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Analgesic and antiinflammatory activities of the capilliposide derived from *Lysimachia capillipes* Hemsl., a traditional Chinese medicinal herb

Zhipan Wu[1], Haote Han[1], Mengting Xu[1], Yuhang Shen[1], Chengcheng Gao[1], Huahua Yuan[1], Shouxin Li[1,2], He Ye[3,*] and Jingkui Tian[1,2,#]

[1]*College of Biomedical Engineering and Instrument Science, Zhejiang University*, Hangzhou 310027, China

[2]*Key Laboratory for Biomedical Engineering of Ministry of Education, Zhejiang-Malaysia Joint Research Center for Traditional Medicine, Zhejiang University*, Hangzhou 310027, China

[3]*Department of Pharmacy, Zhejiang Hospital*, Hangzhou 310013, China

Corresponding authors: *yehe20092009@163.com; #tjk@zju.edu.cn

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Abstract: Pain and inflammation are associated with the pathophysiology of different clinical conditions. The *Lysimachia capillipes* Hemsl. capilliposide (LCc) is the main bioactive component of this Chinese medicinal herb, which is widely used as a remedy for the treatment of colds and arthritis. This study investigated the analgesic and antiinflammatory activities of LCc in an animal model. LCc had no significant influence on the spleen, lung, liver and stomach coefficients in mice. Pharmacological studies showed that LCc at all doses (40, 60 and 90 mg/kg) increased the latency period of paw licking induced by thermal stimulation, and at the dose of 40 mg/kg it significantly suppressed abdominal writhing episodes of mice induced by intraperitoneal (i.p.) injection of acetic acid. LCc also had antiinflammatory effect on inflammation models. Doses of 60 and 90 mg/kg suppressed paw edema induced by subcutaneous (s.c.) injection of carrageenan. Mechanistic studies revealed that the antiinflammatory effect of LCc was associated with inhibition of the production of malondialdehyde (MDA), prostaglandin E2 (PGE2), tumor necrosis factor (TNF-α), cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) in paw tissue of carrageenan-injected mice. These results show that LCc has analgesic and antiinflammatory effects in mice.

Keywords: capilliposide; *Lysimachia capillipes* Hemsl.; antiinflammatory; analgesic

INTRODUCTION

Pain and inflammation are common manifestations of many diseases and are elements of the complex response of the body to harmful stimuli [1,2]. Pain is defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" [3-5]. Inflammation is a beneficial host response of the body that induces physiological adaptations to minimize tissue damage by removing the classic initiators of inflammation, such as pathogens, chemicals or physical tissue injury [6]. However, if the inflammation persists, it may become destructive and cause tissue damage, such as rheumatoid arthritis, atherosclerosis, heart disease, Alzheimer's disease, AIDS, cancer, diabetes, gout, inflammatory bowel disease, aging and others [7].

The secretion of multiple chemicals is a critical component of secondary damage, promoting inflammation and cell death [8]. TNF-α, a proinflammatory mediator, plays an important role in the development of inflammation and oxidative stress [9]. As modulators of inflammation, prostaglandins (PGs) have a major role in the early phase of inflammation [10], with PGE2 a proinflammatory lipid mediator of inflammation [11]. Additionally, PGs are produced by the agency of COX-1 and COX-2 through the arachidonic acid pathway, which has a crucial role in pain and inflammation [2]. The greatest degree of inflammation is observed about 3 h post carrageenan injection and is attributed to PG release [12]. The expression of COX-2 is maximal at the late phase [13], and it has also been proposed that free radicals play an important role in carrageenan-induced acute inflammatory response

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[14]. MDA is the end-product of enzyme- and free radical-catalyzed lipid peroxidation of polyunsaturated fatty acids, including arachidonic acid [15], which accumulates after carrageenan injection [16].

