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Analgesic and anti-inflammatory activity of *Cotinus coggygia* Scop. extracts *in vivo*Kai Lu¹, Chong-Yu Yang², Zhao-Hong Yan³✉¹Department of Anesthesiology, Qiantang Campus Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, 310018, China²Department of Anesthesiology, 3201 Hospital, Hanzhong, 723000, China³Department of Anesthesiology, The Second Affiliated Hospital of the Chinese People's Liberation Army Air Force Medical University, Xi'an, 710038, China

ABSTRACT

Objective: To assess the analgesic and anti-inflammatory effects of standardized extract of *Cotinus coggygia* (*C. coggygia*) in different animal models.

Methods: *C. coggygia* extracts (25, 50, and 100 mg/kg) were administered to rats and mice ($n=6$) during hot plate, tail-flick, acetic acid-induced writhing, and formalin tests to determine its analgesic efficacy. The anti-inflammatory activity of *C. coggygia* extracts was evaluated by histamine and carrageenan-induced paw edema, cotton pellet-induced granuloma, and acetic acid-induced peritoneal capillary dye leakage tests.

Results: *C. coggygia* extracts (50 and 100 mg/kg) significantly alleviated thermal and chemical-induced pain in rodents ($P<0.05$). It also demonstrated notable anti-inflammatory properties by mitigating histamine and carrageenan-induced paw edema, granuloma deposits, and vascular permeability ($P<0.05$). Moreover, *C. coggygia* extracts remarkably reduced TNF- α , IL-1 β , IL-6, COX-2, and oxidative stress in rat paws ($P<0.05$). Carrageenan-induced histological aberrations in hind paw tissues were effectively ($P<0.05$) mitigated by treatment with *C. coggygia* extracts.

Conclusions: *C. coggygia* Scop. extracts show analgesic and anti-inflammatory effects *via* inhibition of COX-2 and inflammatory and oxidative mediators.

KEYWORDS: Antioxidant; Carrageenan; *Cotinus coggygia* Scop.; Anti-inflammatory; Analgesic; Pain; TNF- α ; Fustin

1. Introduction

The International Association for the Study of Pain defines chronic pain as an unfavorable sensory and emotional perception related to

actual or potential tissue damage[1]. Pain involves various receptors, neurotransmitters, neural fibers, pathways, and specific anatomical sites and may arise from inflammatory or non-inflammatory responses to tissue damage. Inflammatory pain largely stems from prostaglandins, primarily produced in response to inflammation and tissue damage by inducible cyclooxygenase-2 (COX-2)[2]. On an individual level, chronic pain emerges as a substantial public health concern associated with a considerable decline in quality of life and significant societal costs, including loss of work productivity. The global prevalence of chronic pain is on the rise, with 1.9 billion experiencing recurrent tension-type headaches, while low back and neck pain remains prominent in the top 10 disabilities[3]. Notably, the prevalence of chronic pain within the Chinese population alone

Significance

Cotinus coggygia Scop. is a well-known medicinal plant with a wide range of phytoconstituents. Although its anti-inflammatory properties have been well recognized, its mechanisms of action remain unexplored. This study shows that *Cotinus coggygia* extracts exhibit analgesic and anti-inflammatory potential in response to thermal and chemical stimuli *via* inhibition of COX-2, inflammatory mediators, and oxide-nitrosative stress. Based on these findings, *Cotinus coggygia* extracts hold promise as a potential analgesic and anti-inflammatory agent.

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surpasses 30%, with an annual total treatment cost exceeding 500 billion yuan[4].

The increasing burden of pain, combined with the growing expenses of medical care and the resulting psychological, physical, and economic consequences for individuals and their families, establishes chronic pain as a noteworthy public health concern[5]. Critically ill patients undergoing numerous nociceptive interventions that result in substantial procedural pain are commonly administered a combination of opioid analgesics (such as morphine and remifentanyl), non-opioid analgesics (including ketamine and acetaminophen), and immune-modulatory drugs (such as corticoids)[6]. Although pre-clinical evidence indicates the effectiveness of COX-2 selective inhibitors, clinical data reveal their link to several side effects, such as kidney-related issues (edema due to salt and water retention) and heart diseases (hypertension). Moreover, non-selective COX inhibitors have been shown to induce gastrointestinal ulcers and kidney injury. The demand for complementary and integrative medicine approaches, including mind-body interventions, acupuncture therapy, and traditional Chinese medicine practices, has notably increased. However, many traditional medicines used for pain management lack sufficient evidence-based support, and their underlying scientific mechanisms remain incompletely elucidated, constraining their application in clinical practice. To investigate novel analgesic interventions, ideal experimental models should mimic clinical conditions by introducing nociception with injuries, chemicals, or manipulations. The inflammation pain animal model induces acute pain through neutrophil recruitment and chronic pain through macrophage infiltration by injecting various irritants into the skin, paws, muscles, joints, and visceral organs[7,8]. Therefore, animal models are indispensable for deepening our understanding of inflammatory pain and amplifying the translational scope of pre-clinical and clinical investigations.

Cotinus coggygia (*C. coggygia*) Scop. (syn.: *Rhus cotinus* L.; family: Anacardiaceae) is a well-known medicinal plant widely distributed through southern Europe and China, the Mediterranean, and the Himalayas. It contains secondary metabolites, including total phenols, flavonoids, and tannins. Fustin, a photogenic flavanol component, is widely distributed in species of the Anacardiaceae family, including *Rhus verniciflua* Stokes[9] and *C. coggygia* Scop.[10]. Researchers have documented the diverse pharmacological properties of fustin, revealing its antiviral, antidiabetic, anticancer, and neuroprotective potential[9,10]. Moreover, a recent study demonstrated that fustin exhibited anti-arthritic effects by reducing the levels of pro-inflammatory cytokines [interleukins (ILs), tumor necrosis factor-alpha (TNF- α), IL-6, and IL-1 β], and oxidative stress markers [malondialdehyde (MDA), superoxide dismutase (SOD), catalase, and glutathione (GSH)], suppressing the production of prostaglandin E₂ and myeloperoxidase (MPO), and enhancing the action of Nrf2[11]. Although the

various pharmacological properties of *C. coggygia* have been acknowledged in rodent models, its mechanisms of action have not been explored. Therefore, the present study investigated the pharmacological potential of standardized extract of *C. coggygia* using various antinociceptive and anti-inflammatory animal models. Additionally, the study examined the influence of standardized extract of *C. coggygia* on regulating pro-inflammatory and oxidative stress mediators, aiming to elucidate the molecular mechanism underpinning its analgesic and anti-inflammatory effects.

