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Original article

# Anti-Inflammatory Activity of *Cymbopogon schoenanthus* Essential Oil in Animal Models

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## Abstract

**Background and objectives**: The species of *Cymbopogon* are generally used as anti-bacterial, anti-fungal, anti-malarial, and anti-spasmodic agents, as well as in cold treatment. Due to the presence of piperitone in *Cymbopogon schoenanthus*, we were prompted to evaluate were prompted to assess the anti-nociceptive and anti-inflammatory properties of its essential Oil. **Methods**: The analgesic activity of *C. schoenanthus* (50, 100, and 200 mg/kg, i.p.) were examined using writhing, hot-plate, and formalin tests. The control and standard groups respectively received vehicle, morphine (5 mg/kg, i.p.), and mefenamic acid (30 mg/kg). The anti-inflammatory effect of *C. schoenanthus* (50, 100, and 200 mg/kg) was then assessed by carrageenan method at time intervals of 30 min and 1, 2, 3, and 4 h. **Results**: *Cymbopogon schoenanthus* essential oil was analyzed by GC-MASS and 31 constituents were identified which represented 86.8% of the oil. The major component of the essential oil was piperitone (62.0%). The administrated doses of *C. schoenanthus* essential oil could not decrease the number of writhes and hot-plate latency in the mice, compared to the control group. However, it exhibited an analgesic effect, especially in the chronic phase of formalin test. In carrageenan test, all administrated doses of *C. schoenanthus* essential oil significantly reduced the paw edema, compared to the control ($p<0.05$). The anti-inflammatory activity of the essential oil (50, 100, and 200 mg/kg) was comparable with that of mefenamic acid (30 mg/kg). **Conclusion**: The results suggest that *C. schoenanthus* essential oil possesses biologically active constituents that have significant activity against acute inflammation.

**Keywords:** anti-inflammatory activity; *Cymbopogon schoenanthus*; essential oil; mice; rat

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## Introduction

*Cymbopogon schoenanthus*, belongs to the family Poaceae, locally known as Izkhir and traditionally named as camel grass which grows wild in tropical and temperate regions of Africa and Asia [1-3]. It has been mentioned in "Alhadith" for its potential applications [4]. In Africa, *C. schoenanthus* is used in salads and traditional meat dishes. Because of its pleasant aroma and taste, it is also consumed as tea [5]. Its medicinal properties were considered to be supportive in the treatment of gout, prostate inflammation, kidney disorders, stomach pain, fever, and rheumatism [6,7]. The previous studies showed that *C. schoenanthus* had anti-spasmodic, anti-malarial, anti-helminthic, antipyretic, spasmolytic and diuretic effects [4,8-11]. It was shown to possess sedative, digestive, anti-

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parasitic, and anti-fungal properties [12,13]. *Cymbopogon schoenanthus* has also been used as anti-abortive, anti-convulsive, laxative, anti-rheumatic, asthmatic, and antipyretic agent [14]. In Saudi traditional medicine, it is mainly used as a diuretic to inhibit kidney stone formation and as an anti-infectious agent in urinary tract infections [1]. The present study attempted to evaluate the analgesic and anti-inflammatory effects of *C. schoenanthus* essential oil using carrageenan, formalin, hot-plate, and acetic acid tests in experimental animals.

## Materials and Methods

### Ethical considerations

All experiments were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 80-23) and were approved by Research and Ethics Committee of Islamic Azad University, Tehran Medical Sciences, Tehran, Iran with ethical code of (IR.IAU.PS.REC.1397.339).

### Plant material and essential oil extraction

*Cymbopogon schoenanthus* was purchased from a local herbal market in Luristan Province, Iran, in February 2018. It was identified under the voucher specimen No. 1602-Aupf by N. Kazemivash, Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran. The aerial parts of the plant were dried in shade and cut into 3 cm pieces. The essential oil of these aerial parts was extracted by hydro-distillation for 4 h using a clevenger-type apparatus. The oil was separated from the aqueous phase and kept in the refrigerator (2-8 °C) until further use.

### Pharmacological Studies

### Animals

Male Wistar rats (weighing 180-250 g) and male NMRI mice (weighing 20-25 g) were used in the present experiment. They were housed in standard environmental conditions and fed with standard rodent diet. The animals were kept in groups of six each in standard cages on a 12 h light/dark cycle at 22±2 °C.

