Original article

Anti-inflammatory effect and the effect on acute pharyngitis rats model of compound Lobelia oral liquid

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A B S T R A C T

Objective: Observe anti-inflammatory effect and the effect on acute pharyngitis rats model induced by ammonia water of compound Lobelia oral liquid, providing experimental basis for its clinical use.

Methods: Use egg white establish foot swelling rats model and use carboxymethyl cellulose establish white blood cell migration rats model. Then observe the anti-inflammatory effect of compound Lobelia oral liquid. Use 15% ammonia spray at pharyngeal establish acute pharyngitis rats model, Visual observation and conduct grading of pharyngeal tissue stimulation in rats, measure the levels of TNF-α and IL-6 in serum. Pharyngeal tissue was taken to observe the morphological changes.

Result: All dose groups of compound Lobelia oral liquid can reduce the rate of foot swelling of rats at all time points (P < 0.01 or P < 0.05), and significantly reduce the number of white blood cells of rats (P < 0.01); And improve the local hyperemia degree, reduce secretion, reduce local swelling of pharyngeal tissue, reduce the serum TNF-α and IL-6 levels of acute pharyngitis rats with different degrees (P < 0.01 or P < 0.05).

Conclusion: Compound Lobelia oral liquid has a good anti-inflammatory effect on foot swelling and white blood cell migration rats model, as well as significant improvement effect on acute pharyngitis rats model.

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1. Introduction

Pharyngitis is a common seasonal disease of the upper respiratory tract. The incidence of pharyngitis is increasing year by year due to environmental factors such as climate change and haze, work pressure and smoking (Faustini et al., 2013; RrincipiI et al., 2013). Many scholars believe that acute pharyngitis is mainly due to viruses, bacteria and other factors that directly stimulate the pharynx, resulting in tissue inflammation (Di et al., 2016). Compound Lobelia oral liquid consists of Lobelia, Sculellaria barbata, Oldenlandia diffusa and others which has the functions of clearing away heat and toxic materials, relieve swelling and pain. Clinical use for external wind induced sore throat, acute pharyngitis, upper respiratory tract infection, and has remarkable curative effect. Studies have found that Lobelia has significant pharmacological effects on anti-tumor, regulating endothelial cells, relieving pain and reducing inflammation, and Sculellaria barbata has significant pharmacological effects on anti-oxidation, anti-tumor, immune regulation (Li et al., 2015; Kou et al., 2011). However, the injection has some adverse reactions in clinical application. The oral dosage form of compound Lobelia oral liquid is the improved dosage form of its injection. Oral dosage form has the advantages that the original injection does not have, such as it is safe to use, easy to carry and store (Tan et al., 2014). In order to verify the anti-inflammatory effect and therapeutic effect on pharyngitis, the anti-inflammatory effect were observed by foot swelling and white blood cell migration rats model, so as to provide the experimental basis for the clinical application of compound Lobelia oral liquid.

2. Experimental materials

2.1. Experimental apparatus

Laryngeal spray apparatus, Taizhou huichun medical equipment co. LTD; Type 680 enzyme marker, USA BIO-RAD co. LTD; PV-200
type rat foot measuring instrument, hengdu taisheng technology co. LTD; Kdc-160 HR high-speed refrigerated centrifuge, Zhong jia branch of keda innovation co. LTD; Fluorescence microscope and imaging system BX61, Olympus of Japan.

2.2. Experiment reagents

Compound Lobelia oral liquid, Henan tongyang pharmaceutical co., LTD. Batch number: 110701; Yinhuang oral liquid, Shaanxi bailu pharmaceutical co., LTD. Batch number: 160805. Sodium carboxymethyl cellulose, Tianjin hengxing chemical reagent manufacturing co., LTD. Batch no.: 20160728. Ammonia water, Luoyang haohua chemical reagent co., LTD. Batch no.: 151019.

