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ORIGINAL ARTICLE

Effect of Total Alkali in Leonuri Herba on rat ear acne model of serum IL-6 level, Thymus and Spleen Tissue Morphology



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KEYWORDS

Total Alkali in Leonuri Herba (TALH);
Epidermis staphylococcus;
Rat;
Acne model;
IL-6;
Thymus and spleen

Abstract To investigate the effect of Total Alkali in Leonuri Herba (TALH) on rat ear acne model of serum IL-6 level, Thymus and Spleen Tissue Morphology. The rats were divided into the TALH group at high, medium and low doses (200 mg kg⁻¹, 100 mg·kg⁻¹, 50 mg·kg⁻¹), the tanshinone group (360 mg·kg⁻¹), model group and the blank group. All the rats were administrated once a day for 14 days. At the same time on the first day, the rats in the blank group were injected normal saline in the stomach and also had intradermal injection of sterile saline in the auricle. The rats in other groups (model group were lavaged with physiological saline) were injected intradermally auricle spare *Staphylococcus epidermidis* bacteria liquid. After the injection, the rat ear swelling rate was calculated for 5 consecutive days in terms of rat auricle thickness measurement. 1 h after the last administration, the eyeball blood was removed, centrifuged to separate serum, and measured to be at serum IL-6 level; The tissue sections of thymus and spleen were observed according to morphology criteria. Compared with the blank group, the level of IL-6 in serum of the rats in the model group was remarkably higher, and those in the thymus and spleen groups were remarkably lower, indicating the rat ear acne model is successful. Compared with the model group, the rats in the TALH group can remarkably make serum IL-6 level decrease, causing significant thymus cortical thickening and increased spleen sections, and remarkably increasing the lymphocytes of thymus and spleen. The effect of large dose in the TALH group is the best and increases with the increase of the dose and curative effect. The TALH treatment of acne may be related to the reduction of serum IL-6 levels and the resistance of the atrophy of the thymus and spleen on the rat ear acne model.

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1. Introduction

Acne is a chronic inflammatory skin disease of the pilosebaceous, often occurring on the face, chest, back and other parts of the body rich in fat. In modern medicine, the disease is usually treated with antibiotics, anti-androgen,

estrogen, retinoids and other drugs, but all these treatments will bring side effects at various degrees, which is not easy for the patient to accept. In order to seek safer and more effective treatment methods, in recent years, the fight against acne with the help of Chinese medicine through research has been strengthened. Leonuri Herba is dry or fresh aerial parts of the *Leonurus heterophyllus* sweet and is commonly used in traditional Chinese medicine. It has a pungent and slightly bitter flavor and makes one feel slightly cold after touching it. When injected into the pericardium or the liver, the latter may swell with regulating menstruation. It also promotes blood circulation and diuresis and has the detoxification effect. Leonuri Herba contains alkaloids, flavonoids, terpene, polysaccharide, ferulic acid, volatile oil and various chemical composition (Li, 2013). Motherwort topical protects and repairs skin photoaging caused by ultraviolet irradiation damage (Xu and Wu, 2012). Large doses of motherwort inhibit certain fungal skin effect, in clinical practice, it is widely used in treating eczema (Miao et al., 2013a,b; Liu et al., 2014; Liu and Wang, 2010; Fan, 2016; Liu, 2010), urticaria, dermatitis, prurigo skin, carbuncles and female skin pruritus. For this project, the effect of Total Alkali in Leonuri Herba (TALH) on rat ear acne model at serum IL-6 level, Thymus and Spleen Tissue Morphology is studied in order to provide experimental evidence for clinical application.

2. Materials and methods

2.1. Animals

Wistar rats, male, whose weight were 180–200 g, were supplied by the Experimental Animal Center of Hebei Province (Animal permit number: 812074). Laboratory Certificate of Conformity: SYXK (Henan) 2010-001.