Common steroids and nonsteroidal drugs are used in the relief of pain and inflammation [2]. However, most of them still present a range of problems in efficacy and undesired effects that limit their usefulness. New research focuses on the development of herbal medicines or forgotten drugs, recommending the use of medicinal plants for the treatment of various diseases [17-21] and in the treatment of pain and inflammation [22,23]. Compounds derived from natural sources such as higher plants are the most important sources of effective and clinically useful drugs [24-26]. For instance, several triterpenoids, including oleanolic acid, betulinic acid, celastrol, pristimerin, lupeol and avicins have been reported to possess antiinflammatory, hepatoprotective, and antitumor properties [27,28].

Lysimachia capillipes Hemsl., a traditional medicinal plant that grows in southeast China, belongs to the Primulaceae family, and was included in the Flora of China and Flora of Zhejiang Province for its biological activities, with the whole plant used for treating coughs, menstrual disorders, rheumatic pain, arthritis and carcinomas [29-32]. The "Manshanxiang tablet" made from *Lysimachia capillipes* Hemsl. is used to treat fever, headache, etc. [32]. Capilliposides, novel oleanane triterpenoid saponins, are the characteristic chemical markers of *L. capillipes* Hemsl. [29,33,34]. The anticancer property of the LCc, the main bioactive component of *L. capillipes* Hemsl., was reported in different cancer cell lines both *in vivo* and *in vitro* [35,36].

Although LC exerts antitumor effects, the analgesic and antiinflammatory activities remain poorly understood. Therefore, this study was conducted to investigate the analgesic and antiinflammatory activities of LCc in mice and its underlying mechanisms.

MATERIALS AND METHODS

Ethics Statement

The experiments were approved by the Animal Ethics Committee of Zhejiang University (ZJU202000044) (Zhejiang, China) according to National Regulations.

Reagents and materials

LCc was provided by Prof. Tian from Zhejiang University (Hangzhou, China), TS101021. Carrageenan was obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China, Cat. #C8830). Glacial acetic acid was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Aspirin tablets were purchased from Astra Zeneca Pharmaceuticals Co., Ltd. (Jiangsu, China, H32026201). Saline was purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China, Lot. #L30A9G69142). MDA, PGE2, TNF-α, COX-1, COX-2 ELISA kits were obtained from Lai Er Bio-Technology Co., Ltd (Hefei, China; LE-08961, LE-08619, LE-08349, LE-08823 and LE-08822, respectively). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Plant material and the LCc extract

Whole *L. capillipes* Hemsl. plants were cultivated in the city of Guiyang of Guizhou Province, China, at 300-500 m a.s.l. The Voucher specimen (No. 20010523) was deposited at the College of Biomedical Engineering and Instrument Science of Zhejiang University, Hangzhou, China. The dried powdered plant materials (5 kg) were extracted with 70% EtOH under reflux twice. After removal of the solvent by evaporation, the combined extracts were subjected to chromatography on an AB-8 resin column, and eluted with H_2O, 40%, 70% and 95% ethanol. The 70% ethanol eluate was evaporated to give LCc (about 300 g).

Experimental animals

Female ICR mice (18±2 g) were purchased from Shanghai SLAC Laboratory. The Animal Co. Ltd. mice were kept in the Animal Laboratory of Zhejiang University in plastic cages at room temperature at a 12 h light-dark cycle with *ad libitum* access to pellet food and water. The animals were acclimatized to the laboratory for one week before commencement of the experiment. Female mice were selected for the experiment because the male mice scrotum would relax and touch the hot plate after heating; as the scrotal skin is heat-sensitive, this would affect the experimental results.

The mice were randomly divided into the vehicle group (normal saline), the positive control group (130 mg/kg,

aspirin), and the LCc (40 mg/kg, 60 mg/kg and 90 mg/kg) groups; each group contained 6 animals. Dose selection was made based on acute toxicity test and pilot experiments. All the drugs were administered daily at a volume of 10 mL/kg through the oral route after dissolving in saline.

