Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Dendrobium officinale Kimura et Migo Improved Dry Eye Symptoms via Promoting Tear Production in an Experimental Dry Eye Rat Model

Qiang Zeng[1,2,3], Wing-Sum Siu[1,2], Chun-Hay Ko[1,2], Chun-Wai Wong[1,2], Clara Bik-San Lau[1,2], Zheng-Zhi Wu[3], Jiang-Miao Hu[4], Ping-Chung Leung[1,2]

[1]Institute of Chinese Medicine, The Chinese University of Hong Kong, [2]State Key Laboratory of Phytochemistry and Plant Resources in West China, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, [3]Shenzhen Institute of Geriatrics, The First Affiliated Hospital of Shenzhen University (Shenzhen Second People's Hospital), Shenzhen, Guangdong,, [4]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, China

Submitted: 30-Sep-2019 Revised: 11-Nov-2019 Accepted: 17-Feb-2020 Published: 15-Jun-2020

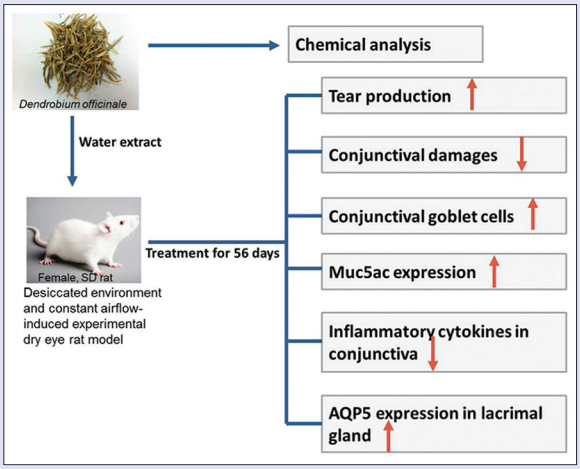
ABSTRACT

Objectives: To evaluate the ameliorative effect of *Dendrobium officinale* Kimura et Migo (DO) on a desiccated environment-induced experimental dry eye rat model and elucidate its underlying mechanisms. **Materials and Methods:** The Sprague-Dawley rats were kept in low-humidity environment and received constant airflow for 8 weeks to establish the experimental dry eye model. DO water extract (DOW, 372 mg/kg/day) was orally administered daily for 8 weeks. Schirmer's test was used to measure the tear fluid production at days 0, 14, 28, 42, and 56. At the end of experiment, lacrimal gland tissues and eyeballs were collected for hematoxylin and eosin staining, PAS staining, and immunohistochemical staining. Inflammatory cytokines in conjunctiva including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and matrix metalloproteinase 9 were measured by real-time PCR. The aquaporin 5 (AQP5) expression in lacrimal gland was also determined using Western blot assay. **Results:** DOW treatment (DOWT) increased the tear production of rats significantly in the desiccated environment at day 42. Histological analysis revealed that DOW could reverse destruction of conjunctiva and increase goblet cell number and mucin expression in the experimental dry eye rats. In dry eye rats, desiccated environment and constant airflow induced TNF-α and IL-1β production in the conjunctiva, whereas DOWT reversed the upregulation of proinflammatory cytokines. Moreover, DOWT increased the expression of AQP5 at protein level in the lacrimal gland tissues in both desiccated and normal environmental conditions. **Conclusion:** The present study suggests that DO has therapeutic potential on dry eye symptoms through upregulating AQP5 expression, increasing tear production, inhibiting conjunctiva destruction and inflammation, as well as promoting mucin production.

Key words: Conjunctival goblet cell, *Dendrobium officinale*, dry eye disease, inflammation, tear production

SUMMARY

- Long-term stay in air-conditioned or desiccated environment could result in conjunctiva destruction and dry eye symptoms. Oral administration of *Dendrobium officinale* Kimura et Migo showed great potential in improving experimental dry eye symptoms via increasing tear fluid, inhibiting conjunctiva destruction and inflammation, restoring the number of conjunctival goblet cells, as well as promoting mucin production.



Abbreviations used: DO: *Dendrobium officinale* Kimura et Migo; DOW: Water extract of *Dendrobium officinale* Kimura et Migo; DED: Dry eye disease; AQP5: Aquaporin 5; MCP-1: Monocyte chemoattractant protein-1; IL-6: Interleukin-6; IL-1β: Interleukin-1β; TNF-α: Tumor necrosis factor-α; MMP9: Matrix metalloproteinase 9.

Correspondence

Dr. Ping-Chung Leung,
5/F, The CUHK Hong Kong Jockey Club, School of Public Health Building, Prince of Wales Hospital, Shatin, New Territories, Hong Kong, China.
E-mail: pingcleung@cuhk.edu.hk
Dr. Jiang-Miao Hu,
Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, China.
E-mail: hujiangmiao@mail.kib.ac.cn
Dr. Zheng-Zhi Wu,
Shenzhen Institute of Geriatrics, The First Affiliated Hospital of Shenzhen University (Shenzhen Second People's Hospital), Shenzhen, Guangdong, China.
E-mail: szwzz001@email.szu.edu.cn
DOI: 10.4103/pm.pm_435_19

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Dry eye disease (DED) is one of the common eye diseases in terms of tear film instability arising from decreased tear secretion or excessive tear evaporation, with essential damage to the ocular surface.[1] Although DED is not lethal, it still strongly affects quality of life of 6%–34% people in the whole world population.[1] With the changing

Cite this article as: Zeng Q, Siu WS, Ko CH, Wong CW, Lau CB, Wu ZZ, *et al.* *Dendrobium officinale* Kimura et Migo improved dry eye symptoms via promoting tear production in an experimental dry eye rat model. Phcog Mag 2020;16:294-302.

in living environment, more and more people are suffering from DED due to excessive video display viewing and reading, long-term stay in air-conditioned environment, and long-term contact lens wearing.[2-5] Tears contain various ingredients which are produced from the lacrimal gland, meibomian gland, cornea, and conjunctiva. These tear ingredients form the tear film to cover ocular surface and exert the function of moistening and protecting the eyes against the changing environmental conditions.[6] Classically, the tear film consists of three layers including inner mucin layer, middle aqueous layer, and outer lipid layer. Specifically, the inner mucin layer is hydrophilic and produced by conjunctival goblet cells. The middle aqueous layer is secreted by lacrimal gland, whereas the outer lipid layer is generated by the meibomian gland.[7] As a result, any conditions destroying the tear film could induce either aqueous deficiency DED or high evaporative DED.

