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Original Article

Buddleja officinalis eye drops alleviate the symptoms of moderate to severe dry eye

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Abstract: Objective: *Buddleja officinalis* is used as a natural Chinese medicine and contains flavonoids that may be beneficial to the treatment of dry eye. Methods: Sixty postmenopausal females diagnosed with dry eye were assigned to treatment with *Buddleja officinalis* eye drops and artificial tears (n=30) or a control treatment with artificial tears only (n=30). The analysis of subjective symptoms of the ocular surface, the Ocular Surface Disease Index (OSDI), tear film function, tear protein levels and confocal scanning microscopy results were analyzed at pre-therapy and following 1, 2, 4, and 8 weeks of therapy. Results: Following 8 weeks of therapy, all the indices of the patients treated with *Buddleja officinalis* eye drops were improved compared with those pre-therapy ($P < 0.05$), but those in the control group were not. All indices at 8 weeks presented statistically significant differences between the two groups (all $P < 0.05$). In addition, the mean density of inflammatory cells and corneal epithelium basal cells of the patients treated with *Buddleja officinalis* eye drops was reduced compared with the men density of the control ($P < 0.05$). Conclusion: These results show that *Buddleja officinalis* eye drops provided a clinical benefit in relieving the symptoms of moderate to severe dry eye in postmenopausal females.

Keywords: *Buddleja officinalis* eye drops, dry eye, postmenopausal women, treatment, androgen

Introduction

Dry eye causes ocular discomfort that can seriously affect a patient's quality of life, and this condition has increased in prevalence [1]. A reduction in androgen levels is considered to be the primary cause of dry eye. Postmenopausal women and aging men typically experience androgen deficiency-induced dry eye [2, 3]. Due to the severity of the symptoms, it is important to identify methods for preventing and treating dry eye in order to prevent adverse effects on the patient's health and ability to function. At present, androgen substitution therapy appears to be the only therapy that is able to treat dry eye caused by a deficiency of androgen; however, this is likely to cause notable side effects if used as a long-term medication [4]. Although other therapies for dry eye are available, these also have certain flaws and limitations for the treatment of dry eye induced by androgen deficiency. Therefore, it is imperative that novel therapeutic drugs for this condition be developed.

Flavonoid compounds are heterocyclic polyphenol compounds that have similar chemical structures to androgens and, therefore, could potentially provide an endogenous androgen effect in the treatment of dry eye caused by androgen deficiency. In traditional Chinese medicine, *Buddleja officinalis* is thought to act on the 'liver meridian', and the flowers of *Buddleja officinalis*, or their active flavonoid components, are often used to treat conditions affecting the eyes [5]. Therefore, the aim of the present study was to evaluate the therapeutic efficiency and feasibility of using *Buddleja officinalis* eye drops to treat moderate and severe dry eye in postmenopausal women.

Materials and methods

Preparation of Buddleja officinalis eye drops

The dry flowers *of Buddleja officinalis* Maxim., which were obtained from a crude drug market in Nanchang, China, were cut into small pieces and extracted twice in hot 80% alcohol with

centrifugal filtration (4,024.8 × g, 4°C, 5 min). The filtrate was fractionated using an HPD-100 macroporous resin column, with an elution using ethanol. The 70% ethanol eluate was collected, and the residue that remained following drying was crushed to provide the *Buddleja officinalis* extract. High-performance liquid chromatography was conducted using a Gemini C_{18} column (Phenomenex, Inc., Torrance, CA, USA), with the mobile phase as previously described [6]. Linarin solution was used as a control to establish a standard UV curve, and the total flavonoid content of the *Buddleja officinalis* extract was measured on the basis of UV measurements [7]. The formulation of the *Buddleja officinalis* eye drops was carried out as previously described [6]. In brief, the *Buddleja officinalis* extract was dissolved in distilled water (water: extract ratio, 1:0.1), carboxymethyl cellulose (1.5%) was added as an ocular lubricant, and potassium bicarbonate and potassium chloride were added as a buffer system (≤ 0.1%). At this time, the physical and chemical properties of the formulation were tested, and the formulation adjusted to ensure that the following criteria were satisfied: pH value, 7.3-7.8; osmotic pressure, 311-350 mOsm; and specific gravity, ~1; refractive index, 1.336. Finally, benzalkonium bromide (0.005%) was added as a preservative.