2. Materials and methods

2.1. Chemicals, kits and reagents

Fustin, carrageenan, pentazocine, and indomethacin were purchased from Otto Chemicals, India. A microliter syringe (Hamilton, Bonaduz, Switzerland) was obtained from Anchrom Enterprises (I) Pvt. Ltd (Mumbai, India). High-performance liquid chromatography (HPLC) spectra were recorded on a Linomat V (Agilent Technologies, Beijing, China). All analytical reagents were purchased from Merck Life Science Pvt Ltd, India. Rat-specific ELISA kits for TNF- α , ILs, and COX-2 were obtained from Bethyl Laboratories Inc., Montgomery, TX, USA.

2.2. Identification of fustin from *C. coggygia*

The *C. coggygia* heartwood (CCH) was collected from northern China, Hebei, in April 2023, and the identified material was deposited (voucher specimen No. P089845) at the Department of Biology, Zhejiang University. The air-dried CCH was milled to a fine powder and extracted using methylene chloride/methanol (1:1, at room temperature for 24 h) three times. The obtained crude extract was fractionated using silica gel chromatography, and semipreparative reversed phased HPLC [Agilent Technologies C₁₈ (250 mm $\times$ 4.6 mm *i.d.*, 5 μ m) and Zorbax Extend C₁₈ guard column (10 mm $\times$ 4.6 mm *i.d.*, 5 μ m)]; mobile phase: water and acetonitrile; gradient program: 20%-37% (0-20 min), 37%-50% (20-21 min), 37%-50% (21-27 min), 50%-100% (27-30 min); flow rate: 1.0 mL/min; detection wavelength: 254 nm; injection volume: 10 μ L at 35 $^{\circ}$ C. Compounds were separated from CCH under optimal conditions. The flavonoids were obtained in a single purification where fustin was collected from 80-85 min.

2.3. Experimental animals

Male Wistar rats (10-12 weeks of age, 150-200 g) and male Swiss albino mice (4 weeks of age, 20-25 g) were procured from the

animal house facility of Xi'an Zhongkai. Animals were housed at $(24 \pm 1)^\circ\text{C}$ and relative humidity of $(65 \pm 10)\%$, and standard environmental conditions (normal light and dark cycle) in the animal house. The animals were fed a standard pellet rodent diet, and water was provided *ad libitum*.

2.4. Analgesic activity

The effects of a standardized extract of *C. coggygia* (hereafter referred to as "SECC") on thermal pain (hot plate test and tail-flick test) and visceral pain (acetic acid-induced writhing and formalin test) were evaluated in male Swiss albino mice according to the previously reported methods[12–15]. Mice were randomly divided into various groups ($n=6$) viz. vehicle control [0.1% carboxymethyl cellulose (CMC), 10 mg/kg, *p.o.*], pentazocine (10 mg/kg, *p.o.*, for hot plate test, formalin test and tail-flick test), or indomethacin (10 mg/kg, *p.o.* for acetic acid-induced writhing), SECC (25, 50 and 100 mg/kg, *p.o.*)[9,11,16]. Animals were pre-treated with either vehicle, pentazocine, indomethacin, or SECC once a day for 7 d and various tests including hot plate test, formalin test, tail-flick test, and acetic acid-induced writhing test were performed on 8th day. The normal group was treated with a vehicle for 7 d. In acetic acid-induced writhing, a mouse was administered acetic acid (0.7%, 10 mL/kg, intraperitoneally), and the number of writhes per mouse was counted for 30 min. In the formalin test, mice were injected with formalin (2.5%, 2 mL) into the subplantar of the hind paw, and the frequency of paw lickings was measured at 0–5 min and 15–20 min. During the hot plate test, mice were placed on a hot plate [constant temperature of $(55 \pm 1)^\circ\text{C}$], and the reaction time in seconds for hind paw licking or jumping was recorded at 15, 30, 45, 60, and 120 min. During the tail-flick test, the mouse's tail (3 cm) was immersed in the water bath [constant temperature of $(54 \pm 1)^\circ\text{C}$], and the time in seconds taken to flick the tail was recorded. The cut-off time was 30 s for the hot plate test and 15 s for the tail-flick test.

2.5. Anti-inflammatory activity

Male Swiss albino mice were used for histamine-induced paw edema and capillary permeability or dye leakage test. They were randomly divided into various groups ($n=6$) viz. vehicle control (0.1% CMC, 10 mg/kg, *p.o.*), indomethacin (10 mg/kg, *p.o.* for histamine-induced paw edema) or pentazocine (10 mg/kg, *p.o.* for capillary permeability), SECC (25, 50 and 100 mg/kg, *p.o.*). Animals were pre-treated with either vehicle, pentazocine, indomethacin, or SECC once a day for 7 d. The normal group was treated with a vehicle for 7 d. Histamine-induced paw edema[17] and capillary permeability test[18] were performed on the 8th day. Briefly, in histamine-induced paw edema, mice were injected with histamine

(1 mg/mL, 0.1 mL, subcutaneously) into the hind paws, and the paw thickness was assessed using a digital vernier caliper (IP67-ZD, Baker Gauges India Pvt. Ltd., India). During the capillary permeability or dye leakage test, mice were injected with acetic acid (0.6%, 0.25 mL, intraperitoneally) followed by administration of Evan's Blue (10%, 10 mL/kg, intravenously) through the tail vein. Then, the mice were killed 30 min after the injection, and the viscera was rinsed with distilled water. The dye leaking out into the peritoneal cavity was measured at 610 nm.

The potential of SECC against cotton pellet-induced granuloma was assessed in male Wistar rats according to the previously reported methods[12,19]. Briefly, sterilized cotton pellets (40 mg) were subcutaneously introduced into the abdominal skin of anesthetized rats. Then they were randomly divided into various groups ($n=6$) viz. vehicle control (0.1% CMC, 10 mg/kg, *p.o.*), indomethacin (10 mg/kg, *p.o.*), SECC (25, 50, and 100 mg/kg, *p.o.*). They were treated with either vehicle, indomethacin, or SECC once a day for 7 d. On the 8th day, the animals were sacrificed, and the cotton pellet-induced granulomas were collected and dried at 60°C overnight to determine the dried weight.

