and residues were subjected to a second round of extraction under identical conditions. The two upper phases were combined and concentrated under vacuum to a final volume of 50 mL using a Büchi rotary evaporator (Switzerland) at 37°C. In this process, 1 mL of the concentrated extract solution, designated as GSME, corresponded to 1 g of *G. stenophylla* fruit pulp. The concentrated extract was then aliquoted into Eppendorf tubes, vacuum-dried using a Savant concentrator (Thermo Fisher Scientific, USA), and stored at -10°C until further analysis.

Qualitative and quantification phytochemical analysis of extract

Following the plant screening method described by Tran *et al*.,[8] the extract was analyzed for various bioactive constituents, including alkaloids, flavonoids, phenolics, steroids, tannins, cardiac glycosides, terpenoids, and saponins.

The total polyphenol content was measured using the Folin-Ciocalteu method described by Tran and Tran.[9] In this process, 1 mL of the extract was mixed with 5 mL of Folin-Ciocalteu reagent, diluted 1:10 with distilled water, and allowed to react for 5 minutes at room temperature. Afterward, 4 mL of 7.5% sodium carbonate solution was added, and the mixture was incubated in the dark at 25°C for 60 minutes. The absorbance was measured at 765 nm using a UV5 spectrophotometer (Mettler Toledo, Switzerland). Gallic acid was used as the standard, with a calibration curve constructed over concentrations of 50-500 µg/mL. The total polyphenol content was calculated from the standard curve equation ($y = 0.0098x + 0.0488$, $R^2 = 0.9971$) and expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

The total flavonoid content was determined using the aluminum chloride colorimetric method described by Nhung and Quoc, with some small modifications.[10] In this assay, 1 mL of the extract was mixed with 4 mL of distilled water, followed by the addition of 0.3 mL of 5% sodium nitrite ($NaNO_2$) solution. After a 5-minute incubation at room temperature, 0.3 mL of 10% aluminum chloride ($AlCl_3$) solution was added, and the mixture was left to stand for 6 minutes. Then, 2 mL of 1M sodium hydroxide (NaOH) solution was added, and the final volume was adjusted to 10 mL with distilled water. The mixture was vortexed thoroughly, and absorbance was measured at 510 nm using a UV5 spectrophotometer (Mettler Toledo, Switzerland). Quercetin was used as the standard, with a calibration curve ranging from 25 to 250 µg/mL. The total flavonoid content was calculated using the standard curve equation ($y = 0.0115x + 0.0545$, $R^2 = 0.9991$) and expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW).

The quantification of terpenoids in plant extracts was performed using the method outlined by Tran and Tran.[11] A 100 mg sample of the plant extract was dissolved in 1 mL of ethanol. A 5% vanillin solution in ethanol was prepared, and 0.5 mL of this solution was combined with 2 mL of concentrated sulfuric acid to form the vanillin-sulfuric acid reagent. Then, 0.5 mL of the sample solution was added to the reagent and incubated at room temperature for 20 minutes to develop the color reaction. The absorbance was measured at 538 nm using a UV5 spectrophotometer (Mettler Toledo, Switzerland). Terpenoid content was quantified by comparing the absorbance of the sample to a calibration curve constructed using lupeol standards (10-100 µg/mL), yielding the equation $y = 1.8214x + 0.1632$ ($R^2 = 0.9946$).

Experimental animals

Healthy Swiss mice weighing between 31 and 33 grams were procured from the Pasteur Institute in Ho Chi Minh City, Vietnam. The mice were individually housed in glass cages at the experimental breeding facility of the Eastern Agriculture and Food Company in Ho Chi Minh City, where they were kept under controlled environmental conditions. The facility maintained a constant temperature of 24 ± 2°C and a relative humidity of 55-60%. The mice were subjected to a 12-hour light/12-hour dark cycle throughout the experimental period, following a 14-day acclimation period to the new caging environment. During the study, the mice had unrestricted access to standard laboratory food and water, with no dietary restrictions imposed. Ethical guidelines for animal research were strictly adhered to as outlined in the Basel Declaration on Animal Research in 2010.[12] All animal care and handling aspects were conducted by trained personnel under the ethical standards set by the Animal Ethics Committee of the Ho Chi Minh City University of Industry.

The analgesic activity of the extract

The acetic acid-induced writhing test

Peripheral analgesic activity was assessed using acetic acid as a chemical irritant. Intraperitoneal (IP) injection of acetic acid induces pain and inflammation, leading to characteristic writhing behavior in mice, including abdominal muscle contractions.[13] Swiss mice were divided into five groups in this study, with five mice per group. Experimental groups received oral administration of GSME at doses of 100, 150, and 200 mg/kg (GSME100, GSME150, and GSME200, respectively). The negative control group was given physiological saline at 5 mL/kg. Aspirin at 100 mg/kg served as the reference drug. Thirty minutes after administration of the test substances and the reference drug, acetic acid (0.6% v/v in normal saline) was injected intraperitoneally. Abdominal contractions were counted over 30 minutes, with observations recorded every 5 minutes. The percentage of pain relief (PRA, %) was calculated using the specified formula.

$$\text{PRA (\%)} = \frac{\text{Mean number of writhes (control} - \text{test)}}{\text{Mean number of times of writhing in the control}} \times 100 \quad \text{(Eq. 1)}$$

