• Original Article •

Effect of Buddleja officinalis eye drops to inflammatory factors of lacrimal gland cells of castrated male rabbit with dry eye

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Abstract

• AIM: To observe the effect of different concentrations Buddleja offcinalis eye drops (BOED) to inflammatory factors of lacrimal gland cells of castrated male rabbit with dry eye.

• METHODS: Forty-two healthy adult New Zealand rabbits were randomly divided into 7 groups, 6 rabbits each group. Group A: blank group, Group B: model group, Group C: low concentrations BOED group, Group D: medium concentrations BOED, Group E: high concentrations BOED, Group F: the placebo group, Group G: testosterone groups. All rabbits of group B to G were cut off bilateral testis and epididymis except group A. The rabbits of group C, D, E and F were dropped relevant eye drops except groups A and B. All rabbits were tested Schimer I test (SIT) and break-up time (BUT) before operation and 4wk after operation. Expressions of inflammatory factors of TNF-α, IL-1β from lacrimal gland cells were checked in immunohistochemical staining method.

• RESULTS: 1) Comparison of SIT and BUT: Compared with the groups D, E and G, group C had statistical significance (*P*<0.01). Compared with group D and E, G groups, there was no significant difference among those group before and after operation (*P*>0.05); 2) Comparison of inflammatory factors of TNF-α, IL-1β after operation: Compared with value of the average optical density of TNF-α and IL-1β of group C, those of group D, E and G were obviously different after operation (*P*<0.05). Compared D and with E, G groups, there was no significant difference between those group after operation (*P*>0.05).

• CONCLUSIONS: 1) Compared with androgen, BOED has the similar effect of depressing the expression of inflammatory factors. But its effect is slightly weaker than the androgen; 2) The depressing effect of medium and high concentration BOED are better than low concentration. Compared with medium and high concentration of BOED, there was no significant difference between two groups.

• KEYWORDS: Buddleja offcinalis eye drops; dry eye; lacrimal gland cell; inflammatory factors

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INTRODUCTION

Dry eye is also known as keratoconjunctivitis sicca, which is a variety of diseases characterized by abnormal quality or quantity of tear due to any reason, or the drop the tear film stability caused by abnormal dynamics and accompanied ocular discomfort and/or ocular surface tissue disease[1]. Dry eye is a common ocular surface disease, most of which are binocular diseases. Epidemiological and clinical studies have been found that the incidence is far higher than what people think. We have confirmed that Buddleja officinalis has a good curative effect on dry eye in previous clinical and animal experiments. In this experiment Buddleja offcinalis eye drops was made by Buddleja officinalis crude drug, for the treatment of animal model with dry eye induced by androgen reducing to investigate the effects of Buddleja offcinalis eye drops (BOED) on lacrimal gland cell inflammatory factor TNF-α and IL-1β of dry eye caused by androgen decreasing.

MATERIALS AND METHODS

Laboratory Animals Forty-two healthy adult New Zealand white rabbits (male) were chosen, body weight between 1.5-2.0kg(Animal experimental center of Hunan University of Chinese Medicine, Laboratory animal quality certificate No.:SCXK (Xiang) 2009-0012).

Laboratory Equipment Slit lamp microscope, hand-held direct ophthalmoscope, GB11241-89 constant temperature water bath box, Leica Paraffin section machine, micro camera

and computer image analysis system, *etc*. Relevant equipment used in the above experiments were provided by Hunan University of Chinese Medicine Laboratory of morphology and Laboratory of Ophthalmology.

Drugs and Reagents Testosterone Propionate Injection: Tianjin Jin Yao Pharmaceutical Co., Ltd. (Code number approved by SFDA:H12020531; specifications:1 mL:25 mg); Benzylpenicillin Sodium for Injection: Guangzhou Baiyun Mountain Tianxin pharmaceutical Limited by Share Ltd (Code number approved by SFDA:H444022446; specifications: 400 thousand units); Sodium Chloride Injection: Jiangsu Yabang Shengyuan Pharmaceutical Co. Ltd. (Code number approved by SFDA:H32024531; specifications:500 mL:4.5 g); chloral hydrate: analysis of pure Tianjin Kermel Chemical Reagent Co. Ltd. (specifications: 250 g); Sterile Water for Injection: Shanghai treeful Jinshan Pharmaceutical Co. Ltd. (Code number approved by SFDA:H31021935; specifications: 500 mL). Buddleja officinalis and placebo eye drops were prepared in modern technology by the department of medicine of Hunan University of Chinese Medicine (Specifications: 3 mL low concentration BOED was equivalent to 0.5 g original medicinal materials; each 3 mL medium concentration BOED was equivalent to 1 g original medicinal material; each 3 mL high concentration BOED was equivalent to 2g original medicinal material). Harris hematoxylin, eosin and goat anti rabbit TNF- antibody (specifications: 200 g/mL) were from Beijing Golden Bridge Biotechnology Co. Ltd. Rabbit anti rat IL-1 antibodies(specifications: 200 g/mL) and 3, 3-diaminobenzidine chromogenic agent were from Wuhan Boster Biological Technology., LTD.