According to the previously reported method, acute paw edema was induced in male Wistar rats by subplantar carrageenan injection (1%, 0.05 mL) in the left hind paw[12]. They were randomly divided into various groups ($n=6$) viz. vehicle control (CMC, 10 mg/kg, *p.o.*), indomethacin (10 mg/kg, *p.o.*), SECC (25, 50 and 100 mg/kg, *p.o.*). Treatment procedures were done as abovementioned. On the 8th day, acute inflammation was induced in the right hind paws of rats by subplantar injection of 0.1 mL carrageenan (1% suspension, using 2% gum acacia). Another group of healthy normal rats received gum acacia (2%, 0.1 mL), followed by treatment with a vehicle (CMC, 10 mg/kg, *p.o.*). The paw edema volume was measured by a Hugo Basile plethysmometer (Gemonio, Italy) at 0, 1, 2, 3, 4, 6, and 24 h after the carrageenan injection. In one set of animals, rats were sacrificed using the cervical dislocation method under ether anesthesia at the 4th hour, and then, the left hind paw was isolated. Another set of animals was continued to measure the paw volume for 24 h.

2.6. Biochemical estimations

One portion of the hind paw was rinsed in ice-cold normal saline and homogenized at 4°C . The resultant supernatant was used to determine the levels of pro-inflammatory cytokines, including TNF- α , ILs, and COX-2, by rat-specific ELISA kits (Bethyl Laboratories Inc., Montgomery, TX, USA) according to the manufacturer's instructions (Bethyl Laboratories Inc., Montgomery, TX, USA).

The levels of SOD, GSH, MDA, and NO were measured according

to earlier reported methods[20,21]. Briefly, the SOD assay was determined in tissue homogenate (0.5 mL) using ice-cold ethanol: chloroform mixture followed by the addition of epinephrine (0.4 mL). The change in optical density/min was measured at 480 nm to measure SOD activity (units/mg protein). During the GSH assay, equal volumes of tissue homogenate supernatant were mixed in trichloroacetic acid (20%), followed by the addition of Ellman's reagent (2.0 mL) to the resultant supernatant. The developed color intensity was measured at 412 nm, and the amount of GSH is expressed as $\mu\text{g}/\text{mg}$ protein. In the malondialdehyde (MDA) assay, tissue homogenate supernatant was added to trichloroacetic acid (10%) and allowed to stand in an ice bath for 15 min. A clear supernatant solution was mixed with thiobarbituric acid (2.0 mL), followed by heating in boiling water and immediately cooled in an ice bath. The developed color intensity was measured at 532 nm, and MDA levels are expressed as nM/mg protein. The NO level was estimated as nitrite by the acidic Griess reaction after the reduction in nitrate to nitrite by vanadium trichloride. The absorbance of the resultant pink azo-product was measured at 543 nm.

2.7. Histopathological study

Another portion of the hind paw was fixed in 10% formalin, and paraffin-embedded tissues were sectioned (5 μm), stained with hematoxylin and eosin, and analyzed histopathologically using light microscopy (Nikon E200, Japan). The intensity of histological aberrations in the hind paw was graded as Grade 0 (not present); Grade 1 (slight/minimal); Grade 2 (mild); Grade 3 (moderate) or Grade 4 (severe) as described in the literature[22].

2.8. Statistical analysis

Data are expressed as mean $\pm$ SEM. Statistical analysis was carried out using one-way ANOVA followed by Tukey's multiple range test (for parametric), and the Kruskal-Wallis test (for non-parametric) was applied for *post-hoc* analysis. During two-way ANOVA, Bonferroni's test was applied to the *post-hoc* analysis.

Histological data are expressed as median (Q1, Q3) and analyzed by the non-parametric Kruskal-Wallis test followed by Mann-Whitney's multiple comparison test. All statistical analyses were performed using Graph Pad Prism software (San Diego, California, USA). The correlation between paw volume and the levels of inflammatory cytokines (TNF- α , IL-1 β and IL-6) and COX-2 was analyzed by simple linear analysis. A value of $P < 0.05$ was considered to be statistically significant.

2.9. Ethical statement

All the experimental protocols used in this study were approved by the Institutional Animal Ethical Committee of Xi'an Zhongkai Animal Experiments Medical Research (Ethical approval No. 0258).

3. Results

3.1. Identification of fustin from *C. coggygia*

The percent yield of methanolic extract of *C. coggygia* was 51.23% *w/w*, and it showed the presence of tannins, flavonoids, and phenolic compounds. The retention time for fustin on the HPLC column was 5.11 min in a total run time of 20 min, with a percent area of 62.78% (Figure 1).

3.2. Analgesic activity

3.2.1. Thermal pain

There was a notable ($P < 0.05$) increase in paw withdrawal latency (from 30 to 120 min) in mice treated with SECC (50 and 100 mg/kg) compared to the control group (Figure 2A). Similarly, the reduction in thermal pain was mitigated by the administration of SECC (50 and 100 mg/kg), demonstrated by a significant ($P < 0.05$) increase in tail withdrawal latency compared to the control group (Figure 2B). Meanwhile, the treatment of SECC (25 mg/kg) did not significantly affect paw and tail withdrawal latency compared to control mice.

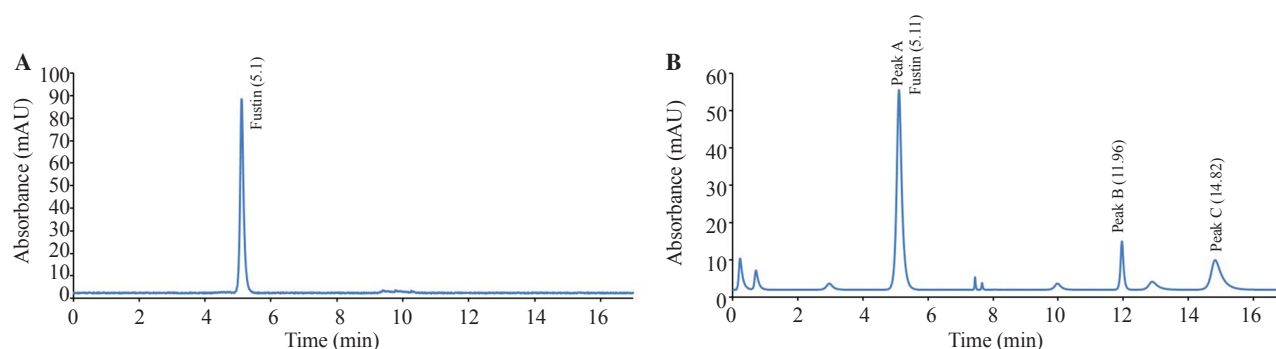


Figure 1. HPLC fingerprint of standard fustin (A) and standardized *Cotinus coggygia* (peak A: fustin, retention time: 5.11 min) (B).

