Effect of Bushen Qingre Yuyin Decoction on Salivary Secretion, Spleen Index, Submandibular Gland Index, Submandibular Gland Histomorphology, and aqp5 Expression in the Nonobese Diabetic Mouse Model

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Abstract

Objective: The objective of this study was to observe the effect of Bushen Qingre Yuyin Decoction on the spleen index, submandibular gland index, salivary secretion, submandibular gland morphology, and aquaporin 5 (AQP5) expression levels in the submandibular gland.

Materials and Methods: Fifty 8-week-old nonobese diabetic (NOD) female mice were randomly divided into the following five groups: control model; hydroxychloroquine; and high-, medium-, and low-dose Bushen Qingre Yuyin Decoction prescription. The blank group consisted of ten 8-week-old Balb/C rats. Bushen Qingre Yuyin Decoction was administered by gavage at 20, 40, and 80 g/kg in the low-, medium-, and high-dose groups, respectively. The hydroxychloroquine group was administered hydroxychloroquine at a dose of 80 mg/kg by gavage. The normal and model groups were administered the same amount of saline. After the different administrations, the amount of salivary secretion in the mice was regularly observed. After 12 weeks, the mice were sacrificed, and their submandibular gland tissues were excised, stained with hematoxylin-eosin, and pathologically scored. AQP5 expression levels in the tissues were detected using real-time polymerase chain reaction.

Results: The spleen index of mice in the high-dose group was higher than that in the model group ($P < 0.05$), and the submandibular gland index of the hydroxychloroquine group and all the Bushen Qingre Yuyin Decoction prescription groups was higher than that in the model group ($P < 0.05$). After 6 weeks, the salivary volume of the hydroxychloroquine group and all the Bushen Qingre Yuyin Decoction prescription groups was significantly higher than that of the model group ($P < 0.05$); the amount of lymphocyte infiltration in mice among each administration group was significantly reduced ($P < 0.05$); and the AQP5 expression levels in the submandibular glands of mice had significantly increased in the hydroxychloroquine and high-dose groups ($P < 0.05$), with the high-dose group showing the best effect. Conclusion: Bushen Qingre Yuyin Decoction can effectively increase the spleen and submandibular gland indexes of NOD mice, increase the amount of salivary secretion, reduce lymphocyte infiltration in submandibular gland tissue, and significantly increase AQP5 expression levels, which may be positively correlated with the administered dose.

Keywords: Aquaporin 5, Bushen Qingre Yuyin Decoction, nonobese diabetic mouse, Sjögren’s syndrome, Submandibular gland histomorphology

Introduction

Primary Sjögren’s syndrome (pSS) is a chronic autoimmune disease characterized by the inflammation of secretory glands such as lacrimal and salivary glands. It leads to keratoconjunctivitis and xerostomia (i.e., dry eye and mouth),[1] and its progression is characterized by unregulated lymphocytic invasion. Notably, some of these lymphocytes organize...
themselves into germinal center-like formations in the glands, causing ductal aggregation, inflammation, and apoptosis. These changes lead to glandular dysfunction, including loss of tears from the lacrimal glands and loss of saliva from the salivary glands.[2-4] The etiology and pathogenesis of pSS are not yet fully understood. It is caused by genetic, viral infections, abnormal sex hormones, and other factors, which lead to abnormal reactions of the body’s cellular and humoral immunity. The main pathological feature is the infiltration of lymphocytes into exocrine glands such as salivary and lacrimal glands. Previous studies have shown that aquaporin 5 (AQP5), which is located on the cell membranes of secretory glands (e.g., salivary, lacrimal, and sweat glands), plays an important role in their secretory process.[5] Moreover, in recent years, AQP5 expression has been significantly related to the rate of saliva flow, and its abnormal expression and distribution are considered the main cause of SS.[6] Professor Yan Xiaoping, a renowned traditional Chinese medicine rheumatologist who is a Chinese Medicine Grandmaster, noted that liver and kidney yin essence is not the basis of this disease; instead, it results from yin deficiency, endogenous dry heat, dry evil, and other standard external attacks. Thus, there is a need to tonify kidney heat and Yin Yu Tang. A preliminary clinical study demonstrated that Bushen Qingre Yuyin Decoction had significant efficacy in treating pSS.[7] The present study used the pSS spontaneous nonobese diabetic (NOD) mouse model as a research object. NOD mice have a tendency to develop spontaneous insulin-dependent diabetes with autoimmune inflammation throughout the body. Notably, they exhibit a large amount of lymphocyte infiltration in submandibular glands, lacrimal glands, and other organs. These lymphocytes primarily include CD4+ T-cells, with smaller populations of CD8+ T- and B-cells. Therefore, NOD mice are an ideal animal model for the present study that investigated the effects of Bushen Qingre Yuyin Decoction on the submandibular gland and spleen indexes, as well as salivary secretion, T-lymphocyte infiltration of the submandibular gland, and aqp5 mRNA expression in NOD mice.