Organ tissue collection

On day 8, mice from the five groups were killed by cervical dislocation. Organ tissues including kidney, spleen, lung, liver and stomach, were rapidly excised on a Petri dish placed on ice and the attached external vessels and connective tissues were removed carefully. The organs were washed with cold saline. After blotting dry with filter paper, the tissues were weighed. The organ coefficients were calculated as follows:

organ coefficient = (tissue weight/body weight) × 100%.

All samples were stored at -80°C.

Hot-plate assay

The analgesic activity of LCc was evaluated in mice by the hot-plate procedure [37-39] with an electrical hot plate (DB026) maintained at 55.0±0.5°C. Oral gavage was performed daily for 5 days. At 15, 30 and 60 min after the last administration, the mice were placed on an electrical hot plate (at a room temperature of 20±2°C), and the time was recorded when the mice exhibited any nociceptive indicators. Analgesia was defined as prolongation of latency without licking or flicking of the hind limb or jumping. A cutoff time of 60 s was used to avoid tissue injury. Before the experiments, the heat stimulation latency of all animals was tested, the licking of the paws was regarded as the pain response index, and individuals displaying a pain response of 5-30 s were selected.

Body torsion assay

The acetic acid-induced writhing test is often used to evaluate the analgesic effect [40]. Briefly, LCc (40, 60 and 90 mg/kg), aspirin (130 mg/kg) or saline were orally administrated daily for one week. At 50 min after the last administration, 0.8% glacial acetic acid was injected i.p. (10 mL/kg) to develop a twisting response. Due to the wide distribution of sensory nerves in the peritoneum, glacial acetic acid administered by i.p. injection into the abdominal cavity of mice can stimulate the visceral layer and peritoneum, resulting in the writhing reaction of mice, the reaction occurring most frequently within 15 min after injection. The writhing response, which consists of contraction of the abdominal muscle together with a stretching of the hind limbs, was therefore determined for 15 min after a latency period of 5 min.

Paw-swelling assay

The carrageenan-induced hind paw edema test was conducted as previously described [41,42]. Oral gavage was performed daily for 8 days, and at 30 min after the last administration, 0.05 mL of freshly prepared 1% carrageenan was administered by hypodermic injection into the plantar surface of the right hind paw of mice to induce inflammation. The left hind paws without injection were used as controls. After 3 h, the mice were killed by cervical dislocation. The left and right hind paws of the mice were aligned and cut along the ankle joint and weighed.

The increase in paw weight of the right hind paw was calculated as follows:

paw edema = B-A,

where A and B are the weight of the left and right hind paw, respectively.

The percent inhibition of edema was calculated in comparison to the vehicle control animals and was calculated using the following formula:

inhibition of paw edema = (B-A)/B × 100%,

where A and B are the average paw edema in the drug-treated group and the vehicle control group, respectively.

Enzyme-linked immunosorbent assay (ELISA)

The mice received normal saline, LCc (40, 60, and 90 mg/kg) and aspirin (130 mg/kg) for 8 successive days. At 30 min after the last treatment, the mice were administered by hypodermic injection 0.05 mL of 1% carrageenan suspension in the right hind paw. After 3 h, all mice were killed by cervical dislocation and

the right hind paws were dissected and stored at -80°C. The concentrations of MDA, PGE2, TNF-α, COX-1 and COX-2 in paw tissues were estimated by ELISA. Assays were performed according to the manufacturer's instructions.

Statistical analysis

The data are expressed as the mean±standard deviation (SD). The statistical significance of the difference was assessed by analysis of variance (ANOVA) followed by the post hoc test with the least significant difference (LSD) method. Values of P less than 0.05 were considered as significant.

RESULTS

The effect of LCc on organ coefficients

The organ coefficient is one of the most common indicators in toxicological experiments [43]. Increases in the organ coefficient are always accompanied by organ congestion, edema or hypertrophy; in most instances, a decreased organ coefficient indicates organ atrophy and other degenerative changes [43].

