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Original Research Article

Analgesic and Antipyretic Activities of Ethanol Extract of *Gardenia jasminoides* Ellis Fruits in Mice

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ABSTRACT

This study investigates the analgesic and antipyretic effects of *Gardenia jasminoides* Ellis fruit in mice. In vivo experiments were conducted using ethanol extract from *Gardenia jasminoides* fruit (EEGJ) at 75, 100, and 125 mg/kg. The positive control group was treated with standard medications, including aspirin (10 mg/kg) and fentanyl (10 mL/kg) for pain experiments, and acetaminophen (10 mg/kg) for fever experiments. In contrast, the negative control group received normal saline (10 mL/kg). The analgesic effectiveness of EEGJ was assessed by measuring response latency and the reduction in writhing responses. Fever was induced by injecting a 20% yeast suspension (10 mL/kg), and rectal temperature was measured both before and after the intervention. The results demonstrated that EEGJ significantly reduced acetic acid-induced writhing responses by 25.8%, 34.78%, and 48.27%, respectively. Furthermore, this extract exhibited a pronounced dose-dependent inhibition of formalin-induced pain during the second phase, with inhibition rates of 15.31%, 25.23%, and 33.57%, respectively. The highest pain inhibition due to mechanical stimulus in the Haffner tail clip test at 15 minutes was observed at a dose of 125 mg/kg, achieving an inhibition rate of 43.08%. Additionally, after 3 hours, rectal temperature significantly decreased in the EEGJ 125 mg/kg group ($37.29 \pm 0.12^{o}C$) ($p < 0.05$). In conclusion, our study unequivocally demonstrates that ethanol extract from *Gardenia jasminoides* fruit exhibits potent analgesic and antipyretic effects, with the highest activity observed at the dose of 125 mg/kg. Phytochemical constituents in EEGJ, such as alkaloids and flavonoids, may be responsible for the observed effects.

Keywords: *Gardenia jasminoides*, plant extract, analgesic, antipyretic, mice, *Mus musculus*

Introduction

Pain and fever are prevalent symptoms of injuries and illnesses. Pain is an uncomfortable sensory and emotional experience linked to tissue damage, acting on either the central or peripheral nervous system. Fever is triggered as a secondary response to infection, tissue damage, inflammation, or other medical conditions. The body, through its natural defense mechanism, establishes an environment where infectious agents or damaged tissue cannot thrive. Diseased or injured tissue initiates the enhanced production of pro-inflammatory mediators like interleukin 1β, α, β, and TNF-α, leading to increased prostaglandin E2 (PGE2) synthesis near the subfornical organ region. This subsequently activates the subfornical organ, elevating body temperature.[1] Current pain relievers and fever reducers, such as non-steroidal anti-inflammatory drugs (NSAIDs), ibuprofen, and acetaminophen, among others, are associated with side effects like gastrointestinal irritation, impaired liver and kidney function, and more. Most fever-reducing medications typically block or inhibit the expression of COX-2 to lower elevated body temperature by curtailing the synthesis of PGE2. Moreover, these synthetic agents, exhibiting high selectivity in inhibiting COX-2, can be toxic to the liver, kidney, brain cortex, and cardiac muscle cells.[2]

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Due to the existing concerns surrounding current pain relievers and fever reducers, the quest for alternative medications is constantly ongoing, particularly from natural sources. Naturally occurring COX-2 inhibitors often possess lower selectivity and fewer side effects. Presently, there is a growing demand for herbal medicines, as they are perceived to be safer compared to synthetic compounds. Furthermore, as healthcare costs continue to rise, the appeal of cost-effective treatment modalities has prompted patients to reconsider the potential of alternative solutions.[3]