Aquaporins (AQPs) are a group of water-transporting proteins mainly expressed in exocrine glands such as salivary gland, lacrimal gland, and sweat gland. They contribute to local fluid production.[8,9] AQP5 is found to be expressed in lacrimal gland and located at the apical membrane of acinar and ductal cells which are responsible for fluid secretion to maintain tear volume in ocular surface.[8] Increasing data provide evidences to support the involvement of AQP5 in tear and saliva secretion. For example, the abnormal expression of AQP5 was observed in the lacrimal gland and salivary gland tissues of Sjögren's syndrome.[10-12] The current mainstay therapy for DED is the application of artificial tears. Nonetheless, they have palliative effect only, but do not affect the underlying pathophysiology of dry eye.[13] Recently, novel therapeutic strategies to treat DED by targeting on AQPs, especially AQP5, have attracted increasing attentions.[14]

Dendrobium officinale Kimura et Migo (DO) is a precious herbal medicine widely used in East Asia, especially in China. According to the theory of Traditional Chinese Medicine (TCM), it is commonly used to treat the state of '*Yin*' deficiency for its diverse effects of enhancing body fluid production, reinforcing stomach, nourishing *Yin,* and removing heat.[15] Recent studies revealed that DO was capable of relieving dry mouth symptom through increasing saliva secretion and improve AQP5 expression in labial gland.[16,17] In some clinical studies, the formulae containing *Dendrobium* were demonstrated to be effective in treating dry eye patients.[18,19] However, the protective effect of DO on DED and the underlying mechanisms have not been well elucidated. Therefore, the present study aimed to evaluate the efficacy of DO in treating DED on an experimental dry eye rat model induced by desiccated environmental stress. We also aimed to investigate the underlying mechanisms involved.

MATERIALS AND METHODS

Chemical and reagents

The DO herbs were collected from Pu er in Yunnan Province of China and authenticated by Professor Jiangmiao Hu at Kunming Institute of Botany, Chinese Academy of Sciences, China. The raw herb was deposited in the museum of the Institute of Chinese Medicine, the Chinese University of Hong Kong (CUHK), with voucher specimen number as 3542. PAS staining kit was obtained from Sigma-Aldrich (St. Louis, USA). Primary antibodies of Muc5ac and AQP5 were purchased from Thermo (Waltham, USA) and Abcam (Cambridge, UK), respectively. The primers of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, matrix metalloproteinase 9 (MMP9), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from BGI Company (Shenzhen, China). All reagents and solvent for chemical study were from Sinopharm (Shanghai, China).

Preparation of herbal extract and chemical analysis

The air-dried DO was pulverized and extracted twice with distilled water (1:10, w/v) for 2 h. The combined aqueous extraction solution was filtered and centrifuged at 1500 rpm to remove undissolved powder. The final water extract of *D. officinale* (DOW) was lyophilized and stored at −20°C for further usage.

The total polysaccharides and protein contents in DOW were determined by chemical reactions. The total polysaccharides were quantified by spectrophotometric phenol-sulfuric acid assay. Briefly, phenol solution (5%) and five-fold volume of sulfuric acid were added into DOW. The mixtures were reacted in boiling water bath for 20 min and then cooled on ice for another 5 min. The OD value was collected at 488 nm by UV-Vis spectrophotometer (BioTek, USA). Serial concentrations of glucose solution were used as standards.

The total protein content was detected by bicinchoninic acid-copper sulfate assay (BCA) using bovine serum albumin (BSA) as standard. According to the manufacturer's instructions, the DOW solution and the equal volume of bicinchoninic acid-copper sulfate solution (50:1, v/v) were added into the same well. After 10-min reaction at 50°C, the OD value was read at 540 nm by a microplate reader (BioTek, USA).

Establishment of experimental dry eye rat model

All animal experiments were performed according to the Animals (control of experiments) Ordinance of Hong Kong, and the protocol was approved by the Animal Experimentation Ethics Committee of the CUHK (Ref: 14-087-MIS). Female Sprague-Dawley rats (200–220 g) were obtained from the Laboratory Animal Services Center of CUHK. The untreated animals were kept in the normal conditions with controlled light (12 h light/12 h dark), ambient temperature at 24°C ± 1°C, and relative humidity at 55%–60%. The rats for the dry eye model were placed in a low-humidity chamber with a relative humidity of 24% ± 2%, temperature of 23°C ± 3°C, and received constant airflow for 8 weeks. The desiccated environment and constant airflow could induce high evaporation of tear fluid and dry eye symptoms could be observed on the rats after a long exposure. The low-humidity chamber was made with reference to the controlled-environment chamber reported by Barabino *et al.*[20]

Groups and treatment protocols

The rats were randomly divided into four groups ($n = 6$ in each group). The rats in the normal control group (Normal) and DOW control group (DOWC) were housed in normal conditions. The rats in the vehicle (distilled water) control group (Model) and DOW treatment group (DOWT) were kept in a low-humidity chamber. Rats in the DOWC and DOWT groups were orally administered with DOW (372 mg/kg/day) daily for 8 weeks, whereas rats in the normal and model groups were administered with the equal volume of distilled water instead of DOW. During the experimental period, body weight and tear production of rats were monitored biweekly.

Tear fluid production

The tear fluid production was measured by Schirmer's test. Briefly, the rats were anaesthetized with pentobarbital (50 mg/kg, i.p., Alfasan, Holland). A standard Schirmer's test strip (Jingming New Technological Development Company, Tianjin, China) was placed in the temporal one-third of the lower eyelid and stayed for 5 min. The length of the wet portion on the strip was then measured. The measurement at day 0 acted as the baseline.

Histological analysis of lacrimal gland, cornea, and conjunctival goblet cells

At the end of experiment, lacrimal gland tissues and eyeballs were collected and fixed in 10% neutral formalin. After embedding in paraffin, the tissues were sectioned at 5 μm. Hematoxylin and eosin (H and E) staining was performed according to routine procedure.

PAS staining was used for the demonstration of glycoproteins in conjunctival goblet cells. Briefly, the slides were dewaxed in xylene and rehydrated in a series of ethanol. Then, the periodic acid solution was added onto the slides, incubating for 5 min. Schiff's reagent was added onto the slides for another 15 min following rinsing off periodic acid with tap water. The nuclei were counterstained in hematoxylin. The number of conjunctiva goblet cells was counted in ×200 microscopic fields. Two slides were used and two fields of each section were analyzed.

Detection of Muc5ac expression in conjunctiva

Immunohistochemical staining was performed to test the expression of Muc5ac in conjunctiva. Briefly, the sectioned tissues were dewaxed in xylene and rehydrated in a series of ethanol. Antigen heat retrieval was performed in rodent Decloaker reagent (Biocare Medical, USA). After washing with TBST, 0.3% hydrogen peroxide was used to inactivate peroxidase in the tissue. The sections were rinsed with TBST and blocked with 10% normal goat serum (Abcam, UK) for 1 h. Then, the primary antibody against Muc5ac (1:100, Thermo Fisher, USA) was added on the section and incubated overnight at 4°C. After washing three times with TBST, goat secondary antibodies against rabbit and mouse immunoglobulins coupled with peroxidase (Dako, Denmark) were added to slides and incubated for 1 h at room temperature. The chromogenic reaction-locating antigen was performed by Dako REAL™ DAB chromogen diluted in substrate buffer (Dako, Denmark). Finally, the nuclei were counterstained in hematoxylin. Tissues stained without primary antibody were used as negative control. The quantification of Muc5ac expression was performed by ImageJ software and expressed as percentage of total area.