Materials and Methods

Animals

Fifty 8-week-old specific pathogen-free NOD mice, weighing 20–22 g, and 10 healthy BALB/c mice of the same age, weighing 18–20 g, were provided by Beijing Huakang Experimental Animal Co., Ltd., animal license No.: SCXK (Beijing) 2014–0004. The experimental animals were housed in the animal laboratory of the Clinical Research Institute of China-Japan Friendship Hospital for 12 weeks. They were raised in five cages, maintained at a temperature of 20°C, allowed food and water ad libitum, and acclimatized for 1 week before the experiment. The animal experiment was approved by the Ethics Committee of the China-Japan Friendship Hospital (Batch number: 150008).

Medicine

Bushen Qingre Yuyin Decoction was prepared with the following: Dihuang (Rehmannia Root) 10 g, Shanzhuyu (Macrocarpium Fruit) 12 g, Shanyao (Raw Yam) 15 g, Fuling (Tuckahoe) 20 g, Mudanpi (Moutan Cortex) 10 g, Zelan (Herba Lycopii) 15 g, Zexie (Rhizoma Alismatis) 15 g, Shenggancao (Radix Glycyrrhizae) 10 g, Danzhuuye (Lophatherum gracile) 10 g, Yuanshen (Filipwart Root) 10 g, Tiandong (Radix Asparagi) 12 g, Maidong (Radix Ophiopogonis) 12 g, Tianhuafen (Snakegourd Root) 15 g, Qingfengteng (Caulis Sinomenii Orimenten) 20 g, and Sharen (Fructus Amomi) 10 g. Drugs were prepared by taking the appropriate amount of the abovementioned drug decoction components (the drug use standard refers to the dose converted from the equivalent dose of human [body weight/human clinical drug dose] and mouse) and adding 10-fold amount of ultrapure water. This was followed by stirring and soaking the mixture for 30 min in boiling fire, simmering for 30 min, obtaining the soup juice, filtering residues, and concentrating the solution (while simmering) to obtain final concentrations of 2, 4, and 8 g/mL Bushen Qingre Yuyin Decoction for administering low-, medium-, and high-dose groups, respectively. The decoction was sterilized and stored at 4°C for future use. Chinese medicine decoction ingredients were provided by the Pharmacy Department of the China-Japan Friendship Hospital, and the decoction was prepared by the technical staff of the Pharmacy Department of the China-Japan Friendship Hospital and members of the research team. In addition, in the hydroxychloroquine group, we used hydroxychloroquine sulfate tablets produced by Shanghai Zhongxi Pharmaceutical Co., Ltd.; Specification: 0.1 g/tablet, batch number: H19990263.

Reagents and Instruments

Polymerase chain reaction (PCR) reagents included a spin column RNA extraction kit (Catalog DP431, TIANGEN); a reverse transcription system (Catalog A3500, Promega); a SYRB Green real-time PCR master mix (Catalog QPK-201, TOYOBO); synthetic primers (Invitrogen); a real-time fluorescence PCR instrument (Catalog ABI7500, Invitrogen); DEPC-treated water (Beijing Solabao Technology Co., Ltd.); and a centrifuge (Catalog 5804R, Eppendorf). Histology reagents included paraformaldehyde; hematoxylin-eosin (HE) staining reagent (Sinopharm Group Beijing Chemical Reagent Company); a Millex-GP filter (0.2 μm, Millipore); a rotary tissue dewatering machine (Catalog 4634, SAKURA); a tissue embedding machine (Catalog 5235, SAKURA); a paraffin wax box (Catalog PM401, SAKURA); Shu slicer (Catalog CRM440, SAKURA); and a double microscope (Catalog BH2, OLYMPUS).

