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Anti-Inflammatory Effects of Different Elution Fractions of Er-Miao-San on Acute Inflammation Induced by Carrageenan in Rat Paw Tissue

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Background: Er-Miao-San (EMS) is used in traditional Chinese medicine. This study aimed to investigate the effect of different elution fractions of EMS on acute inflammation induced by carrageenan in the rat paw and the possible mechanisms of action.

Material/Methods: Different aqueous fractions of EMS added to an AB-8 macroporous resin column and eluted with 0, 30%, 60%, and 90% ethanol. The content of berberine was evaluated by ultra-performance liquid chromatography (UPLC). Following injection of carrageenan and elution fractions of EMS into the rat paw, the volume of edema, levels of prostaglandin E2 (PGE2), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-10 in the rat tissue were quantified by enzyme-linked immunosorbent assay (ELISA). Myeloperoxidase (MPO) activity and nitric oxide (NO) levels were measured by spectrophotometry.

Results: The 60% and 90% ethanol elution fractions of EMS contained berberine, and both inhibited edema after carrageenan injection, with inhibitory rates of 31.04–40.86% and 48.84–52.18%, respectively, and with a significant reduction in MPO activity and NO production. The 60% ethanol elution fraction of EMS significantly decreased IL-1β levels and increased IL-10 levels, and the 30%, 60%, and 90% ethanol EMS elution fractions considerably reduced the levels of TNF-α. The 60% and 90% ethanol EMS elution fractions significantly reduced PGE2 levels in the rat paw.

Conclusions: The 60% and 90% ethanol elution fractions of EMS had an anti-inflammatory effect following injection of carrageenan in the rat paw.

MeSH Keywords: Antirheumatic Agents • Inflammation • Nitric Oxide Synthase

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Background

Inflammation is part of the immune defense system and is an acute tissue response to injury that involves the activation of a cascade of events, including the production of inflammatory mediators [1]. Non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids are used to suppress inflammation are associated with side effects. In cases of chronic inflammation, these drugs are used on a long-term basis, reducing their efficacy and increasing the risk of side effects. Allergic and autoimmune conditions, cancer, and chronic inflammatory diseases are associated with chronic inflammation and require the use of long-term drug treatment, which can cause side effects [2,3]. Traditional Chinese medicine (TCM) uses natural compounds derived from herbs, and these medicines are of interest because of their reduced side effects in some cases.

Herbal medicines are the basis of Chinese medicine, which has been used for thousands of years. Er-Miao-San (EMS) is a traditional medicine that was originally recorded by Dan-Xi-Xin-Fa in Danxi's Experiences in Medicine, from the Yuan dynasty. EMS consists of equal amounts of *Phellodendri cortex* and *Atractylodis rhizoma*. EMS has been used clinically for 500 years to treat bi zheng, which describes conditions that include rheumatoid arthritis and gouty arthritis [4]. A previously reported study showed that EMS exerts its anti-inflammatory effect by inhibiting NF-κB-regulated genes that encode for IL-1β and IL-8, and tumor necrosis factor-α (TNF-α)-induced matrix metalloproteinase-1 (MMP-1) expression, which reduced nuclear p65 protein expression in human dermal fibroblasts [5]. EMS has also been shown to suppress the production of nitric oxide (NO) and inflammatory mediators in lipopolysaccharide (LPS)-stimulated RAW264.7 murine macrophages [6].

The pharmacological activity of *Phellodendri cortex* is mediated by its main component, berberine, which has been reported to suppress IL-33-induced inflammatory responses in mast cells [7]. Berberine has also been shown to reduce amyloid β25–35-induced inflammatory responses in human neuroblastoma cells [8], to inhibit IL-21 and IL-21R-mediated proliferation of fibroblast-like synoviocytes [9], and to reduce the expression of pro-inflammatory factors and protect against neuronal damage [10]. Berberine has a variety of other effects on a range of chronic diseases. However, there have been few studies to investigate which components of EMS are associated with the anti-inflammatory effects and whether these effects are associated with berberine.

Therefore, this study aimed to investigate the effect of different elution fractions of EMS on acute inflammation and edema induced by carrageenan injection in the rat paw and the possible mechanisms of action.

Material and Methods

Drugs and reagents

Carrageenan was purchased from Shanghai ZhongQin Chemical Reagent Co. (Shanghai, China). Tumor necrosis factor-α (TNF-α), IL-1β, IL-10, and prostaglandin E2 (PGE2) enzyme-linked immunosorbent assay (ELISA) kits were obtained from CusaBio Biotech Co., Ltd. (Wuhan, China). Myeloperoxidase (MPO) and nitric oxide (NO) detection kits were obtained from China Sun Specialty Products Co., Ltd. (Jiangsu, China). Aspirin was purchased from Nanjing Baijingyu Pharmaceutical Co., Ltd. (Nanjing, China). Dried *Phellodendri cortex* and *Atractylodis rhizoma* were obtained from the Bozhou medicinal materials market (Anhui, China). All reagents were authenticated by Dr SJ Liu, Department of Pharmacology, Anhui University of Chinese Medicine.