Experimental Methods

Experimental animal group According to the random arrangement table method 42 rabbits were divided into 7 groups, each group of 6 rabbits. A: blank group; B: model group; C: low concentration BOED group; D:medium concentration BOED group; E: high concentration BOED group; F: placebo group; G: testosterone group.

The establishment of animal model of dry eye All rabbits were treated by bilateral testicular and epididymis resection with reference to the relevant literature[2-3] except blank group.

Postoperative administration In addition to A, B group, from the third day after the operation group C, D, E were respectively treated with corresponding concentration BOED, 3 times one day. Group F was treated with placebo eyedrop, 3 times one day Group G was injected Testosterone Propionate Injection in the thigh muscles by 0.5 mL/kg, once every 3d.

Dry Eye Routine Inspection Method Each test was completed by the same group of experimental personnel division. All the rabbits were made 1d before and the fourth week after operation for Schirmer I test (SIT) and tear film BUT determination.

Specimen Collection and Processing After 4wk all rabbits after conventional breeding, were scarified by air embolism. Lacrimal gland of both eyes were removed, and specimens were fixed in 4% paraformaldehyde for 24h, embedded in paraffin sections.

Index Detection The removal lacrimal gland tissue were stained in eosin staining and immunohistochemical staining, then inflammatory factors of lacrimal gland cells after staining were observed in 400 times optical microscope, then taken photos and preserved.

Statistics Treatment All the experimental data were processed by SPSS16.0 statistical software. The measurement data were expressed by mean plus or minus standard deviation (s), and the normal distribution and homogeneity of variance were tested, and the variance analysis was used to meet the normality and homogeneity of variance. Non parametric multiple comparisons were used to satisfy the normality and variance homogeneity. Enumeration data were analyzed with χ^2 test, and other homogeneity of variance with rank sun test. P was less than 0.05 that was considered to be statistically significant. P was less than 0.01 that the difference had a significant meaning.

RESULT

The Results of Routine Examination of Dry Eye

Value of Schirmer I test Compared with the placebo group and the model group, the value of SIT decreased significantly (P<0.01). Compared with the model group, the values of SIT in each concentration BOED group and the testosterone group did not significantly decreased (P<0.01). Compared with the low concentration BOED group, the SIT values of the medium, high concentration BOED group and testosterone group did not significantly reduced(P<0.01). Compared with the placebo group, the SIT values of the testosterone group did not decrease significantly (P<0.01; Table 1).

Tear break-up time Compared with the placebo group and the model group, the value of BUT decreased significantly (P<0.01). Compared with the model group, the values of BUT in each concentration BOED group and the testosterone group did not significantly decreased (P<0.01). Compared with the low concentration BOED group, the BUT values of the medium, high concentration BOED group and testosterone group did not significantly reduced (P<0.01). Compared with the placebo group, the BUT values of the testosterone group did not decrease significantly (P<0.01; Table 2).

The expression of TNF-α,IL-1β were observed in rabbit lacrimal gland cells after operation Compared with the blank group, the expression of the model group and the placebo group increased significantly (P<0.01). Compared with the model group, the expression of each concentration BOED group and the testosterone group did not increase significantly (P<0.01). Compared with the low concentration

Table 1 SIT values of rabbits before and after operation (mean±SD; *n*=12; mm)

Time	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Preoperative	14.75±2.26	14.83±2.04	14.92±2.07	14.75±1.66	14.58±1.51	14.67±1.92	14.83±1.59
Postoperative	14.50±1.45	7.75±1.54[a]	12.82±1.19[b]	14.17±1.11	14.17±1.19	7.50±1.62[a]	14.33±1.15

Compared with each group before the surgery, there was not statistically significant (P>0.05). They were comparable. Compared with group B and group F before and after operation, there were significant differences (P<0.01). Compared with the other groups before and after surgery, there was no statistical significance (P>0.05). There was no statistical significance between group B and F after operation (P>0.05). Compared with the other groups, group B had statistical significance ([a]P<0.01). Compared with the group D, E and G, group C had statistical significance ([b]P<0.01) Compared with group E and G, the group D had no statistical significance.