However, the effects of SECC on the paw or tail withdrawal latency in mice were comparably ($P < 0.05$) lower than pentazocine.

3.2.2. Visceral pain

Subplantar injection of formalin resulted in a noticeable ($P < 0.05$) increase in the frequency of paw lickings (0-5 and 15-20 min) in control mice compared to the normal group (Figure 2C). SECC (50 and 100 mg/kg) treatment showed a significant ($P < 0.05$) reduction in paw licking frequency (15-20 min) compared to control mice, which was less effective ($P < 0.05$) compared to pentazocine.

Furthermore, following acetic acid administration, the control group showed a noticeable ($P < 0.05$) elevation in the number of writhing (from the 5th to 20th minutes) compared to the normal group. SECC (50 and 100 mg/kg) treatment remarkably ($P < 0.05$) decreased the number of writhing compared to the control group. However, the effect of SECC in reducing the number of writhing was significantly ($P < 0.05$) lower than indomethacin (Figure 2D).

3.3. Anti-inflammatory activity

3.3.1. Effect of SECC on histamine-induced paw edema

No variation was observed in the paw volume of mice before treatments. However, subcutaneous injection of histamine (1 mg/mL, 0.1 mL) into the hind paws resulted in a pronounced ($P < 0.05$) increase in paw edema of control mice (Figure 3B) compared to normal mice (Figure 3A). In contrast, treatment with SECC (50 and 100 mg/kg) significantly ($P < 0.05$) alleviated paw edema (Figure 3E and 3F), and this effect was significantly ($P < 0.05$) lower compared to indomethacin. However, SECC (25 mg/kg) did not significantly affect paw edema (Figure 3D and G).

3.3.2. Effect of SECC on cotton pellet-induced granuloma

The control group showed a notable ($P < 0.05$) rise in the granuloma formation. The weight of granuloma was significantly ($P < 0.05$) suppressed after SECC (50 and 100 mg/kg) administration compared

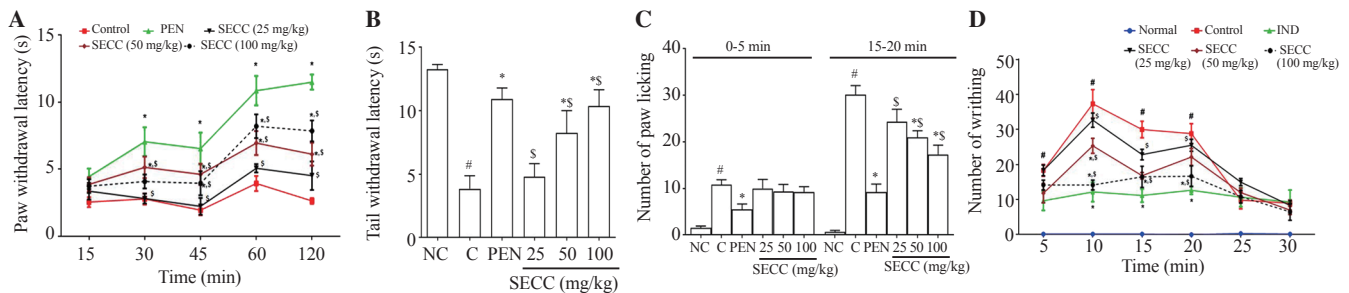


Figure 2. Effect of SECC on thermal pain by hot plate (A) and tail-flick (B) tests, and visceral pain by formalin test (C) and acetic acid-induced writhing (D) in mice. The data are expressed as mean $\pm$ standard error mean (SEM) ($n=6$). Two-way ANOVA followed by Bonferroni's *post hoc* test is used for hot plate and acetic acid-induced writhing test while one-way ANOVA followed by Tukey's *post hoc* test for tail-flick and formalin tests. $^{\#}P < 0.05$, $^{*}P < 0.05$, and $^{\$}P < 0.05$ compared with the normal, control, and pentazocine or indomethacin groups, respectively. NC: normal control; C: control; IND: indomethacin; PEN: pentazocine; SECC: standardized extracts of *Cotinus coggygria*.