Gene expression of inflammatory cytokines in conjunctiva by real-time polymerase chain reaction

DEDs are usually accompanied with inflammation in conjunctiva. Inflammatory cytokines are usually associated with conjunctival goblet cell apoptosis, epithelial cell metaplasia, and tissue destruction. Therefore, the messenger RNA (mRNA) levels of inflammatory cytokines including IL-1β, IL-6, TNF-α, and MMP9 in conjunctiva were measured. According to the manufacturer's instruction, total RNA in conjunctiva was extracted using TRIzol reagent (Invitrogen, Italy) and TransZol UP Plus RNA Kit (TransGen Biotech, China). Real-time polymerase chain reaction (RT-PCR) was performed using QuantiFast™ SYBR Green RT-PCR Master Mix (Qiagen, German). Briefly, 100 ng of total RNA was amplified in a 35 μl reaction system. PCR reactions were carried out in triplicates using Qiagen Quick 96-well SYBR only protocol: 1 cycle of 50°C for 10 min and 95°C for 5 min, 49 cycles of 95°C for 10 s, 60°C for 30 s, and 72°C for 30 s, followed by 1 cycle of 95°C for 1 min. Rat GAPDH was used as a housekeeping gene for quantity normalization. The fold changes of gene expression among groups were normalized to rat GAPDH and calculated by the comparative threshold method.

Aquaporin 5 expression in lacrimal gland by western blotting

Total proteins in lacrimal glands were extracted with radioimmunoprecipitation buffer containing protease inhibitor cocktail (Roche, Switzerland). The protein concentration was determined by BCA assay. About 80 μg of protein was subjected onto 10% resolving sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel and 5% stacking gel. Then, the protein was transferred to methanol-activated PVDF membrane. After blocking with 5% skim milk in PBS for 1 h, the membrane was incubated with primary antibody against AQP5 (1:1000) overnight at 4°C. After removing primary antibody and washing the membrane with Tris-buffered saline adding 0.1% Tween-20 (TBST), the secondary antibody was added and incubated for 2 h. The unconjugated secondary antibody was washed with TBST three times. Subsequently, the signal was developed using chemiluminescence ECL assay Kit (GE Healthcare, USA) and the chemiluminescent bands were captured on a Bio-Rad ChemiDoc™ XRS + imaging system. β-actin was served as a control and protein expression levels were normalized to β-actin.

Statistical analysis

All data were expressed as mean ± standard error of the mean and analyzed with GraphPad Prism 5.0 (GraphPad Software, San Diego, USA). One-way ANOVA with *Bonferroni* test was used to examine differences among groups. $P < 0.05$ was considered statistical significance.

RESULTS

Chemical analysis of water extract of *Dendrobium officinale* Kimura et Migo

Chemical analysis showed that DOW mainly contained polysaccharides and protein. The contents of total polysaccharides and protein in DOW were calculated as 73.1% (w/w) and 5.2% (w/w), respectively, according to standard curves.

Changes of body weight

All rats had stable increase in body weight within 8 weeks. The rats in the desiccated environment increased slower than those of rats in normal conditions, but without significant difference among groups [Figure 1a]. In addition, the rats in the desiccated chamber demonstrated normal behavior. This observation implied that the rats staying in the desiccated environment for 56 days might not affect their basal living condition.

Water extract of *Dendrobium officinale* Kimura et Migo promoted tear production in dry eye rats

The results of the Schirmer's test showed that the rats in the model group had remarkable decrease of tear production at day 42 and day 56 compared with those in the normal group. This indicated that desiccated stress and constant airflow induced dry eye symptom on the rats successfully. DOWT for 42 days showed a significant increase of tear production in dry eye rats (DOWT group vs. model group, $P < 0.05$), whereas no significant difference was found when the DOWC group was compared with the normal group ($P > 0.05$). These data suggested that oral administration of DOW could promote tear fluid production in rats with DED, but not in normal rats [Figure 1b].

Histological changes of lacrimal gland, cornea, and conjunctiva

The representative sections of lacrimal gland, cornea, and conjunctiva by H and E staining are shown in Figure 2. Typical histology of lacrimal gland showed the tubule-acinar structure with a regular shape for acinar cells and basally located nuclei. However, mild inflammatory changes with some infiltrating plasma cells were observed in the lacrimal gland of dry eye rats (model group). The lacrimal glands of rats in other groups showed normal structure of acinar cells [Figure 2a]. The cornea showed normal cell layers, without alterations in epithelial morphology [Figure 2b]. In addition, there were intact goblet cells inserting in conjunctival

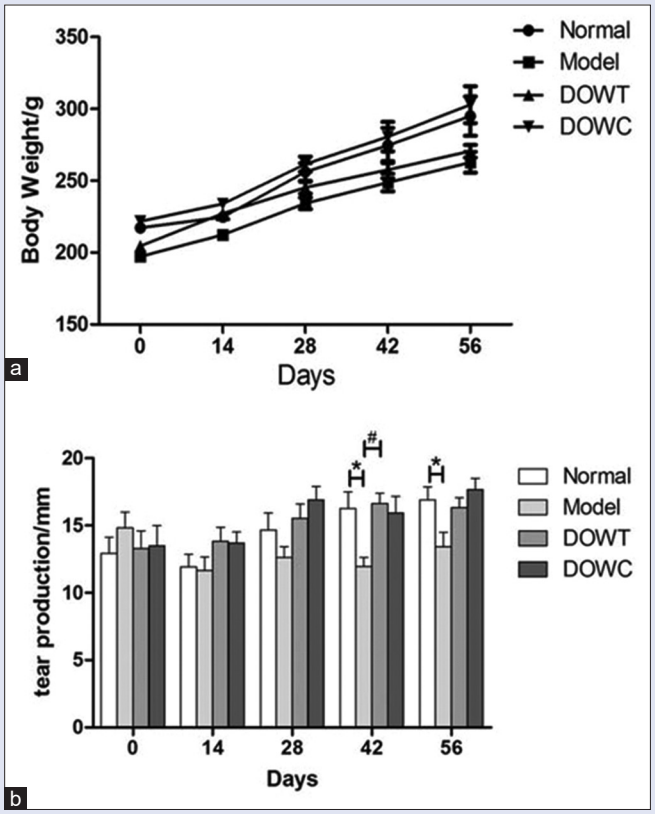


Figure 1: Changes of body weight and tear production. (a) Changes of body weight. The body weight of rats in all the groups increased stably and was not affected by the desiccated stress and constant airflow blowing. (b) Changes of tear production. Oral administration of DOW promoted tear production in dry eye rats at day 42 (DOWT). Data were expressed as mean ± standard error of the mean from six rats in each group. $^{*}P < 0.05$ when compared with normal control group, $^{\#}P < 0.05$ when compared with model group

epithelium in the rats placed in normal conditions. However, structure destruction of conjunctival epithelium was observed in the rats placed in the desiccated environment and received constant airflow(model group). DOWT reversed destruction of conjunctiva slightly [DOWT vs. model group, Figure 2c].

Water extract of *Dendrobium officinale* Kimura et Migo increased goblet cell number and muc5ac expression in conjunctiva of dry eye rats

PAS staining demonstrated that the number of goblet cells distributed in conjunctival epithelium was significantly decreased in the model group. The conjunctival goblet cells showed remarkable higher density in DOWT group than that of model group. In addition, goblet cells in DOWC group showed a similar density compared to the normal control group, indicating that DOWT could prevent from conjunctival goblet cells loss in the rats with DED but not in healthy rats [Figure 3a and b].

A significant reduction of Muc5ac expression was observed in the conjunctival epithelium of dry eye rats (model group vs. normal group, $P < 0.05$) [Figure 3c and d]. However, oral administration of DOW increased the expression level of Muc5ac in the conjunctival epithelium of dry eye rats evidently (DOWT group vs. model group, $P < 0.05$), suggesting that DOWT could prevent from the loss of mucin expression in the desiccated environment.