2.2. Experimental reagents and drugs

The TALH, provided by the chemical room, concentration is 52%; tanshinone capsule, Hebei Xinglong Force Pharmaceutical Co., Ltd., production batch number 20090819; *S. epidermidis* strain No. 12228, provided by Zhengzhou University microbiology laboratory, experimental passages before backup; IL-6 radiation immunoassay kit, produced by Beijing Ear Cause Biological Technology Co., Ltd. production batch number 20091226.

2.3. Experimental instruments

Electronic balancer, Mettler-Toledo Instruments Co., LTD, model AL204; Micrometer screw, Jiangyin Production Throttling Device Factory Co., LTD. Automatic γ Immune Counter, State-run Twenty-six Plant Nuclear Medical Instrument Factory, model FJ2003PS.

2.4. Modeling and administration

According to the weight balance, 60 Wistar male rats, weighing 180–200 g. were randomly divided into the TALH group at high, medium and low doses (200 mg·kg⁻¹, 100 mg·kg⁻¹,

50 mg·kg⁻¹), the tanshinone group (360 mg·kg⁻¹) model group and the blank group (Xu et al., 2009). The corresponding drugs were administered in each group. The model group and blank group were given volumes of distilled water. All the rats were administrated once a day for 14 days. At the same time on the first day, the rats in the blank group were injected normal saline in the stomach and also had intradermal injection of sterile saline in the auricle. The rats in other groups (model group were lavaged with physiological saline) were injected intradermally auricle spare *S. epidermidis* bacteria liquid. After the injection, the rat ear swelling rate was calculated for 5 consecutive days in terms of rat auricle thickness measurement. 1 h after the last administration, the eyeball blood was removed (without anesthesia), centrifuged at 3000 r/min for 15 min to separate serum, and measured according to the IL-6 immunoradiometric analysis kit instruction manual operation. Meanwhile the thymus and spleen are weighed, and the biopsy was stained with HE (Miao et al., 2013a,b).

2.5. Method statistical analysis

SPSS 13.0 for windows has been used for statistical processing. The measurement data are represented by mean \pm variance ($\bar{x} \pm s$), and group comparison has adopted analysis of variance; and the Ridit test has been used to rank the data.

3. Results

Previous studies had shown that the effect of Total Alkali in Leonuri Herba on *S. epidermidis* rats has been caused by acne model (Miao et al., 2016). Through our research, we have found that the effect of Total Alkali in Leonuri Herba (TALH) on rat ear acne model of serum IL-6 level is related to Thymus and Spleen Tissue Morphology.

3.1. Effect on thymus tissue morphology of rat ear acne model

For the rats in the blank group, the thymic lobule cortex, medulla clear boundaries, cortical lymphocytes were normal (Fig. 1A); For the rats in the model group, the thymic lobule cortex, the medulla clear boundaries, the cortical atrophy thinning, lymphocytes decreased significantly (Fig. 1B); The rats in the tanshinone group rat, the thymic lobule cortex, medulla, clear boundary, the cortical thickening, denser lymphocyte (Fig. 1C); For the rats in the high dose TALH group, the cortical skin, the medullary thymic lobule, the clear boundary, the cortical thickening, denser lymphocyte (Fig. 1D); The rats in the medium dose of TALH group, the cortex, medullary thymic lobule, clear boundary, cortical thickening, denser lymphocyte (Fig. 1E); The rats in the low dose TALH group, the cortical, medullary thymic lobule, clear boundary, cortical atrophy thinning dense, lymphocyte (Fig. 1F).