### Acetic acid-induced writhing test

The abdominal constrictions were produced according to the procedure described previously by [12]. The number of stretches or writhes (arching of the back, development of tension in the abdominal muscles, and elongation of body extension of the forelimbs) of each animal was counted during a 30 min period starting 30 min after the administration of 0.01 mL/g of an aqueous acetic acid solution (1%V/V, i.p.). The vehicle (almond oil), mefenamic acid (30 mg/kg), morphine (7 mg/kg) or essential oil (50, 100, and 200 mg/kg) were administered (i.p. injection) 30 min before the acetic acid injection in separated groups.

### Hot-plate test

The hot-plate test measured the response latencies according to the method described by Siegmund et al. [16]. The animals were placed on a thermostatically controlled hot-plate (Borj Sanat, Iran) maintained at 55 ± 0.5 °C and the time between the placement of the animal on the hot-plate and the occurrence of either licking the hind paws or shaking or jumping off from the surface was recorded as the response latency. The cut-off time for hot-plate latencies was set at 12 s. The reaction time of each group was recorded when the animals licked their hind paws and jumped before (0) and 15, 30, 60, and 120 min after i.p. administration of 200 and 300 mg/kg of essential oil. Morphine (7 mg/kg) was used as the reference drug.

### Carrageenan-induced rat paw edema test

The anti-inflammatory activity of *C. schoenanthus* essential oil was examined by the carrageenan-induced edema test in the hind paws of rats [17]. Male Wistar rats (six per each group), weighing 180-220 g, were used with free access to water and food. The groups were then administered i.p. with the essential oil with doses of 50, 100, and 200 mg/kg and mefenamic acid at the dose of 30 mg/kg. Drugs or drugless vehicle were injected 30 min before the carrageenan treatment. The paw volume was measured using plethysmometer (Borj Sanat, Iran) before and after carrageenan injection at different time intervals of 30 min, 1, 2, 3, and 4 h. The anti-inflammatory activity was revealed as the inhibition percent of the edema, compared to the control group which was measured by the following equation:

$$\text{Inhibition of edema (\%)} = 100 \times [(V_{control} - V_{test})/V_{control}]$$

**Formalin test**

This method used in the present study was similar to that described previously [18]. *Cymbopogon schoenanthus* (50, 100, and 200 mg/kg i.p.), mefenamic acid (30 mg/kg), and morphine (7 mg/kg i.p.) were all administered 30 min before formalin injection. The control animals received the same volume of almond oil intraperitoneally. An aliquot of 2.5% (50 µL) was injected subcutaneously into the right hind paw of the mice. Scoring of nociceptive behaviors began immediately after formalin injection and continued for 60 min. A nociceptive score was determined for each 5-min time block by measuring the amount of time spent in each of the following behavioral categories: 0 = the injected paw was not favored; l = the injected paw had little or no weight placed on it; 2 = the injected paw was elevated and was not in contact with any surface; and 3 = the injected paw was licked, bitten or shaken [19]. The weighted average nociceptive score (pain rating), ranging from 0 to 3, was calculated by multiplying the time spent in each category and the category's weight and then dividing by the total time for each 5-min time block. Individual time course determinations in formalin test were converted to area-under-the-curve values 0 to 10 min (AUC first phase) and 10 to 60 min after formalin injection (AUC second phase).

**Statistical analysis**

Comparative analyses were conducted by one-way ANOVA analysis followed by the post hoc Tukey's test, and $p<0.05$ was considered as being significant. The data were analyzed using the statistical software GraphPad Prism 5.

## Results and Discussion

The oil sample was analyzed on an HP 6890 gas chromatograph equipped with an FID and a DB-5 capillary column (30 m × 0.25 mm) with 0.25-µm film thickness and the temperature of 60 to 240 °C at a rate of 4 °C/min. The flow rate of the carrier gas N2 was 2.0 mL/min and the temperatures of the injector port and detector were 250 and 300 °C, respectively. The samples were injected by splitting with a split ratio of 1:10. The analysis of GC-MS was performed on a Hewlett Packard 6890/5972 system with the same DB-5 capillary column. The operating conditions were the same but the carrier gas was He. Mass spectra were taken at 70 eV. The range of scan mass was from 40 to 400 m/z at a sampling rate of 1.0 scan/s. The quantitative data were obtained from the electronic integration of the FID peak areas. The components of the oil were identified through their retention time and indices, relative to $C_9$-$C_{28}$ n alkanes, computer matching with WILEY275.L library, and comparing their mass spectra with those of the authentic samples or the data already available in the literature [20,21]. The percentage composition of the identified compounds was computed from the GC peaks areas without any correction factors and was calculated relatively.

