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Effects of compound lobelia oral liquid on acute pharyngitis rabbits model



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ABSTRACT

Aims: Explore the effect of compound lobelia oral liquid on acute pharyngitis rabbits model caused by ammonium hydroxide, evaluate its curative effect.**Methods:** Acute pharyngitis rabbit model was prepared by ammonia spray at pharyngeal. After 1 h of the last administration, score the pharyngeal tissue symptoms of rabbits under the condition of naked eyes (score according to the score criteria). Then, take blood from the marginal auricular vein for routine blood tests and serum TNF- α level measure. Take pharyngeal tissue for HE staining, and observe its pathological changes under the microscope.**Results:** Compared with the blank group, the pharyngeal tissue symptom score and serum TNF- α level in the model group were significantly increased ($P < 0.01$). The results of blood routine showed that the number of white blood cells and lymphocytes in the model group increased significantly ($P < 0.01$), and neutrophilic granulocyte percentage decreased significantly ($P < 0.01$). The infiltration of inflammatory cells in the pharynx tissue was obvious and pathological changes were observed. Compared with the model group, large, middle and small doses of compound lobelia oral liquid group could reduce the scores of pharyngeal tissue symptoms in rabbits with acute pharyngitis induced by ammonia. The large and middle dose compound lobelia oral liquid group could significantly reduce serum TNF- α level and neutrophilic granulocyte percentage, significantly increase the number of white blood cells and lymphocytes ($P < 0.01$); The small dose compound lobelia oral liquid group could significantly reduce the number of white blood cells and lymphocytes ($P < 0.01$), there was an increasing tendency to neutrophils and a decreasing tendency to serum TNF- α , but no statistical significance. The large, middle and small doses of compound lobelia oral liquid group all could improve the pathological changes of pharyngeal tissue inflammatory infiltration.**Conclusion:** Compound lobelia oral liquid has a good effect on rabbit acute pharyngitis model caused by ammonia.© 2019 Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Acute pharyngitis is an acute non-specific inflammation of the pharyngeal mucosa and submucosal tissue, which were mainly caused by viruses or bacteria, often involving the lymphatic tissue of the pharynx, characterized by redness, swelling and pain of the pharynx. Acute pharyngitis is a common clinical disease and

frequently-occurring disease, the incidence of about 5% (Zhang et al., 2018; Harris et al., 2016). Western medicine mainly treats acute pharyngitis with antibiotics, antiviral drugs and glucocorticoid hormones, which are prone to drug resistance and double infection (Zhang et al., 2017). The dialectical therapy of traditional Chinese medicine (TCM) has its own unique advantages in the treatment of acute pharyngitis, which to some extent avoids the abuse of antibiotics. Clinical application shows that compound lobelia injection is effective in the treatment of upper respiratory tract infection, pneumonia, tracheitis and other diseases, but there are certain adverse reactions (Shi et al., 2011). The oral dosage form of compound lobelia oral liquid is the improved dosage form of its injection. The oral dosage form has the advantages that the original injection does not have, for example, it is easy to take, relatively safe, easy to store and has low side effects (Shen, 2018). In

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order and evaluate its curative effect, This experiment used ammonia spray to replicate the rabbit acute pharyngitis model to observe the effect of compound lobelia oral liquid on acute pharyngitis, so as to provide basis for the clinical treatment of compound lobelia oral liquid on acute pharyngitis.

2. Experimental materials

2.1. Experimental apparatus

Laryngeal spray apparatus, Taizhou huichun medical equipment co. LTD; FA(N)/JA(N) series electronic balance, Shanghai minqiao precision instrument co. LTD; HWS12 electric thermostatic water bath, Shanghai yiheng scientific instrument co. LTD; Kdc-160 h high-speed refrigerated centrifuge, Zhongjia branch of keda innovation co. LTD; Adjustable pipette, Shanghai leibo analytical instruments co. LTD; Type 680 enzyme marker, bio-rad company, USA. Adjustable pipette, Shanghai rebo analytical instrument co. LTD.