The hot plate test

The hot plate test is a widely utilized method for evaluating responses to high-intensity nociceptive stimuli, focusing on the transmission of pain via thermal stimulation through the central nervous system.[14] In this study, Swiss mice were allocated into five groups, each comprising five individuals. The mice were individually placed on a hot plate set to a constant temperature of 50 ± 2°C, and their reaction latencies -such as paw lifting, licking, or jumping - were recorded. Baseline latency was measured before drug administration. The negative control group received oral physiological saline at a dose of 5 mL/kg. Experimental groups were treated with plant extract doses of 100, 150, and 200 mg/kg (designated as GSME100, GSME150, and GSME200, respectively), while tramadol at 5 mL/kg was used as the reference drug. Mice were assessed on the hot plate at 15, 30, 45, and 60 minutes after treatment, and the duration of pain responses (paw licking or jumping) was noted. The percentage of pain inhibition (PPI, %) was then calculated using the designated formula:

$$\text{PPI (\%)} = \frac{\text{Time after treatment} - \text{Time before treatment}}{\text{Time after treatment}} \times 100 \quad \text{(Eq. 2)}$$

The antipyretic effect of the extract

The antipyretic activity was evaluated by inducing fever in mice through yeast injection, based on a modified protocol by Nhung and Quoc.[14] Mice were fasted overnight with access to water before treatment. Each of the six treatment groups, comprising five mice each, received a subcutaneous injection of 20% yeast solution (10 mL/kg) to induce fever. The extract groups were administered oral doses of GSME at 100, 150, and 200 mg/kg (Yeast+GSME100, Yeast+GSME150, and Yeast+GSME200, respectively). The negative control group received yeast injection alone (10 mL/kg, Yeast treatment), the standard control

group was treated with oral paracetamol (100 mg/kg) (Yeast+PCM treatment), and the normal control group received physiological saline (5 mL/kg) (Saline treatment). Rectal temperatures were measured before and 24 hours after yeast injection using a digital rectal probe (USA). Post-treatment temperatures were recorded at 1, 2, 3, and 4 hours. The percentage of fever reduction (PFR) was calculated based on these measurements.

$$\text{PFR}\ (\%) = \frac{\text{Temperature after causing fever} - \text{Temperature after 1,2,3 ,4 hours}}{\text{Temperature after causing fever} - \text{The normal body temperature}} \times 100 \quad \text{(Eq. 3)}$$

Assessment of anti-inflammatory of GSME
Carrageenan-induced paw oedema
The anti-inflammatory efficacy of GSME was evaluated using the carrageenan-induced paw edema model in mice, following a modified procedure by Nhung and Quoc.[14] Six groups of healthy Swiss mice, each weighing between 31-33 grams and comprising five individuals, were used. After an overnight fast with free access to water, the initial volume of the right hind paw was recorded. Then, 50 µL of carrageenan (CGN) (1% w/v in physiological saline) was injected into the subplantar region of the right hind paw. The treatment groups received oral GSME at doses of 100, 200, and 300 mg/kg (CGN+GSME100, CGN+GSME200, and CGN+GSME300, respectively). The positive control group was treated with oral indomethacin (10 mg/kg) (CGN+IND), the negative control group received carrageenan alone (CGN), and the normal control group was given physiological saline (5 mL/kg) (Saline). Paw volume changes, reflecting inflammation, were measured in millimeters using a digital caliper (Mitutoyo, Japan) at baseline (0 hours) and at 1, 2, and 3 hours post-carrageenan injection. The percentage of edema inhibition (PEI) was calculated according to the specified formula.

$$\text{PEI}\ (\%) = \frac{\text{Paw edema in the control group} - \text{Paw edema in the test group}}{\text{Paw edema in the control group}} \times 100 \quad \text{(Eq. 4)}$$

Evaluation of the serum levels of cytokines in mice
Quantification of TNF-α, IL-6, and IL-1β was performed using an enzyme-linked immunosorbent assay (ELISA) combined with an immunoassay technique, as described by Nhung and Quoc.[15] In this procedure, capture antibodies for TNF-α, IL-6, and IL-1β were immobilized on individual wells of a 96-well plate and allowed to adhere overnight. Following incubation with standard antigens, a biotin-labeled secondary antibody was added. The addition of streptavidin resulted in a color change from purple to yellow, which was measured at 450 nm using a spectrophotometer. The concentrations of TNF-α, IL-6, and IL-1β in the samples were determined and reported in pg/mL.

Statistical analysis
The data are expressed as the mean ± standard deviation (mean ± SD). Statistical significance is denoted by a p-value < 0.05, and a Tukey's post hoc test was employed for the analysis of multiple treatment groups, with ANOVA calculated using Statgraphics Centurion software version XIX.

Results and Discussion

Screening and quantification of phytochemicals in extracts
Table 1 presents the phytochemical profile of GSME, revealing the presence of alkaloids, flavonoids, phenolics, steroids, tannins, terpenoids, and saponins. However, cardiac glycosides were not detected in GSME. The total phenolic content of GSME was quantified at 65.19 ± 2.01 mg GAE/g, indicating a substantial presence of phenolic compounds known for their antioxidant properties. The total flavonoid content was 39.27 ± 1.89 mg QE/g, suggesting the extract contains significant amounts of flavonoids, which are associated with anti-inflammatory and antioxidant effects. Additionally, the terpenoid content was measured at 71.32 ± 2.49 mg TAE/g, highlighting the presence of terpenoids with potential anti-inflammatory and antimicrobial activities.

Table 1: Qualitative and quantitative analysis of phytochemicals in extract

Phytoconstituents	Present in GSME	Quantification of phytochemicals
Tannins	+	NT
Flavonoids	+	39.27 ± 1.89 mg QE/g
Terpenoids	+	71.32 ± 2.49 mg TAE/g
Polyphenol	+	65.19 ± 2.02 mg GAE/g
Saponins	+	NT
Steroids	+	NT
Cardiac glycosides	-	-
Alkaloids	+	NT

Presence of phytochemicals in GSME: "+": Present, "-": Absent, "NT": Not tested, "GAE": Gallic acid equivalents, "QE": Quercetin equivalents, and "TAE": Tannic acid equivalents.