Animals

Eighty male Sprague-Dawley rats weighing between 140–180 g, and between 6–8 weeks of age were purchased from the Experimental Animal Center of the Anhui Medical University (Hefei, China). Rats were maintained under standard conditions at 22±2°C and 55±5% relative humidity with 12-hour light and dark cycle and with free access to food and water. All animal experiments were conducted according to international ethical guidelines. All experiments were approved by the Ethics Review Committee for Animal Experimentation of Anhui University of Chinese Medicine (Hefei, China).

Preparation of aqueous extracts and elution of Er-Miao-San (EMS)

Equal parts of the herbs, *Phellodendri cortex* and *Atractylodis rhizoma* were crushed and mixed. An extract of Er-Miao-San (EMS) was prepared by decocting *Phellodendri cortex* and *Atractylodis rhizoma* with boiling water for one hour, and the extraction was performed three times. The suspension was separated by filtration and concentrated to a 0.5 g/ml solution, which was added to an AB-8 macroporous resin column and eluted with 0, 30%, 60%, and 90% ethanol. Four different fractions, the 0% ethanol elution fraction, 30% ethanol elution fraction, 60% ethanol elution fraction, and 90% ethanol elution fraction, were obtained using gradient elution.

Ultra-performance liquid chromatography (UPLC) analysis of the EMS fractions

The presence of berberine was determined using an ACQUITY H-Class UPLC system (Waters, Milford, MA, USA) with a Discovery-C18 analytical column of 2.1×100 mm, with a 1.7 μm particle size (Supelco Analytical, Sigma-Aldrich,

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St. Louis MO, USA). The mobile A phase was acetonitrile, and mobile B phase consisted of 0.1% formic acid in water, using the following gradient and elution timings: 0–2 min, 8% A; 2–5 min, 8–12% A; 5–15 min, 12% A; 15–25 min, 30–40% A. The mobile phase was run at a flow rate of 0.2 mL/min, the detection wavelength was 284 nm, the column temperature was 30°C, and the injection volume was 2 µL. These chromatography conditions were used to analyze both experimental and control samples.

Preparation of the rat model of paw edema and carrageenan injection

Carrageenan-induced inflammation in the right rat paw was used to determine the anti-inflammatory activity of different elution fractions of EMS. Eighty rats were randomly divided into eight groups: the normal control rats (N=10), the rat model (N=10), the rat model treated with the 0% ethanol elution fraction (N=10); the rat model treated with the 30% ethanol elution fraction of EMS (N=10); the rat model treated with the 60% ethanol elution fraction of EMS (N=10); the rat model treated with the 90% ethanol elution fraction of EMS (N=10); the EMS control (3 g/kg) (N=10); and the positive control treated with aspirin (100 mg/kg) (N=10). The EMS elution fractions, EMS, or aspirin were administered once per day for six days by gavage using a stomach tube. One hour after the last gavage, 50 µL of carrageenan (1%) was administered by intraplantar injection into the right posterior paw of the rat.

Paw tissue volume measurements

The paw volume was measured before carrageenan injection and 1 hour, 2 hours, 3 hours, and 4 hours after the injection of carrageenan using a PV-200 plethysmometer (Chengdu Taimeng Technology Co. Ltd., China). The difference in the paw volume before and after the injection of carrageenan was used as the measurement of edema due to inflammation. The percentage of inhibition of edema for each experimental group was calculated using the following formula:

$$\text{edema inhibition (\%)} = 1-(Vt/Vc)\times 100\%,$$

where Vt represents mean paw volume in rats treated with EMS fractions, EMS, or aspirin, and Vc represents mean paw volume of rats in the control group.

After 4 hours, the rats were euthanized by carotid artery bleeding under anesthesia, and the right hind paws were dissected immediately to analyze the inflammatory factors.

MPO activity

MPO activity in the rat paw tissue was measured based on a modified version of the method used by Bradley et al. [11]. At a specified time following the intraplantar injection of carrageenan, biopsies of inflamed paws were weighed. Each piece of tissue was finely chopped in 1 mL of 50 mM phosphate-buffered saline (PBS) containing 0.5% hexadecyltrimethylammonium bromide (HTAB) buffer and centrifuged for 30 min at 20,000×g (4°C). An aliquot of the supernatant was then added to a solution of 1.6 mM tetramethylbenzidine and 0.1 mM H_2O_2. The absorbance of the reaction mixture was determined at 450 nm using a spectrophotometer. The activity of MPO was determined and reported in units (U) per gram (mass of wet tissue).

Cytokine, prostaglandin E2 (PGE2), and nitric oxide (NO) assay

Rat paw tissue was homogenized in ice-cold PBS (1: 9, v/w) to obtain a 10% homogenate suspension. Supernatants were then removed, and levels of cytokines, IL-1β, IL-10, and TNF-α, and PGE2 were quantified by enzyme-linked immunosorbent assay (ELISA), according to the protocol supplied by the manufacturer (Becton Dickinson, Franklin Lakes, NJ, USA). NO levels were measured with a NO assay kit (Jiancheng Bioengineering Institute, Nanjing, China).