2.3. Experimental animals

SD rats, weighing 180–220 g, half male and half female, Shandong experimental animal center. Laboratory animal certificate: no. 37009200003339; Used to establish acute pharyngitis rats model.

SD rats, weighing 180–200 g, male, Hebei experimental animal center, laboratory animal certificate: 1109003. Laboratory qualification certificate: SYXK (Yu) 2015–0005; Used to establish foot swelling and white blood cell migration rats model.

3. Experimental method

3.1. Foot swelling rats model induced by egg white (Shu et al., 2018; Motevalian et al., 2017)

A total of 40 rats weighing 180–200 g were randomly divided into saline group, Yinhuang group (6 ml/kg) and large, middle and small compound Lobelia oral liquid groups (30 g/kg, 15 g/kg, 7.5 g/kg) with 8 rats in each group. In addition to the blank group and model group, the corresponding drugs were given for intervention. The saline group were given the same volume of saline, the remaining groups were given corresponding drugs. Before the administration on the 3rd day, the normal left hind foot volume of the rat was measured with the foot pedal tester, and the linear position of the rat ankle was adjusted to make the normal foot volume of the rat around 1 ml. After 30 min of administration, each rat was subcutaneously injected with 0.1 ml newly prepared 10% fresh egg white solution. The volume of the left hind foot of the rat was measured again with a foot plantar meter at 60 min, 120 min, 240 min and 360 min after given egg white, and the swelling rate of the foot was calculated.

3.2. White blood cell migration rats model induced by carboxymethyl cellulose (Ma et al., 2013; Zeng et al., 2018)

A total of 50 rats weighing 180–200 g were randomly divided into saline group, Yinhuang group (10 ml/kg) and large, middle and small compound Lobelia oral liquid groups (30 g/kg, 15 g/kg, 7.5 g/kg) with 10 rats in each group. Administration is the same as 2.1. After the second day of administration, the backs of rats in each group were depilated by about 2 cm * 2 cm, and 5 ml of air was injected subcutaneously, forming an air bag. At 8 o’clock in the evening, start fasting for 12 h. At 1 h after administration on day 3, 5 ml 1.5% carboxymethyl cellulose was injected into the air bag under the skin of rats. After 3 h and 7.5 h of injection of carboxymethyl cellulose, the intracellular fluid was extracted, diluted 20 times with 0.9% sodium chloride injection, placed on a blood cell counting plate, and observed under light microscope to calculate the number of white blood cells.

3.3. Acute pharyngitis induced by ammonia in rats (Wu et al., 2018)

12 rats were selected from a total of 72 rats as blank group, remaining rats were used to establish acute pharyngitis rats model. Acute pharyngitis model was established by spraying 15% ammonia water on the pharynx of rats in the morning from day 1 to day 3 (Spray 3 thrush with sprayer every time. Causes the pharynx mucosa to be hyperemia swollen due to acute stimulation, forms the acute inflammation). The rats were randomly divided into model group, Yinhuang group (10 ml/kg) and large, middle and small dose compound Lobelia oral liquid groups (30 g/kg, 15 g/kg, 7.5 g/kg) on the 3rd day. Give the corresponding drugs once a day for 5 days. After 1 h of the last administration, the pharynx tissues of rats were integrated (according to the score standard), blood was taken from the eyeball, serum was separated to measure TNF-α and IL-6 levels. The pathological changes of pharyngeal tissue were observed by staining with HE.

Score standard: According to the tissue color, gloss, number of secretions, hyperemia and swelling of pharynx, it can be divided into 4 grades. “−”: Pharyngeal tissue was light red, with moist and shiny surface, no secretion, no hyperemia, swelling and other pathological phenomena; “+”: Some of the rats had poor glossiness of pharyngeal mucosa, a small amount of secretions and mild acute hyperemia; “++”: Pharyngeal tissue color is dark red, glossiness is poor, secretion appears, and accompanied by acute hyperemia, mild swelling and other phenomena; “+++”: The pharyngeal tissues of rats were dark red and dull, with increased mucous secretions and obvious acute hyperemia and swelling.