From Table 1, it can be seen that compared with the blank group and the model group, the thymic cortex thickness and the number of lymphocytes decreased significantly ($P < 0.01$), indicating the rat ear acne model was successful; Compared with the model group, the high and medium dose TALH groups and the tanshinone group significantly increased the thymus cortical thickness and the number of lymphocytes on rat ear acne model ($P < 0.01$); the low dose of

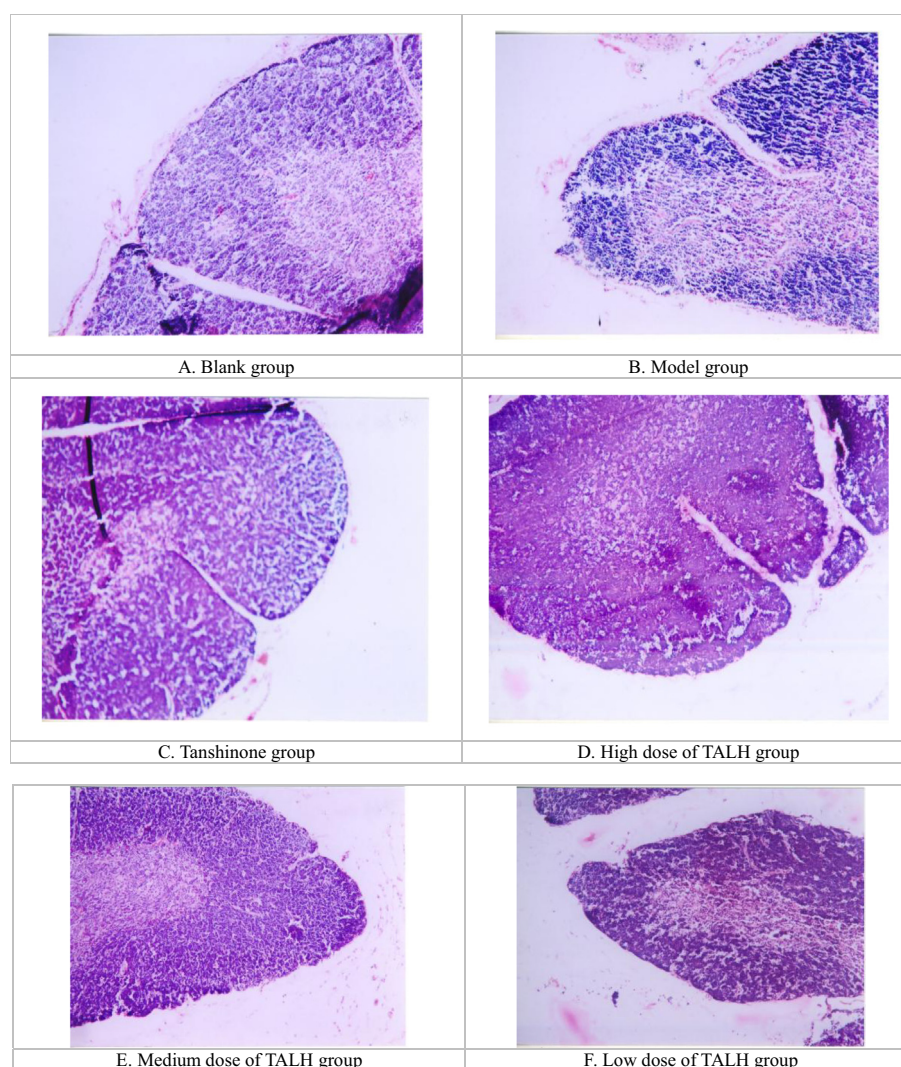


Figure 1 Effect of TALH model of thymus tissue of rats (HE×100).

Table 1 Effect of Leonurine rats caused by acne model thymic tissue morphology of *Staphylococcus epidermidis*.

Group	N	The thickness of thymic cortex (μm)	Number of lymphocyte
Blank group	10	23.46 ± 4.32**	37.21 ± 9.25**
Model group	10	14.22 ± 6.24	21.30 ± 8.12
Tanshinone group	10	35.47 ± 6.20**	40.28 ± 7.26**
High dose of TALH group	10	31.28 ± 5.43**	38.12 ± 6.21**
Medium dose of TALH group	10	28.30 ± 6.26**	33.25 ± 6.17**
Low dose of TALH group	10	17.20 ± 4.26*	29.16 ± 5.14*

Note: Compared with the model group.