Statistical analysis

All data were expressed as the mean ± standard deviation (SD) and were analyzed with SPSS version 17.0 software for Windows. One-way analysis of variance (ANOVA) with a post hoc Bonferroni–Dunn test was used for multiple comparisons. Results were considered statistically significant at $p<0.05$.

Results

Ultra-performance liquid chromatography (UPLC) analysis and quantitative evaluation of Er-Miao-San (EMS) fractions

The results of ultra-performance liquid chromatography (UPLC) analysis of Er-Miao-San (EMS) are shown in Figure 1. A comparison of UPLC chromatograms of individual herbal medicines with standard reference substances confirmed that berberine was a major component of EMS (Figure 1A). According to the UPLC results, the 90% ethanol elution fraction contained a small amount of berberine and a small polar part of the extract, and the 60% ethanol elution fraction contained more berberine (Figure 1D, 1E), whereas the 0% and 30% ethanol elution fractions did not contain berberine using detection at 284 nm (Figure 1B, 1C).

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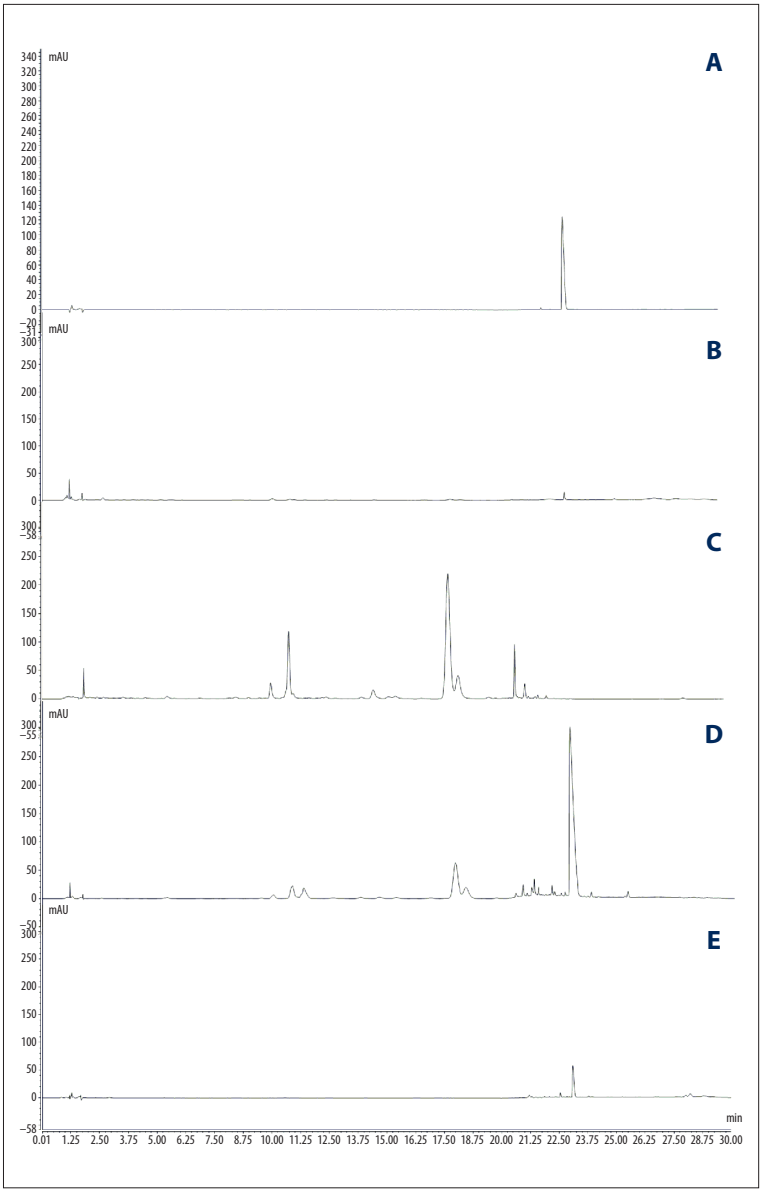

Figure 1. (**A–E**) The berberine content in different elution fractions of Er-Miao-San (EMS) based on ultra-performance liquid chromatography (UPLC).