Gardenia jasminoides Ellis, a flowering plant belonging to the Rubiaceae family, holds significant botanical compounds such as methyl ester, iridoids, crocins, organic acids, crocetin, rutin, isoquercitrin, and nicotiflorin.[4] These compounds exhibit diverse biological properties, and their extracts have been utilized in traditional medicine for pain relief, fever reduction, liver treatment, high blood pressure, and gastric inflammation, among others.[5] The fruit of *G. jasminoides*, which grows naturally, is employed as a food colorant in East Asian countries. Furthermore, several bioactive constituents within the *G. jasminoides* fruit extracts, including iridoids, crocins, and organic acids, demonstrate anti-inflammatory and antioxidant effects.[6] Currently, the pain-relieving and fever-reducing activities of *G. jasminoides* fruit remain unexplored. Hence, the present study was conducted to assess the analgesic properties of ethanol extract from *G. jasminoides* fruit using animal analgesia models in mice (through acetic acid-induced writhing, formalin pain, and tail-flick tests). The study also investigated the antipyretic effects caused by yeast injection in mice.

Material and methods

Plant material: Fresh *G. jasminoides* fruits were collected in Chau Thanh district, Long An province (Coordinates: 10°27′52″N 106°30′0″E), in October 2022. The voucher specimen (sample code GJ131022VST) was deposited at the Department of Plant

Biotechnology of the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Vietnam. The collected fruits were then thoroughly washed with water and subsequently rinsed with distilled water to remove any surface contaminants. The fruits were shade-dried for 2 days and further dried in a Memmert drying oven at 60°C until the moisture content reached < 12%. The dried samples were finely powdered and stored in a desiccator until used for extraction.

Preparation of *G. jasminoides* ethanol extract: The extraction process was carried out using the Soxhlet apparatus for 10 h with absolute ethanol as the solvent. The solvent was subsequently evaporated using a rotary evaporator, and the crude extract was collected and stored in a desiccator.

Chemicals and reagents

All chemicals used were of analytical grade, including 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ascorbic acid, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), water-soluble quercetin, gallic acid, anhydrous sodium carbonate (Na_2CO_3), aluminum trichloride, potassium acetate, sodium acetate, ferric chloride hexahydrate ($FeCl_3.6H_2O$), Folin-Ciocalteu reagent, Dragendorff reagent, mercuric chloride, potassium iodide, and iodine were purchased from Sigma-Aldrich. Ethanol, methanol, hydrochloric acid (HCl), sulfuric acid (H_2SO_4), chloroform, ammonia, glacial acetic acid, and sodium hydroxide (NaOH) were purchased from Merck, and potassium peroxodisulfate was purchased from Fluka.

Phytochemical screening and determination of the total phenolic and flavonoid content of G. jasminoides fruit ethanol extract

Phytochemical screening in *G. jasminoides* fruit ethanol extract: The ethanol extract of *Gardenia jasminoides* fruit (EEGJ) was tested for the presence of alkaloids, steroids, tannins, saponins, and glycosides. Qualitative results were indicated by (+) for the presence and (-) for the absence of plant chemicals. The presence of alkaloid (Mayer's test), tannin (Ferric chloride test), and saponin (Froth test) was determined according to the procedure described by Banso and Adeyemo,[7] while glycoside (Borntrager's test), steroid and terpenoid (Liebermann-Burchard test) were identified according to the procedure described by Joshi *et al*.[8]

The total polyphenol content (TPC) and total flavonoid content (TFC) were analyzed using the Folin-Ciocalteu colorimetric method and the aluminum colorimetric method as described by Chlopicka *et al.* and Stankovic with some minor modifications, respectively.[9,10]