Natural herbs are increasingly recognized for their potential to alleviate pain, reduce fever, and provide anti-inflammatory benefits.[16] *Gardenia stenophylla* (GSME) stands out due to its ethanol extract, which encompasses a broad spectrum of phytochemicals, including alkaloids, flavonoids, phenolics, steroids, tannins, terpenoids, and saponins. These components collectively underpin GSME's potential as an effective remedy for pain, fever, and inflammation.[3] The observed therapeutic effects of GSME, which include alleviating pain, reducing fever, and combating inflammation, are attributed to its diverse chemical profile.[17] Alkaloids present in GSME are known for their potent analgesic properties, potentially through modulation of neurotransmitter systems and inhibition of pain signals.[18] Steroidal compounds contribute to GSME's anti-inflammatory effects by targeting and inhibiting inflammatory mediators and reducing edema.[19] Tannins recognized for their anti-inflammatory and analgesic properties, stabilize cell membranes and inhibit inflammatory enzymes, while saponins enhance these effects by modulating immune responses and cytokine release.[6] The interplay of these diverse phytochemicals suggests a synergistic mechanism, amplifying GSME's efficacy as a natural treatment. The quantitative analysis further supports GSME's therapeutic potential.[15] The total phenolic content of GSME, measured at 65.19 ± 2.01 mg GAE/g, underscores the presence of compounds with well-established antioxidant and anti-inflammatory properties. Similarly, the total flavonoid content of 39.27 ± 1.89 mg QE/g reinforces GSME's efficacy, given the role of flavonoids in inhibiting inflammatory pathways and modulating pain perception. Additionally, the terpenoid content of 71.32 ± 2.49 mg TAE/g highlights the presence of terpenoids with broad-spectrum anti-inflammatory and analgesic effects.
The quantified chemical constituents of GSME underscore its potential therapeutic efficacy in pain relief, fever reduction, and inflammation management. The presence of these bioactive compounds is consistent with findings from previous research on plant extracts and their therapeutic applications. For instance, Sherif *et al*. demonstrated that phenolic compounds in *Oxystelma esculentum* extracts play a significant role in mitigating oxidative stress and inflammation, thereby contributing to pain relief and fever reduction.[20] Similarly, flavonoids, which are present in GSME, have been extensively documented for their capacity to inhibit inflammatory pathways and modulate pain perception. This is corroborated by studies on plant extracts in pain and inflammation models conducted by Nhung and Quoc.[14] Terpenoids, known for their broad-spectrum anti-inflammatory and analgesic properties, have also been shown to effectively reduce inflammation and alleviate pain in experimental models, as demonstrated by McDougall and McKenna.[21] These findings align with the broader literature, which consistently supports the therapeutic potential of plant extracts rich in phenolic compounds, flavonoids, and terpenoids.

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Numerous studies have affirmed the efficacy of such extracts in alleviating pain and inflammation in animal models.[3,6,22,23] The congruence between GSME's chemical profile and established research highlights its potential as a potent natural remedy. Thus, the concentrations of phenolics, flavonoids, and terpenoids in GSME reinforce its efficacy in pain relief, fever reduction, and anti-inflammatory applications, as supported by existing research.

Assessment of analgesic activity of the extract

The hot plate test

The hot plate test results (Table 2, Figure 1) show significant differences in reaction time (RT) and pain inhibition percentage (PPI) among treatment groups ($p < 0.05$). Mice treated with saline had the lowest RT, peaking at 3.72 ± 0.06 seconds at 30 minutes, then dropping to 3.21 ± 0.05 seconds at 60 minutes ($p < 0.05$). The PPI also peaked at 21.55% at 30 minutes, declining to 9.27% at 60 minutes ($p < 0.05$), indicating minimal analgesic effect. Tramadol significantly increased RT from 3.23 ± 0.05 seconds at 0 minutes to 11.07 ± 0.07 seconds at 60 minutes ($p < 0.05$), with PPI rising from 67.58% to 70.80% ($p < 0.05$), confirming strong analgesic effects. GSME100 increased RT from 3.44 ± 0.06 seconds to 9.55 ± 0.05 seconds, and PPI from 57.84% to 63.97% ($p < 0.05$). GSME150 showed greater efficacy, with RT increasing from 3.31 ± 0.06 seconds to 10.28 ± 0.06 seconds and PIP from 61.16% to 67.80% ($p < 0.05$). GSME200 produced RT and PIP comparable to tramadol, with RT increasing from 3.98 ± 0.05 seconds to 10.56 ± 0.04 seconds, and PPI from 67.35% to 71.76% ($p > 0.05$).

The hot plate test evaluated the central analgesic properties of GSME by assessing its impact on pain transmission and perception inhibition. The results indicate that GSME can interfere with pain signals, activating the gray matter to release endogenous peptides, which contribute to the descending pain inhibitory pathway.[24] The dose-dependent increase in reaction time (RT) observed in GSME-treated groups suggests that this extract effectively delays the onset of pain sensation in mice, an important indicator of its analgesic potential. Notably, the GSME200 group exhibited an increase in RT nearly equivalent to that of tramadol, indicating strong analgesic effects at higher doses. Additionally, the pain inhibition percentage (PPI) also increased with GSME dosage, further reinforcing the extract's analgesic efficacy. Both GSME200 and tramadol demonstrated significant analgesic effects ($p < 0.05$) after 60 minutes compared to the control group, with nearly equivalent pain inhibition percentages. This dose-dependent increase in PPI highlights that higher concentrations of GSME are more effective in suppressing pain responses, possibly due to the increased bioavailability of active compounds in the extract.