Table 2 BUT values of rabbits before and after operation （mean±SD; *n*=12; s)

Time	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Preoperative	14.33±2.42	14.42±1.38	14.42±1.51	14.58±1.98	14.67±1.61	14.25±2.42	14.75±2.22
Postoperative	14.08±1.88	6.75±1.66[a]	12.92±1.08[b]	14.00±1.28	14.08±1.24	6.83±1.70[a]	14.50±1.24

Compared with each group before the surgery, there was not statistically significant (P>0.05). They were comparable. Compared with group B and group F before and after operation, there were significant differences (P<0.01).Compared with the other groups before and after surgery, there was no statistical significance (P>0.05).There was no statistical significance between group B and F after operation (P>0.05). Compared with the other groups, group B had statistical significance ([a]P<0.01).Compared with the group D, E and G, group C had statistical significance ([b]P<0.01). Compared with group E and G, the group D had no statistical significance.

Table 3 Value of the average optical density of TNF-α and IL-1β in each rabbit lacrimal gland cells after operation (mean±SD, *n*=12)

Time	Group A	Group B	Group C	Group D	Group E	Group F	Group G
TNF-α	0.18±0.10	0.68±0.14	0.35±0.14	0.24±0.09	0.24±0.11	0.65±0.13	0.23±0.14
IL-1β	0.24±0.12	0.59±0.17	0.41±0.16	0.30±0.08	0.24±0.13	0.57±0.28	0.17±0.21

Comparison of TNF-alpha, IL-1 beta value: Compared with group B and F after operation, there was no statistical significance (P>0.05). Compared with the other groups , group B and F had statistical significance (P<0.01). Compared with group D, E and G, group C had statistical significance (P<0.05). Compared with groups D, E and G, there was no statistical significance (P>0.05).

BOED group, the expression of medium, high concentration BOED group and testosterone group did not significantly increased (P<0.05). There was a significant increase in the placebo group (P<0.01), and compared with placebo group, the expression of testosterone group did not increase significantly (P<0.01; Table 3).

Observation of TNF-α and IL-1β Groups A and G: lacrimal gland structure was clear and no expression of TNF-α and IL-1β; groups B and F: lacrimal gland structure blurred, lots of TNF- α and IL-1β expressed in cell membrane and cytoplasm showing brown yellow granules; groups C, D and E: lacrimal gland structure was clear, scattered expression of TNF-α and IL-1β were observed (Figures 1, 2).

CONCLUSION

The new definition of dry eye has been reported by the Tear Film and Ocular Surface Society Dry Eye Workshop (TFOS DEWS) in 2017 that is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles. The disease was accompanied by inflammation of the ocular surface and increase of tear film permeability[4]. Compared with the traditional definition of dry eye, the definition emphasized the important role of inflammation in the pathogenesis of dry eye disease. Although the initial cause of dry eye caused by different mechanisms, once it enters the stage, inflammation became the key factor in the pathogenesis of dry eye. Compared with normal people, a variety of cytokines in tear and conjunctival epithelium of patients with dry eye including IL-1, IL-6, IL-8, alpha TNF- alpha and TGF-beta level changed significantly, and were related with the severity of dry eye[5].

In recent years, especially the sex hormone androgen has played an important role in tear secretion. Androgen exerts its immunosuppressive effect by stimulating the synthesis of TGF- beta to decrease TNF-alpha, IL-1 beta in lacrimal gland level, effectively protect the lacrimal gland from inflammation and degeneration, accelerate metabolism, regulate morphology and secretion function of lacrimal and meibomian gland.

Dry eye belongs to “Bai She Zheng” of traditional Chinese medicine, is also called “Ganshe Hunhua Zheng”, “Shen Shui Jiang Ku Zheng”, “Shen Qi Ku Cui”. Generally dry eye is caused by liver and kidney deficiency, yin and blood loss, being lack of nourishment. Buddleja officinalis granules is consisted of Buddleja officinalis, Chinese wolfberry,