As shown in table 1, thirty one components were identified in *C. schoenanthus* essential oil, which represented about 86.8% of the total chromatographical material. The remained (13.2%) is related to the unknown constituents of the essential oil. These constituents couldn't be characterized by gas chromatography-mass spectrometry (GC/MS).

**Table 1.** GC-MS analysis of *Cymboogon shoenanthus* essential oil

| Compound[a] | KI[b] | RKI[c] | % |
|---|---|---|---|
| 1. Tricyclene | 922 | 926 | 0.1 |
| 2. α-Pinene | 933 | 939 | 0.2 |
| 3. Camphene | 950 | 954 | 0.3 |
| 4. α-Terpinene | 1023 | 1018 | 7.1 |
| 5. ρ-Cymene | 1028 | 1026 | 2.9 |
| 6. Limonene | 1030 | 1031 | 2.3 |
| 7. *cis*-β-Ocimene | 1047 | 1040 | 0.3 |
| 8. *trans*-β-Ocimene | 1053 | 1050 | 0.3 |
| 9. Fenchone | 1091 | 1087 | 0.2 |
| 10. Linalool | 1102 | 1098 | 0.5 |
| 11. *cis*-ρ-Mentha-2,8-dien-1-ol | 1136 | 1138 | 0.2 |
| 12. *trans*-ρ-Menth-2-en-1-ol | 1137 | 1140 | 0.8 |
| 13. Dill ether | 1187 | 1184 | 0.3 |
| 14. α-Terpineol | 1193 | 1189 | 1.0 |
| 15. Nerol | 1232 | 1228 | 0.1 |
| 16. Piperitone | 1288 | 1282 | 62.0 |
| 17. Thymol | 1294 | 1290 | 0.5 |
| 18. Carvacrol | 1301 | 1298 | 0.1 |
| 19. α-Cubebene | 1348 | 1345 | 0.1 |
| 20. Neryl acetate | 1367 | 1365 | 0.1 |
| 21. α-Copaene | 1380 | 1376 | 0.5 |
| 22. *trans*-Caryophyllene | 1422 | 1418 | 0.8 |
| 23. α-Humulene | 1446 | 1440 | 0.1 |
| 24. β-Selinene | 1487 | 1485 | 0.1 |
| 25. α-Selinene | 1499 | 1494 | 0.3 |
| 26. δ-Cadinene | 1528 | 1524 | 0.7 |
| 27. Elemol | 1551 | 1547 | 1.4 |
| 28. Caryophyllene oxide | 1579 | 1583 | 1.7 |
| 29. 10-epi-γ-Eudesmol | 1600 | 1602 | 0.3 |
| 30. Hinesol | 1640 | 1638 | 0.5 |
| 31. α-Eudesmol | 1656 | 1652 | 1.0 |
| Total | | | 86.8 |

[a]Compounds have been listed in order of elution.; [b]KI (Kovats index) was measured relative to *n*-alkanes ($C_9$-$C_{28}$) on the non-polar DB-5 column under the conditions mentioned in the materials and methods section.; [c]RKI, (Kovats index) from the existing literature

In the gas chromatography-mass spectrometry (GC-MS) analyses, *C. schoenanthus* essential oil was observed to possess a mixture of monoterpenes like piperitone (62 %), α-terpinene (7.1%), ρ-cymene (2.9 %), and limonene (2.3%) as its main compounds. (table 1).

*Cymbopogon shoenanthus* essential oil with doses of 200 and 300 mg/kg did not increase the reaction time of the animals (figure 1). Morphine indicated significant analgesic activity in the hot-plate test.