Groups

Fifty 8-week-old NOD mice were randomly divided into five groups: model; low-, medium-, and high-dose Bushen Qingre Yuyin Decoction, and hydroxychloroquine. Ten 8-week-old BALB/c mice were used as the control group.

Method of Administration

The low, medium, and high doses of Bushen Qingre Yuyin Decoction were 20, 40, and 80 g/kg, respectively, and these
doses were equivalent to 6, 12, and 24 times the adult dosage. Once daily, the hydroxychloroquine group was intragastrically administered 80 mg/kg hydroxychloroquine in a volume of 0.2 mL. The control and model groups were administered the same volume of normal saline. The duration of treatment for each group was 12 weeks.

**Measurement of submandibular gland and spleen indexes**

After the 12-week treatment, the mice were sacrificed under anesthesia and their organs were weighed at a low temperature to calculate the organ indexes as follows: submandibular gland weight (mg)/mouse body mass (g); thymus index (mg/g) = thymus weight (mg)/mouse body mass (g); and spleen index (mg/g) = spleen weight (mg)/mouse body mass (g).

**Measurement of salivary secretion**

The amount of salivary secretion in mice was evaluated once every 2 weeks (to 20 weeks of mouse). The mice were fasted for 1 h before the test but allowed to drink water. Dry cotton balls were placed within the cheek of the mouse using a tweezer, removed after 5 min, and then weighed on an electronic balance (weight is W2). W2-W1 was recorded as the amount of salivary secretion in mice.

**Morphological observation of submandibular gland tissue**

Conventional hematoxylin and eosin staining was performed. The stained samples were visualized using a 400x microscope and scored under a light microscope by three participants according to the following rules: zero score, occasional lymphocyte infiltration; one score, a few scattered lymphocyte infiltration foci; three scores, zero-one lymphocyte infiltration foci (>50 lymphocytes infiltrated)/five low-power visual fields occasionally observed; and four scores, easy to observe two-three lymphocyte infiltration foci.[8]

**Aquaporin 5 real-time quantitative polymerase chain reaction**

**RNA extraction**

RNA was extracted using the TIANGEN RNA isolation and extraction kits according to the manufacturer’s instructions. A spectrophotometer was used to check the optical density of the RNA to calculate the total RNA concentration and ensure that the purity was within the A260/λ280 ratio of 1.7–2.1.

**Reverse transcription reaction**

cDNA synthesis was performed using the Bio-Rad iScript cDNA synthesis kit and cDNA reverse transcription kit according to the manufacturer’s instructions. Reaction conditions were as follows: 25°C for 5 min, 42°C for 30 min, 85°C for 5 min, and 4°C for 5 min. After the reaction was completed, the cDNA was stored at −20°C.

**Real-time quantitative polymerase chain reaction**

The reaction mixture was prepared as follows: 1 μL of cDNA was added to a 0.2 mL PCR tube, followed by the addition of 1 μL of upstream and downstream primers and 12.5 μL of SYRB Green real-time PCR master mix, and then raising to a final volume of 40 μL with DEPC water. The amplification conditions were as follows: predenaturation at 95°C for 2 min, followed by two-step cycle reaction (95°C for 10 s and 60°C for 1 min) for 40 cycles. The melting curve was 95°C for 15 s, 60°C for 60 s, and 95°C for 15 s. The Gapdh internal reference gene was used to obtain the Ct value of the target gene, and the relative expression level of the gene was expressed as the ΔΔCt value. The primers were synthesized by Invitrogen with the sequences shown in Table 1.

**Statistical analysis**

The results were statistically analyzed using SPSS18.0 statistical software (IBM, SPSS, STATISTICS 18.0, Armonk, New York, U.S.). The measurement data are expressed as x ± standard deviation. Univariate analysis of variance was used to compare the differences between the groups. P < 0.05 was considered to be statistically significant.