2.2. Experiment reagents

Compound lobelia oral liquid, Henan tongyuan pharmaceutical co, LTD, batch number: 110701; Yinhuang oral liquid, Shaanxi bailu pharmaceutical co, LTD, batch number: 160805. TNF- α ELISA kit, Suzhou Calvin biotechnology co. LTD; Ammonia water, Luoyang haohua chemical reagent co, LTD, batch no: 151019.

2.3. Experimental animals

New Zealand rabbit, weighing 1.8–2.2 kg, half male and half female, Shandong experimental animal center. Laboratory animal certificate: No.3700970000246. Laboratory qualification certificate: SYXK (Yu) 2015-0005.

3. Experimental method (Miao et al., 2018; Zhou et al., 2017)

Ten rabbits were randomly selected as blank group. The other rabbits were prepared with acute pharyngitis model with ammonia water. Spraying rabbit pharynx with 6% ammonia water 0.05 ml/kg; six days in a row, once a day. The model rabbits were randomly divided into 5 groups: model group, Yinhuang oral liquid group (6 ml/kg), large, middle and small doses of compound lobelia oral liquid group (18 g/kg, 9 g/kg, 4.5 g/kg). The blank group and the model group were given normal saline of the same volume according weigh. Other rats were given corresponding drugs according to their groups once a day for 5 days. After 1 h of the last administration, score the pharyngeal tissue symptoms of rabbits under the condition of naked eyes (score according to the score criteria). Then, take blood from the marginal auricular vein for routine blood tests and serum TNF- α level measure. Take pharyngeal tissue for HE staining, and observe its pathological changes under the microscope.

Score standard: According to the pharynx tissue color, luster obvious or not, secretion, degree of congestion and swelling, there are 4 levels. “–” Pharynx tissue reddish, the surface of pharynx is moist and shiny, no secretions, no congestion, no swelling and other phenomena; “+” Pharyngeal tissue mucosa glossiness is dark, a small amount of exudate and mild hyperemia can be seen under the naked eye; “++” Pharyngeal tissue dark red, glossiness is poor, there are slight of secretions, accompanied by congestion, mild swelling and other phenomena; “+++” Pharyngeal tissue dark red, mucous membrane is lackluster, secretions increased with severe congestion and swelling.

Table 1

Comparison of visual observation of pharyngeal tissue of rabbits in each group.

Group	Dose	n	–	+	++	+++
Blank group	–	10	10	0	0	0
Model group	–	10	0	0	2	8
Yinhuang group	6 ml/kg	10	0	2	8	0
Compound lobelia	18 g/kg	10	0	6	4	0
	9 g/kg	10	0	4	6	0
	4.5 g/kg	10	0	2	8	0

4. Experimental results

4.1. General state of animals

As the number of mold-inducing increased, the rabbits gradually scratched the muzzle; The amount of drinking water increased gradually, and the mucous secretion accumulated in the oral cavity; Rabbit overall mental fatigue, independent activities are increasingly reduced, eating less; Some rabbits pharyngeal mucosa superficial formed superficial ulcer. The blank group had normal autonomic activity and no ulceration of pharyngeal mucosa.

4.2. Visual observation of pharyngeal tissue 1 h after the last administration

According to the scoring standard, the pharyngeal tissues of rabbits in different groups were observed. As we can see from [Table 1](#): In the blank group, there was no inflammatory infiltration, hyperemia and edema in the pharynx, and the glossiness was good and all in “–” level. In the model group, the pharyngeal secretions of rabbits increased, and the phenomena of hyperemia and edema were obvious, all in “+++” and “+++” level. In large, middle and small doses of compound lobelia oral liquid group, the pharyngeal secretions decreased, and the phenomena of hyperemia and edema were significantly improved.

4.3. Effects of compound lobelia oral liquid on TNF- α level of acute pharyngitis rabbit model

As we can see from [Table 2](#): Compared with the blank group, the level of TNF- α was significantly increased in the model group ($P < 0.01$); Compared with the model group, the level of TNF- α in Yinhuang oral liquid group and the large- and middle-dose compound lobelia oral liquid group could be significantly decreased ($P < 0.01$).

4.4. Effects of compound lobelia oral liquid on blood routine of acute pharyngitis rabbit model

As we can see from [Table 3](#): Compared with the blank group, the number of white blood cell and lymphocytes in the model

Table 2

Effects of compound lobelia oral liquid on TNF- α level of acute pharyngitis rabbit model.