The effect of systemic administration of different doses of *C. shoenanthus* essential oil (50, 100, and 200 mg/kg) on the behavioral responses were studied during the first and second phases of the formalin test. In formalin test, morphine revealed anti-nociceptive effects in the both phases (figure 2). However, *C. shoenanthus* essential oil showed anti-nociceptive effects in the second phase, compared to mefenamic acid (table 2). *Cymbopogon schoenanthus* essential oil with doses of 50, 100, 200 mg/kg did not statistically decrease the number of writhes during the test (30-min), compared to the control group (figure 3).

The acute anti-inflammatory effect of *Cymbopogon schoenanthus* essential oil (i.p. administration) on the rat paw edema induced by carrageenan was observed (table 3). *Cymbopogon schoenanthus* essential oil (50, 100 and 200 mg/kg) and mefenamic acid (30 mg/kg) significantly inhibited the carrageenan paw edema development ($p<0.05$, $p<0.01$) at the first hours of the experiment by 62.16, 56.7, 48.6 and 59.45%, respectively. At the fourth hour of the experiment, the edema inhibition reached 40.7, 25.9, 40.752.92, and 22.22 %, respectively (table 3).

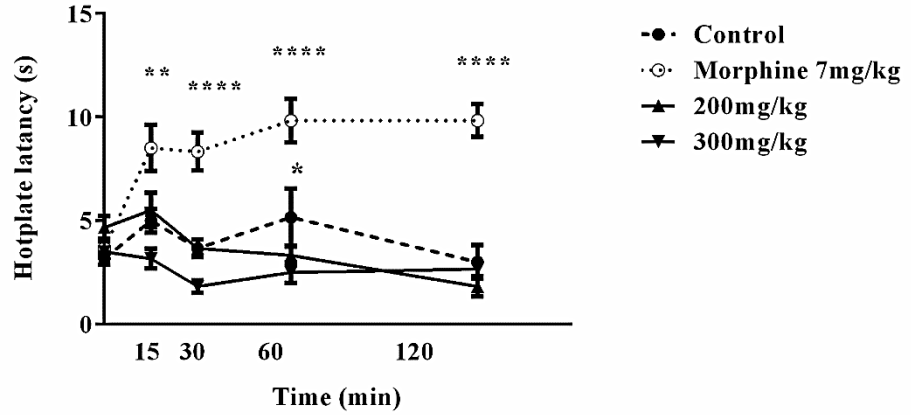


**Figure 1.** Analgesic activity of *Cymbopogon schoenanthus* essential oil in the hot-plate test. Values indicate mean±SEM (n=6-8); $^{*}p<0.05$, $^{**}p<0.01$, $^{****}p<0.0001$: significant difference, compared to the control

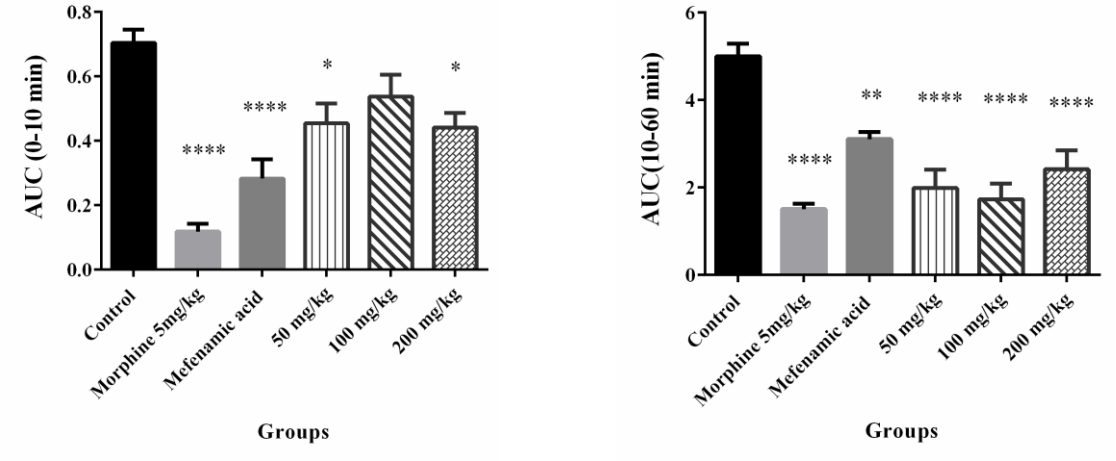


**Figure 2.** Effect of *Cymbopogon schoenanthus* essential oil on nociceptive responses in phases I (A) and II (B) of the formalin test. Values indicate mean±SEM (n=6–8). $^{*}p<0.05$, $^{**}p<0.01$, $^{****}p<0.0001$: significant difference, compared to the control