* $P < 0.05$.

** $P < 0.01$.

TALH group can be obviously increased the thymus cortical thickness and number of lymphocytes on rat ear acne model ($P < 0.05$).

3.2. Effect on spleen tissue morphology of rat ear acne model

For the rats in the blank group, the red pulp of spleen white pulp clear boundaries, splenic corpuscle and lymphocyte were normal (Fig. 2A); The rats in the model group, the red pulp of spleen white pulp clear boundaries, splenic nodule was obviously reduced, lymphocyte sparse decreased obviously (Fig. 2B); The rats in the tanshinone group, the spleen red and white pulp, clear boundary, splenic corpuscle increased significantly, lymphocyte dense (Fig. 2C); The rats in the high dose of TALH group, the spleen red and white pulp, clear boundary, splenic corpuscle increased obviously, lymphocyte dense (Fig. 2D); The rats in the medium dose of TALH group, the red and white pulp, clear boundary, splenic corpuscle increased, lymphocytosis (Fig. 2E); The rats in the low dose of TALH group, the spleen red and white pulp, clear boundary, splenic corpuscle increased, lymphocytosis (Fig. 2F).

From Table 2, compared with the blank group, in model group the spleen section and the number of lymphocytes decreased significantly ($P < 0.01$), indicating the rat ear acne model successfully; Compared with model group, the high