The results of this study align with previous research on the use of plant extracts for pain relief in hot plate tests using rodent models. Numerous studies have documented the analgesic effects of various plant-derived compounds, demonstrating that natural extracts can modulate pain pathways and increase pain tolerance in animal models. Similar findings have been reported with extracts from plants like *Gompho purpurascens*,[22] *Trametes versicolor*,[25] and Black shallot,[6] which also showed dose-dependent increases in reaction time and pain inhibition percentage in hot plate tests. These studies support the hypothesis that phytochemicals, particularly those found in ethanol extracts, may interact with central pain receptors, leading to delayed pain perception and enhanced pain inhibition. The results obtained with *Gardenia stenophylla* extract (GSME) contribute to this growing body of evidence, suggesting that GSME's analgesic properties are comparable to those of well-studied medicinal plants.

Table 2: Analgesic effects of *G. stenophylla* fruit extract in mice using the hot plate test method

Treatments	Reaction time (sec)				
	0 min	15 min	30 min	45 min	60 min
Saline treatment	2.92 ± 0.07^{a}	3.39 ± 0.08^{a}	3.72 ± 0.06^{a}	3.49 ± 0.07^{a}	3.21 ± 0.05^{a}
Tramadol treatment	3.23 ± 0.05^{b}	9.97 ± 0.08^{e}	10.62 ± 0.08^{e}	10.65 ± 0.07^{e}	11.07 ± 0.07^{e}
$GSME_{100}$ treatment	3.44 ± 0.06^{c}	8.16 ± 0.05^{b}	9.27 ± 0.04^{b}	8.81 ± 0.07^{b}	9.55 ± 0.05^{b}
$GSME_{150}$ treatment	3.31 ± 0.06^{b}	8.53 ± 0.06^{c}	9.71 ± 0.08^{c}	9.99 ± 0.08^{c}	10.28 ± 0.06^{c}
$GSME_{200}$ treatment	23.98 ± 0.05^{d}	9.13 ± 0.06^{d}	10.08 ± 0.05^{d}	10.37 ± 0.06^{d}	10.56 ± 0.04^{d}

The values are expressed as Mean ± SD, where the letters (a, b, c, d, and e) indicate differences between treatments ($p < 0.05$).

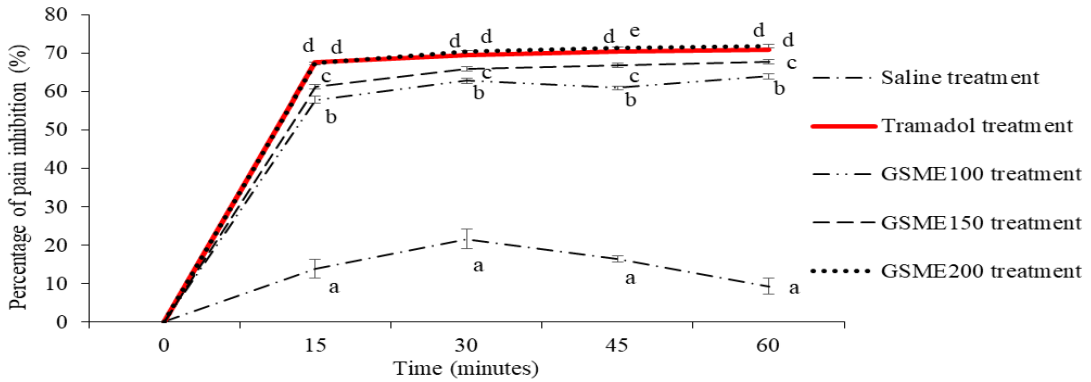

Figure 1: Effect of *G. stenophylla* fruit extract on the percentage of pain inhibition in hot plate test. Values are expressed as Mean ± SD, and letters (a, b, c, d, and e) represent the difference between treatments ($p < 0.05$).

Acetic acid-induced writhing test

The results in Table 3 indicate that saline treatment led to the highest number of writhing episodes (45.76 ± 0.06 times) ($p < 0.05$), suggesting minimal analgesic effect. In contrast, aspirin treatment significantly reduced the number of writhing episodes to 19.93 ± 0.05 times ($p < 0.05$), demonstrating its strong analgesic efficacy. Among the GSME treatments, a dose-dependent decrease in the number of writhing episodes was observed ($p < 0.05$), with GSME100 showing 28.76 ± 0.04 times, GSME150 showing 26.64 ± 0.05 times, and GSME200 showing 21.04 ± 0.03 times. GSME200 exhibited the greatest efficacy among the extract treatments, approaching the effectiveness of aspirin. Regarding the pain inhibition percentage (PIW), saline treatment, showed no inhibition (0.00 ± 0.00%), while aspirin showed the highest PIW at 56.44 ± 0.13% ($p < 0.05$). The GSME treatments demonstrated dose-dependent increases in PIW ($p < 0.05$), with GSME100 at 37.15 ±

0.03%, GSME150 at 41.78 ± 0.07%, and GSME200 at 54.01 ± 0.06%. Notably, GSME200's PIW was very close to that of aspirin ($p > 0.05$), highlighting its strong analgesic potential at higher doses.