4. Experimental results

4.1. Effect of compound Lobelia oral liquid on foot swelling rats model induced by egg white

As we can see from Table 2: Compared with the model group, after 60 min, 120 min, 240 min and 360 min of injected egg white, the large dose of compound Lobelia oral liquid could significantly reduce the foot swelling rate of rats (P < 0.01); Yinhuang group and compound Lobelia oral liquid middle and small dose group can obviously reduce the foot swelling rate of rats (P < 0.01). The results showed that compound Lobelia oral liquid had a strong anti-inflammatory effect.

4.2. Effect of compound Lobelia oral liquid on white blood cell migration rats model induced by carboxymethyl cellulose

As we can see from Table 2: Compared with the model group, large, middle and small doses of compound Lobelia oral liquid and Yinhuang group can significantly reduce the number of white blood cells of rats at 3 h and 7.5 h (P < 0.01), which indicating that compound Lobelia oral liquid had a strong anti-inflammatory effect.

4.3. Effect of compound Lobelia oral liquid on acute pharyngitis rats model induced by ammonia water

4.3.1. General status observation of acute pharyngitis rats model induced by ammonia water

After modeling, rats gradually began to scratch their mouths, and they drank water frequently but in small amounts, oral secretion gradually increased, mental fatigue, food intake and spontaneous activity decreased; There was redness and swelling in pharynx, mucous secretions were accumulated, and superficial ulcer was formed in some rats. The blank group did not show the above situation.
Pharyngitis is clinically common multiple respiratory infection disease, incomplete treatment will lead to repeated attacks. According to the theory of traditional Chinese medicine, acute pharyngitis is usually caused by the invasion of exogenous...
pathogens, or the heat of the lungs and stomach, attack of the throat. Therefore, the main clinical manifestations are throat pain, redness and fever (Zhang et al., 2017). And it is advisable to nourish the lung and kidney, clear the heat and detoxify. Compound Lobelia oral liquid is composed of Lobelia, Sculellaria barbata, Oldenlandia diffusa, etc. Lobelia has the functions of clearing heat and detoxify, dispersing blood stasis and relieving pain (Tao and Balunas, 2016); Sculellaria barbata and Oldenlandia diffusa all can clear heat and detoxify, and have more applications in the treatment of tumor, inflammation, and often in the form of drug pairs (Zheng et al., 2018; Fan et al., 2010; Peng et al., 2018). Combined with the indications and the composition of prescription of compound Lobelia injection, it is suggested that the oral liquid of compound Lobelia oral may have curative effect on acute pharyngitis.

It is reported that acute pharyngitis may appear within one week of chemical stimulation. In this study, the acute pharyngitis model of rats was prepared by spraying ammonia water on the pharynx of rats (Miao et al., 2018). The stimulation of ammonia water can make the blood vessels of pharynx dilate and hyperemia, causing a large number of inflammatory cells infiltrate under the mucosa. "++" means that the mucosa of pharynx and submucosa are normal; "+" indicates that the scaly epithelium of pharynx has little hyperplasia and a small amount of inflammatory cells infiltrate under the mucosa. "+++" means the pharynx mucosa scaly epithelium hyperplasia, a large number of inflammatory cells infiltrated under the mucosa.