Study design

A total of 60 female patients were diagnosed with moderate to severe dry eye in the Outpatient Department of the First Affiliated Hospital of Nanchang University (Nanchang, China) from March 2016 to July 2018. To minimize the inhomogeneity of the study, criteria for important non-experimental factors, such as age, were assigned. Case histories were obtained, and complete ocular surface examinations were performed to determine the eligibility of each participant. The average age of the patients was 54.2±2.4 years (range, 50-56 years). In addition, 30 young women were 52.5±4.6 years (range, 50-58 years) who without dry eye disease were recruited as the control group. The cases with moderate or severe symptoms were randomly divided into two groups, A and B. The patients in Group A were treated with a combination of *Buddleja officinalis* eye drops plus artificial tears (Hypromellose 2910, Dextran 70 and Glycerol Eye Drops; Alcon Corporation, Fort Worth, TX, USA) and those in Group B were treated only with artificial tears. The administration was continued for 8 weeks, three times a day. None of the enrolled patients had a history of systemic illness, other ocular problems, or a history of anti-hypertensive or anti-depressant intake, and the cohort did not include pregnant or lactating women. Prior to treatment, as well as 1, 2, 4, and 8 weeks later, the Ocular Surface Disease Index (OSDI) questionnaire, the visual acuity, the subjective symptoms, the 4-terms tear film, the tear protein measurements, and the corneal confocal scanning were conducted. Based on the equation n=15.6R+1.6, a sample size of 30 per group was chosen to achieve 80% confidence.

Ethical considerations

The principles of the Declaration of Helsinki were fully followed, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University (Nanchang, China). The study protocol and procedures were fully explained to each patient, and each signed an informed consent form.

Patients

Patients were eligible if they had undergone bilateral oophorectomy, or were 50-56 years old, had been menopausal for > 1 year, had not received chemotherapy, had not undergone treatment with tamoxifen or toremifene, had not received any other treatment that suppresses ovarian function, had follicle-stimulating hormone (FSH) and estradiol levels within the postmenopausal range (basic FSH > 40 IU/l; estradiol, 40-100 pmol/l); were 50-56 years old, had undergone tamoxifen or toremifene treatment, or had FSH and estradiol levels within the postmenopausal range.

Recruitment criteria

The recruitment criteria were as previously described [8]. Criterion 1, chronic symptoms (> I term): Visual tiredness, dry and unsmooth sensation, foreign body sensation, burning sensation, photophobia, pain, red eye, and tears. Criterion 2, fluorescein staining (FL): The cornea was divided into four quadrants, and the pigmentation/staining of each quadrant was classified into non-staining, mild, moderate and severe (scored 0-3 points, respectively), so the entire corneal FL had a score of 0-12 points. Criterion 3, tear film break-up time

(BUT): < 10 sec. Criterion 4, Schirmer I test (SIT): SIT value ≤ 10 mm/5 min. Patients with chronic dry eye symptoms and any two positive results from criteria 2, 3 and 4 were diagnosed as having dry eye. Patients who had only one positive result from criteria 2, 3 and 4 were considered to be suspected dry eye cases. For these cases, a subsequent check of tear lactoferrin concentration was performed, and patients with chronic dry eye symptoms and tear lactoferrin concentrations < 1.04 mg/mL were diagnosed as having dry eye.