The effect of different elution fractions of EMS on carrageenan-induced edema in rat paws

As shown in Figure 2A, edema appeared in the paw one hour after the intradermal injection of carrageenan, and the tissue volume increased in a time-dependent manner before reaching a peak at 4 hours. Paw edema volumes followed the same trend after treatment with the 0% and 30% ethanol elution fractions at all time points. However, paw tissue edema was significantly reduced in the 90% ethanol elution fraction, EMS, and aspirin treatment groups in all phases ($P<0.01$, $P<0.05$). Treatment with the 60% ethanol elution fraction of EMS significantly reduced paw edema at 3 hours and 4 hours after carrageenan injection compared to that in the model group ($P<0.01$, $P<0.05$). As shown in Figure 2B, paw edema was reduced by the 60% and 90% ethanol elution fractions, with inhibitory rates of 31.04–40.86% and 48.84–52.18%, respectively, 1–4 hours after carrageenan treatment. Also, EMS and aspirin inhibited the development of edema by 56.74–50.26% and 57.06–56.82%, respectively.

During the six-day drug administration period, prior to carrageenan injection, water and food intake, movement, appearance of the hair, behavior, and control of urination were not significantly different between the control group and the treatment groups. As shown in Figure 2C, the body weight of the animals gradually increased over time and the weight curves in the control and the drug treatment groups showed similar trends with no significant difference between them throughout the study period. These results indicated that treatment with EMS, and the different elution fractions of EMS, was safe.

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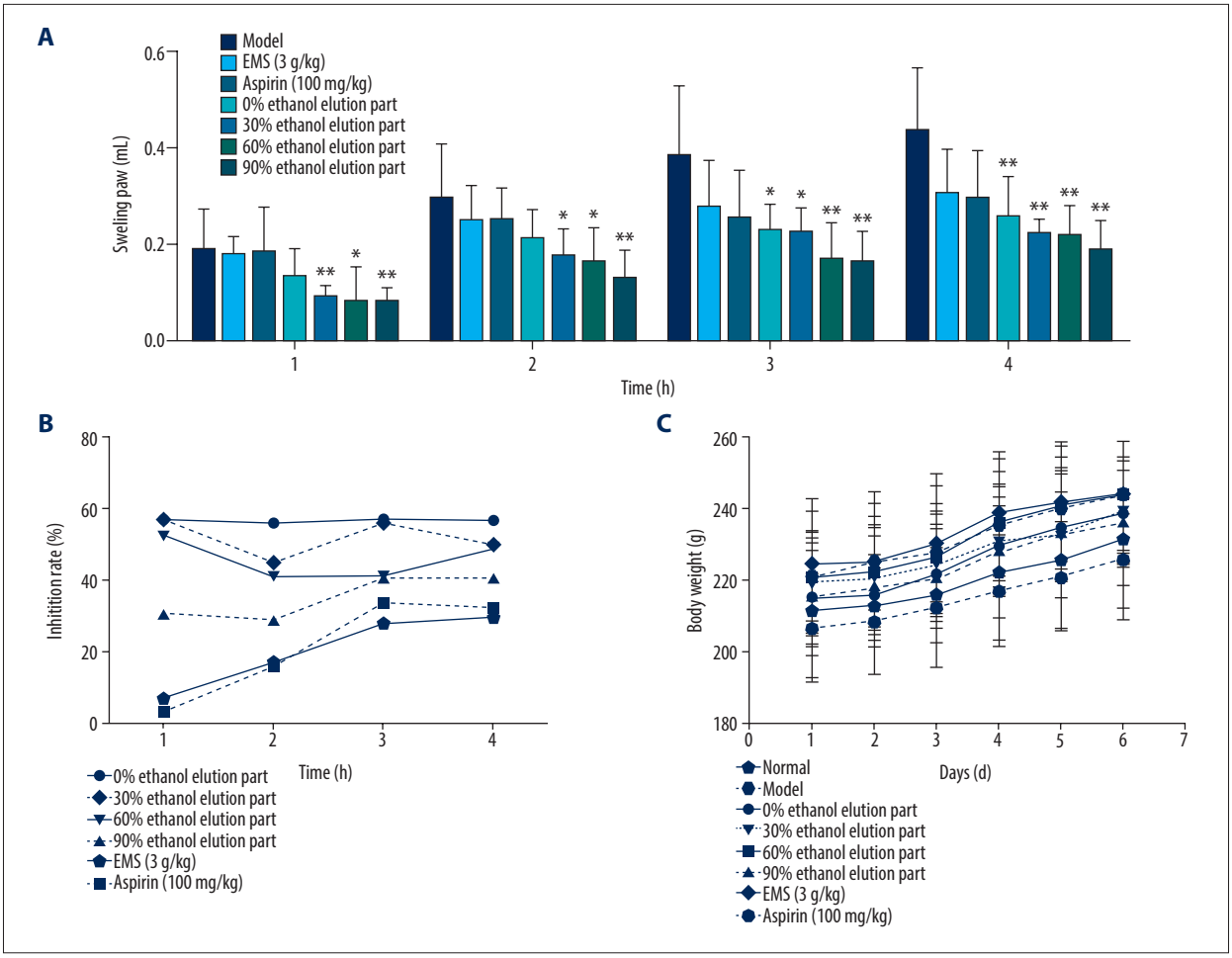

Figure 2. Effect of different elution fractions of Er-Miao-San (EMS) on carrageenan-induced edema in the rat paws. (**A**) The degree of edema in the rat paw tissue was calculated as the ratio of the change in paw volume between the basal volume (0 h) and different time intervals of 1, 2, 3, and 4 h after carrageenan treatment. (**B**) The inhibition of paw edema (%) is represented as the difference in paw volume between the model group and the treatment groups. (**C**) Curves of the change in body weight change after the administration of different elution fractions of EMS. The vehicle control was administered with EMS (3 g/kg). Aspirin was administered at 100 mg/kg. Data are expressed as the mean ±SD (n=10); * p<0.05 and ** p<0.01 versus the model group.