In this study, the effects of compound Lobelia oral liquid on acute pharyngitis rats model were investigated by observing the

Table 5
Histopathological grading table of pharyngeal tissue of rats in each group.

| Group          | Dose    | Pharyngeal histopathologic classification |
|---------------|---------|-----------------------------------------|
|               |         | _  | *      | ++  | +++ |
| Blank group   | –       | 12 | 0      | 0   | 0   |
| Model group   | –       | 0  | 0      | 0   | 12  |
| Yinhuang group| 10 ml/kg| 2  | 8      | 3   | 0   |
| Compound lobelia| 30 g/kg| 2  | 9      | 1   | 0   |
|               | 15 g/kg | 1  | 8      | 3   | 0   |
|               | 7.5 g/kg| 0  | 8      | 4   | 0   |

"_" means that the mucosa of pharynx and submucosa are normal; "+" indicates that the scaly epithelium of pharynx has little hyperplasia and a small amount of inflammatory cells infiltrate under the mucosa. "+++" means scaly epithelium hyperplasia of pharynx mucosa, and inflammatory cell infiltration is seen in the lower layer. "+++

"+" means the pharynx mucosa scaly epithelium hyperplasia, a large number of inflammatory cells infiltrated under the mucosa.

Fig. 1. Pathological changes of pharyngeal tissues in each group (HEX100).
anti-inflammatory effect, as well as the apparent symptoms, TNF-α and IL-6 levels and pharyngeal histopathological changes of acute pharyngitis rats. Results showed that compound Lobelia oral liquid could improve the infiltration of inflammatory cells in the pharynx and repair the pharynx mucosa of acute pharyngitis rats, so as to improve the symptoms of pharyngitis. It also could reduce the degree of foot swelling and the number of white blood cells in rat model of white blood cells migration. The experimental results show that compound Lobelia oral liquid has a good effect on improving acute pharyngitis model in rats, as well as good anti-inflammatory effect which provides an experimental basis for the clinical medicine of compound Lobelia oral liquid.