The analgesic effects of the ethanol extract from *Gardenia stenophylla* fruits (GSME) are demonstrated by the observed reductions in the number of writhing episodes and increases in pain inhibition percentage (PIW) during the acetic acid-induced writhing test, a well-established method for assessing peripheral analgesic activity. The results show that GSME exerts dose-dependent analgesic effects, with higher doses, particularly GSME200, significantly reducing writhing frequency and approaching the efficacy of aspirin, a standard analgesic. This reduction in writhing is indicative of effective pain suppression, due to GSME's interference with pain pathways. The increase in PIW with higher GSME doses further supports its effectiveness in pain inhibition. Specifically, the GSME200 treatment achieved a PIW close to that of aspirin, suggesting that at higher concentrations, GSME inhibits pain responses nearly as effectively as conventional analgesics. The underlying mechanism of this effect is related to the bioactive compounds in GSME, which likely modulate the release of inflammatory mediators triggered by acetic acid injection, such as histamine, prostaglandin, bradykinin, substance P, TNF-*α*, IL-6, and COX.[26] These mediators are known to be involved in peripheral pain mechanisms, and GSME's ability to interfere with their pathways underscores its substantial peripheral analgesic potential.[27] The dose-dependent nature of its effects highlights the importance of optimal dosing in maximizing the analgesic benefits of GSME. The findings of this study are consistent with previous researchs on the analgesic effects of plant extracts in acetic acid-induced writhing tests on mouse models. Studies by Nhung and Quoc, Tesfaye *et al*., and Kim *et al*. on the analgesic effects of *Gardenia jasminoides*, *Echinops kebericho*, and *Symplocos chinensis* extracts in acetic acid-induced writhing tests have demonstrated that various plant-derived compounds effectively reduce pain responses by interfering with inflammatory mediators and pain pathways, similar to the results observed with GSME.[3,28,29] The dose-dependent analgesic effects of GSME, particularly at higher concentrations, further reinforce the potential of plant extracts as viable alternatives or complements to conventional analgesics.

Table 3: Analgesic effects of *G. stenophylla* fruit extract in acetic acid-induced writhing pain

Parameters	Saline treatment	Aspirin treatment	$GSME_{100}$ treatment	$GSME_{150}$ treatment	$GSME_{200}$ treatment
Number of times writhing (times)	45.76 ± 0.06^{e}	19.93 ± 0.05^{a}	28.76 ± 0.04^{d}	26.64 ± 0.05^{c}	21.04 ± 0.03^{b}
PIW (%)	0.00 ± 0.00^{a}	56.44 ± 0.13^{e}	37.15 ± 0.03^{b}	41.78 ± 0.07^{c}	54.01 ± 0.06^{d}

The values are expressed as Mean ± SD, where the letters (a, b, c, d, and e) indicate differences between treatments ($p < 0.05$).

Antipyretic activity

Table 4 presents the mean body temperatures across various treatment groups, highlighting the effects of carrageenan and the potential influence of *G. stenophylla* extract on fever. The Saline group maintained a stable temperature, ranging from 36.74 ± 0.11 ℃ at 0 hours to 36.81 ± 0.14 ℃ at 2 hours ($p > 0.05$), indicating no significant fever response. In contrast, the CGN group exhibited a marked increase in temperature, peaking at 39.85 ± 0.14 ℃ at 4 hours ($p < 0.05$), demonstrating a significant febrile response due to carrageenan. The yeast + PCM group showed a moderate elevation, reaching 37.32 ± 0.14 ℃ by 4 hours ($p < 0.05$), suggesting some anti-inflammatory effect from indomethacin. Meanwhile, the yeast + GSME100, yeast + GSME150, and yeast + GSME200 groups experienced slight increases, with final temperatures of 37.59 ± 0.21 ℃, 37.51 ± 0.16 ℃, and 37.39 ± 0.21 ℃ ($p < 0.05$), respectively, indicating mild antipyretic effects of *G. stenophylla* extracts. About these temperature changes, Figure 2 illustrates the percentage of fever reduction (PFR%) across treatment groups at 1, 2, 3, and 4 hours, further reflecting the inflammatory response and treatment efficacy. The Saline group maintained a PFR% of 0.00%, indicating no response. Conversely, the yeast group showed significant negative changes in PFR%, with values of -19.74 ± 1.82% at 1 hour, declining to -73.39 ± 2.70% by 4 hours, reflecting a substantial inflammatory response. In contrast, the yeast + PCM group exhibited a marked improvement in PFR%, increasing from 13.75 ± 2.48% at 1 hour to 62.79 ± 0.48% at 4 hours, showcasing the potent anti-inflammatory effects of indomethacin. The yeast + GSME100, yeast + GSME150, and yeast + GSME200 groups also displayed significant increases in PFR%, with values reaching 52.96 ± 0.83%, 55.47 ± 1.22%, and 60.19 ± 1.29%, respectively, at 4 hours.

The antipyretic effects of ethanol extract from *G. stenophylla* fruits (GSME) were assessed by evaluating its impact on rectal temperature and fever reduction percentage (PFR) in a yeast-induced fever model. The initial observations revealed a significant elevation in rectal temperature in the untreated yeast group, indicating a robust febrile response. Conversely, the administration of GSME led to a dose-dependent decrease in rectal temperature, demonstrating its efficacy in fever mitigation. Higher doses of GSME were associated with more pronounced reductions in temperature and significant improvements in PFR values. These findings suggest that GSME exhibits notable antipyretic activity, with higher doses producing increasingly significant effects. The mechanism of action appears to align with the physiological processes of fever regulation. Yeast-induced fever typically elevates prostaglandin E2 (PGE2) levels, increasing the hypothalamic thermoregulatory set-point and elevating body temperature.[30] Conventional antipyretics, such as paracetamol (PCM), function by inhibiting cyclooxygenase (COX) enzymes and subsequently reducing PGE2 concentrations in the hypothalamus.[31] GSME's antipyretic action appears to follow a similar mechanism, suggesting its potential as an effective fever-reducing agent.