The effect of different elution fractions of EMS on myeloperoxidase (MPO) activity and nitric oxide (NO) production in carrageenan-induced edema of the rat paws

The intraplantar injection of carrageenan enhanced the activity of MPO when compared with the control group (P<0.01). Also, when compared with the model group, a significant reduction in MPO activity was found in the groups treated with 60% and 90% ethanol elution fractions, EMS, and aspirin (P<0.01, P<0.05). Treatment with the 60% ethanol elution fraction reduced MPO activity, similar to that observed with EMS and aspirin (Figure 3A).

As shown in Figure 3B, nitric oxide (NO) production was also significantly increased in the model group when compared with that in the normal group (P<0.01). However, the 60% and 90% ethanol elution fractions significantly reduced NO production. EMS and aspirin also significantly reduced NO production (P<0.05).

The effect of different EMS elution fractions on the levels of prostaglandin E2 (PGE2), tumor necrosis factor-α (TNF-α), IL-1β, and IL-10 in carrageenan-induced edema of the rat paw

As shown in Figure 4A and 4C, in the model group, IL-1β concentrations were significantly increased (P<0.05), and IL-10 concentrations were significantly reduced (P<0.01). Treatment with the 60% ethanol elution fraction significantly reduced IL-1β levels and increased IL-10 levels (P<0.05). Also, EMS and aspirin

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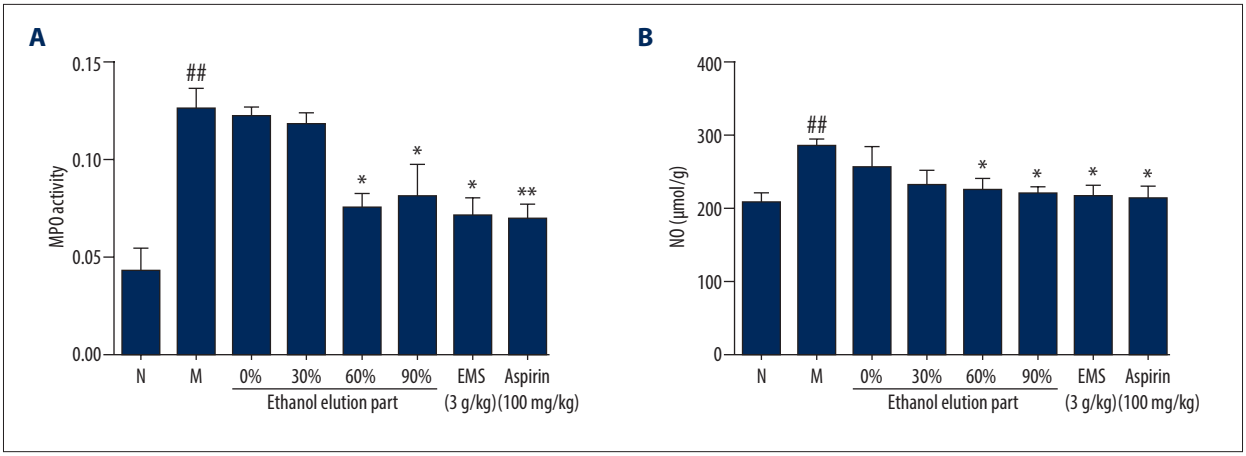

Figure 3. The effect of different elution fractions of Er-Miao-San (EMS) on myeloperoxidase (MPO) activity (**A**) and nitric oxide (NO) (**B**) production in carrageenan-induced rat paw tissue, quantified by spectrophotometry. Data are expressed as the mean ±SD (n=10); ## p<0.01 versus the normal group, * p<0.05 and ** p<0.01 versus the model group.