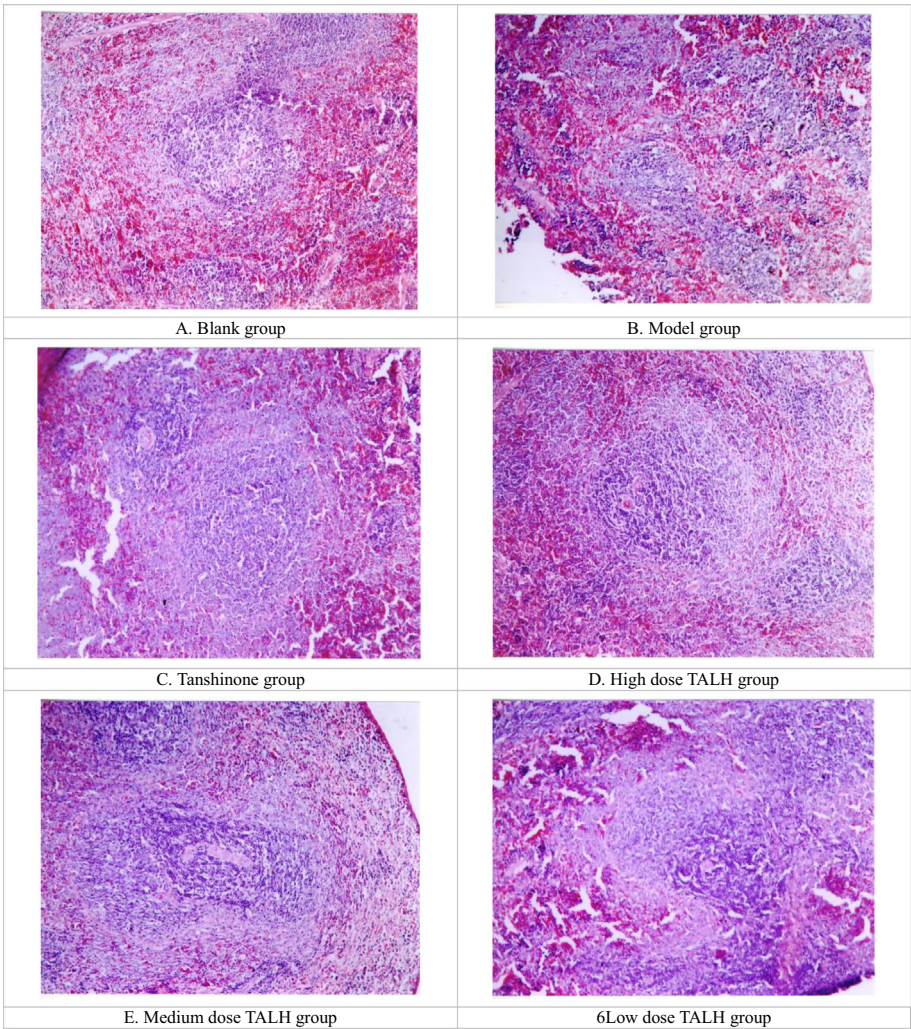


Figure 2 Effect of TALH model of spleen tissue of rats (HE×100).

Table 2 The results of experimental groups with different pathological changes of spleen determination.

Group	N	The spleen section (μm)	Number of lymphocyte (μm)
Blank group	10	13.26 ± 2.35**	22.18 ± 3.25**
Model group	10	8.34 ± 1.27	12.37 ± 2.15
Tanshinone group	10	22.16 ± 3.15**	36.14 ± 5.20**
High dose of TALH group	10	18.25 ± 2.43**	27.24 ± 3.35**
Medium dose of TALH group	10	16.34 ± 2.36**	24.18 ± 2.27**
Low dose of TALH group	10	10.17 ± 3.24*	18.26 ± 3.24*

Note: Compared with the model group.
 * $P < 0.05$.
 ** $P < 0.01$.

and medium doses of TALH group and tanshinone group can significantly increase the size of spleen section and number of lymphocytes on rat ear acne model ($P < 0.01$); the low dose of TALH group can be obviously increased the size of spleen

Table 3 The effect of TALH on rat ear acne model of serum IL-6 level ($\bar{x} \pm s$, $N = 10$).

Group	N	IL-6 (ng/ml)
Blank group	10	0.17 ± 0.03**
Model group	10	0.29 ± 0.07
Tanshinone group	10	0.18 ± 0.02**
High dose of TALH group	10	0.19 ± 0.03**
Medium dose of TALH group	10	0.20 ± 0.03**
Low dose of TALH group	10	0.25 ± 0.03*

Note: Compared with the model group.
 * $P < 0.05$.
 ** $P < 0.01$.

section and number of lymphocytes on rat ear acne model ($P < 0.05$).

3.3. Effects of serum IL-6 levels on rat acne model

From Table 3, compared with the blank group, the rats in the model group increased significantly in terms of the IL-6 serum level ($P < 0.01$), indicating that the rat ear acne model is suc-

cessful; Compared with the model group, the high and medium dose TALH group and the tanshinone group can significantly decrease the level of IL-6 in serum on rat ear acne model ($P < 0.01$); the low dose of TALH group can significantly decrease the level of IL-6 in serum on rat ear acne model ($P < 0.05$).

4. Discussion

Acne often occurs on the head and face as hair follicles and sebaceous glands. It may also occur on the chest and back of the neck. It may cause acute purulent infection, which is easy to expand into subcutaneous tissue, subjecting the skin to varying degrees of damage. Other consequences of acnes include pimples, nodules, pus blisters, cysts, scars. According to relevant statistics, the incidence of adolescent suffering from acne is as high as 82.7% and the prevalence rate of male was higher than female (Huang et al., 2012). The current treatment of acne is usually through the use of antibiotics, anti-androgen drugs such as retinoic acid -based, but this method may cause certain therapeutic effects, adverse reactions of antibiotics drug and strong toxicity in the liver and the kidney (Xu, 2011); Anti-androgen drugs can cause human hormone disorders, particularly affecting the female menstrual regularity, and in severe cases can cause infertility; Retinoids to skin desquamation is serious, and had severe liver toxicity, teratogenicity and ocular toxicity (Yan and Chen, 2010; Wang and Hedner, 2016). Therefore, many scholars are studying how to use traditional Chinese medicine to treat acne, and have achieved satisfactory result.