Water extract of *Dendrobium officinale* Kimura et Migo inhibited gene expression of inflammatory cytokines in conjunctiva of dry eye rats

As shown in Figure 4, the mRNA levels of IL-1β, IL-6, and TNF-α increased by about 25-folds, 3-folds, and 1.7-folds, respectively in the conjunctiva of dry eye rats induced by desiccated environment and constant airflow (model group vs. normal group). However, DOWT for 8 weeks significantly inhibited mRNA levels of IL-1β and TNF-α in dry eye rats. However, DOWT could not significantly suppress the mRNA level of IL-6 compared with the model group. It was noteworthy that the mRNA level of MMP9 did not have obvious changes after desiccated environment induction and DOWT.

Water extract of *Dendrobium officinale* Kimura et Migo promoted AQP5 expression in lacrimal gland

To evaluate the relationship between DOW-treated tear production and AQP5, the total AQP5 expression level in lacrimal gland was detected in the current study. As shown in Figure 5, the AQP5 levels in the total lysates of lacrimal gland showed no significant difference between normal group and model group. However, the results demonstrated that the AQP5 levels after DOWT increased evidently both in normal rats and desiccated stress-induced dry eye rats (DOWC group vs. normal group, $P < 0.01$, DOWT group vs. model group, $P < 0.05$). These results implied that DOW could promote tear production in DED possibly relating to the upregulation of AQP5 levels in lacrimal gland.

DISCUSSION

Nowadays, the incidence of DED is gradually increasing worldwide. More and more people are suffering from this chronic disease with a descending quality of life.[2] Currently, medical cares for DED is palliative because the pathological mechanisms of DED have not been clearly understood. It is of great importance to develop effective treatments or health supplements for this disease. With respect to low toxicity and whole system actions, TCM has drawn more and more attentions. Modern pharmacological and biological evaluation revealed that several Chinese Medicines had promising results and great potential in relieving DED through different working mechanisms such as inhibiting inflammation and increasing tear production.[21-23] According to the theory of TCM, DED is defined as *Zaozheng* resulting from *Yin* deficiency. Therefore, herbal medicines with functions of nourishing *Yin* and promoting body fluid production were usually used to treat DED.[24]

DO is a famous Yin-nourishing herb in the history of TCM and mainly contains polysaccharides. Water-soluble polysaccharides from DO exhibited diverse activities, particularly immunoregulatory and antioxidative effects.[25-27] Other ingredients such as flavonoids and sterols in the water extract of DO had antiosteoporotic effect.[28] Recently, polysaccharides of DO were demonstrated to ameliorate inflammation and apoptosis in mouse salivary gland and to promote AQP5 expression in the patients with Sjögren's syndrome.[17] In addition, the glucosides isolated from peony could upregulate AQP5 expression in salivary gland and improve pathological damages in the salivary gland of Sjögren's syndrome in mouse, but the AQP5 expression in lacrimal gland was not detected.[22] These reports indicated that polysaccharides or glucosides in herbs played important roles in improving dry eye and dry mouth symptoms. In the present study, chemical analysis showed that DOW used in our study contained 73.1% (w/w) polysaccharides and 5.2% (w/w) protein. Thus, it is speculated that polysaccharides may serve as the main active ingredients in the DOW to prevent dry eye. At present, the efficacy of DO intervention on DED remains unclear. Therefore, the curative effect of aqueous extract of DO on DED was investigated

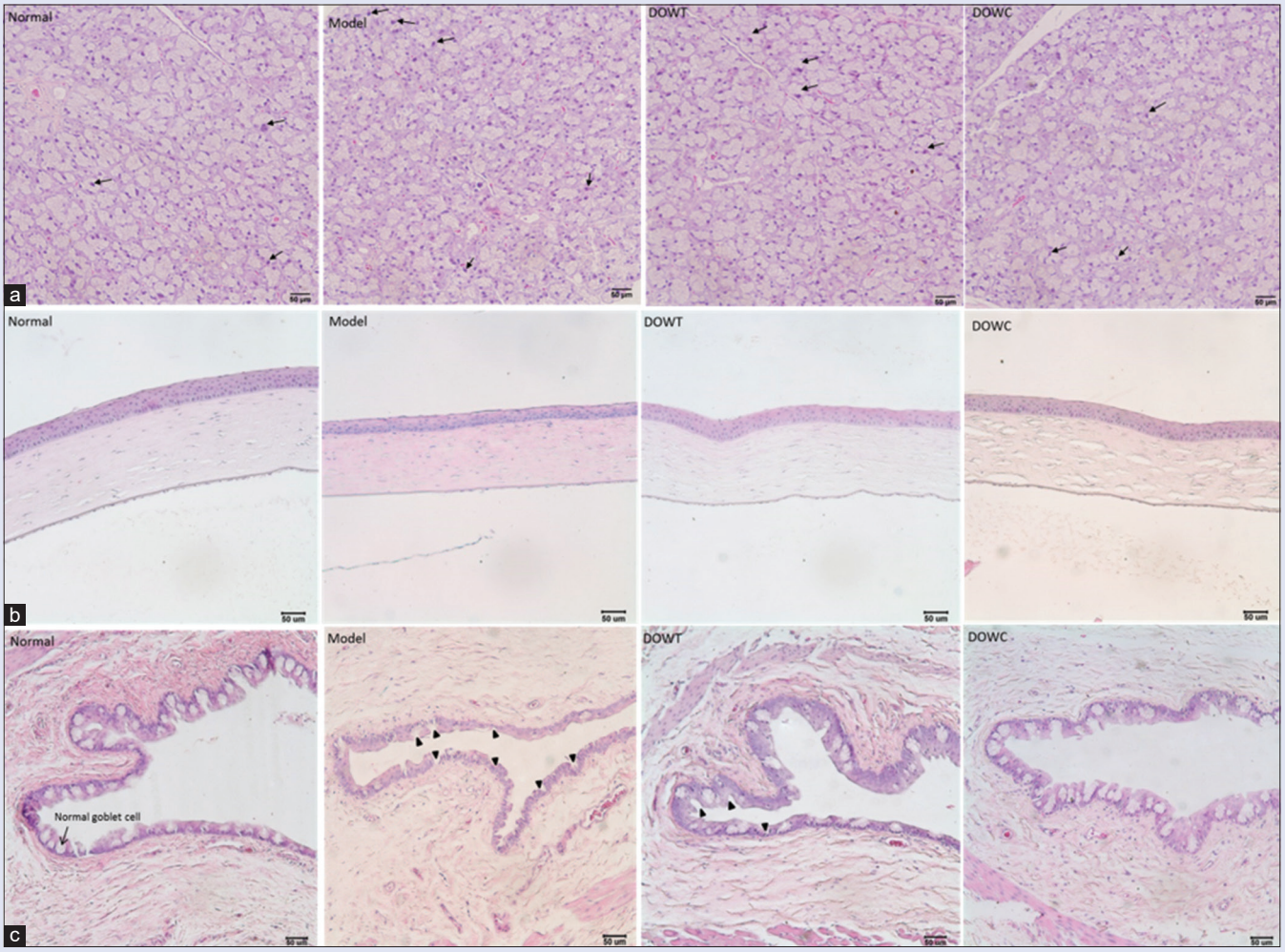


Figure 2: Morphological changes of lacrimal gland, cornea and conjunctiva by hematoxylin and eosin staining. (a) Morphology of lacrimal gland. (b) Morphology of cornea. (c) Morphology of conjunctiva. The conjunctiva showed structure destruction in conjunctival epithelium of model group rats, which relieved slightly after water extract of *Dendrobium officinale* Kimura et Migo treatment. Arrowheads showed destruction of goblet cells. Original magnification, ×200, scale bar: 50 μm

using experimental dry eye rat model in this study. We found that DOW could increase tear production, inhibit the destruction of conjunctival goblet cells and inflammation in conjunctiva, as well as restore Muc5ac expression.