Experimental animals

Swiss mice (26 ± 5 g, 7-8 weeks old) were procured from the Pasteur Institute of Ho Chi Minh City. The animals were maintained under standard laboratory conditions with a room temperature of (23 ± 2°C), humidity (55 ± 5%), and a 12-hour light-dark cycle. Sterilized glass cages containing a mixture of wood shavings were used as bedding material. The mice were fed standard pellet food for rodents and provided with RO-filtered water ad libitum. All animals were acclimatized for seven days to the new housing conditions before the commencement of the study. The mice were randomly divided into experimental and control groups. Water was available ad libitum, but the food was restricted 12 h before experiments. Throughout the experiment, the animals were treated humanely following the principles of the Basel Declaration on animal research (2010) and the Livestock law in Vietnam (No. 32/2018/QH14).[11,12] The experimental protocol adhered to the Guidelines for Preclinical and Clinical Trials of Oriental Medicines and Herbal Medicines (Decision No. 141/QD-K2DT, 2015).[13] All experimental animals were cared for and handled by trained personnel, adhering to the ethical principles of animal research set by the Ethics Committee for Animal Research at Ho Chi Minh City University of Industry, Vietnam.

Evaluation of analgesic activity of G. jasminoides fruit ethanol extract

Experimental design: 75 mice were randomly divided into five groups for each pain model, with five mice in each group. The first group served as the negative control (negative control group) and received normal saline (10 mL/kg). The second group served as the positive control (positive control group) and was administered the standard drugs aspirin (10 mg/kg) and fentanyl (10 mg/kg) orally in a tail-flick test, formalin-induced pain test, and acetic acid-induced writhing test. The experimental groups receiving EEGJ ($EEGJ_{75}$, $EEGJ_{100}$, and $EEGJ_{125}$ groups) were treated with EEGJ at doses of 75, 100, and 125 mg/kg, respectively.

Formalin-induced pain test: The formalin-induced pain test was conducted following the method by Akindele *et al.* with slight modifications.[14] 25 mice were fasted overnight and randomly divided into different groups. The various animal groups were treated with normal saline (10 mL/kg), EEGJ (75, 100, and 125 mg/kg), ASA (10 mg/kg), and fentanyl (10 mL/kg). Thirty minutes after oral administration, formalin (20 µL of 1% solution) was injected into the right hind paw of the mice. The time (in seconds) spent in licking and biting the injected paw (indicative of pain response) was recorded for each animal. The mouse responses were observed during the 0 - 5 min period (early phase) and the 15 - 30 min period (late phase) following formalin injection. The animal's response time was compared to the control group and represented as a percentage of inhibition:

$$\%\ Inhibition = \frac{\text{Latency (test} - \text{control)}}{\text{Latency test}}\ x\ 100$$

Haffner tail clip test: Metal artery clips were attached to the base of the mouse tail to induce pain. The Haffner tail clip test was conducted based on the method described by Chang *et al.* with slight modifications.[15] A pre-test sensitivity assessment was performed to select animals for the experiment, and mice that did not attempt to escape the clip within 10 sec were excluded. 25 selected mice were randomly allocated to different groups. The tail clip was applied 30 min after oral administration of EEGJ (75, 100, and 125 mg/kg), fentanyl (10 mL/kg), and normal saline (10 mL/kg). The mouse's reaction time was observed and recorded after attaching the tail clip for 5, 10, and 15 min. The analgesic effect was considered present if no effort was made to escape the clip within 10 sec. The percentage of pain inhibition by EEGJ was calculated using the formula:

$$\%\ Inhibition = \frac{\text{Latency (test)} - \text{Latency (control)}}{\text{Latency (test)}}\ x\ 100$$

Acetic acid-induced writhing test: The acetic acid-induced writhing test on mice followed the method described by Surbakti *et al.* with minor modifications.[16] The method for preparing the acetic acid solution involved diluting 0.2 mL of acetic acid into 20 mL of water in a 100 mL volumetric flask, and then adding water to reach a final volume of 100 mL, resulting in a 0.2% acetic acid solution. 25 experimental mice received EEGJ at the doses for their respective groups (75, 100, and 125 mg/kg), aspirin 10 mg/kg, or normal saline (10 mL/kg). Thirty minutes after being treated with EEGJ, physiological saline, or aspirin, the pain induction was carried out by injecting 0.2 mL of the 0.2% acetic acid solution into the peritoneal cavity. Five minutes after induction, the experimental mice were observed for 30 min for writhing activity. Subsequently, the percentage of average abdominal constriction inhibition was calculated using the formula:

$$\%\ Inhibition = \frac{\text{Mean number of writhes (control} - \text{test)}}{\text{Mean number of times of writhing in the control}}\ x\ 100$$