The changes in organ coefficients were used to evaluate the effect of LCc on female ICR mice. Analysis of the organ weight revealed that kidney weight (kidney weight = (left kidney + right kidney)/2) decreased significantly at a dose of 90 mg/kg in LCc group (P<0.05). Our experimental data showed that no significant differences were found in other organ coefficients (P>0.05); we noted only one significant decrease (P<0.05) in the kidney coefficient in the LCc 90 mg/kg treatment group while the organ coefficients of the spleen, lung and liver did not change significantly after LCc administration (P>0.05) (Table 1).

Table 1. Effects of *Lysimachia capillipes* capilliposide (LCc) on organ coefficients in mice.

	Vehicle	Aspirin 130 mg/kg	LC 40 mg/Kg	LC 60 mg/Kg	LC 90 mg/Kg
Kidney	0.737±0.054	0.707±0.051	0.722±0.037	0.723±0.041	0.669±0.062*
Spleen	0.466±0.073	0.442±0.091	0.382±0.098	0.416±0.048	0.424±0.081
Lung	0.997±0.197	0.970±0.170	0.941±0.132	1.070±0.095	0.911±0.207
Liver	6.393±0.787	6.453±0.686	6.964±0.503	6.354±0.449	6.931±0.573
Stomach	0.800±0.091	0.731±0.079	0.907±0.182	0.950±0.244	0.760±0.048

Values are expressed as means±SD (n=6). *P<0.05, **P<0.01, vs the vehicle control group

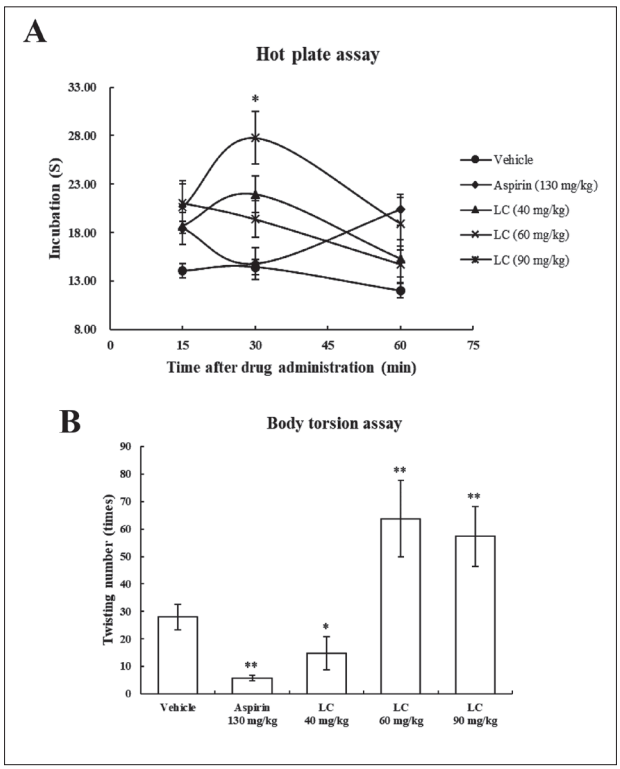

Fig. 1. A – Analgesic effect of *Lysimachia capillipes* Hemsl. capilliposide (LCc) at different dosages on the electrical hot-plate-induced paw-lick reaction of mice. The values are presented as the mean±SD (*n*=6). *P<0.05 compared with vehicle-treated mice at the corresponding time point; **B** – Analgesic effect of LCc at different dosages in the acetic acid-induced writhing model in mice. The values are presented as the mean±SD (*n* = 6). *P<0.05, **P<0.01 compared with vehicle-treated mice.

LCc can prolong the paw-licking time evoked by the electrical hot plate

In the present study, we used the paw-lick test evoked by electrical hot plate in mice to evaluate the analgesic effects of drugs. The effect of LCc on the hot-plate reaction is shown in Fig. 1A. The paw-licking time induced by the hot plate was increased after treatment with aspirin and LCc. The paw-lick reaction time of the vehicle control animals was about 14 s when saline was orally administered. LCc at a dose of 60 mg/kg exhibited an analgesic effect at 30 min, which was a markedly prolonged paw-lick reaction time. Moreover, the effective peak of the 60 mg/kg LCc group was at 30

min after oral administration, with a stronger pharmacological intensity than that of aspirin, which then gradually decreased. Aspirin, as a positive analgesic agent, prolonged the reaction time of the animals after 30 min administration.