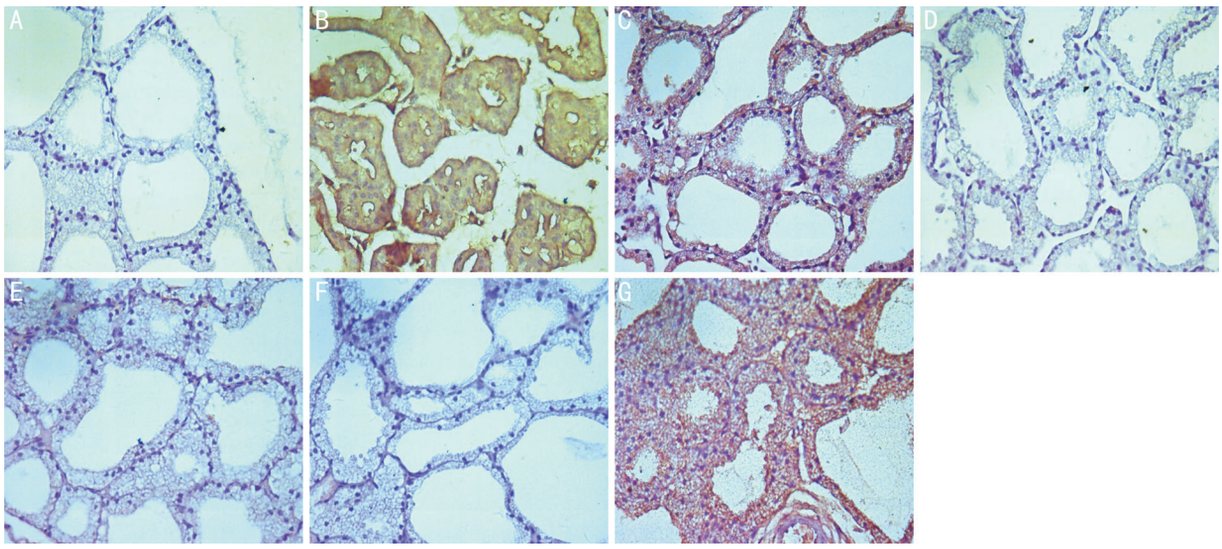

Figure 1 Expression of TNF-α in each group after operation (200 times) A: Lacrimal gland cells arranged orderly, clear structure, no inflammatory cell infiltration, no apoptotic cells, no expression of TNF-α. B: Lacrimal gland cells arranged irregularly, large structure degeneration, inflammatory cell infiltration and cell apoptosis, lots of TNF-α expression in cell membrane and cytoplasm showing brownish yellow granules. C: Lacrimal gland cells arranged orderly, clear structure, a small amount of inflammatory cell infiltration and apoptosis, TNF-α expression in cell membrane and cytoplasm showing brownish yellow granules. D: The lacrimal gland cells arranged irregularly, large degeneration of cell structure , inflammatory cell infiltration and cell apoptosis, TNF-α expressed in cell membrane and cytoplasm showing brownish yellow granules. E: Lacrimal gland cells arranged orderly, clear structure, the infiltration of scattered inflammatory cells and cell apoptosis, TNF-α expression scattered in the cell membrane and cytoplasm showing brownish yellow granules. F: Lacrimal gland cells arranged orderly, clear structure, the infiltration of scattered inflammatory cells and cell apoptosis, TNF-α expression scattered in the cell membrane and cytoplasm showing brownish yellow granules. G: Lacrimal gland cells arranged irregularly, large structure degeneration, a lot of inflammatory cell infiltration and cell apoptosis, lots of TNF-α expression in cell membrane and cytoplasm showing brownish yellow granules.