The pathogenesis of DED is tightly associated with two basic changes of ocular surface: aqueous deficiency because of reduced tear production and excessive evaporation due to desiccated environment or alteration of tear film.[29] The selected animal model of the current study greatly and stably manipulated the environmental factors responsible for dry eye symptoms including destroyed tear film, reduced goblet cell number, and destruction in the corneal and conjunctival epithelium.[30,31] In addition, the mRNA levels of pro-inflammatory cytokines such as IL-17, IL-23, IL-6, IL-1β, and TNF-α were higher in the conjunctiva of desiccated environment-induced dry eye animal model than those of normal mice, which was in line with our present results.[32] Moreover, this model can be used to study the exocrine secretion effect of lacrimal gland because environmental factors usually affect ocular surface but rarely affect lacrimal gland.

Conjunctival goblet cells play an important role in maintaining ocular health because they produce mucins to form the inner layer of tear film, which keeps the eye wetting and prevents adhesion of pathogens.[7] Environmental stresses such as low humidity and constant airflow make the tears highly evaporated and conjunctival epithelium vulnerability to irritation.[33] Thereby, the morphological destructions such as inflammatory cascades and apoptosis of corneal and conjunctival cells are triggered, leading to further cell death. The loss of conjunctival goblet cell exacerbates tear film destruction and drives the vicious cycle of dry eye condition.[34] PAS staining is a commonly used method to visualize conjunctival goblet cell in numerous studies.[35,36] The number of conjunctival goblet cells is correlated with the density of PAS-positive cells. The destruction of conjunctival epithelium and loss of goblet cells were observed in the current study, as evidenced by desiccated environment-induced dry eye animal model in the previous studies.[20,30] Muc5ac, one of the major secreted large gel-forming mucins, acts as a surfactant for the ocular surface and comprises the inner layer of tear film to maintain the hydrophobic epithelium wet and function.[37] The previous studies have unveiled the relationship between Muc5ac expression in the tears and dry eye symptoms. In fact, the dry eye patients had an evidently lower Muc5ac concentration in their tears than in healthy individuals.[38] In the present study, the expression of Muc5ac in conjunctiva was detected by immunohistochemistry staining and semiquantified by measuring its positive area. It was intriguing that the expression of Muc5ac significantly

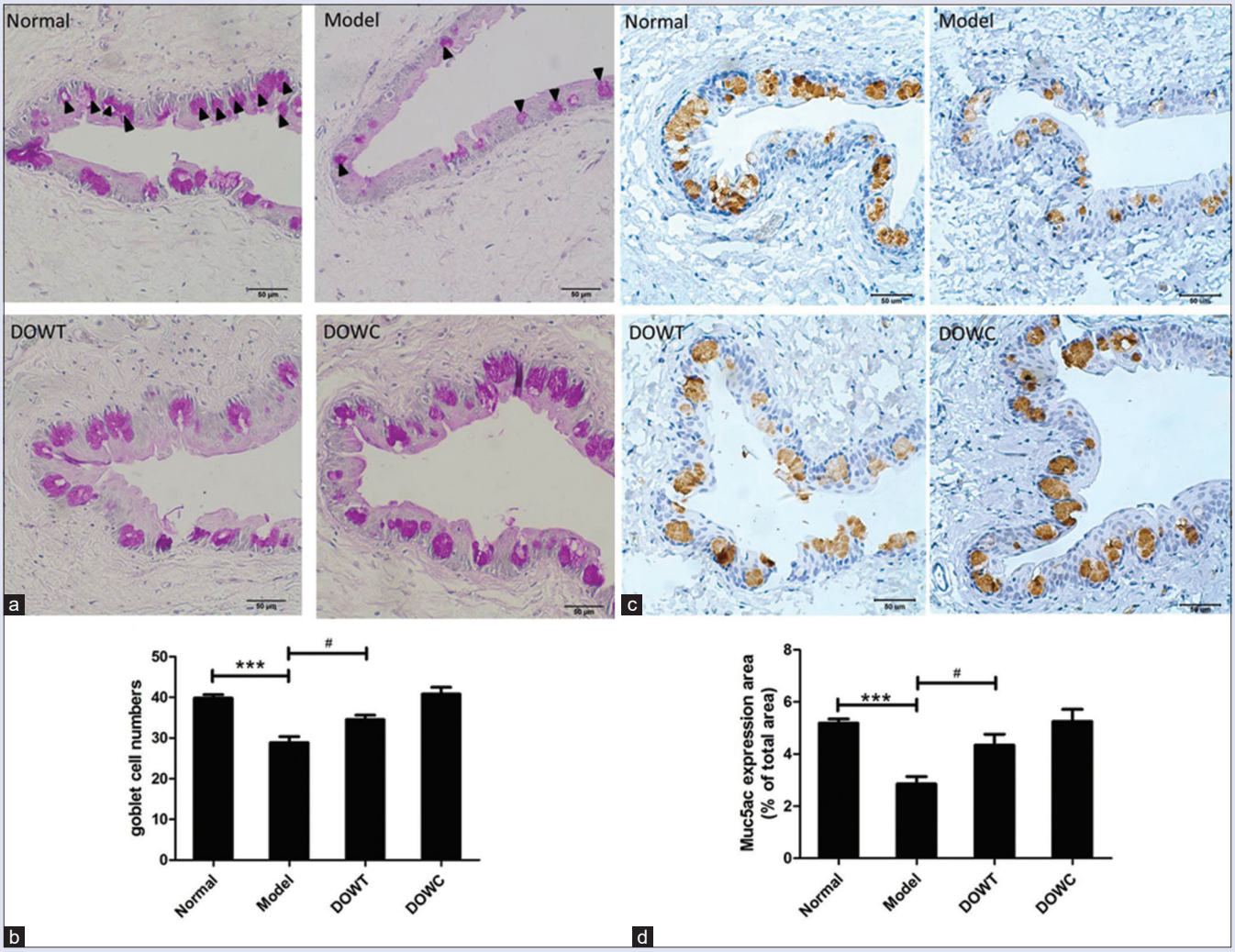


Figure 3: Water extract of *Dendrobium officinale* Kimura et Migo increased goblet cell number and Muc5ac expression in conjunctiva of dry eye rats. (a) PAS staining demonstrated significant decrease in conjunctival goblet cells (arrowheads) in model group compared with the normal group. However, the number of goblet cells increased significantly after 8-week treatment of water extract of *Dendrobium officinale* Kimura et Migo. (b) The number of conjunctival goblet cells was counted under ×200 magnification. (c) Immunohistochemical staining showed that oral administration of water extract of *Dendrobium officinale* Kimura et Migo increased expression level of Muc5ac in the conjunctival epithelium of dry eye rats significantly. (d) The quantification of Muc5ac expression was expressed as percentage of total area. The representative photos were at ×400 original magnification, scale bar: 50 μm. Data were expressed as mean ± standard error of the mean (n = 5-6), $^{***}P < 0.001$ when compared with normal control group, $^{\#}P < 0.05$ when compared with model group

decreased in the experimental dry eye rats. In contrast, increased number of conjunctival goblet cells and elevated Muc5ac expression were observed in the DOWT group, indicating that DO was able to relieve alterations in the conjunctiva of dry eye.