LCc suppresses the abdominal writhing episodes induced by acetic acid

Fig. 1B shows the number of abdominal writhing episodes that were evoked by an i.p. injection of acetic acid. The number of writhes induced by glacial acetic acid in the positive control group was decreased significantly when compared with the vehicle control group ($P<0.01$). The oral administration of 40 mg/kg LCc caused significant inhibition ($P<0.05$) of the nociception induced by acetic acid; however, the 60 and 90 mg/kg LCc groups exhibited no analgesic effect in this peripheral analgesic model.

Inhibition of paw edema of mice by LCc treatment

To confirm the antiinflammatory effect of LCc, we utilized the carrageenan-induced paw-swelling model. As shown in Tables 2 and 3, after administration of LCc and aspirin, the increase in weight of the right hind paws of mice in each administration group were alleviated to different degrees when compared to the vehicle control group. These results implied that LCc dose-dependently displayed an acute antiinflammatory effect in mice. The percentage inhibition provided by 40 mg/kg, 60 mg/kg and 90 mg/kg LCc was 3.10%, 12.68% and 21.13% respectively, which occurred at 3 h after carrageenan injection. The 90 mg/kg dose of LCc showed a comparable effect when compared to aspirin.

Table 2. Effects of *Lysimachia capillipes* capilliposide (LCc) on carrageenan-induced paw edema.

Group	Increase of paw weight (g)
Vehicle	0.012±0.003
Aspirin (130 mg/kg)	0.009±0.004
LCc (40 mg/kg)	0.011±0.003
LCc (60 mg/kg)	0.010±0.006
LCc (90 mg/kg)	0.009±0.005

Values are expressed as means±SD (n=3). *P<0.05, **P<0.01 vs the vehicle control group.

Table 3. Percentage inhibition of *Lysimachia capillipes* capilliposide (LCc) on carrageenan-induced paw edema.

Group	Inhibition of paw edema (%)
Vehicle	0
Aspirin (130 mg/kg)	24.23
LCc (40 mg/kg)	3.10
LCc (60 mg/kg)	12.68
LCc (90 mg/kg)	21.13

Inhibition effects of LCc on MDA, PGE2, TNF-α, COX-1 and COX-2 in carrageenan-induced edema of the mice paw

In order to estimate the antiinflammatory mechanisms of LCc, several mediators involving MDA, TNF-α, PGE2, COX-1 and COX-2 in mice paw tissues were examined by ELISA (Fig. 2). The results showed that the levels of MDA, TNF-α, PGE2, COX-1 and COX-2 in the vehicle control group increased markedly.

After treatment with LCc (40 and 60 mg/kg), the relative PGE2 levels were significantly reduced ($P<0.01$) when compared with the vehicle control group. Examination of the MDA levels revealed that LCc markedly inhibited ($P<0.01$) the production of MDA in a dose-dependent manner.

Previous studies have shown that TNF-α and IL-1β are the major products of inflammation that contribute to further progression of inflammation [44]. Additionally, COX is a key enzyme in the biosynthetic pathway that leads to the formation of PGs [11]. We analyzed the levels of TNF-α to investigate whether use of LCc can affect the release of inflammatory factors. As shown in Fig. 2, compared to the vehicle group, LCc at 40 mg/kg, 60 mg/kg and 90 mg/kg significantly lowered the level of TNF-α. Furthermore, compared with the vehicle-treated mice, 40 mg/kg, 60 mg/kg and 90 mg/kg LCc and 130 mg/kg aspirin markedly reduced the levels of COX-1 and COX-2 in mice paw tissues.