Group	Dose	n	TNF- α (pg/ml)
Blank group	–	10	156.06 \pm 9.42
Model group	–	10	196.34 \pm 23.28 ^{##}
Yinhuang group	6 ml/kg	10	168.94 \pm 13.33 ^{**}
Compound lobelia group	18 g/kg	10	169.23 \pm 11.14 ^{**}
	9 g/kg	10	178.73 \pm 10.11 ^{**}
	4.5 g/kg	10	192.46 \pm 18.92

Note: Compared with the model group, ^{**}represent $P < 0.01$, ^{*}represent $P < 0.05$. Compared with the blank group, ^{##}represent $P < 0.01$, [#]represent $P < 0.05$.

Table 3
Effects of compound lobelia oral liquid on blood routine of acute pharyngitis rabbit model.

Group	Dose	WBC ($10^9/L$)	Percentage of neutral cells (%)	Lymphocyte number ($10^9/L$)
Blank group	–	$11.25 \pm 0.54^{**}$	44.70 ± 2.93	4.52 ± 0.32
Model group	–	12.78 ± 0.41	$38.18 \pm 1.81^{##}$	$5.82 \pm 0.33^{##}$
Yinhuang group	6 ml/kg	$11.07 \pm 0.30^{**}$	$43.90 \pm 3.19^{**}$	$4.52 \pm 0.20^{**}$
Compound lobelia	18 g/kg	$11.44 \pm 0.40^{**}$	$41.91 \pm 2.02^{**}$	$4.84 \pm 0.16^{**}$
	9 g/kg	$11.62 \pm 0.72^{**}$	$41.15 \pm 2.61^{**}$	$4.99 \pm 0.27^{**}$
	4.5 g/kg	$11.73 \pm 0.48^{**}$	39.44 ± 2.21	$5.22 \pm 0.26^{**}$

Note: Compared with the model group, **represent $P < 0.01$, *represent $P < 0.05$. Compared with the blank group, ##represent $P < 0.01$, #represent $P < 0.05$.

group increased significantly ($P < 0.01$) and the percentage of neutrophils decreased significantly ($P < 0.01$). Compared with the model group, both Yinhuang oral liquid group and compound lobelia oral liquid large and middle dose group could significantly increase the percentage of neutrophils ($P < 0.01$), and decrease the number of white blood cells and lymphocytes ($P < 0.01$). Compound lobelia oral liquid small dose group could decrease the number of white blood cell and lymphocytes significantly ($P < 0.01$), there was an increasing tendency to neutrophils and a decreasing tendency to serum TNF- α , but no statistical significance ($P > 0.01$).

4.5. Effects of compound lobelia oral liquid on pathological changes of pharyngeal tissue of acute pharyngitis rabbit model

As we can see from Fig. 1: In blank group, the oral mucosal squamous epithelium and submucosal glands of rabbits were normal. In model group, a large number of inflammatory cell infiltration was observed in the squamous epithelium and submucosa of the oral mucosa. There are also scattered inflammatory cells in the gland of rabbits. In Yinhuang oral liquid group, the oral mucosal

squamous epithelium of the rabbit has slight hyperplasia and a large number of inflammatory cell infiltration can be seen in the submucosal layer. There are also small amounts of scattered inflammatory cells in the gland of the rabbits. In large dose compound lobelia oral liquid group, the oral mucosal squamous epithelium showed obvious hyperplasia and a small amount of inflammatory cell infiltration was seen in the submucosal layer, and the glands were normal. In middle dose compound lobelia oral liquid group, oral mucosal squamous epithelium slightly hyperplasia, a small amount of inflammatory cell infiltration in the submucosal layer, and the glands were normal. In small dose compound lobelia oral liquid group, oral mucosal squamous epithelium of rabbits showed obvious hyperplasia and a small amount of inflammatory cells infiltrated in the submucosal layer, and the glands were normal. Histopathological grading see Table 4.