Evaluation of antipyretic activity of G. jasminoides fruit ethanol extract

Fever-reducing activity was assessed using fever induced by yeast in mice, following the method described by Muhammad *et al*.[17] Fever was induced by subcutaneously injecting 10 mL/kg of a 20% yeast suspension (yeast group). Selected animals were subjected to overnight fasting and provided with water before the experimental procedure. The initial rectal temperature of the animals was recorded using a UNI-T A61 digital thermometer (China). After 18 h of subcutaneous injection, animals exhibiting an increase in rectal temperature by 0.3 - 0.5°C were selected for the fever-reducing activity study. Ethanol extract of *Gardenia jasminoides* fruit (EEGJ) was administered orally at doses of 75, 100, and 125 mg/kg ($EEGJ_{75}$, $EEGJ_{100}$, $EEGJ_{125}$ groups). Paracetamol (10 mg/kg, oral) (paracetamol group) was used as a reference drug, while the control group received only normal saline (10 mL/kg) (normal saline group). Rectal temperature was recorded for 1 h

within 3 h after treatment. The percentage reduction in fever (PR) was calculated using the formula: $PR\ (\%) = \frac{B-Cn}{B-A} \times 100$. Where B is the temperature after causing fever; Cn is the temperature after 1, 2, 3 h and A is the normal body temperature.

Statistical analysis

The values are presented as mean ± standard deviation (SD). Statistical analysis was conducted using One-Way ANOVA. Subsequently, the Least Significant Difference (LSD) test was employed to compare the experimental groups with the control group. Differences with $p < 0.05$ were considered statistically significant.

Results and discussion

Screening of plant chemicals and determination of the total phenolic and flavonoid content of G. jasminoides fruit ethanol extract

The phytochemical screening of *G. jasminoides* fruit ethanol extract revealed the presence of alkaloids, steroids, tannins, saponins, and terpenoids, with the absence of glycosides. The total phenolic content (TPC) and total flavonoid content (TFC) in *G. jasminoides* fruit ethanol extract were determined as 69.74 ± 3.22 mg GAE/g and 43.56 ± 1.97 mg QE/g, respectively.

Alkaloids have been reported to possess anti-inflammatory, antibacterial, antimalarial, antispasmodic, and other pharmacological effects. Similarly, steroids derived from plants have cardio-tonic effects, and exhibit antibacterial properties, and insecticidal activities. Tannins exhibit antimicrobial, anticancer, and antiviral activities, functioning by precipitating microbial proteins, thereby making essential nutrients unavailable to them. The identified plant chemical compounds are responsible for the observed biological activities attributed to *G. jasminoides*, which substantiates its traditional use by the local population.[18]

Plant polyphenols scavenge free radicals. It is thus imperative to determine the polyphenol content in plant extracts. Polyphenols, featuring aromatic benzene rings with hydroxyl groups, including derivatives, have the capability to absorb free radicals and chelate metal ions, thereby inhibiting the formation of reactive oxygen species (ROS) and promoting lipid peroxidation. Among polyphenols, flavonoids hold immense significance due to their role in disease prevention. The antioxidant potency of flavonoids depends on molecular structure, hydroxyl group positioning, and other characteristics in their chemical structure. The results regarding of the total polyphenol and flavonoid content in EEGJ are 69.74 ± 3.22 mg GAE/g and 43.56 ± 1.97 mg QE/g, respectively. Polyphenols and flavonoids may synergize with other plant chemicals present in *G. jasminoides*, rendering it medically activity.[19]