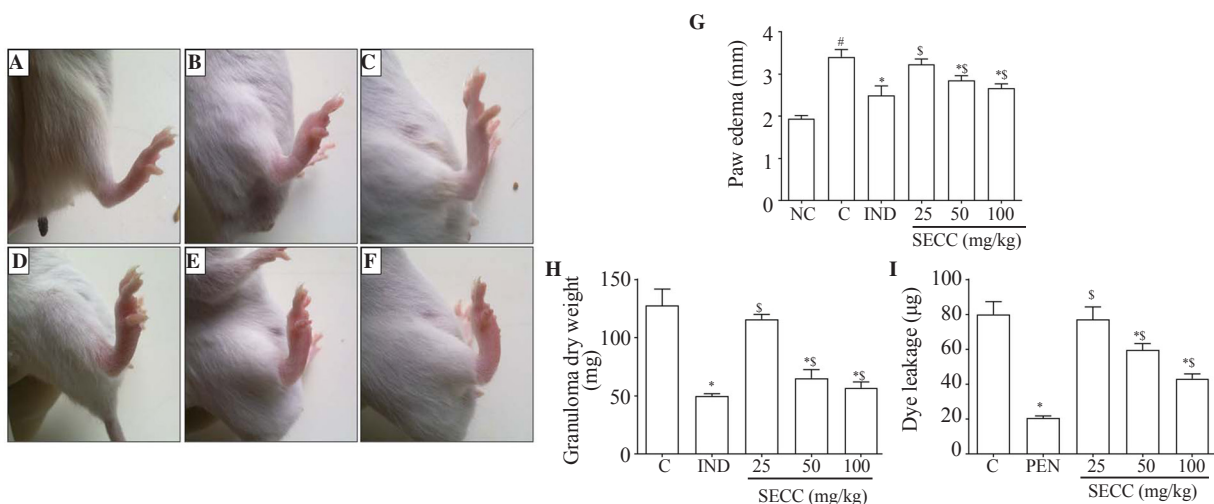


Figure 3. Effect of SECC on histamine-induced paw edema in mice. Representative images of the paw from (A) normal, (B) control, (C) indomethacin (10 mg/kg), (D) SECC (25 mg/kg), (E) SECC (50 mg/kg) and (F) SECC (100 mg/kg) treated mice. (G) The quantitative representation of paw edema in mice ($n=6$). (H) Effect of SECC on cotton pellet-induced granuloma in rats and (I) acetic acid-induced peritoneal capillary dye leakage test in mice. The data are expressed as mean $\pm$ SEM ($n=6$) and analyzed by one-way ANOVA followed by Tukey's *post hoc* test. $^{\#}P < 0.05$, $^{*}P < 0.05$, and $^{\$}P < 0.05$ compared with the normal, control, and indomethacin or pentazocine groups, respectively.

to the control group rats. No pronounced effect was observed in the group treated with SECC (25 mg/kg) ($P>0.05$) (Figure 3H).

3.3.3. Effect of SECC on capillary permeability by acetic acid-induced peritoneal capillary dye leakage test

The control group exhibited increased vascular permeability, as evidenced by a significant ($P<0.05$) elevation in dye concentration. Mice treated with SECC (50 and 100 mg/kg) exhibited a significant

($P<0.05$) reduction in dye leakage compared to the control group, indicating that vascular permeability was inhibited (Figure 3I).

3.3.4. Effect of SECC on carrageenan-induced paw edema in rats

Following carrageenan administration, rats in the control group (Figure 4B) showed a notable ($P<0.05$) increase in paw edema as compared to the normal rats (Figure 4A and G). Compared to the

Table 1. Effect of SECC on alterations in paw histopathology using hematoxylin and eosin staining ($n=3$).

Parameters	Normal	Control	Indomethacin (10 mg/kg)	SECC (25 mg/kg)	SECC (50 mg/kg)	SECC (100 mg/kg)
Inflammatory infiltration	0 (0, 0.75)	3 (3, 3.75) [#]	1 (0.25, 1.75) [*]	3 (3, 3.75) [§]	1 (1, 1.75) ^{§§}	0 (0, 0.75) ^{§§}
Edema	0 (0, 0)	4 (3.25, 4) [#]	1 (1, 1.75) [*]	3 (3, 3.75) [§]	1 (1, 1.75) ^{§§}	1 (0.25, 1) ^{§§}
Disturbance of synovial membrane	0 (0, 0.75)	4 (3.25, 4) [#]	0 (0, 0.75) [*]	3 (3, 3.75) [§]	1 (1, 1.75) ^{§§}	0 (0, 0.75) ^{§§}

The data are expressed as median (Q1, Q3) and analyzed by the non-parametric Kruskal-Wallis test followed by Mann-Whitney's multiple comparison test.

[#] $P<0.05$, ^{*} $P<0.05$, and [§] $P<0.05$ compared with the normal, control and indomethacin groups, respectively. SECC: standardized extracts of *Cotinus coggygia*.