The results of the study on the antipyretic effects of *G. stenophylla* fruit extract (GSME) are contextualized with previous research on plant extracts used to treat yeast-induced fever in rodent models. Several studies have demonstrated that plant extracts provide antipyretic effects comparable to traditional antipyretics. For instance, Tegegne and Alehegn reported that *Bersama abyssinica* extract effectively reduced fever in a yeast-induced model by decreasing prostaglandin E2 (PGE2) levels.[32] Similarly, Tesfaye *et al*. found that *Echinops kebericho* extract reduced fever through cyclooxygenase inhibition, leading to lowered PGE2 levels in the central nervous system.[33] Ilmi *et al*. also demonstrated that *Andrographis paniculata* extract alleviates fever by modulating cyclooxygenase activity and PGE2 levels.[34] These findings are consistent with our observations of GSME, where GSME decreased rectal temperature and improved fever reduction percentage (PFR) in a dose-dependent manner, suggesting a potential mechanism involving PGE2 inhibition. Furthermore, the study by Nhung and Quoc also showed that *Hedyotis capitellata* leaf extract also effectively reduced yeast-induced fever by modulating cyclooxygenase enzymes, similar to the mechanism of GSME.[14] These results support the hypothesis that GSME acts through a similar mechanism, regulating elevated PGE2 levels in the body. Thus, the evidence from this study not only supports the antipyretic efficacy of GSME but also contributes to the broader understanding of plant extracts in fever management, particularly yeast-induced fever.

Carrageenan-induced paw oedema

Figure 3 and Table 5 collectively illustrate the changes in paw volume across various treatment groups over 3 hours, highlighting significant differences in inflammatory response and treatment efficacy. In the saline group, the paw volume remained stable at 0.00% throughout, indicating no inflammatory response. Conversely, the CGN group demonstrated a significant negative change in paw volume, decreasing

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from 0.00% at 0 hours to -101.43 ± 0.61% at 3 hours ($p < 0.05$), suggesting substantial variability in inflammatory responses among subjects. In terms of paw volume, the CGN group showed a dramatic increase from 4.31 ± 0.06 mm to 8.73 ± 0.05 mm ($p < 0.05$), confirming the inflammatory effect of carrageenan. The CGN + IND group exhibited a strong positive response, with paw volume increasing from 0.00% at 0 hours to 62.45 ± 7.36% at 3 hours ($p < 0.05$), while the corresponding values decreased significantly from 4.34 ± 0.03 mm to 1.61 ± 0.05 mm ($p < 0.05$), demonstrating the potent anti-inflammatory effect of indomethacin. The CGN + GSME100 and CGN + GSME200 groups also showed moderate reductions in paw volume, with increases of 40.82 ± 6.39% ($p < 0.05$) and 48.85 ± 3.90% ($p < 0.05$) respectively, paralleling reductions in absolute values from 4.35 ± 0.04 mm to 2.66 ± 0.04 mm ($p < 0.05$) and from 4.34 ± 0.06 mm to 2.35 ± 0.04 mm ($p < 0.05$). The CGN + GSME300 group exhibited the most pronounced reduction, with paw volume increasing from 0.00% to 59.45% ($p < 0.05$) and absolute values decreasing from 4.36 ± 0.04 mm to 1.82 ± 0.07 mm ($p < 0.05$), indicating that higher doses of *G. stenophylla* extract are highly effective in mitigating inflammation.

Table 4: Effect of *G. stenophylla* fruit extract on yeast-induced fever

Treatments	Initial (ºC)	Fever (ºC)	1 h (ºC)	2 h (ºC)	3 h (ºC)	4 h (ºC)
Saline treatment	36.74 ± 0.12^{a}	36.77 ± 0.11^{a}	36.76 ± 0.14^{a}	36.81 ± 0.14^{a}	36.78 ± 0.14^{a}	36.77 ± 0.13^{a}
Yeast treatment	36.84 ± 0.14^{a}	38.58 ± 0.12^{e}	38.92 ± 0.14^{e}	39.21 ± 0.13^{d}	39.49 ± 0.15^{d}	39.85 ± 0.14^{d}
Yeast+PCM treatment	36.83 ± 0.15^{a}	38.14 ± 0.13^{b}	37.96 ± 0.14^{b}	37.74 ± 0.15^{b}	37.51 ± 0.14^{b}	37.32 ± 0.14^{b}
Yeast+ $GSME_{100}$ treatment	36.83 ± 0.14^{a}	38.44 ± 0.14^{de}	38.22 ± 0.14^{d}	38.05 ± 0.14^{d}	37.81 ± 0.17^{c}	37.59 ± 0.21^{c}
Yeast+ $GSME_{150}$ treatment	36.82 ± 0.13^{a}	38.36 ± 0.12^{cd}	38.17 ± 0.12^{cd}	37.94 ± 0.14^{cd}	37.75 ± 0.14^{c}	37.51 ± 0.16^{bc}
Yeast+ $GSME_{200}$ treatment	36.84 ± 0.12^{a}	38.23 ± 0.14^{bc}	38.03 ± 0.12^{bc}	37.82 ± 0.15^{bc}	37.64 ± 0.14^{bc}	37.39 ± 0.13^{bc}

Values were expressed as Mean ± SD and the letters (a, b, c, d, and e) denote the significant difference among treatments ($p < 0.05$).