Evaluation of antipyretic activity of G. jasminoides fruit ethanol extract

Treatment of mice with the ethanol extract of *G. jasminoides* fruit demonstrated antipyretic activity against febrile conditions induced by yeast, as evidenced by rectal temperature reduction (Table 1). The initial average rectal temperature of the mice was approximately 36°C. Within the first hour post-treatment, the plant extract at a dose of 125 mg/kg exhibited the highest antipyretic activity with a remarkable activity of 21.46% among the tested EEGJ doses, compared to paracetamol (the reference drug) at 21.49% ($p > 0.05$) (Figure 1). In the second hour, EEGJ at doses of 70, 100, and 125 mg/kg displayed significant rectal temperature reduction to 38.18 ± 0.11°C, 37.99 ± 0.11°C, and 37.64 ± 0.12°C, respectively (Table 1). During the third hour, EEGJ at the dose of 125 mg/kg produced remarkable antipyretic activity, achieving a reduction efficacy of 53.62%, comparable to the reference drug's 59.85% (Figure 1).

Yeast was administered in Swiss mice to trigger fever. The fever was observed 19 h after administering the yeast solution (this time frame accounted for the total duration required for the yeast solution to elicit a body temperature increase). Subcutaneous injection of the yeast solution induced fever by elevating prostaglandin synthesis. The fever caused by yeast solution is referred to as pyrexia, and its etiology might involve prostaglandin production. Suppression of prostaglandin synthesis is achieved by inhibiting cyclo-oxygenase (COX) enzyme activity.[20] Oral administration of EEGJ significantly lowered the rectal temperature in mice. Hence, it can be hypothesized that EEGJ contains pharmacological active principles that hinder prostaglandin release. Following three hours of experimentation, EEGJ exhibited notable antipyretic activity against yeast-induced pyrexia in mice. The 125 mg/kg body weight dose caused the greatest rectal temperature-reducing effect. These findings align with the effects of other herbal agents on experimental animals.[21]

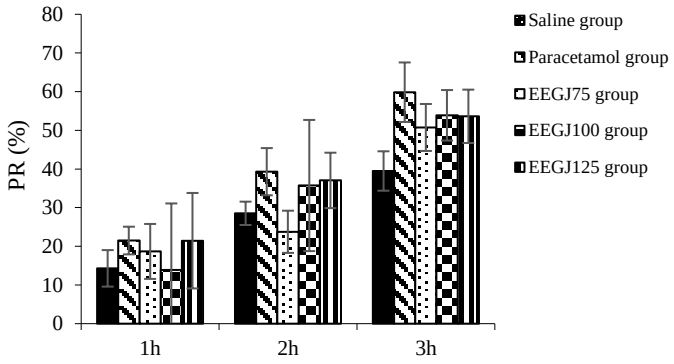

Figure 1: The percentage reduction in fever in mice

Table 1: Effect of *G. jasminoides* fruit ethanol extract on yeast-induced fever

Experimental group	Initial (°C)	Fever (°C)	1 h (°C)	2 h (°C)	3 h (°C)
Saline group	36.29 ± 0.24^{a}	39.79 ± 0.17^{d}	39.29 ± 0.14^{c}	38.79 ± 0.14^{d}	38.41 ± 0.16^{d}
Paracetamol group	36.31 ± 0.21^{a}	38.27 ± 0.15^{a}	37.84 ± 0.11^{a}	37.51 ± 0.15^{a}	37.11 ± 0.13^{a}
$EEGJ_{75}$ group	36.28 ± 0.14^{a}	38.77 ± 0.22^{c}	38.36 ± 0.21^{b}	38.18 ± 0.11^{c}	37.51 ± 0.11^{c}
$EEGJ_{100}$ group	36.32 ± 0.19^{a}	38.61 ± 0.15^{bc}	38.21 ± 0.16^{b}	37.99 ± 0.11^{b}	37.38 ± 0.11^{bc}
$EEGJ_{125}$ group	36.3 ± 0.25^{a}	38.42 ± 0.13^{ab}	37.98 ± 0.18^{a}	37.64 ± 0.12^{a}	37.29 ± 0.12^{b}

Values are expressed as Mean ± SD, letters (a, b, c, d) represent the difference between groups ($p < 0.05$)