**RESULTS**

**General situation of mice**

Before the experiment, there was no difference in body hair, weight, food intake, drinking water, and activity. After 12 weeks of treatment, the normal group of BALB/C mice grew well, and the hair gloss, food intake, and activity were favorable. Some mice in the model group showed weight loss, increased water consumption, dry hair, and slow activity. By the end of the experiment, four mice died. The mice in the Chinese medicine group had slightly darker hair gloss, and their weight, food intake, drinking water, and activity were acceptable. By the end of the experiment, two mice died in each of the low- and medium-dose groups, whereas three mice died in the high-dose group, because improper operation of the gavage resulted in the drug being injected into the trachea. In the hydroxychloroquine group, hair gloss decreased, water intake increased, and their body hair, weight, food intake, and activity levels were acceptable [Table 2].

Organ indexes of the mice are shown in Table 3.

Compared with the spleen index in the model group, that in the high-dose group was significantly greater (P < 0.05). Spleen indexes in the Western medicine and low- and medium-dose groups were also greater than those in the model group, but this difference was not statistically significant. Compared with the spleen index in the low-dose group, that in the high-dose group was significantly increased (P < 0.05).

Compared with submandibular gland index in the model group, that in the hydroxychloroquine group and all the Bushen Qingre Yuyin Decoction groups was significantly greater (P < 0.05). Compared with submandibular gland index in the Western medicine and low-dose group, that in the high-dose group was significantly greater (P < 0.05).

**Comparison of salivary secretion**

With the exception of the control group, salivary secretion in all other groups significantly decreased after the 2nd and 4th weeks of the experiment (P < 0.05). After the 12th week of the experiment,
Table 1: Primer sequences

<table>
<thead>
<tr>
<th>Name</th>
<th>Upstream</th>
<th>Length</th>
<th>Downstream</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP5</td>
<td>TCAATCCGCTCAGCAACAAC</td>
<td>20</td>
<td>AGTGTGACCAGCAAGCCAAT</td>
<td>20</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GTGGACCTCGACCTGCCGTCT</td>
<td>20</td>
<td>GGAGGAGTGGGTGTTGCGGCTG</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 2: General situation of mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Death (number)</th>
<th>Survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Model group</td>
<td>4</td>
<td>60</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Low-dose group</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td>Medium-dose group</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td>High-dose group</td>
<td>3</td>
<td>70</td>
</tr>
</tbody>
</table>

Table 3: Comparison of organ indices among groups (x±s, mg/g)

<table>
<thead>
<tr>
<th>Group</th>
<th>Spleen index</th>
<th>Submandibular gland index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>3.43±0.47</td>
<td>3.67±0.46*</td>
</tr>
<tr>
<td>Model group</td>
<td>3.22±0.28</td>
<td>2.50±0.14</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>3.65±0.11</td>
<td>3.92±0.70*</td>
</tr>
<tr>
<td>Low-dose group</td>
<td>3.26±0.12</td>
<td>3.91±0.51*</td>
</tr>
<tr>
<td>Medium-dose group</td>
<td>3.63±0.124</td>
<td>4.18±0.29*</td>
</tr>
<tr>
<td>High-dose group</td>
<td>4.21±0.20†‡</td>
<td>5.84±0.50†‡</td>
</tr>
</tbody>
</table>

Compared with the model group, *P<0.05; compared with the low-dose group, †P<0.05; compared with the hydroxychloroquine group, ‡P<0.05