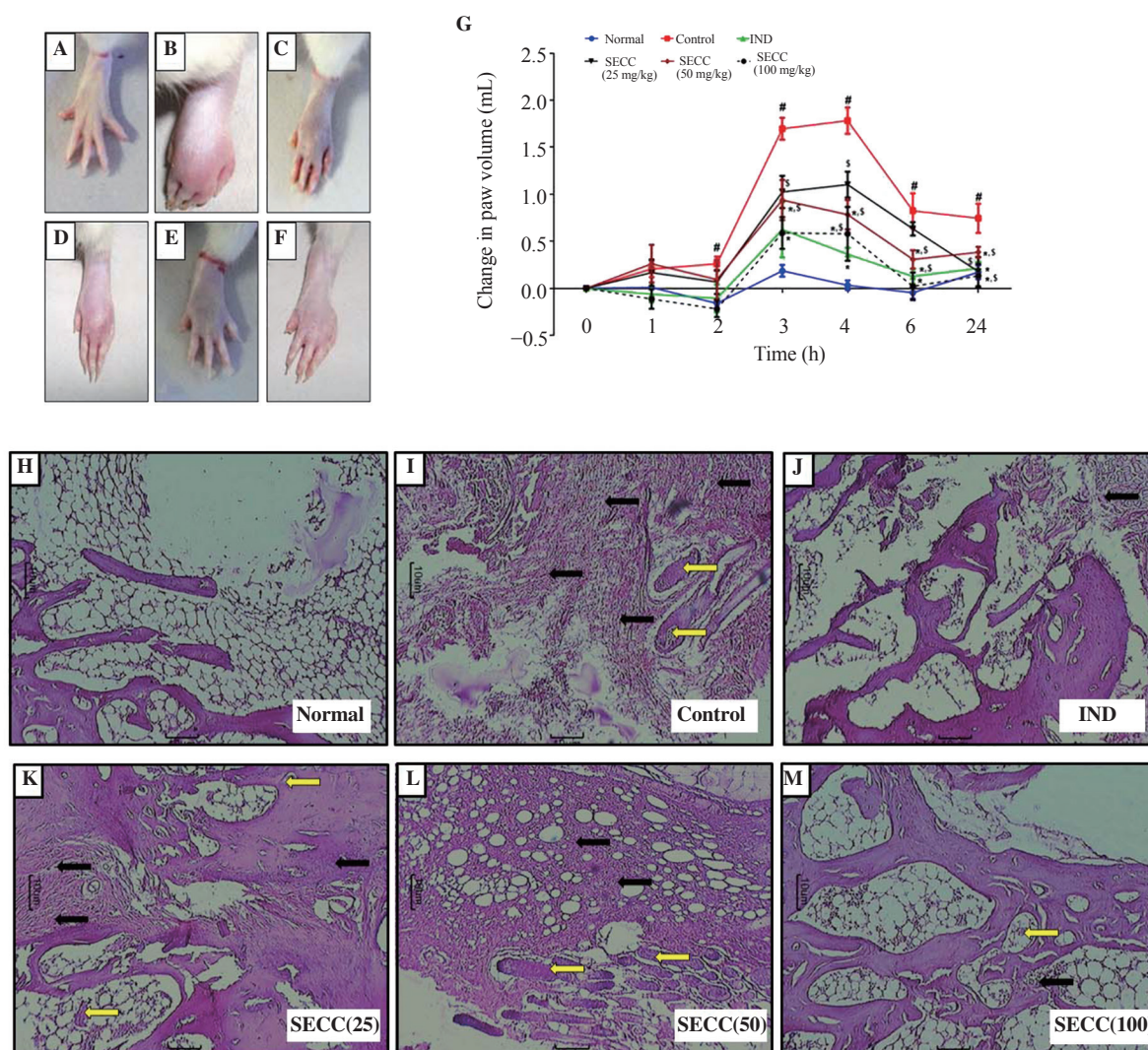


Figure 4. Effect of SECC on alterations in paw volume of carrageenan-administered rats. Representative images of the paw from (A) normal, (B) control, (C) indomethacin (10 mg/kg), (D) SECC (25 mg/kg), (E) SECC (50 mg/kg) and (F) SECC (100 mg/kg) treated rats. The quantitative representation of change in paw volume (G) in rats. The data are expressed as mean $\pm$ SEM ($n=3$) and analyzed by two-way ANOVA followed by Bonferroni's *post hoc* test. (H-M) Effect of SECC on alterations in paw histology of carrageenan-administered rats (magnification: $\times 40$). Edema (yellow arrow) and inflammatory infiltration (black arrow). [#] $P<0.05$, ^{*} $P<0.05$, and [§] $P<0.05$ compared with the normal, control and indomethacin groups, respectively.

control group, treatment with SECC (50 and 100 mg/kg) mitigated the carrageenan-induced increase in paw volume (Figure 4E-F). However, no notable change was observed in rats treated with SECC (25 mg/kg) (Figure 4D).

3.3.5. Effect of SECC on carrageenan-induced paw histopathology in rats

In Figure 4H, the histological analysis revealed a well-organized structure in the hind paw of normal rats, characterized by minimal infiltration of inflammatory cells, no discernible alterations in the synovial membrane, and the absence of edema. Conversely, Figure 4I illustrates a significant histological abnormality induced by subplantar carrageenan injection, as reflected by a substantial ($P<0.05$) increase in the histological score (Table 1), featuring abnormal inflammatory infiltration, edema, and notable disruption in the synovial membrane when compared to the histological characteristics observed in normal rats. A noteworthy protective effect was observed in the hind paw of rats treated with SECC (50 and 100 mg/kg) compared to control rats (Figure 4L and 4M). However, SECC (25 mg/kg) treatment did not exhibit any significant effect against carrageenan-induced histological aberrations (Figure 4K and Table 1).

3.3.6. Effect of SECC on carrageenan-induced alterations in inflammatory cytokines, COX-2 and oxido-nitrosative levels in the rat paw

Subplantar injection of carrageenan caused a significant ($P<0.05$) elevation in TNF- α , IL-1 β , IL-6, COX-2, and oxidative stress (MDA and NO) in the paw of control rats compared to normal rats (Table

2). Treatment with SECC (50 and 100 mg/kg) conferred significant ($P<0.05$) protection by mitigating the elevated TNF- α , IL-1 β , IL-6, COX-2, and oxidative stress levels in rats. Furthermore, the results obtained in this study were substantiated by a positive correlation (Figure 5) between paw volume and the levels of TNF- α ($R^2=0.731$), IL-1 β ($R^2=0.638$), IL-6 ($R^2=0.675$), and COX-2 ($R^2=0.716$).

4. Discussion

Pain and inflammation represent complex physiological responses often linked to diverse diseases and governed by complex molecular networks, posing significant challenges due to their subjective nature and diverse origins, with profound implications for human health. Recent decades have seen increased research on natural inflammation and pain management medicines, driven by their potential to address challenges in conventional treatments. Researchers seek safer, more effective alternatives, offering promising avenues for novel therapies leveraging natural compounds[23]. Flavonoids are natural substances widely used as analgesic, anti-inflammatory, and antioxidant agents due to their safe pre-clinical and clinical profiles[24]. The present study noted that SECC demonstrated significant analgesic and anti-inflammatory effects in various pain and inflammation animal models. These effects may be modulated by inhibiting oxidative stress, COX-2, and inflammatory cytokines.

Hot plate tests assess analgesic activity by measuring the latency time to jumping and paw-licking onset following thermal stimuli. Furthermore, the tail-flick test is another thermal-induced pain analysis that involves exposing the tail to either heat or water, and

Table 2. Effect of SECC on inflammatory cytokines, COX-2, and oxido-nitrosative levels in hind paws of carrageenan-administered rats ($n=6$).

Parameters	Normal	Control	Indomethacin (10 mg/kg)	SECC (25 mg/kg)	SECC (50 mg/kg)	SECC (100 mg/kg)
TNF- α (pg/mg protein)	10.97 $\pm$ 1.06	29.32 $\pm$ 1.41 [#]	15.46 $\pm$ 1.48 [*]	28.65 $\pm$ 1.28 ^s	20.99 $\pm$ 1.17 ^{ss}	17.22 $\pm$ 1.47 ^{ss}
IL-1 β (pg/mg protein)	12.98 $\pm$ 1.27	36.35 $\pm$ 1.71 [#]	15.40 $\pm$ 1.25 [*]	35.23 $\pm$ 1.34 ^s	28.63 $\pm$ 1.41 ^{ss}	22.68 $\pm$ 1.42 ^{ss}
IL-6 (pg/mg protein)	15.35 $\pm$ 6.30	534.00 $\pm$ 6.01 [#]	95.17 $\pm$ 5.75 [*]	523.00 $\pm$ 6.66 ^s	430.00 $\pm$ 5.51 ^{ss}	260.10 $\pm$ 5.37 ^{ss}
COX-2 (pg/mg protein)	33.96 $\pm$ 1.53	149.20 $\pm$ 2.07 [#]	65.00 $\pm$ 2.21 [*]	136.00 $\pm$ 2.01 ^s	112.50 $\pm$ 2.29 ^{ss}	78.21 $\pm$ 2.19 ^{ss}
SOD (U/mg of protein)	5.38 $\pm$ 0.34	2.67 $\pm$ 0.37 [#]	4.67 $\pm$ 0.22 [*]	2.62 $\pm$ 0.27 ^s	3.42 $\pm$ 0.28 ^{ss}	3.99 $\pm$ 0.38 ^{ss}
GSH (μ g/mg of protein)	3.33 $\pm$ 0.22	0.77 $\pm$ 0.15 [#]	2.88 $\pm$ 0.15 [*]	1.16 $\pm$ 0.18 ^s	2.16 $\pm$ 0.20 ^{ss}	2.51 $\pm$ 0.26 ^{ss}
MDA (nM/mg of protein)	3.06 $\pm$ 0.51	11.97 $\pm$ 0.71 [#]	4.65 $\pm$ 0.28 [*]	11.12 $\pm$ 0.54 ^s	9.60 $\pm$ 0.40 ^{ss}	6.71 $\pm$ 0.29 ^{ss}
NO (μ g/mg of protein)	2.53 $\pm$ 0.39	5.55 $\pm$ 0.36 [#]	2.41 $\pm$ 0.37 [*]	5.16 $\pm$ 0.38 ^s	3.84 $\pm$ 0.43 ^{ss}	3.23 $\pm$ 0.43 ^{ss}