Table 2: Analgesic effects of *G. jasminoides* fruit ethanol extract on acetic acid-induced writhing pain

Parameters	Saline group	Aspirin group	$EEGJ_{75}$ group	$EEGJ_{100}$ group	$EEGJ_{125}$ group
No. of times writhing (times)	45.18 ± 0.13^{d}	19.45 ± 0.13^{a}	33.52 ± 0.12^{c}	29.46 ± 0.13^{b}	23.368 ± 0.12^{b}
% pain inhibition of EEGJ (%)	0.00 ± 0.00^{a}	56.95 ± 0.17^{e}	25.80 ± 0.11^{b}	34.78 ± 0.13^{c}	48.27 ± 0.30^{d}

Values are expressed as Mean ± SD, letters (a, b, c, d, e) represent the difference between groups ($p < 0.05$)

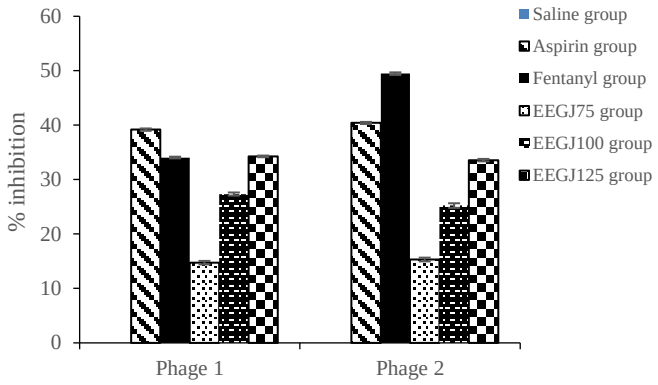

Figure 2: Percentage of formalin-induced pain inhibition in mice by *G. jasminoides* fruit ethanol extract

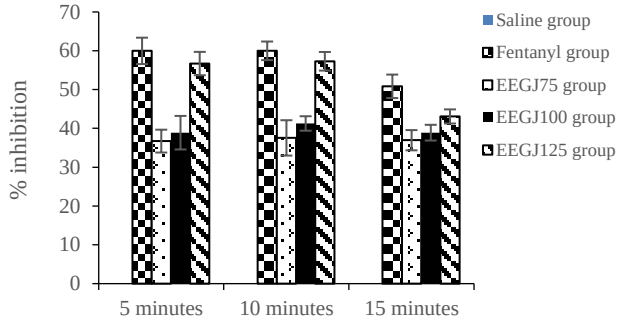

Figure 3: Percentage of pain inhibition by *G. jasminoides* fruit ethanol extract in mice in Haffner tail clip test

Non-steroidal anti-inflammatory drugs cause antipyresia by inhibiting prostaglandin synthetase in the hypothalamus. Qualitative screening of plant chemical compounds in this study revealed the presence of tannins, saponins, polyphenols, alkaloids, flavonoids, and more. Some of these plant chemicals have been demonstrated to inhibit cyclooxygenase (COX) enzymes, thereby exerting antipyretic effects by either impeding prostaglandin formation or elevating endogenous antipyretic components.[22] Flavonoids are known to target fever-related pathways. Therefore, the presence of flavonoids in EEGJ contributes to its antipyretic activity. The presence of alkaloids and saponins in this extract is associated with inhibiting prostaglandin synthesis and might also be accountable for antipyretic activity.[21]

Evaluation of analgesic activity of G. jasminoides fruit ethanol extract
Acetic acid-induced writhing test

A notable number of writhing episodes were induced using 0.2 mL of 0.2% acetic acid in the control mice (45.18 ± 0.13) administered with normal saline (10 mL/kg). EEGJ (75, 100, and 125 mg/kg) exhibited dose-dependent reduction in the number of acetic acid-induced writhing episodes (Table 2). At 75 mg/kg, there was a significant decrease of 33.52 ± 0.12 times ($p < 0.05$), corresponding to a pain inhibition percentage of 25.8%. With the use of 100 and 125 mg/kg doses, the writhing episodes were reduced to 29.46 ± 0.13 times and 23.368 ± 0.12 times respectively ($p < 0.05$), with pain inhibition of 34.78% and 48.27%. Similarly, aspirin (10 mg/kg) significantly reduced the number of writhing episodes induced by 0.2 mL of 0.2% acetic acid (19.45 ± 0.13), with a pain reduction of 56.95%.