Discussion

pSS has no corresponding disease name in Chinese medical literature, and thus, it is described as “dryness syndrome,” “sickness syndrome,” and “dryness,” based on its clinical manifestations. In recent years, the National Committee of Traditional Chinese Medicine has proposed the name “dryness.” It is more appropriate to attribute the Sjogren’s syndrome to the name “dryness,” as it can guide clinical practice. Professor Yan Xiaoping has speculated that the disease is located in the lung, spleen, stomach, and particularly, the liver and kidney. The pathogenesis is yin deficiency, whereas heat is the standard. As treatment, Professor Yan Xiaoping proposed the use of kidney and heat on the basis of years of clinical experience with respect to condensing. The method of Yin Yin is based on the method of tonifying kidney and yin and supplementing with warm kidney yang to promote the formation of kidney yin. This approach should take into consideration the clearing of heat and yin, accompanied by Shengjin Runzao and clinically banned bitter cold. Concomitantly, venous stagnation, poor blood, and blood flow can be adjusted in the spleen and lungs using addition and subtraction, accompanied by Huoxuetongluo, in combination with the source of the disease. Professor Yan Xiaoping created Bushen Qingre Yuyin Decoction, which could replenish the liver and kidney, clear heat and nourish yin, and make the water biochemically active, infuse the chemistry regularly.

The main drugs for tonifying kidney and clearing Yin Yin are Dihuang (Rehmannia Root), Shanzhuyu (Macrocarpium Fruit), Shanyao (Raw Yam), Fuling (Tuckahoe), Mudanpi (Moutan Cortex), Zexie (Rhizoma Alismatis), Zelan (Herba Lycopii), Maidong (Radix Ophiopogonis), Tiandong (Radix Asparagi), Yuanshen (Figwort Root), Tianhuafen (Snakegourd Root), and Sharen (Fructus Amomi). This prescription uses Liwei Dihuang Pill as a monarch drug, thus nourishing the liver and kidney. With respect to the use of Dihuang for nourishing kidney yin, Shanzhuyu is used to nourish the liver and kidney, Shanyao spleen yin is used to solidify the kidney, and Zexie can clear damp and promote diuresis. Fuling can relieve heat deficiency, cool liver, relieve Yin and subdue fire, and constraint Shanzhuyu’s warmth and astringency.

The results of real-time PCR showed that the aqp5 expression in the submandibular gland of the model group was lower than that in the normal group; however, this difference was not statistically significant. On intervention with Bushen Qingre Yuyin Decoction, the expression level of aqp5 in the submandibular gland significantly increased. Compared with that in the model group, the expression levels in the Western medicine and high-dose groups were significantly different (P < 0.05), and the effect in the high-dose group was optimal [Figure 3].
moistens the Shanyao spleen, but also purges kidney turbidity together with Zexie, thus enabling the true yin to recover its position. Supplemented with Maidong for clearing heat and moistening the lung, generation between the metal and water, Tiandong for nourishing yin to moisten dryness, Yuanshen for tonifying kidney and decreasing internal heat as a minister, and Tianhuafen for clearing heat and purging fire, help produce saliva and slake thirst; in addition, Zelan can be used for water swelling and can promote blood circulation. Qingfengteng dispels wind dampness by moving through channels and collaterals. Shenren prevents dampness and stagnation and guides medicines to the kidney. Medicines can be used in combinations such as nourishing and not cold, warm and not dry, nourishing without leaving evil, reducing sputum without hurting the positive, tonifying the kidney and clearing heat, Yu Yin and moistening dryness, promoting blood circulation and collaterals, and preserving fluids and slow drying.

A previous multicenter randomized controlled clinical study showed that the use of Bushen Qingre Yuyin Decoction can effectively improve the symptoms of dry mouth and dryness in patients with kidney, qi, and yin deficiencies. This approach could inhibit immune inflammation.[7,10-13] The results of the present study showed that the Bushen Qingre Yuyin Decoction and Western medicine treatments could significantly improve salivation in NOD mice. After the 6th week of experiment, salivary secretion was significantly increased in the hydroxychloroquine

Table 4: Saliva secretion in each group of mice (average value, mg)