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References

Di, M.F., Barucco, M., Guerriero, F., 2016. Diagnosis and treatment of acute pharyngitis/tonsillitis: a preliminary observational study in General Medicine. Eur. Rev. Med. Pharmacol. Sci. 20 (23), 4950–4954.
Duan, F., Zhao, M.Y., Wang, X.X., 2015. Effect of ori gel on anti-inflammatory and IL-8 in esophagus of reflux esophagitis model rats. Tradition. Chin. Drug Res. Clin. Pharmacol. 26 (1), 64–67.
Fan, Y.S., Wen, C.P., Xie, Z.J., 2010. Effects of three Chinese herbal antidotes (Herba artemisiae annuae, Herba hedysotis diffusa and Rhizoma cimicifugae) and their different combinations on regulated on activation normal T cell expressed and secretead expression in MRL/lpr mice. Chinese J. Integr. Tradit. Western Med. 30 (12), 1306–1309.
Faustini, A., Stafoggia, M., Colais, P., Berti, G., Bisanti, L., Cadum, E., Cernigliaro, A., Mallone, S., Scarnato, C., Forastiere, F., 2013. Air pollution and multiple acute respiratory outcomes. Eur. Respir. J. 34 (5), 304–313.
Kou, P.C., Hwang, T.L., Lin, Y.T., Kuo, Y.C., Leu, Y.L., 2011. Chemical constituents from lobelia chinensis and their anti-virus and anti-inflammatory bioactivities. Arch. Pharmacal Res. 34 (5), 715–722.
Lee, S.H., Shin, J.H., Choi, S., 2018. Label-free monitoring of inflammatory tissue conditions using a carrageenan-induced acute inflammation rat model. Microsc. Res. Tech. 81 (6), 544–550.
Li, K.C., Ho, Y.L., Huang, G.J., Chang, Y.S., 2015. Anti-oxidative and anti-inflammatory effects of lobelia chinensis in vitro and in vivo. Ann. J. Chin. Med. 43 (2), 269–287.
Ma, H., Huang, H., Qang, L.L., Wang, X., Liu, Y., Yao, H., Yang, Q.L., Shi, Y.Z., Zhang, L., 2013. Lishizhen Medicine and Materia Medica Research, 24(10), 2378–2380.
Ma, J.L., Li, C.T., Li, X., Liu, Q., Liang, L., Piao, J.H., 2017. Effect of biaoyan oral solution on matrix metallo proteins-9 IL-1α and TNF-α expression in rats with acute pharyngitis. Pharmaceut. J. Chin. People’s Liberation Army 33 (2), 138–141.
Matsumoto, J., Dohgu, S., Takata, F., Machida, T., 2018. TNF-α-sensitive brain pericytes activate microglia by releasing IL-6 through cooperation between I kappa beta-IF kappa B and JAK-STAT3 pathways. Brain Res. 1692, 34–44.
Miao, M.S., Chang, B.J., Bai, M., Bai, L., 2018. Specification for preparation of animal models of acute pharyngitis [Draft]. Pharmacol. Clin. Chin. Materia Medica 34 (1), 175–178.
Motevalian, M., Shiri, M., Shiri, S., Shiri, Z., Shiri, H., 2017. Anti-inflammatory activity of Elaeagnus angustifolia fruit extract on rat paw edema. J. Basic Clin. Physiol. Pharmacol. 28 (4), 377–381.
Peng, M.F., Bai, M., Miao, M.S., 2018. Application and analysis of Chinese patent drougs containing hedysotis diffusa. J. Hunan Univ. Chin. Med. 38 (7), 829–833.
Principi, N., Bianchini, S., Baggi, E., Esposito, S., 2013. No evidence for the effectiveness of systemic corticosteroids in acute pharyngitis, community acquired pneumonia and acute otitis media. Eur. J. Clin. Microbiol. Infect. Dis. 32 (12), 151–160.
Shu, Y.C., Zhu, X.X., Chen, Y.J., Qin, K.M., Cai, B.C., 2018. Study on spectrum-effect relationship of anti-inflammatory active of Yinquaosan Decoction at different decoct time. China J. Tradition. Chin. Med. Pharm. 33 (9), 4161–4166.
Song, T., Li, L.J., Liu, J.L., Geng, S.J., 2018. Study on the relationship between changes of immune cells and TNF-α in peripheral blood of patients with multidrug-resistant and extensively drug-resistant tuberculosis. Eur. Rev. Med. Pharmacol. 22 (4), 1061–1065.
Tan, L.J., Wang, M., Zhu, Y., 2014. Research progress of adverse reactions of traditional Chinese medicine injections. China J. Chinese Materia Medica 39 (20), 3889–3898.
Tan, G.Y., Balunas, M.J., 2016. Current therapeutic role and medicinal potential of Scutellaria barbata in Traditional ChineseMedicine and Western research. J. Ethnopharmacol. 182, 170–180.
Wu, M.M., Li, C.Y., Li, F.Y., Xue, S.Y., Shi, S.L., Xue, P.F., 2018. Treatment of herbal medicine flos hostae on rats with acute pharyngitis and its influence on cell factors. J. Inner Mongolia Univ. (Natural Science Edition) 49 (5), 507–514.
Zeng, J.W., Li, R.M., He, Q.Y., Liu, S.Z., Chen, X., 2018. The treatment effect of chinese eupatorium root mixture in an acute pharyngitis rat model. Pharm. Today 28 (2), 84–87.
Zhang, Y.Y., Wang, A., Wang, L., Yang, R., Ni, X., Shan, L., 2018. Nitric oxide inhibits the release of TNF-alpha and IL-6 by down-regulating the expression of connexin 40 (Cx40) in rat T lymphocytes 34 (5), pp. 385–389.
Zhang, X., Xie, Y.M., Li, G.X., Gao, Y., Zhao, Y.C., Tang, J.J., Yao, X.Y., Li, M., 2017. Advantages and problems of traditional Chinese medicine in treatment of acute pharyngitis. China J. Chinese Materia Medica 42 (19), 3819–3825.
Zheng, X., Kang, W., Liu, H.H., Guo, S.Y., 2018. Inhibition effects of total flavonoids from Scutellaria barbata D. Don on human breast carcinoma bone metastasis via downregulating TcTHP pathway. Int. J. Mol. Med. 41 (6), 3137–3146.