The experimental model has chosen ear sebaceous glands of the rat, intradermal injection of *S. epidermidis* infections and then expanded to form a micro acne hair follicle cavity. Due to the fact that excessive sebaceous gland secretion is an important condition for the formation of acne, and acne are the final performance of hair follicle enlarged model. This method is in accordance with the clinical symptoms of acne and is easy to operate, and achieve high repeatability. It had been reported that the qingrecuochuang soup (Pugongying, wild chrysanthemum, honeysuckle, violet, giant knotweed etc.) can significantly inhibit the delayed type hypersensitivity and inflammation and significantly reduce the peripheral T lymphocyte of spleen and the immune organ index, has anti-inflammatory effect, inhibit cellular over expression in a certain extent (Kou et al., 2003). In order to further investigate the relationship between acne and the immune regulation, in this paper, on the basis of effect of TALH on rat ear acne model, we have observed the local tissue pathology of thymus, spleen, thymus cortex thickness, spleen section size, the corresponding number of lymphocytes, and the influence of the level of serum IL-6. To observe the change of immune organs of rats, we have often selected the thymus and spleen. General observation of the thickness of thymus cortical is conducted by measuring the narrowest and widest point cortex, the mean value for the thickness, and then calculating the pressure in the micrometer reticle number of lymphocytes. General observation of the spleen is conducted in the spleen size section, with eyepiece micrometer reticle and central arteriole of spleen as the midpoint to calculate pressure in lines on both sides of the lymphocyte count, meaning a central small periarterial

lymphatic sheaths of the lymphocyte count (Miao et al., 2010); serum IL-6, mainly produced by macrophages, natural-mediated immune factors, also known as pro-inflammatory cytokines, is the key to the innate immune response of the start. There were reports of some significance of inflammatory cytokines in the pathogenesis of acne (Wang et al., 2013). As the thymic cortex thickening reduced, the spleen section increased, and cortical lymphocytes and spleen lymphocytes increased, the serum IL-6 level decreased, which suggested an improvement in immune function. Meanwhile, this paper has a positive control of drug selection tanshinone because the main active ingredient is Cryptotanshinone, which has a strong inhibitory effect (Li et al., 2012). In addition, there is a mild estrogen-like activity, and anti-male hormone-like effect (Wang, 2011). It had been reported that the cure rate of acne was 31.7%, and the total efficiency was 90% (Zheng, 2011). There was a clinical report (Wu and Liu, 2006) that Danshen Mixture (*Hedyotis diffusa*, *Scutellaria baicalensis* Georgi, *Salvia miltiorrhiza*, *Prunella vulgaris* etc.) could significantly or obviously change the peripheral blood IgG and IL-2 levels of acne patients, suggested that the regulation of the mixture of cellular and the humoral immunity may be one of the mechanisms of clinical treatment.

The results of the study indicate that the acne model has made the spleen and thymus of the rat atrophy significantly, the ear squamous epithelium hyperplasia was thicker obviously, and less emergence of a large number of inflammatory cell infiltration, and reduced the number of lymphocytes. The TALH can make the acne model caused by rat thymus and spleen atrophy reduce significantly, significantly thicken the thymus cortex, significantly increase the spleen sections, the number of the cortex of thymus lymphocyte and spleen lymphocytes, and significantly reduce the serum IL-6 level in serum. In the high dose TALH group, the antagonism thymus and the spleen have atrophied, the serum levels of serum IL-6 have decreased strongly, the TALH increases with the increase of the dose and efficacy, and its concentration-response relationship was positively correlated. This article, based on the serum levels of IL-6, local tissue pathology in thymus and spleen, measures the size and corresponding number of the spleen lymphocytes and thymus cortical, which is the first analysis of the immune system with acne relevance. The Motherwort is a common gynecological medicine. This study also provides new ideas for the treatment of acne.

Acknowledgments

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