DISCUSSION

Inflammation and pain are the most common and main symptoms of many diseases [45], and the current treatment is to use steroidal and nonsteroidal drugs [46,47]; however, most of these treatments have side effects [2]. *Lysimachia capillipes* Hemsl., a Chinese herb

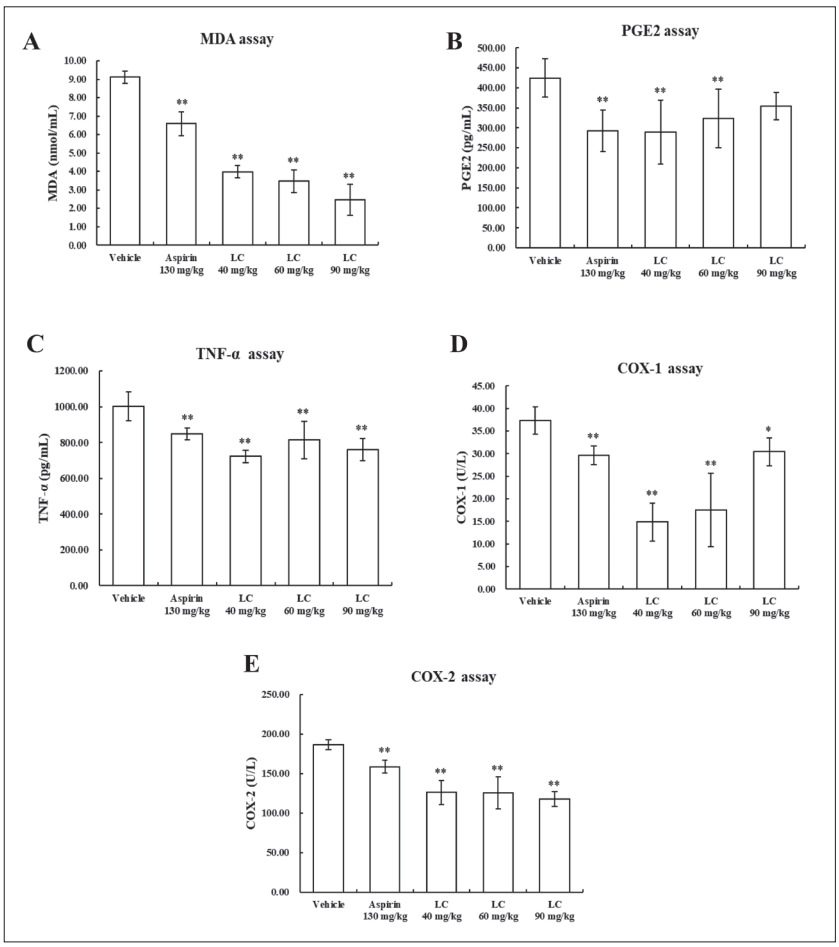

Fig. 2. The effect of *Lysimachia capillipes* Hemsl. capilliposide (LCc) on the levels of malondialdehyde (MDA) (**A**), prostaglandin E2 (PGE2) (**B**), tumor necrosis factor (TNF-α) (**C**), cyclooxygenase-1 (COX-1) (**D**) and cyclooxygenase-2 (COX-2) (**E**) in carrageenan-treated mice paw tissues, quantified by ELISA. The data are expressed as the mean±SD (n=6); *P<0.05, **P<0.01 compared with vehicle-treated mice.

and medicinal plant, is widely used as a remedy for colds and arthritis [33,48]. LCc, a natural compound extracted from the *L. capillipes* Hemsl. plant, exhibits an inhibitory effect on cell proliferation in various cancers [49]; however, its analgesic and antiinflammatory activities have not been sufficiently studied. In this paper, we show the analgesic and antiinflammatory activities of LCc in different animal models.