The data are expressed as mean $\pm$ standard error mean (SEM) and analyzed by one-way ANOVA followed by Tukey's *post hoc* test. [#] $P<0.05$, ^{*} $P<0.05$, and ^s $P<0.05$ compared with the normal, control and indomethacin groups, respectively. TNF- α : tumor necrosis factor-alpha; ILs: interleukins; COX-2: cyclooxygenase-2; SOD: superoxide dismutase; GSH: glutathione; MDA: malondialdehyde; NO: nitric oxide.

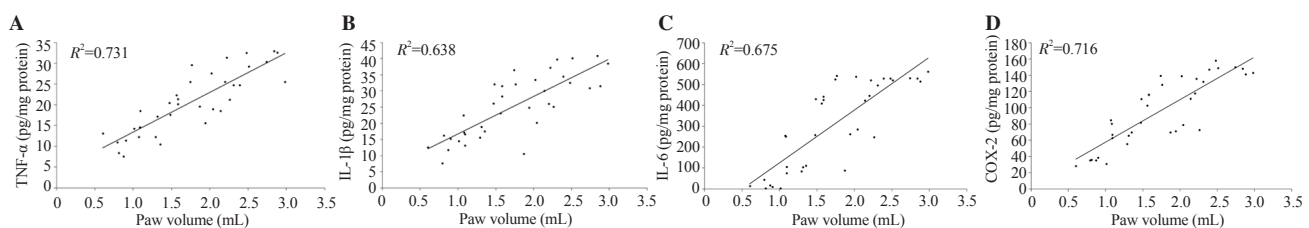


Figure 5. A simple regression of TNF- α (A), IL-1 β (B), IL-6 (C), and COX-2 (D) levels with paw volume of carrageenan-administered rats. Correlation coefficients were determined using a two-sided Fisher test. TNF- α : tumor necrosis factor-alpha; IL: interleukin; COX-2: cyclooxygenase-2.

the duration needed for a withdrawal response to occur is measured. Our study revealed that following the induction of thermal pain in the paw and tail, treatment with SECC exhibited significant analgesic activity, as evidenced by the delayed paw and tail withdrawal latency in mice. These findings align with previous reports indicating that flavonoids such as quercetin, luteolin, hesperidin, hyperin, mangiferin, kaempferol, linarin, and pectolinarin[25] demonstrate promising analgesic properties by elevating the pain threshold in a thermal pain model.

The writhing test is a chemical technique employed to induce peripheral pain in mice by injecting irritant substances such as acetic acid and phenylquinone. The acetic acid-induced abdominal writhing test is utilized as a preliminary assessment tool to evaluate the peripheral analgesic or anti-inflammatory properties of experimental drugs. It is widely acknowledged that acetic acid plays a role in releasing endogenous pain mediators, activating neurons responsible for pain perception and responsiveness to analgesic and anti-inflammatory agents. The analgesic activity of the test compound is deduced from a reduction in the frequency of writhing episodes[26]. According to the current findings, SECC demonstrates a notable analgesic effect by alleviating the writhing induced by intraperitoneal acetic acid injection. This reduction in the writhing effect may be attributed to SECC's ability to inhibit the contraction of abdominal muscles initiated by releasing various inflammatory endogenous mediators such as histamine, prostaglandins, bradykinin, and serotonin[27]. These findings align with the research indicating significant analgesic effects of several flavonoids, such as quercetin, luteolin, kaempferol, hesperidin, apigenin, myricetin, gossypin, procumbentin, amentoflavone, ginketin, and vitexin on reducing the writhing frequency caused by acetic acid administration[25].

The cotton pellet-induced granuloma model serves as a valuable representation of the pathological processes linked to chronic inflammation and is widely employed for appraising the chronic anti-inflammatory efficacy of newly developed compounds. This model enables assessing both the transudative and proliferative components of chronic inflammation, characterized by fibroblast proliferation, monocyte infiltration, exudation, and angiogenesis[11,28]. Granulomatous tissue formation, signifying chronic inflammation, involves the proliferation of macrophages, neutrophils, fibroblasts, and small blood vessels. These result in a highly vascularized reddish mass referred to as granulation tissue. The moisture absorbed by the cotton pellet markedly impacts the granuloma's wet weight. This granuloma formation resulting from transudate and granulomatous tissue formation is associated with the cotton pellet's wet and dry weights[24]. Numerous studies have demonstrated that commonly occurring flavonoids, such as apigenin, rutin, morin, hesperidin, hesperetin, and quercetin, exhibit a similar effect in preventing the deposition of granuloma weight[24]. Our findings align with these reports, indicating that SECC prominently reduces the deposits

of granuloma weight, suggesting its probable mechanism of anti-inflammatory action *via* mitigating inflammatory cell infiltration, fibroblast proliferation, angiogenesis, and exudation. Furthermore, our investigation substantiates this mechanism by illustrating the ability of SECC to diminish the infiltration of inflammatory cells as observed from the histological tissue of the hind paw.