In the present study, EEGJ significantly inhibited acetic acid-induced writhing. Satyanarayana *et al.* indicated that acetic acid induces writhing by stimulating prostaglandin production.[23] Aspirin has been proven to inhibit prostaglandin synthesis. Therefore, it is not surprising that aspirin significantly reduced the writhing sensation caused by acetic acid. Since EEGJ also reduced acetic acid-induced writhing, it is plausible that *G. jasminoides* may exert its antinociceptive activity by influencing the prostaglandin system.

Formalin-induced pain test

Administration of EEGJ (75, 100, and 125 mg/kg), 30 min before formalin injection, demonstrated a significant analgesic effect through the increase in paw-licking latency in both the early and late phases of the central and peripheral formalin test. Table 3 and Figure 2 depict the significant increase in paw licking latency during phase 1 at all three doses of EEGJ, namely 22.73 ± 0.16 sec, 26.68 ± 0.16 sec, and 29.49 ± 0.15 sec, resulting in pain inhibition percentages of 14.73%, 27.35%, and 34.26% respectively compared to the saline control (19.38 ± 0.11 sec and 39.17%) ($p < 0.05$); and Phase 2, which was 20.69 ± 0.13 sec, 23.43 ± 0.18 sec, and 26.37 ± 0.11 sec, with pain inhibition percentages of 15.31%, 25.23%, and 33.57% significantly different from the saline control group at 17.52 ± 0.1 sec and 40.4%. The inhibition of paw licking by ethanol extract of *G. jasminoides* can be compared to standard aspirin and fentanyl ($p < 0.05$).

The formalin-induced pain behavior is evident in both early (Phase 1) and late (Phase 2) phases. The early phase, immediately following formalin injection, lasts for about 5 min due to direct chemical stimulation of pain receptors (peripheral pain). The second phase lasts from 15 to 30 min after formalin injection. The distinct two phases in the formalin test are attributed to the direct effect of formalin on pain sensation and the inflammatory response involving the release of serotonin, histamine, bradykinin, prostaglandin, and the sensitization of central pain-sensing nerve cells. Stimulation of opioid receptors has been suggested as a mechanism of action against nociceptive pain. The effects of analgesics are manifested differently in the two phases of the formalin test. Analgesics acting on both central and peripheral sites produce pain relief effects in both phases of the formalin test.[24]

Table 3: Analgesic effects of *G. jasminoides* fruit ethanol extract on formalin-induced pain

Experimental phase	Saline group	Aspirin group	Fentanyl group	$EEGJ_{75}$ group	$EEGJ_{100}$ group	$EEGJ_{125}$ group
Phase 1	19.38 ± 0.11^{a}	31.87 ± 0.12^{f}	29.39 ± 0.12^{d}	22.73 ± 0.16^{b}	26.68 ± 0.16^{c}	29.49 ± 0.15^{e}
Phase 2	17.52 ± 0.1^{a}	29.39 ± 0.11^{e}	34.05 ± 0.16^{f}	20.69 ± 0.13^{b}	23.43 ± 0.18^{c}	26.37 ± 0.11^{d}

Values are expressed as Mean ± SD, letters (a, b, c, d, e) represent the difference between groups ($p < 0.05$)

Table 4: Analgesic effect of *G. jasminoides* fruit ethanol extract on pain in Haffner tail clip test on pain in Haffner tail clip test