Increasing evidences demonstrated that dry eye was related to the inflammation of ocular surface in the basic, clinical, and translational researches involving patients, animal models, and cell cultures.[39] Inflammation can be triggered by chronic stimulation such as desiccated environment, contact lens wearing, microbe infection, or a systemic inflammatory autoimmune disease like Sjögren's syndrome and rheumatoid arthritis. Consequently, destroyed tear film and decreased tear production enhance the production of inflammatory mediators. Therefore, inflammation of ocular surface is believed as both the cause and the consequence of DED.[40] It was reported that the levels of IL-1β, IL-6, IL-8, TNF-α, and IFN-γ in the tears of dry eye patients were higher than healthy controls, particularly in the patients with aqueous deficiency.[41,42] These inflammatory cytokines were also report to overexpress in the conjunctiva of the evaporative dry eye and aqueous tear-deficient dry eye model.[43,44] Some cytokines had correlations with clinical diagnostic parameters for dry eye, suggesting that inflammatory cytokines could be used as a potential biomarker of DED.[45] In consistency with the results of the previous studies, the current study showed that IL-1β, IL-6, and TNF-α were significantly upregulated in the conjunctiva of dry eye rats, but reversed by DO treatment. MMP9 has been demonstrated to promote corneal epithelial regeneration in the healing process by modulating the inflammatory response.[46] Although MMP9 is more highly expressed in Sjögren's syndrome than evaporative DED, it is dubious whether the level of MMP9 is higher in evaporative DED than healthy controls.[47] Our data suggested that MMP9 level in conjunctiva was not changed in the dry eye rat model induced by desiccated conditions.

Aquaporins contribute to fluid secretion in exocrine glands. Among the aquaporins family, AQP5 is ubiquitous in many fluid-secreting

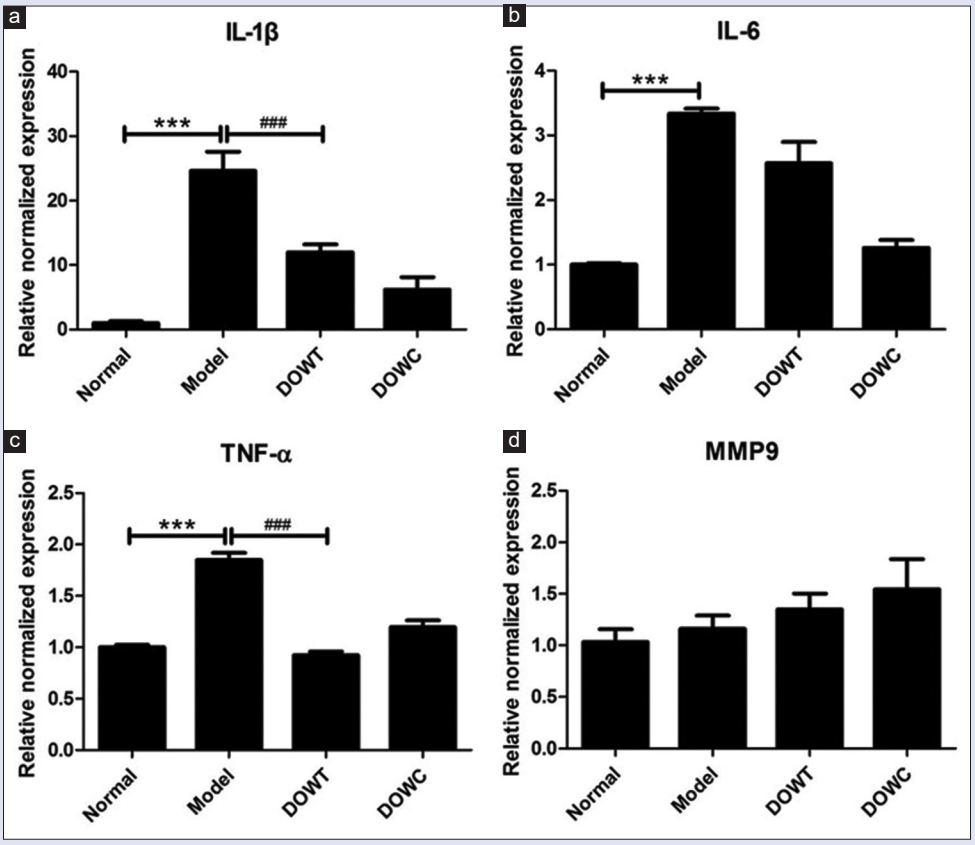


Figure 4: Water extract of *Dendrobium officinale* Kimura et Migo inhibited the mRNA levels of inflammatory cytokines in conjunctiva of dry eye rats. Water extract of *Dendrobium officinale* Kimura et Migo treatment significantly inhibited mRNA levels of interleukin-1β and tumor necrosis factor-α in dry eye rats. Data were from 3 to 6 rats in each group and expressed as mean ± standard error of the mean, $^{***}P < 0.001$ when compared with the normal group. $^{\#\#\#}P < 0.001$ when compared with the model group. (a, b, c, d) The mRNA levels of IL-1β, IL-6, TNF-α, MMP9

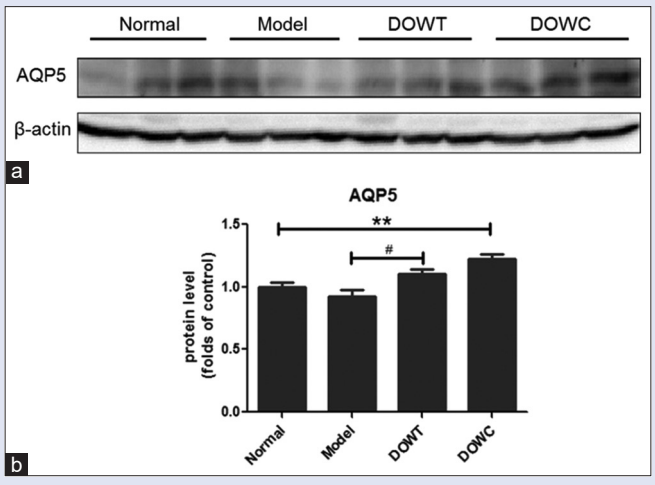


Figure 5: Water extract of *Dendrobium officinale* Kimura et Migo promoted AQP5 expression in lacrimal gland. (a) The total AQP5 expression level in lacrimal gland was detected by western blot analysis. The bands represented three independent samples for each group. (b) The semi-quantified results were detected by densitometry using ImageJ software and represented as folds of control. β-actin served as an internal control. The data were from six rats and expressed as mean ± standard error of the mean $^{**}P < 0.01$, compared with the normal group. $^{\#}P < 0.05$ compared with the model group