The acute inflammatory response is manifested by three primary vascular effects involving the dilation of blood vessels with augmented blood flow, increased vascular permeability, and the migration of leukocytes into the injured tissues[29]. Intraperitoneal injection of acetic acid significantly enhances vascular permeability, allowing leakage of diagnostic dyes such as Evans blue, Chicago blue, and many others. This process is associated with endothelial cell shrinkage in local capillary beds during the initial stages of inflammation, creating gaps between cells that enhance vascular permeability, facilitating fluid and cellular extravasation, and contributing to localized edema in the inflammatory response. Our experimental findings reveal that acetic acid injection significantly elevated vascular permeability in mice, leading to extensive leakage of Chicago blue dye in peritoneal fluid. The current findings suggested that SECC attenuated this dye exudation, which indicates its inhibitory effects on fluid and cellular extravasation, suggesting potential anti-inflammatory activity. Consistent with our results, a previous study showed that flavonoids alleviated the vascular permeability effects induced by biological dyes in various animal models, emphasizing their potential anti-inflammatory efficacy. For instance, rutin and quercetin inhibited lipopolysaccharide (LPS) and acetic acid-induced endothelial cell permeability in mice[30]. Luteolin demonstrated anti-inflammatory effects by reducing Evan's blue dye leakage in the peritoneal cavity, justifying its protective role against vascular permeability[31]. Additionally, a flavone glycoside, diosmin, exhibited potential anti-inflammatory properties by protecting against vascular leakage and inhibiting Evans blue dye permeability in the Iliac vein stenosis mouse model[32]. Other flavonoids, including fisetin, kaempferol, baicalin, baicalein, and wogonin, demonstrated anti-inflammatory effects by inhibiting Evans blue vascular permeability induced by high glucose administration[24].

Chemical-induced paw edema assays are commonly employed to assess the anti-inflammatory efficacy of compounds, utilizing inducers such as histamine, carrageenan, dextran, 5-hydroxytryptamine, LPS, and bradykinin in rodents. Following chemical stimulation, diagnostic parameters like paw withdrawal latency, edematous paw volume and thickness, and expression of inflammatory mediators are monitored to investigate the anti-inflammatory effects of medicinal agents[33]. The present investigation explored the anti-inflammatory activity of SECC against chemically induced edema, utilizing histamine and carrageenan as inflammation inducers in rodent models. The histamine-induced paw edema model may serve as a supplementary

approach to validate the outcomes of the carrageenan-induced paw edema model, particularly for pharmaceutical agents demonstrating efficacy during the initial phase of carrageenan-induced inflammation. In contrast, carrageenan, a polysaccharide, is an inflammatory agent extensively employed to elicit an acute, non-immune, and highly replicable inflammatory context in laboratory animals[34]. Meanwhile, subplantar injection of carrageenan rapidly produces inflammation, followed by mechanical and thermal allodynia, making it a promising model for exploring the anti-inflammatory and/or antinociceptive properties of novel drugs[35]. Studies have demonstrated that certain widely recognized flavonoids, such as silymarin, quercetin, and isokaempferide, exhibit potential anti-inflammatory effects by mitigating carrageenan- and histamine-induced paw edema. The findings from the present study show that SECC has the same anti-inflammatory properties as demonstrated by other widely recognized flavonoids, illustrating the potential of SECC to attenuate the augmented edematous paw volume and thickness in rodents. COX-2 is a central enzyme in the inflammatory cascade, catalyzing the conversion of arachidonic acid into prostaglandins. These lipid mediators play integral roles in physiological and pathological processes, notably inflammation[36]. Hence, COX-2 is a therapeutic target for many anti-inflammatory drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and newer COX-2 selective inhibitors[37]. SECC has exhibited potential effects in the later phase of carrageenan-induced inflammation, where it inhibits COX-2, an enzyme associated with inflammation, pain, and platelet aggregation[38]. These observations are consistent with our research findings, which substantiate the anti-inflammatory potential of SECC. However, further study is needed to confirm the phytoconstituents responsible for this anti-inflammatory potential.

With an increasing global requirement for enhanced therapeutic strategies, flavonoids represent a natural compound group undergoing extensive pre-clinical and clinical investigations[24]. A clinical study involving 24 patients indicated that daily administration of hesperidin (500 mg) for three weeks stimulated nitric oxide production in endothelial cells and reduced inflammatory markers in patients with metabolic syndrome[39]. Other clinical studies demonstrated that the administration of Daflon® tablets (500 mg) containing hesperidin and diosmin improved severe inflammation, congestion, edema, and reduced severity of hemorrhoidal episodes[40]. In a double-blind, randomized controlled trial, daily administration of quercetin supplement (500 mg, for 8 weeks) in female patients with rheumatoid arthritis showed notable improvements in clinical symptoms such as early morning stiffness and pain[41]. Based on these clinical reports, it is evident that flavonoid compounds have potential effects for managing pain and inflammation. Based on findings from the present investigation and other studies, SECC can be considered a potential candidate for future clinical interventions in controlling pain and inflammation.

This study exhibits several limitations. Firstly, there is a lack of clinical studies to identify the safety of SECC before its use for human consumption. Secondly, formalin used in the determination of visceral pain may cause additional irritation in experimental animals as formalin itself is a severe irritant that might produce additional pain. Thirdly, this study did not evaluate the dose-dependent potential of SECC, although different dosages were used. Future investigations need to consider the dose-dependent evaluation of the anti-inflammatory and analgesic activity of SECC. Lastly, this study did not evaluate the levels of various anti-inflammatory agents such as TNF- α , IL-1 β , and IL-6 at different time points during carrageenan-induced paw edema.

In conclusion, the current findings indicated that *C. coggygia* Scop. exhibits analgesic and anti-inflammatory potential in experimental animals in response to thermal (hot plate, tail flick test, cotton pellet-induced granuloma) and chemical (acetic acid-induced writhing, formalin test, histamine and carrageenan-induced paw edema) stimuli. SECC exerts these effects *via* inhibition of COX-2, inflammatory mediators (TNF- α , IL-1 β , and IL-6) and oxidonitrosative stress (MDA and NO). Based on these findings, SECC can be used to develop analgesic and anti-inflammatory agents.

Conflict of interest statement

The authors have no conflicts of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

KL conceptualized the work and performed data analysis and interpretation. CYY collected the data. ZHY performed the data

analysis and interpretation, and drafted the article. All the authors critically revised the article, and approved the final version of the manuscript.

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