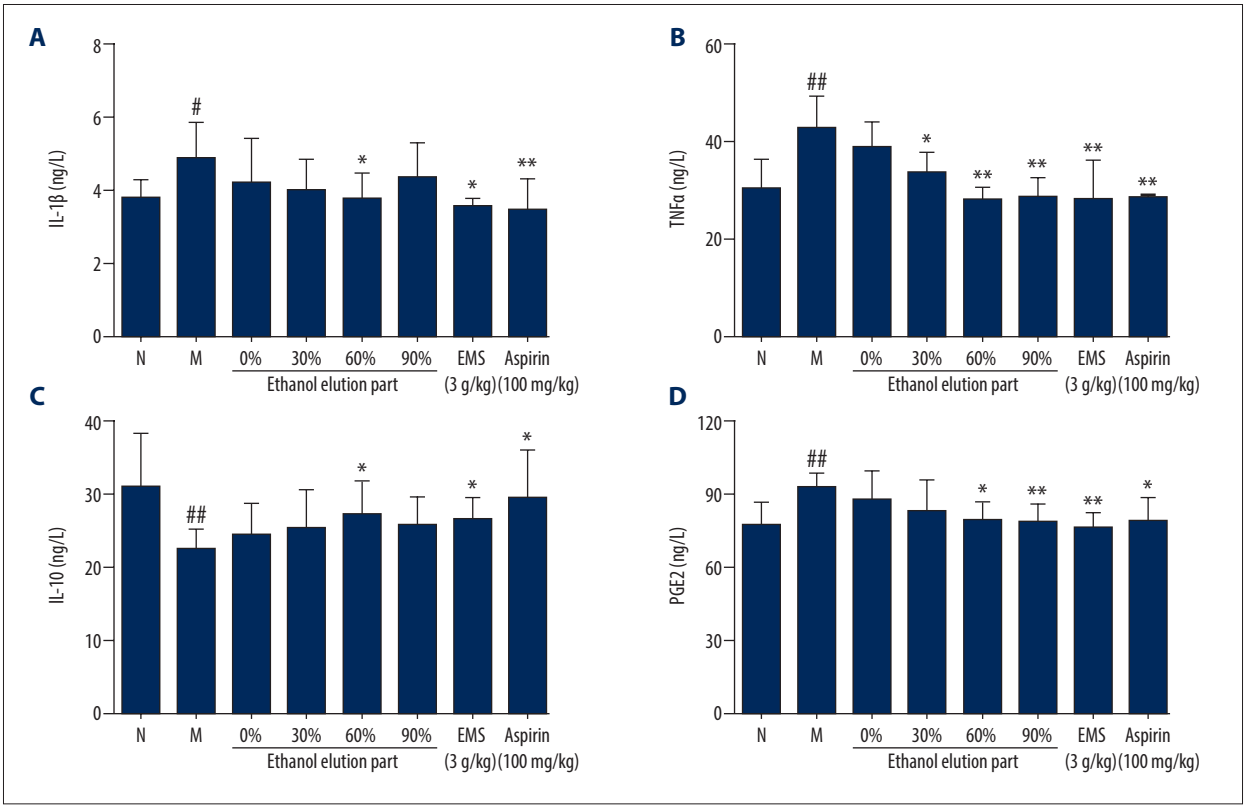

Figure 4. The effect of different elution fractions of Er-Miao-San (EMS) on the levels of IL-1β (**A**), TNF-α (**B**), IL-10 (**C**), and PEG2 (**D**) in carrageenan-treated rat paw tissues, quantified by enzyme-linked immunoassay (ELISA). Data are expressed as the mean ±SD (n=10); # p<0.05 and ## p<0.01 versus the normal group, * p<0.05 and ** p<0.01 versus the model group.

treatment both reduced IL-1β levels and increased IL-10 levels in carrageenan-treated rat paws (P<0.01, P<0.05). TNF-α concentrations were also significantly increased in carrageenan-treated rat paws compared with the normal group (P<0.01). However, all of the ethanol elution fractions other than the 0% fraction, in addition to EMS and aspirin, considerably reduced TNF-α levels in carrageenan-treated rat paws (P<0.01, P<0.05) (Figure 4B). Compared with the normal group, PGE2 levels were significantly increased in the model group (P<0.01), but were significantly reduced by the 60% and 90% ethanol elution fractions, EMS, and aspirin groups (P<0.01, P<0.05) (Figure 4D).

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Discussion

The search for new substances that modulate inflammation remains an area of intense research interest. Currently available anti-inflammatory drugs are only partially effective due to their associated side effects that limit their use. In this study, using a rat model of inflammation of the paw, modern pharmacological analysis applied to traditional Chinese medicine (TCM) showed that Er-Miao-San (EMS) had anti-inflammatory and analgesic properties. These properties have been previously recorded in the state Pharmacopoeia of the People's Republic of China for the successful treatment of rheumatoid and gouty arthritis. However, the components of EMS that have anti-inflammatory effects have not been previously investigated. The present study, in an animal model of acute inflammation, provides support for the anti-inflammatory properties of certain EMS fractions.

EMS is composed of the herbs *Phellodendri cortex* and *Atractylodis rhizoma*, and the active ingredients of EMS include berberine, phellodendrine, β-eudesmol, and hinesol. However, berberine is the main active component of EMS and this component is most likely to contribute to most of its anti-inflammatory properties. Previous studies have reported that berberine has an anti-inflammatory effect on many diseases [12–14]. In this study, we found that the 60% ethanol elution fraction of EMS contained more berberine and had greater anti-inflammatory activity when compared with other fractions. We also found that the 90% ethanol elution fraction contained a small amount of berberine together with a small polar component of the EMS extract and had an even stronger anti-inflammatory effect. Based on this finding, it is possible to speculate that berberine and the small polar part of the extract could work synergistically to enhance the anti-inflammatory effects. Further studies are needed to determine the identity and characteristics of the chemical components present in the fractions with the greatest anti-inflammatory activity, which were the 60% and 90% ethanol fractions identified in this study.