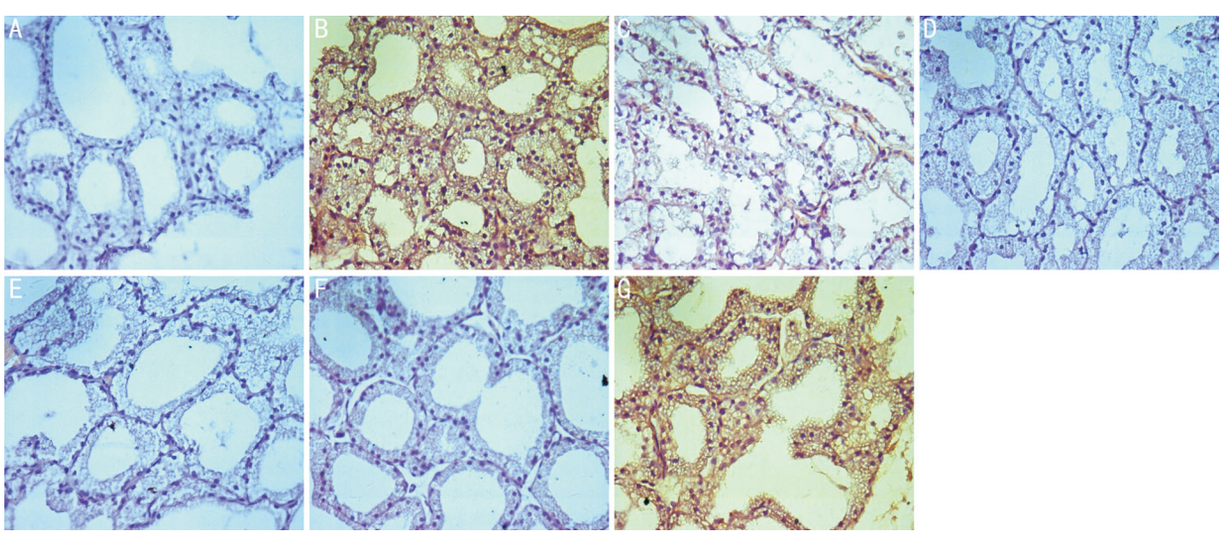

Figure 2 IL-1βexpression in each group after operation (200 times) A: Lacrimal gland cells arranged orderly, clear structure, no inflammatory cell infiltration, no apoptotic cells, no expression of IL-1 beta. B: Lacrimal gland cells arranged irregularly, large structure degeneration, inflammatory cell infiltration and cell apoptosis, lots of IL-1 beta. Expression in cell membrane and cytoplasm showing brownish yellow granules. C: Lacrimal gland cells arranged orderly, clear structure, a small amount of inflammatory cell infiltration and apoptosis, IL-1 beta. Expression in cell membrane and cytoplasm showing brownish yellow granules. D: The lacrimal gland cells arranged irregularly, large degeneration of cell structure, inflammatory cell infiltration and cell apoptosis, IL-1 beta. -αexpressed in cell membrane and cytoplasm showing brownish yellow granules. E: Lacrimal gland cells arranged orderly, clear structure, the infiltration of scattered inflammatory cells and cell apoptosis, IL-1 beta Expression scattered in the cell membrane and cytoplasm showing brownish yellow granules. F: Lacrimal gland cells arranged orderly, clear structure, the infiltration of scattered inflammatory cells and cell apoptosis, IL-1 beta Expression scattered in the cell membrane and cytoplasm showing brownish yellow granules. G: Lacrimal gland cells arranged irregularly, large structure degeneration, a lot of inflammatory cell infiltration and cell apoptosis, lots of IL-1 beta Expression in cell membrane and cytoplasm showing brownish yellow granules.

Chrysanthemum and so on. Buddleja officinalis is sweet, slightly cold, belonging to liver meridian, which has effect of clearing heat-fire, nourishing liver, and removing nebula for improving eyesight[6]. It is drug to treat red eye, blurred vision, eye dryness, cloudy vision due to liver deficiency and so on[7-8]. According to modern research the active ingredients of Buddleja officinalis are flavonoids[9], and some flavonoids have androgen like effect[10] which can be used to treat certain diseases induced by sex hormone levels decreased, such as bone loss[11].

We have confirmed previous experimental research of Buddleja officinalis, the main components of Buddleia flavonoids extract could play the androgenic like effect, and reduce the occurrence of dry eye in rats after androgen levels decreased , inhibit apoptosis of lacrimal gland cell , and maintain the stability and volume basic secretion of lacrimal gland[12-15].

In this study, the effects of 3 kinds of concentration eye drops on tear secretion, tear break-up time, the expression of inflammatory cytokines TNF-alpha and IL-1beta in the male rabbits were studied. Then we have drawn some conclusions: BOED has been androgen-like inhibition of expression of TNF-alpha and IL-1 beta, could inhibit the inflammatory reaction induced by androgen deficiency in rabbit lacrimal gland cells, but the effect has been weaker than that of androgen. The inhibitory effect of medium and high concentration of BOED on TNF- alpha and IL-1beta was better than that of low concentration of BOED, but there was not obvious difference in the inhibitory effect of medium and high concentration of BOED.

At present the dry eye are treated mainly by drugs. Clinical commonly used drugs are artificial tears, which mainly relieve eye discomfort, can not change the pathological state of dry eye fundamentally. This study has been confirmed that BOED has had hormone like effect, could inhibit expression of inflammatory factor TNF- alpha and IL-1 induced androgen deficiency in rabbit lacrimal gland cells, maintained the stability and volume of basic secretion of lacrimal gland. A new natural drug has been provided for the treatment of dry eye disease, at the same time it has avoid the side effects caused by long-term use of and rogen, which has been broad application prospects.

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