tissues such as lacrimal gland, salivary gland, and other exocrine or endocrine tissues.[8] AQP5 is located at the apical membrane of lacrimal acinar and ductal cells. In addition, AQP5 also exists in conjunctival epithelium and increases in the rabbit after excision of lacrimal gland.[48] It was observed that the protein level of AQP5 was upregulated in response to pilocarpine-induced tear secretion.[49] AQP5 was also found to be involved in normal tear secretion and pathogenesis of Sjögren's syndrome.[10,12] Due to the important biological roles of aquaporins and their involvement in pathophysiological conditions, it is likely to develop a remedy targeting aquaporins to treat DED. For example, reinforcement of aquaporin function by upregulating aquaporin expression or gene transfer may provide effective treatment for aquaporin function deficiency-related diseases such as salivary and lacrimal gland dysfunction, renal disease, and nephrogenic diabetes insipidus.[50] In the present study, DO upregulated AQP5 levels of dry eye rats, indicating that upregulation of AQP5 may be the mechanisms of DO to increase tear fluid. In another study, polysaccharides from DO were discovered to stimulate AQP5 expression in the airway submucosal glands of patients suffering from chronic obstructive pulmonary disease and in the cigarette smoke-treated lung epithelial cells, although the underlying mechanism had not been elucidated.[51] These studies all support the notion that modulation of AQP5 expression or function can be an effective approach for treating DED. However, further mechanistic studies are required to provide more evidences between DO extract induction and AQP5 expression.

CONCLUSION

Oral administration of DO showed great potential in improving experimental dry eye symptoms via increasing tear fluid, inhibiting conjunctiva destruction and inflammation, restoring the number of conjunctival goblet cells, as well as promoting mucin production. In addition, upregulation of AQP5 expression by DOW might be a possible mechanism relating to its tear-promoting action in treating DED. Our study provided preclinical evidence on the efficacy of DO as dry eye therapy, especially for the dry eye patients resulted from long-term stay in air-conditioned or desiccated environment and excessive video display viewing and reading. DO is deserved to be further studied and developed as an alternative or adjuvant therapy for DED.

Acknowledgements

The authors would like to thank Ms. Liu Yang, Ms. Ling Cheng, Mr. Ching-Po Lau, and Ms. Anita Shum for their technical support.

Financial support and sponsorship

The present study was financially supported by the Project of TCM Bureau of Guangdong Province (20191286), Guangdong Basic and Applied Basic Research Foundation (2020A1515010758), the Science and Technology Innovation Projects of Shenzhen (JCYJ 20190812171611467) Shenzhen Sanming Project of Medicine and Health (SZSM201612049), the funding of State Key Laboratory of Phytochemistry and Plant Resources in West China (P2018-KF07), and Yunnan Provincial Science and Technology Department (2017ZF003, 2015HB093).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Valim V, Trevisani VF, de Sousa JM, Vilela VS, Belfort R Jr. Current approach to dry eye disease. Clin Rev Allergy Immunol 2015;49:288-97.
2. Gayton JL. Etiology, prevalence, and treatment of dry eye disease. Clin Ophthalmol 2009;3:405-12.
3. Kojima T, Ibrahim OM, Wakamatsu T, Tsuyama A, Ogawa J, Matsumoto Y, *et al.* The impact of contact lens wear and visual display terminal work on ocular surface and tear functions in office workers. Am J Ophthalmol 2011;152:933-4000.
4. Yang WJ, Yang YN, Cao J, Man ZH, Yuan J, Xiao X, *et al.* Risk factors for dry eye syndrome: A retrospective case-control study. Optom Vis Sci 2015;92:e199-205.
5. Young G, Chalmers RL, Napier L, Hunt C, Kern J. Characterizing contact lens-related dryness symptoms in a cross-section of UK soft lens wearers. Cont Lens Anterior Eye 2011;34:64-70.
6. Stern ME, Beuerman RW, Fox RI, Gao J, Mircheff AK, Pflugfelder SC. The pathology of dry eye: The interaction between the ocular surface and lacrimal glands. Cornea 1998;17:584-9.
7. Rolando M, Zierhut M. The ocular surface and tear film and their dysfunction in dry eye disease. Surv Ophthalmol 2001;45 Suppl 2:S203-10.
8. Delporte C. Aquaporins in secretory glands and their role in Sjögren's syndrome. Handb Exp Pharmacol 2009;190:185-201.
9. Delporte C, Bryla A, Perret J. Aquaporins in Salivary Glands: From Basic Research to Clinical Applications. Int J Mol Sci 2016;17: 166-178.
10. Ohashi Y, Tsuzaka K, Takeuchi T, Sasaki Y, Tsubota K. Altered distribution of aquaporin 5 and its C-terminal binding protein in the lacrimal glands of a mouse model for Sjögren's syndrome. Curr Eye Res 2008;33:621-9.
11. Tsubota K, Hirai S, King LS, Agre P, Ishida N. Defective cellular trafficking of lacrimal gland aquaporin-5 in Sjögren's syndrome. Lancet 2001;357:688-9.
12. Ding C, Lu M, Huang J. Changes of the ocular surface and aquaporins in the lacrimal glands of rabbits during pregnancy. Mol Vis 2011;17:2847-55.
13. Bhavsar AS, Bhavsar SG, Jain SM. A review on recent advances in dry eye: Pathogenesis and management. Oman J Ophthalmol 2011;4:50-6.
14. Nakamachi T, Ohtaki H, Seki T, Yofu S, Kagami N, Hashimoto H, *et al.* PACAP suppresses dry eye signs by stimulating tear secretion. Nat Commun 2016;7:12034.
15. Chinese Pharmacopoeia Commission. Chinese Pharmacopoeia. 2015, Beijing: China Medical Science Press; 2015.
16. Xiao L, Ng TB, Feng YB, Yao T, Wong JH, Yao RM, *et al.* Dendrobium candidum extract increases the expression of aquaporin-5 in labial glands from patients with Sjögren's syndrome. Phytomedicine 2011;18:194-8.
17. Lin X, Shaw PC, Sze SC, Tong Y, Zhang Y. *Dendrobium officinale* polysaccharides ameliorate the abnormality of aquaporin 5, pro-inflammatory cytokines and inhibit apoptosis in the experimental Sjögren's syndrome mice. Int Immunopharmacol 2011;11:2025-32.
18. Li X, Zhang M, Wang C, Li J. Clinical observation of dry eye treated with *Dendrobium* luminous pills and tears naturale forte. Liaoning J Tradit Chin Med 2012;39:8-10.
19. Wei D, Li YH, Zhou WY. Observation on therapeutic effect of runmushu oral liquid in treating xerophthalmia in postmenopausal women. Zhongguo Zhong Xi Yi Jie He Za Zhi 2009;29:646-9.
20. Barabino S, Shen L, Chen L, Rashid S, Rolando M, Dana MR. The controlled-environment chamber: A new mouse model of dry eye. Invest Ophthalmol Vis Sci 2005;46:2766-71.
21. Zhang C, Li K, Yang Z, Wang Y, Si H. The Effect of the aqueous extract of *Bidens pilosa* L. on androgen deficiency dry eye in rats. Cell Physiol Biochem 2016;39:266-77.
22. Wu GL, Pu XH, Yu GY, Li TY. Effects of total glucosides of peony on AQP-5 and its mRNA expression in submandibular glands of NOD mice with Sjogren's syndrome. Eur Rev Med Pharmacol Sci 2015;19:173-8.
23. Yao XL, Peng QH, Peng J, Tan HY, Wu QL, Wu DL, *et al.* Effects of extract of Buddleja officinalis on partial inflammation of lacrimal gland in castrated rabbits with dry eye. Int J Ophthalmol 2010;3:114-9.
24. Lim P, Lan WW, Ping WQ, Tong L. Traditional Chinese medicine in dry eye disease. In: Eye Diseases and Disorders: A Complete Guide. Hong Kong: IConcept Press; 2015.
25. Tang H, Zhao T, Sheng Y, Zheng T, Fu L, Zhang Y. *Dendrobium officinale* Kimura et Migo: A review on its ethnopharmacology, phytochemistry, pharmacology, and industrialization. Evid Based Complement Alternat Med 2017;2017:1-19.
26. Xie SZ, Liu B, Zhang DD, Zha XQ, Pan LH, Luo JP. Intestinal immunomodulating activity and structural characterization of a new polysaccharide from stems of *Dendrobium officinale*. Food Funct 2016;7:2789-99.
27. Zeng Q, Ko CH, Siu WS, Li LF, Han XQ, Yang L, *et al.* Polysaccharides of *Dendrobium officinale* Kimura Migo protect gastric mucosal cell against oxidative damage-induced apoptosis *in vitro* and *in vivo*. J Ethnopharmacol 2017;208:214-24.
28. Wang Q, Zi CT, Wang J, Wang YN, Huang YW, Fu XQ, *et al. Dendrobium officinale* orchid extract prevents ovariectomy-induced osteoporosis *in vivo* and inhibits RANKL-induced osteoclast differentiation *in vitro*. Front Pharmacol 2017;8:966.
29. The definition and classification of dry eye disease: Report of the definition and classification subcommittee of the International dry eye WorkShop (2007). Ocul Surf 2007;5:75-92.
30. Chen W, Zhang X, Zhang J, Chen J, Wang S, Wang Q, *et al.* A murine model of dry eye induced by an intelligently controlled environmental system. Invest Ophthalmol Vis Sci 2008;49:1386-91.
31. She Y, Li J, Xiao B, Lu H, Liu H, Simmons PA, *et al.* Evaluation of a Novel artificial tear in the prevention and treatment of dry eye in an animal model. J Ocul Pharmacol Ther 2015;31:525-30.
32. Xiao B, Wang Y, Reinach PS, Ren Y, Li J, Hua S, *et al.* Dynamic ocular surface and lacrimal gland changes induced in experimental murine dry eye. PLoS One 2015;10:e0115333.
33. Peng CC, Cerretani C, Braun RJ, Radke CJ. Evaporation-driven instability of the precorneal tear film. Adv Colloid Interface Sci 2014;206:250-64.
34. Baudouin C, Aragona P, Messmer EM, Tomlinson A, Calonge M, Boboridis KG, *et al.* Role of hyperosmolarity in the pathogenesis and management of dry eye disease: Proceedings of the OCEAN group meeting. Ocul Surf 2013;11:246-58.
35. Chen YT, Lazarev S, Bahrami AF, Noble LB, Chen FY, Zhou D, *et al.* Interleukin-1 receptor mediates the interplay between CD4+T cells and ocular resident cells to promote keratinizing squamous metaplasia in Sjögren's syndrome. Lab Invest 2012;92:556-70.
36. Lee H, Kim CE, Ahn BN, Yang J. Anti-inflammatory effect of hydroxyproline-GQDGLAGPK in desiccation stress-induced experimental dry eye mouse. Sci Rep 2017;7:7413.
37. Mantelli F, Argüeso P. Functions of ocular surface mucins in health and disease. Curr Opin Allergy Clin Immunol 2008;8:477-83.
38. Uchino Y, Uchino M, Yokoi N, Dogru M, Kawashima M, Okada N, *et al.* Alteration of tear mucin 5AC in office workers using visual display terminals: The Osaka Study. JAMA Ophthalmol 2014;132:985-92.