**Table 2.** Comparison of *Cymbopogon schoenanthus* inflammatory inhibition of essential oil in formalin test

| Groups | AUC 0-10 (Inhibition %) | AUC 10-60 (Inhibition %) |
|---|---|---|
| Morphine (7 mg/kg) | 83% | 70% |
| Mefenamic acid (30 mg/kg) | 60% | 38% |
| *C. shoenanthus* essential oil (50 mg/kg) | 36% | 60% |
| *C. shoenanthus* essential oil (100 mg/kg) | 23% | 65% |
| *C. schoenanthus* essential oil (200 mg/kg) | 38% | 52% |

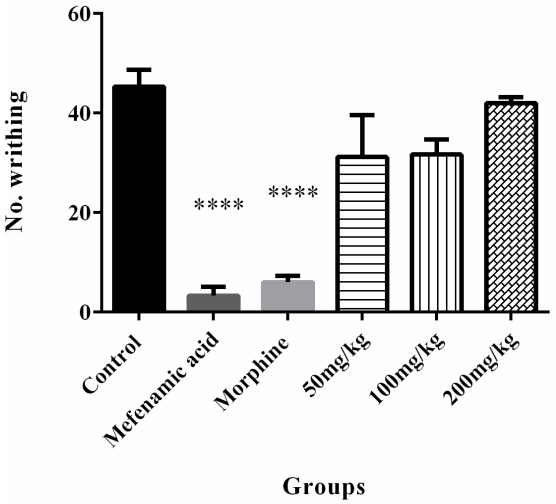


**Figure 3.** Analgesic activity of *Cymbopogon schoenanthus* essential oil in the acetic acid writhing test. Values indicate mean±SEM (n=6–8). $^{****}p<0.0001$: significant difference, compared to the control

Chemical drugs are widely being used to relieve pain and reduce inflammation. However, they have many undesirable side effects that may limit their use and prompt the researchers and scientists to find new high efficiency compounds with fewer side effects. The analgesic and anti-inflammatory activities of *C. schoenanthus* essential oil were evaluated in the present study. It is the first report studying the anti-inflammatory activities of *C. schoenanthus* in acute inflammation.

Carrageenan-induced edema has been usually offered as an acute inflammation model in the animal studies. It is well known that carrageenan paw edema is observed as a biphasic period with the involvement of inflammatory mediators. In the first phase (the first 2 h after carrageenan injection) such chemical mediators as histamine and serotonin play a role, while in the second phase (3 to 4 h after carrageenan injection) kinins and prostaglandins are observed [22]. The findings of the present study indicated that *C. schoenanthus* essential oil at doses of 50, 100, and 200 mg/kg had adequate anti-inflammatory effect in carrageenan test in the first two hours.

Heat-induced and formalin-induced pain models were used in the present study in order to assess the anti-nociceptive effect of *C. schoenanthus* essential oil in experimental mice. Formalin test consists of two phases. The first phase (neurogenic pain) is caused by direct chemical stimulation of nociceptive afferent fibers, predominantly C fibers, which can be inhibited by opiates like morphine [23]. The second phase is produced by the action of such inflammatory mediators as prostaglandins, serotonin, and bradykinin in peripheral tissues and also from functional changes in the spinal dorsal horn [24]. It was observed that different doses of *C. schoenanthus* essential oil (50, 100, and 200 mg/kg) had considerable analgesic effects in the second phase of formalin test. Also a thermal nociception model (hot-plate test) was used to define the central analgesic activity of the essential oil. It did not significantly change the reaction time of the animals against the thermal stimulus. Acetic acid writhing test was also experienced on the mice and indomethacin was used as the reference drug [15].