Time (min)	Saline group	Fentanyl group	$EEGJ_{75}$ group	$EEGJ_{100}$ group	$EEGJ_{125}$ group
5	2.46 ± 0.18^{a}	6.15 ± 0.28^{d}	3.88 ± 0.14^{b}	4.02 ± 0.13^{b}	5.68 ± 0.19^{c}
10	2.49 ± 0.15^{a}	6.22 ± 0.2^{e}	3.99 ± 0.22^{b}	4.23 ± 0.12^{c}	5.82 ± 0.19^{d}
15	2.31 ± 0.16^{a}	4.71 ± 0.22^{d}	3.67 ± 0.13^{b}	3.78 ± 0.14^{b}	4.06 ± 0.15^{c}

Values are expressed as Mean ± SD, letters (a, b, c, d, e) represent the difference between groups ($p < 0.05$)

The activity of the ethanol extract of *Gardenia jasminoides* was observed in both phases of the formalin test, phase 1 and phase 2, demonstrating comparability with aspirin and fentanyl, respectively, and pain relief activity occurring at the level of both peripheral and central nervous systems. EEGJ exhibited the highest analgesic efficacy at the 125 mg/kg dose in both the early and late phases. These findings suggest a direct analgesic effect on nociceptive receptor blockade and inhibition of synthesis or release of pain-inducing mediators such as prostaglandins. This result is consistent with previous studies on evaluating the analgesic activities of herbal extracts. The aqueous extract from *Aloe volkensii* leaves demonstrated a reduction in paw-licking time caused by formalin in both phases.[21]

Haffner tail clip test

The reaction times and pain inhibition percentages of EEGJ after conducting the tail-flick test for all mice at different time intervals are presented in Table 4 and Figure 3. In the control group, at 5, 10, and 15 min after tail clamp application, the average reaction times were 5.97 ± 0.19 sec, 6.09 ± 0.13 sec, and 4.54 ± 0.18 sec, respectively. The average reaction time increased in the experimental groups treated with 75, 100, and 125 mg/kg EEGJ. The maximum reaction times were 3.99 ± 0.22 sec, 4.23 ± 0.12 sec, and 5.82 ± 0.19 sec, observed at the corresponding doses at the 10-minute mark, significantly higher than the distilled water control at 2.49 ± 0.15 sec ($p < 0.05$). However, after 15 min of tail clamp, which is 45 min after extract administration, the reaction time delay gradually decreased in the experimental and standard drug groups ($p < 0.05$). The pain inhibition percentage of EEGJ also peaked at the 10-minute mark after commencing the test, with the highest percentage seen in the group administered 125 mg/kg of the extract (57.29%), significantly different from the distilled water control (0%) ($p < 0.05$).
The brain and spinal cord play a pivotal role in central pain mechanisms. The dorsal part of the spinal cord contains numerous substances such as substance P, endogenous opioids, somatostatin, and other inhibitory hormones, all of which are targets for pain modulation. Tail flick models have been established as effective methods to assess the central analgesic effects of drugs acting through opioid receptors.[25] Tail flick testing proves valuable in elucidating the central antinociceptive mechanisms, primarily focusing on changes in spinal levels. The significant increase in pain threshold caused by EEGJ in the tail-flick test implies the involvement of central pain pathways. Central pain is orchestrated through intricate processes. Analgesic effects are mediated via central mechanisms involving receptor systems or through peripheral mechanisms linked to the inhibition of prostaglandins and other endogenous substances that play pivotal roles in pain processes.[26] Experimental animals treated with EEGJ exhibited prolonged reaction times at the 5, 10, and 15-minute intervals post-extract administration, compared to baseline levels. This observation is statistically significant ($p < 0.05$) at the 125 mg/kg dose when compared to the control group, demonstrating the pain-relieving activity of EEGJ.

Conclusion

This study validates the analgesic and antipyretic potential of the ethanol extract from *G. jasminoides* fruits in Swiss mice. EEGJ can inhibit both central and peripheral pain sensations.
The phytochemical constituents present in EEGJ played a role in its analgesic and antipyretic activities

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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