Table 5: Effects of *G. stenophylla* fruit extract against carrageenan-induced paw edema model in mice

Treatments	Diameter paw (mm)			
	0 h	1 h	2 h	3 h
Saline treatment	4.31 ± 0.07^{a}	4.34 ± 0.06^{e}	4.29 ± 0.06^{e}	4.33 ± 0.03^{e}
CGN treatment	4.31 ± 0.06^{a}	5.84 ± 0.08^{f}	6.97 ± 0.05^{f}	8.73 ± 0.05^{f}
CGN + IND treatment	4.34 ± 0.03^{a}	2.34 ± 0.05^{a}	1.82 ± 0.05^{a}	1.61 ± 0.05^{a}
CGN + $GSME_{100}$ treatment	4.35 ± 0.04^{a}	3.17 ± 0.04^{d}	2.87 ± 0.06^{d}	2.66 ± 0.04^{d}
CGN + $GSME_{200}$ treatment	4.34 ± 0.06^{a}	2.83 ± 0.06^{c}	2.56 ± 0.04^{c}	2.35 ± 0.04^{c}
CGN + $GSME_{300}$ treatment	4.36 ± 0.04^{a}	2.57 ± 0.05^{b}	2.19 ± 0.05^{b}	1.82 ± 0.07^{b}

Values were expressed as Mean ± SD and the letters (a, b, c, d, e, and f) denote the significant difference among treatments ($p < 0.05$).

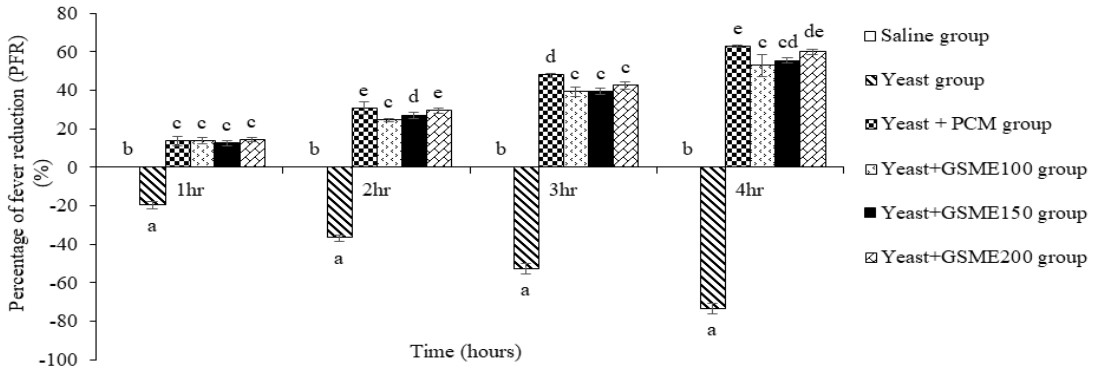

Figure 2: Effect of *G. stenophylla* fruit extract on the percentage of fever reduction in mice. Values are expressed as Mean ± SD. Letters (a, b, c, d, and e) denote significant differences between treatments ($p < 0.05$).

The results in Table 6 illustrate the variation in cytokine levels (TNF-α, IL-1β, and IL-6) across different experimental groups. In the saline-treated control group, cytokine levels remained low, with TNF-α at 149.62 ± 9.18 pg/mL, IL-1β at 285.17 ± 12.82 pg/mL, and IL-6 at 25.97 ± 2.69 pg/mL ($p < 0.05$). Upon treatment with CGN, these parameters significantly increased, with TNF-α reaching 311.71 ± 12.97 pg/mL, IL-1β rising to 663.19 ± 19.13 pg/mL, and IL-6 elevating to 96.19 ± 3.65 pg/mL ($p < 0.05$), indicating a pronounced inflammatory response

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induced by CGN. However, co-treatment with CGN and indomethacin (CGN + IND) resulted in a marked reduction in cytokine levels, with TNF-α reduced to 178.76 ± 11.21 pg/mL, IL-1β to 360.98 ± 14.01 pg/mL, and IL-6 to 32.06 ± 2.11 pg/mL ($p < 0.05$), demonstrating the anti-inflammatory effect of indomethacin. Treatment with *G. stenophylla* fruit extract (CGN + GSME) also led to reduced inflammation, with the anti-inflammatory effect depending on the dosage. In the highest dose group (GSME300), TNF-α levels decreased to 199.49 ± 10.16 pg/mL, IL-1β to 390.69 ± 14.40 pg/mL, and IL-6 to 36.11 ± 3.25 pg/mL ($p < 0.05$), indicating the extract's anti-inflammatory potential.

The ethanol extract from *G. stenophylla* fruits (GSME) demonstrated significant anti-inflammatory effects in the carrageenan-induced inflammation model, as evidenced by a marked reduction in paw swelling, comparable to that observed with indomethacin, a standard anti-inflammatory drug. Inflammation typically involves the activation of immune cells and the secretion of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6 through pathways involving pattern recognition receptors (PRRs) and transcription factors like NF-κB.[35] These cytokines amplify the immune response, leading to symptoms such as redness, swelling, and pain.[36] GSME effectively reduces the levels of TNF-α, IL-1β, and IL-6, indicating that its anti-inflammatory mechanism involves the inhibition of NF-κB activation. This inhibition results in decreased cytokine production, subsequently reducing the recruitment and activity of immune cells at the site of inflammation. As a result, there is a significant reduction in paw swelling and an overall attenuation of the inflammatory response.