**Table 3.** Effect of *Cymbopogon schoenanthus* essential oil on the inflammation induced by carrageenan

| | **Paw edema ($cm^3$) at various time intervals (%Inhibition)** | | | | |
|---|---|---|---|---|---|
| **Groups** | **30 min** | **1hr** | **2hr** | **3hr** | **4hr** |
| Control (Almond Oil) | 0.15±0.03 | 0.37±0.06 | 0.38±0.05 | 0.32±0.05 | 0.27±0.05 |
| Mefenamic acid (30mg/kg) | 0.07±0.03 (53.3%) | 0.15±0.05** (59.4%) | 0.20±0.04* (47.4%) | 0.23±0.05 (28.1%) | 0.21±0.05 (22.2%) |
| *C. schoenanthus* essential oil (50mg/kg) | 0.09±0.02 (40.0%) | 0.14±0.02** (62.2%) | 0.19±0.04* (50.0%) | 0.24±0.04 (25.0%) | 0.16±0.04 (40.7%) |
| *C. schoenanthus* essential oil (100mg/kg) | 0.11±0.02 (26.7%) | 0.16±0.02** (56.7%) | 0.19±0.03* (50.0%) | 0.24±0.03 (25.0%) | 0.20±0.05 (25.9%) |
| *C. schoenanthus* essential oil (200mg/kg) | 0.07±0.02 (53.3%) | 0.19± 0.03* (48.6%) | 0.18±0.05* (52.6%) | 0.19±0.04 (40.6%) | 0.16±0.04 (40.7%) |

Each value represents the mean ± SEM (% inhibition) of 6 rats; $^{*}p<0.05$, $^{**}p<0.01$; significant difference, compared to the control

*Cymbopogon schoenanthus* essential oil at doses of 50, 100, and 200 mg /kg did not show any significant analgesic properties. Furthermore, in agreement with the results of acetic acid-induced writhing and hot-plate tests, the essential oil did not show an analgesic activity. Thus, it can be concluded that the essential oil does not have anti-nociceptive effects.

The second phase of formalin test indicates the effect of the drug on inflammation process and pain behavior. In the present study, a reduction was observed in pain behaviors, which is in agreement with the results of carrageenan paw edema test. It is supposed that the essential oil of *C. schoenanthus* contains a substance and/or substances that block(s) the inflammatory process and thus, the inhibition of pain behaviors.

The phytochemical results indicated that the anti-inflammatory effects of *C. schoenanthus* essential oil may be due to its piperitone content. Several studies have reported the anti-inflammatory activities of plants enriched with piperitone [25]. Zhenliang Sun et al. (2014) evaluated the anti-inflammatory effect of *M. piperita* essential oil grown in China [25]. *Mentha* essential oil exhibited potent anti-inflammatory effects in a croton oil-induced mouse ear edema model. They also reported that it could effectively prevent the production of nitric oxide (NO) and prostaglandin E2 (PGE2). These results are similar with the current findings suggesting that *C. schoenanthus* essential oil showed significant anti-inflammatory activities.

Studies have reported that *Cymbopogon* essential oil has anti-inflammatory activities [26]. In a study conducted by Leite et al., it was shown that *Cymbopogon winterianus* had anti-inflammatory effect [26]. In a research study in 2014, the anti-inflammatory effect of *Cymbopogon citratus* (a lemon grass) essential oil was evaluated [27]. It has also been associated with analgesic effects [28]. In 2013, the anti-inflammatory effect of essential oil of *Cymbopogon proximus* (rich in piperiton, alumol, and alphatropinol) was reported [29]

Since some studies have shown that carrageenan injection into the rat paw releases bradykinin which later induces the inflammation of prostaglandin and other autacoids, responsible for the formation of the inflammatory exudates [30], it is possible that the existing piperitone in *C. schoenanthus* essential oil inhibits the biosynthesis of prostaglandins. Also, in the formalin test, the essential oil caused a graded inhibition of the second phase of the formalin-induced pain. Therefore, *C. schoenanthus* essential oil could be a potential candidate as an anti-inflammatory drug.

Several studies have so far reported the immunomodulatory and anti-inflammatory properties of other compounds such as α-terpinene, ρ-cymene, and limonene found in *C. schoenanthus* essential oil and/or other Plants rich in these compounds [31,32]. These studies have concluded that the existing compounds in *C. schoenanthus* essential oil may have either direct or indirect anti-inflammatory activities.

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## Author contributions

Mahshid Golestaneh Talaei has done the tests, analyzed the data and wrote the manuscript. Zahra Mousavi designed the animal studies and edited the manuscript. Maryam Jahandideh participated in plant extraction**.** All authors have read and approved the final manuscript.

## Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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