Changes in organ coefficients are used as an indicator of toxic effects caused by substance testing [50]. Most analgesic drugs cause damage to the liver and kidneys [51]. In addition, the function of liver and kidney is an indicator of the toxicity of drugs [52]. Our results clearly showed that the coefficient of the kidney of mice treated with 90 mg/kg of LCc were decreased. However, the values of the liver coefficient revealed that LCc produced no adverse effects on the liver. In addition, the spleen and lung of the LCc-treated groups showed no changes in organ coefficients as compared with the vehicle control group. Hence, the nephrotoxicity of the LCc was only evident when used at higher concentrations. These data suggest that LCc has a limited influence on mice health.

The hot-plate test was used to evaluate central analgesic activity, and peripheral analgesic activity was assessed by the acetic acid-induced writhing test [53]. LCc and aspirin increased the pain threshold of mice, indicating that LCc had a central analgesic effect. Moreover, the number of writhes induced by acetic acid in the 40 mg/kg LCc-group was significantly inhibited, implying that LCc had a potential peripheral analgesic effect. The carrageenan-induced inflammation model is the standard experimental model for acute inflammation [5,54]. In the carrageenan-induced mice paw edema study, the LCc groups displayed a dose-dependent inhibition of paw edema. The results with these animal models suggested that LCc might act as a central and peripheral analgesic agent, and that it possesses potent inhibitory effects against acute inflammation.

Inflammatory mediators possess strong biological activity and activate other systems that produce a series of cascade amplification reactions promoting further development of inflammation [55,56]. TNF-α is a major mediator in inflammatory responses, inducing innate immune responses by activating T cells and macrophages and stimulating the secretion of other inflammatory cytokines [57]. TNF-α was shown to be one of the proinflammatory mediators of the carrageenan-induced inflammatory reaction, and it

can induce a further release of kinins and leukotrienes with a possible role in the maintenance of a long-lasting nociceptive response [58]. PGs (especially PGE2) are important for inflammation diagnosis and PGE2 is the main metabolite of arachidonic acid, related to many pathophysiological processes, such as inflammation, tissue destruction and tumor development [59,60]. PGE2, the main PG produced during the inflammatory response, participates in the initiation of inflammation and emergence of inflammatory symptoms such as oedema, fever and pain [61-64]. Free-radical generation at the site of inflammation is proposed to be the major cause of the tissue damage induced in many inflammatory disorders [16]. MDA is believed to be one of the most important markers of free-radical generation and subsequent development of oxidative stress [65,66]. It was demonstrated that free radicals are released after carrageenan injection, and that as a result of attack of the plasma membrane, the accumulation of MDA occurs [16]. In the present study, the level of MDA, PGE2 and TNF-α declined in inflammatory tissues after LCc treatment, indicating that LCc exerts an antiinflammatory effect, at least in part, by downregulating the release of MDA, PGE2 and TNF-α.

Cyclooxygenases (COX), which are involved in PG synthesis, are rate-limiting enzymes in PG synthesis [67]. COX has two isoforms, COX-1 and COX-2. COX-1 is constitutively expressed in virtually all tissues that are involved in physiological gastrointestinal, renal and cardiovascular activities [68]. Unlike COX-1, COX-2 is usually expressed at very low levels in most tissues under physiological conditions, but it is upregulated in inflammatory conditions [69]. The contents of COX-1 and COX-2 were increased in the carrageenan-treated group and decreased significantly in the LCc- and aspirin-treated groups, indicating that the antiinflammatory effect of aspirin and LCc was related to the decrease in COX-1 and COX-2 levels. However, some of the indicators were not dose-dependent, which may be related to the mild nephrotoxicity interference caused by LC.

CONCLUSION

LCc produces analgesic and antiinflammatory effects; compared to the reference drug aspirin, the effect was equal to some extent. The results of the present study show that its potential antiinflammatory mechanism can be attributed to the decrease in MDA, PGE2, TNF-α, COX-1 and COX-2 levels. These results demonstrate that LCc could serve as a natural resource for the discovery of novel compounds for pain management and inflammatory conditions; however, its usage should be properly monitored, and its antiinflammatory and analgesic activities require further pharmacological examination.

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Conflicts of interest disclosure: The authors have no conflicts of interest to declare.

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