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 6</th>
<th>Week 8</th>
<th>Week 10</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>0.017</td>
<td>0.015</td>
<td>0.016</td>
<td>0.015</td>
<td>0.016</td>
<td>0.016</td>
</tr>
<tr>
<td>Model group</td>
<td>0.011*</td>
<td>0.011*</td>
<td>0.009</td>
<td>0.008</td>
<td>0.009</td>
<td>0.01</td>
</tr>
<tr>
<td>Hydroxychloroquine group</td>
<td>0.016*</td>
<td>0.012*</td>
<td>0.011*</td>
<td>0.012*</td>
<td>0.013*</td>
<td>0.014*</td>
</tr>
<tr>
<td>Low-dose group</td>
<td>0.013*</td>
<td>0.012*</td>
<td>0.014*</td>
<td>0.013*</td>
<td>0.013*</td>
<td>0.018*</td>
</tr>
<tr>
<td>Medium-dose group</td>
<td>0.016*</td>
<td>0.013*</td>
<td>0.013*</td>
<td>0.017*</td>
<td>0.014*</td>
<td>0.028*</td>
</tr>
<tr>
<td>High-dose group</td>
<td>0.015*</td>
<td>0.012*</td>
<td>0.011*</td>
<td>0.013*</td>
<td>0.016*</td>
<td>0.026*</td>
</tr>
</tbody>
</table>

Compared with the normal group, *P<0.05; Compared with the model group, #P<0.05; Compared with the hydroxychloroquine group, △P<0.05

Figure 1: Hematoxylin-eosin staining of the submandibular gland (×400). NG: Control group; MG: Model group; HCQG: Hydroxychloroquine group; LDG: Low-dose Bushen Qingre Yuyin Decoction group; MDG: Medium-dose Bushen Qingre Yuyin Decoction group; HDG: High-dose Bushen Qingre Yuyin Decoction group

Figure 2: Hematoxylin-eosin staining pathological score. Compared with the normal group, *P < 0.05; compared with the model group, #P < 0.05

Figure 3: H&E stain of control, model, positive, Bushen Qingre Yuyin Decoction group (L), (M), (H).
group and all the Bushen Qingre Yuyin Decoction groups. With extended administration, the improvement increased. At the end of the 12th week of experiment, the amount of salivary secretion in the medium- and high-dose Bushen Qingre Yuyin Decoction groups has significantly increased, relative to that in the hydroxychloroquine group. Thus, Bushen Qingre Yuyin Decoction can significantly increase the amount of salivary secretion in mice and improve dry mouth symptoms.

The spleen is one of the most important immune organs in the human body. It is rich in lymphocytes and macrophages and has a close relationship with humoral immunity. The spleen index can reflect its developmental and functional status and indirectly reflect the strength of the body’s immune function to a certain extent. Therefore, the spleen index has been commonly used as an objective indicator for assessing immune function. Testing the spleen index can also help in understanding the drug’s immunity function, especially the influence of cellular immunity. In the present study, the spleen index of mice in the high-dose Bushen Qingre Yuyin Decoction group was higher than that in the model group, and the submandibular gland index of the model group was significantly greater, and the high-dose group had the best effect on its expression. Aqp5 expression was significantly increased in all the Bushen Qingre Yuyin Decoction groups, indicating that Bushen Qingre Yuyin Decoction can significantly increase aqp5 expression in the submandibular gland of NOD mice. Notably, aqp5 content was negatively correlated with the lymphocyte infiltration score of the labial gland, which is consistent with the findings of a prior study. [17] These findings suggest that aqp5 may control the production and composition of saliva, regulate salivary secretion by increasing the permeability of salivary gland acinar cell membrane and volume of individual cells, and modulate lymphocytic inflammation within the labial glands. The microenvironment formed by lymphocyte-secreted inflammatory factors within the labial gland may contribute to the reduction in aqp5 expression at the acinar cell surface.

There were some limitations to this study. Reportedly, aqp5 is involved in salivary gland dysfunction in pSS, but the specific distribution of aqp5 in acinar cells has not been thoroughly studied. Furthermore, there is a need to analyze the humoral factors and/or cytokines that lead to unique changes in aqp5.

AQP5 is the main water channel protein involved in salivary secretion in mice and improve dry mouth symptoms.
CONCLUSION
In summary, the present study showed that Bushen Qingre Yuyin Decoction can effectively improve the spleen and submandibular gland indexes in NOD mice. Moreover, it can increase salivary secretion, reduce lymphocytic infiltration in submandibular gland tissue, and significantly increase aqp5 expression. Importantly, these benefits may be dose dependent.

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Conflicts of interest
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