5. Discuss

Acute pharyngitis is acute inflammation of the pharyngeal mucosa, involving submucosa and lymphatic tissue, caused by

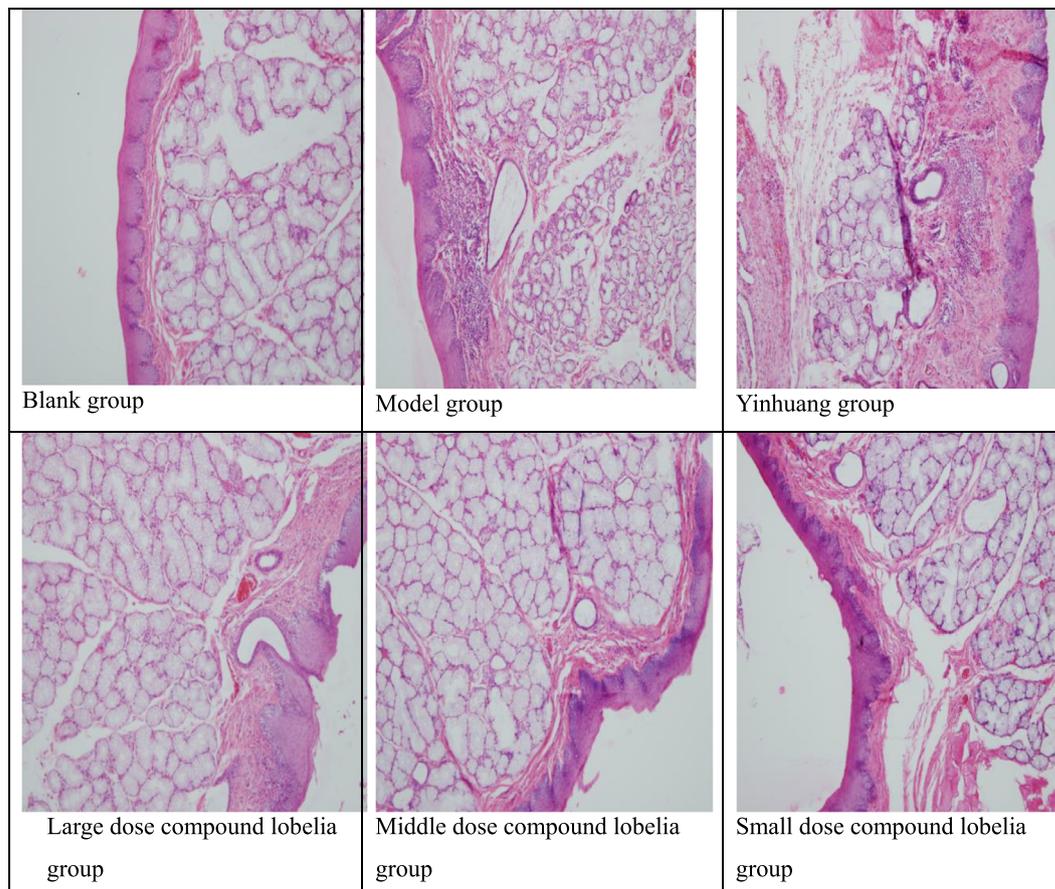


Fig. 1. Pathological changes of pharyngeal tissues in each group (HEx100).

Table 4
Histopathological grading table of pharyngeal tissue of rabbits in each group.

Group	Dose	n	Pharyngeal histopathologic classification			
			–	+	++	+++
Blank group	–	10	10	0	0	0
Model group	–	10	0	0	0	10
Yinhuang group	6 ml/kg	10	0	8	2	0
Compound lobelia group	18 g/kg	10	0	9	1	0
	9 g/kg	10	0	7	3	0
	4.5 g/kg	10	0	6	4	0

“–” means that the pharyngeal tissue was normal and no lesion was observed; “+” indicates that the squamous epithelium of pharyngeal mucosa showed mild hyperplasia and a small amount of inflammatory cells infiltrated under the mucosa. “++” means the mucosal squamous epithelium of the pharynx was obviously hyperplasia with inflammatory cells infiltrating into the lower layer. “+++” means the pharyngeal mucosal squamous epithelium shows moderate or severe hyperplasia with submucosal inflammatory cells.