Carrageenan-induced edema of the rat paw is a classic animal model of acute inflammation and is often used to evaluate or screen the anti-inflammatory effects of drugs. In humans, carrageenan can induce a series of reactions similar to acute inflammation, such as local telangiectasia, increased vascular permeability, plasma extravasation, and rapid edema. The findings from the present study showed that certain EMS fractions reduced carrageenan-induced swelling of the rat paw, indicating that specific EMS components may exert anti-inflammatory effects that could be useful for the treatment of acute inflammation. Furthermore, the anti-inflammatory fractions of EMS, the 60% and 90% ethanol fractions, had no effect on animal body weight, which suggested that EMS had no toxicity in this rat model.

Following carrageenan-induced edema, hyperalgesia and redness occurred due to the action of pro-inflammatory agents released into the tissue. Mediators such as histamine, prostaglandins, bradykinin, tachykinins, cytokines, and substance P are involved in this process [15]. In the first few hours after carrageenan injection, neutrophils are the main cells that are recruited and reach the site of inflammation. The infiltration of neutrophil polymorphs into inflamed tissues can be identified by measuring the levels of the enzyme myeloperoxidase (MPO), which is present on neutrophil intracellular granules [16].

In the present study, the specific effective EMS fractions significantly decreased MPO activity in carrageenan-injected tissue. This reduction in myeloperoxidase (MPO) activity by effective EMS fractions indicates that this inhibited neutrophil migration into the inflamed tissue. A few hours after neutrophil migrate into the tissue, macrophages and lymphocytes also accumulate at the site of inflammation and play pivotal roles in the evolution of the inflammatory response, producing cytokines. Among these cytokines, TNF-α and IL-1β exert a diverse range of biological effects that influence and participate in the development and maintenance of acute and chronic inflammatory conditions [17]. IL-10, as an important anti-inflammatory and immunosuppressive cytokine that strengthens the peripheral tolerance of regulatory T cells and plays an anti-inflammatory role in many inflammatory diseases [18–20]. The results of this study showed that TNF-α and IL-1β levels induced by carrageenan were reduced, suggesting an inhibitory effect, whereas IL-10 levels were increased by the effective fractions of EMS, similar to results obtained after aspirin treatment.

Pro-inflammatory mediators, including prostaglandin E2 (PGE2) and nitric oxide (NO), are produced by the induction of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). PGE2 is an important inflammatory marker that can synergize with histamine and bradykinin to cause inflammation, edema, exudate formation, erythema, hyperemia, pain, and fever [21]. PGE2 reportedly causes pain and inflammation when injected into the hind metatarsal feet of rats [22]. However, iNOS is not expressed without inflammation, but on stimulation by cytokines or microorganisms, its expression increases resulting in the production of NO, which can induce inflammatory responses such as vasodilation, increased vascular permeability, the formation of exudate, and activation of prostaglandin synthase [23]. Also, NO associated with inflammation and can promote matrix metalloproteinase (MMP) and cytokine production, as well as mitochondrial dysfunction, which can accelerate the inflammatory process [24]. The results from this study, using a rat model, showed that effective EMS fractions significantly inhibited PGE2 and NO production. The anti-inflammatory activity of effective EMS fractions might be associated with inhibitory effects on the release of inflammatory mediators and pro-inflammatory cytokines.

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Conclusions

Previous studies have shown that Er-Miao-San (EMS) has anti-inflammatory effects, but the active components were unclear. To the best of our knowledge, this is the first study to demonstrate the anti-inflammatory effect of EMS fractions using the carrageenan-induced rat paw edema model. The findings from this preliminary study may provide a scientific basis to develop applications for the use of EMS further. However, future pharmacological studies are required to clarify the mechanisms responsible for the anti-inflammatory effects of EMS fractions.

Conflict of interest

None.

References:

1. Lee SA, Park BR, Moon SM et al: Anti-inflammatory potential of *Trifolium pratense* L. leaf extract in LPS-stimulated RAW264.7 cells and in a rat model of carrageenan-induced inflammation. Arch Physiol Biochem, 2018; 15: 1–8
2. Ronchetti S, Migliorati G, Bruscoli S, Riccardi C: Defining the role of glucocorticoids in inflammation. Clin Sci (Lond), 2018; 132(14): 1529–43
3. Baranowski DC, Buchanan B, Dwyer HC et al: Penetration and efficacy of transdermal NSAIDs in a model of acute joint inflammation. J Pain Res, 2018; 11: 2809–19
4. Guo Y, Jiang Q, Gui D, Wang N: Chinese Herbal Formulas Si-Wu-Tang and Er-Miao-San synergistically ameliorated hyperuricemia and renal impairment in rats induced by adenine and potassium oxonate. Cell Physiol Biochem, 2015; 37(4): 1491–502
5. Bae S, Jung Y, Choi YM, Li S: Effects of er-miao-san extracts on TNF-alpha-induced MMP-1 expression in human dermal fibroblasts. Biol Res, 2015; 48: 8
6. Chen G, Li KK, Fung CH et al: Er-Miao-San, a traditional herbal formula containing *Rhizoma atractylodis* and *Cortex phellodendri* inhibits inflammatory mediators in LPS-stimulated RAW264.7 macrophages through inhibition of NF-κB pathway and MAPKs activation. J Ethnopharmacol, 2014; 154(3): 711–18
7. Li W, Yin N, Tao W et al: Berberine suppresses IL-33-induced inflammatory responses in mast cells by inactivating NF-κB and p38 signaling. Int Immunopharmacol, 2019; 66: 82–90
8. Xu J, Wu W, Zhang H, Yang L: Berberine alleviates amyloid β25–35-induced inflammatory response in human neuroblastoma cells by inhibiting proinflammatory factors. Exp Ther Med, 2018; 16(6): 4865–72
9. Dinesh P, Rasool M: Berberine inhibits IL-21/IL-21R mediated inflammatory proliferation of fibroblast-like synoviocytes through the attenuation of PI3K/Akt signaling pathway and ameliorates IL-21 mediated osteoclastogenesis. Cytokine, 2018; 106: 54–66
10. Wang H, Liu C, Mei X et al: Berberine attenuated pro-inflammatory factors and protect against neuronal damage via triggering oligodendrocyte autophagy in spinal cord injury. Oncotarget, 2017; 8(58): 98312–21
11. Bradley PP, Priebat DA, Christensen RD, Rothstein G: Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. J Invest Dermatol, 1982; 78(3): 206–9
12. Zhou J, Yu Y, Yang X et al: Berberine attenuates arthritis in adjuvant-induced arthritic rats associated with regulating polarization of macrophages through AMPK/NF-κB pathway. Eur J Pharmacol, 2019; 852: 179–88
13. Zhu L, Gu P, Shen H: Protective effects of berberine hydrochloride on DSS-induced ulcerative colitis in rats. Int Immunopharmacol, 2019; 68: 242–51
14. Jing Y, Peng F, Shan Y, Jiang J: Berberine reduces the occurrence of neonatal necrotizing enterocolitis by reducing the inflammatory response. Exp Ther Med, 2018; 16(6): 5280–85
15. Mert T, Sahin M, Sahin E, Yaman S: Anti-inflammatory properties of Liposome-encapsulated clodronate or Anti-Ly6G can be modulated by peripheral or central inflammatory markers in carrageenan-induced inflammation model. Inflammopharmacology, 2019; 27(3): 603–12
16. Sadeghi H, Hajhashemi V, Minaiyan M et al: Further studies on anti-inflammatory activity of maprotiline in carrageenan-induced paw edema in rat. Int Immunopharmacol, 2013; 15(3): 505–10
17. Li CL, Tan LH, Wang YF et al: Comparison of anti-inflammatory effects of berberine, and its natural oxidative and reduced derivatives from *Rhizoma coptidis in vitro* and *in vivo*. Phytomedicine, 2019; 52: 272–83
18. Soukou S, Brockmann L, Bedke T et al: Role of IL-10 receptor signaling in the function of CD4+ T-regulatory type 1 cells: T-cell therapy in patients with inflammatory bowel disease. Crit Rev Immunol, 2018; 38(5): 415–31
19. Mielle J, Audo R, Hahne M et al: IL-10 producing B cells ability to induce regulatory T cells is maintained in rheumatoid arthritis. Front Immunol, 2018; 9: 961
20. Imbrechts M, Avau A, Vandenhaute J et al: Insufficient IL-10 production as a mechanism underlying the pathogenesis of systemic juvenile idiopathic arthritis. J Immunol, 2018; 201(9): 2654–63
21. Guay J, Bateman K, Gordon R et al: Carrageenan-induced paw edema in rat elicits a predominant prostaglandin E2 (PGE2) response in the central nervous system associated with the induction of microsomal PGE2 synthase-1. J Biol Chem, 2004; 279(23): 24866–72
22. Eldahshan OA, Abdel-Daim MM: Phytochemical study, cytotoxic, analgesic, antipyretic and anti-inflammatory activities of *Strychnos nux-vomica*. Cytotechnology, 2015; 67(5): 831–44
23. Tennyson AG, Lippard SJ: Generation, translocation, and action of nitric oxide in living systems. Chem Biol, 2011; 18(10): 1211–20
24. Shan L, Tong L, Hang L, Fan H: Fangchinoline supplementation attenuates inflammatory markers in experimental rheumatoid arthritis-induced rats. Biomed Pharmacother, 2018; 111: 142–50

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