39. Wei Y, Asbell PA. The core mechanism of dry eye disease is inflammation. Eye Contact Lens 2014;40:248-56.
40. Baudouin C, Irkeç M, Messmer EM, Benítez-Del-Castillo JM, Bonini S, Figueiredo FC, *et al.* Clinical impact of inflammation in dry eye disease: Proceedings of the ODISSEY group meeting. Acta Ophthalmol 2018;96:111-9.
41. Boehm N, Riechardt AI, Wiegand M, Pfeiffer N, Grus FH. Proinflammatory cytokine profiling of tears from dry eye patients by means of antibody microarrays. Invest Ophthalmol Vis Sci 2011;52:7725-30.
42. Massingale ML, Li X, Vallabhajosyula M, Chen D, Wei Y, Asbell PA. Analysis of inflammatory cytokines in the tears of dry eye patients. Cornea 2009;28:1023-7.
43. Sung MS, Li Z, Cui L, Choi JS, Choi W, Park MJ, *et al.* Effect of topical 5-Aminoimidazole-4-car boxamide-1-β-d-ribofuranoside in a mouse model of experimental dry eye. Invest Ophthalmol Vis Sci 2015;56:3149-58.
44. Corrales RM, Villarreal A, Farley W, Stern ME, Li DQ, Pflugfelder SC. Strain-related cytokine profiles on the murine ocular surface in response to desiccating stress. Cornea 2007;26:579-84.
45. Jackson DC, Zeng W, Wong CY, Mifsud EJ, Williamson NA, Ang CS, *et al.* Tear interferon-gamma as a biomarker for evaporative dry eye disease. Invest Ophthalmol Vis Sci 2016;57:4824-30.
46. Mohan R, Chintala SK, Jung JC, Villar WV, McCabe F, Russo LA, *et al.* Matrix metalloproteinase gelatinase B (MMP-9) coordinates and effects epithelial regeneration. J Biol Chem 2002;277:2065-72.
47. Aragona P, Aguennouz M, Rania L, Postorino E, Sommario MS, Roszkowska AM, *et al.* Matrix metalloproteinase 9 and transglutaminase 2 expression at the ocular surface in patients with different forms of dry eye disease. Ophthalmology 2015;122:62-71.
48. Bhattacharya D, Yu L, Wang M. Expression patterns of conjunctival mucin 5AC and aquaporin 5 in response to acute dry eye stress. PLoS One 2017;12:e0187188.
49. Ishida N, Hirai SI, Mita S. Immunolocalization of aquaporin homologs in mouse lacrimal glands. Biochem Biophys Res Commun 1997;238:891-5.
50. Soveral G, Casini A. Aquaporin modulators: A patent review (2010-2015). Expert Opin Ther Pat 2017;27:49-62.
51. Song TH, Chen XX, Tang SC, Ho JC, Lao LX, Ng TB, *et al. Dendrobium officinale* polysaccharides ameliorated pulmonary function while inhibiting mucin-5AC and stimulating aquaporin-5 expression. J Funct Foods 2016;21:359-71.