cold, fatigue, or decreased resistance (Yang, 2017). Western medicine mainly adopts symptomatic treatment such as systemic treatment with antibiotics, local closure, atomization inhalation and tablets containing chemicals. Although it has curative effect, there is a risk of cross-infection (Kettunen et al., 2018; Chiappini et al., 2011). According to the theory of traditional Chinese medicine, “heat leads to swelling”. Yang syndrome ulcers is caused by heat accumulation and qi stagnation and blood stasis. Heat and poison build up, heat accumulation leads to blood stasis and meat decay, and turned into abscess and putrid blood eventually. If the heat poison is concentrated on the skin, it will cause skin ulcers; The heat poison knot in the throat, then cause tonsillitis, acute chronic pharyngitis; The heat poison accumulate in liver and stomach, lacteal collaterals is blocked, bring about mastitis (Zhang et al., 2017). All the above diseases are caused by heat and poison, so different diseases are treated the same way, and their treatment should be clear away heat and toxic materials, reduce swelling and resolve mass.

The oral liquid of compound lobelia is developed on the basis of the injection which is composed of lobelia, *Sculellaria barbata*, *Oldenlandia diffusa*, *Fructus Gardeniae*, *radix digitalis*, liquorice, etc. Lobelia tastes bitter, cold in natural, and channel tropism lungs, liver and kidney. It can not only clear heat and detoxify to treat its heat and toxin accumulation, but also disperse blood stasis and pain relief to treat its swelling and pain. One medicine for two purposes to eliminate the cause of disease, so as to be a royal medicine; *Sculellaria barbata* tastes sweet, neutral in nature, and channel tropism heart and lung. It has the function of clear heat and detoxify, and is a common medicine for treating the symptoms of ulcers caused by toxic heat. *Oldenlandia diffusa* tastes bitter and sweet, cold in natural, and channel tropism heart, liver, spleen, which has the function of clear heat and detoxify. *Sculellaria barbata* and *Oldenlandia diffusa* all can clear heat and detoxify, so as to enhance the power of royal medicine, and used as minister medicine. *Fructus Gardeniae* tastes bitter, cold in natural, and channel tropism heart, liver, lungs, stomach and trifocal. Good at purging fire detoxification, can clear heat and humidity, promote the hot poison out of body from the urine, so it is used as an assistant medicine; Licorice tastes sweet, neutral in nature, it can not only assist royal and minister medicine in antipyretic detoxification, but also ease the bitter cold of various drugs. All drugs can be used together to clear heat and detoxify, and blood vessels freely, and achieve the function of swelling and pain relief.

In this study, high concentration of ammonia was used to spray on the pharynx of rabbits. The stimulation of ammonia rapidly caused local blood vessels to expand and congest, damaged the integrity of laryngopharyngeal tissue, and caused a large number of inflammatory cell infiltration (Liang et al., 2017). Inflammation plays an important role in the course of pharyngitis. TNF- α is an important inflammatory indicator related to acute inflammation,

which can regulate the immune response, inflammatory reaction and endotoxin regulation of the body, and has a damaging effect on relevant tissues in autoimmune diseases. TNF- α is also a pro-fibrotic cytokine, which is involved in the formation of fibrosis and causes the fibrotic tissue to secrete TNF- α itself, which can promote white blood cells to the inflammatory site and further deepen the inflammation (Liu et al., 2018; Song et al., 2018; Li and Pozzi, 2011). Modern medicine considers acute pharyngitis to be the acute inflammation of pharynx mucosa, submucosal tissue and lymphoid tissue, in the inflammatory reaction, lymphocytes and leukocytes increase (Svensson et al., 2017; Chen et al., 2017). Studies have shown that ammonia can cause damage to the immune system in animals, leading to the reduction of neutrophils (Xu et al., 2018). In addition, most studies on the evaluation of the curative effect of acute pharyngitis are focused on the observation of pathological tissues and the biochemical indicators of inflammation, and less on the apparent indicators of the disease itself (Tian et al., 2017; Khan et al., 2018; Kalantari et al., 2017). Therefore, the pharyngeal symptoms directly observed by the naked eye were also taken as an index in this study as the most intuitive manifestation to evaluate the curative effect.

In conclusion, combined with serum biochemical indexes, blood routine examination, pharyngeal tissue symptom score and pathological section observation, it is shown that compound lotus oral liquid has a good intervention effect on animal model of acute pharyngitis caused by ammonia.

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