The anti-inflammatory effects of GSME, as evidenced by reduced paw swelling and decreased levels of TNF-α, IL-1β, and IL-6, align with findings from studies on other plant extracts with similar properties. For instance, Tran *et al*. demonstrated that the ethanol extract of *Plukenetia volubilis* significantly reduced paw diameter in a CFA-induced inflammation model, reflecting its potential as an anti-inflammatory agent.[8] Similarly, *Boswellia serrata* extract, as reported by Majeed *et al*., effectively decreased paw edema in various animal models, indicative of its ability to modulate inflammatory pathways.[37] Esmaeili *et al*. also observed that extracts from *Cinnamomum zeylanicum* and *Syzygium aromaticum* led to a significant reduction in paw diameter, attributed to their polyphenol content, which helps regulate inflammation.[38] This is comparable to GSME's effects on paw swelling. Moreover, other plant extracts have shown efficacy in reducing pro-inflammatory cytokine levels. Wei *et al*. reported that *Echinacea purpurea* extract lowered TNF-α, IL-1β, and IL-6 levels in inflammation models, underscoring its anti-inflammatory potential.[39] Similarly, curcumin, a compound in turmeric, was shown by Boarescu *et al*. to reduce these cytokines, highlighting its potent anti-inflammatory properties.[40,41] Additionally, Nhung and Quoc found that *Caryota urens* extract reduced cytokine levels, attributed to its polyphenol content.[15] These findings collectively underscore that GSME's anti-inflammatory effects, observed through both reduced paw swelling and lowered cytokine levels, are consistent with the mechanisms and outcomes reported for other plant extracts. This suggests that GSME, like these other extracts, offers significant promise as a natural alternative for managing inflammation.

Table 6: Inhibition of TNF-α, IL-1β, and IL-6 levels in edematous paws by *G. stenophylla* fruit extract

Parameters	Saline treatment	CGN treatment	CGN + IND treatment	CGN + $GSME_{100}$ treatment	CGN + $GSME_{200}$ treatment	CGN + $GSME_{300}$ treatment
TNF-α (pg/mL)	149.62 ± 9.18^{a}	311.71 ± 12.97^{e}	178.76 ± 11.21^{b}	248.13 ± 14.11^{d}	241.33 ± 13.14^{d}	199.49 ± 10.16^{c}
IL-1β (pg/mL)	285.17 ± 12.82^{a}	663.19 ± 19.13^{f}	360.98 ± 14.01^{b}	459.95 ± 14.97^{e}	438.72 ± 16.01^{d}	390.69 ± 14.41^{c}
IL-6 (pg/mL)	25.97 ± 2.69^{a}	96.19 ± 3.65^{f}	32.06 ± 2.11^{b}	48.59 ± 2.75^{e}	44.33 ± 2.39^{d}	36.11 ± 3.25^{c}

Values were expressed as Mean ± SD and the letters (a, b, c, d, e, and f) in a row denote the significant difference among treatments ($p < 0.05$).

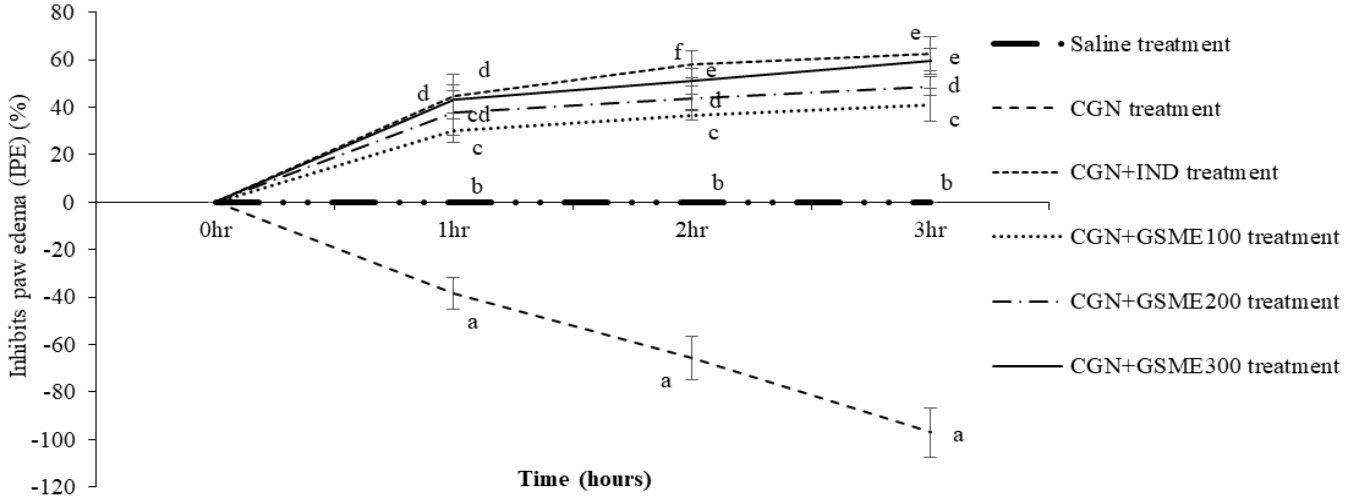

Figure 3: Effect of *G. stenophylla* fruit extract on the inhibits paw edema in mice. Values are expressed as Mean ± SD. Letters (a, b, c, d, e, and f) denote significant differences between treatments ($p < 0.05$).

Conclusion

The ethanol extract of *Gardenia stenophylla* fruit (GSME) demonstrates significant therapeutic potential in the management of pain, fever, and inflammation in experimental mice. In pain assessment models, GSME effectively alleviated nociceptive responses in both the hot plate test and acetic acid-induced writhing. The extract also consistently reduced rectal temperature in the yeast-induced fever model, confirming its antipyretic activity. In the carrageenan-induced inflammation model, GSME markedly diminished paw edema, with the most pronounced effect observed at 3 hours post-injection. Additionally, GSME significantly inhibited the serum levels of pro-

inflammatory cytokines IL-1*β*, IL-6, and TNF-*α*, underscoring its anti-inflammatory efficacy. These results highlight GSME's promise as a multifaceted therapeutic agent, offering potential applications in the treatment of pain, fever, and inflammatory conditions.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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