Exclusion criteria

The exclusion criteria were [9]: i) No evident symptoms; ii) a cornea FL score of 0 points; iii) a BUT of ≤ 1 or > 10 sec; and iv) a SIT value of ≤ l or > 10 mm/5 min. For each patient, the eye with the less severe symptoms served as a control eye and did not receive any drug treatment. Patients were evaluated pretreatment and at 1, 2, 4, and 8 weeks post-therapy. For the classification of moderate to severe dry eye, the patients were stratified according to the evaluation and classification standard of dry eye proposed by Jacobi [10]. Mild dry eye was classified as follows: A dry eye symptom score of 0-2 points; a cornea FL score of 0-4 points; a BUT of 5-10 sec; and a SIT value of 5-10 mm/5 min. Moderate to severe dry eye was classified as follows: A dry eye symptom score of 2 or 3 points; a cornea FL score of 5-12 points, a BUT of < 5 sec; and a SIT value of < 5 mm/5 min. All measurements were conducted from 9:00-11:00 a.m. on the test day.

Efficacy evaluation

Subjective symptom score of the ocular surface: All the patients involved in this study were asked whether they had any discomfort from dryness, abnormal sensation or asthenia; a score 0, 0.5, 1 or 2 was assigned for the severity of the symptoms, as previously described [10]. For stringency, patients also completed the Chinese version of the OSDI questionnaire [11]. A self-administered questionnaire was used to assess ocular symptoms during the 2-4-week period prior to the examination, encompassing a total of 12 items (blurred vision; poor vision; difficulty in reading or driving at night; photophobia; gritty sensation; eye pain; difficulty working with a computer or automatic teller machine, or watching television; discomfort in windy conditions, in air-conditioned rooms or in places with low humidity). The scores ranged from 1 to 100, with higher scores representing greater disability. The OSDI questions were described in detail previously [11]. The scoring followed the following pattern: 0, seldom; 1, sometimes; 2, half of the time; 3, the majority of the time; and 4, always. In cases where the patients were uncertain, the questions were not answered. Patients were not required to answer questions if they were uncertain. OSDI score = sum of the scores of all questions/number of answers × 25.

Tear film test with four terms

The corneal FL test was conducted using fluorescent strips that were inserted into the inferior conjunctival fornix of each patient, followed by blinking. The staining was recorded using a 0-12-point scale, by dividing the cornea into four quadrants, and scoring each with 0-3 points according to the staining level and proportion. The National Ophthalmology Research clinical dry eye scoring system [12] was used to score the results as follows: 0, no staining; 1, few disseminated stains; 2, moderate stains (between class 1 and class 3); and 3, severely fused stains. A slit-lamp microscope was used to measure the height of the tear meniscus (HTM) in the center of the lower eyelid. BUT evaluation was used to assess the tear stability, by calculating the time interval between a blink and the appearance of a break in the tear film. After placing a fluorescein strip into the fornix of the lower eyelid and removing it, the patient blinked three times and then looked straight ahead without blinking, during which a slit-lamp microscope with a cobalt-blue filter was used to investigate the tear film. Three measurements were performed for each eye, and the averages used for further analysis. To conduct SIT, a thin filter paper strip (35 × 5 mm) was placed at the junction of the intermediate and lateral thirds of the lower eyelid to quantify the amounts of tears produced in 5 min. The patients were instructed to look forward and blink normally during the test.

Tear protein measurements

Between 9:00 and 11:00 a.m., all tear samples were collected. A capillary pipette was used to collected non-irritating tears from the patients' tear meniscus (20 μl) and the collected tears

Table 1. Characteristics of the participants included in the study

Variables	*Buddleja officinalis* eye drops group	Artificial tears group	t-value (χ^2)	*P*-value
Age (years)	54.28±6.18 (50 to 56)	53.96±8.68 (50 to 56)	0.428	> 0.05
Spherical equivalent refractive error (diopters)	-1.36±2.09 (-3.5 to -3.75)	-1.32±2.06 (-3.75 to -3.75)	0.326	> 0.05
Interval between onset of symptoms and treatment (weeks)	44.53±20.32 (27 to 63)	43.22±22.18 (28 to 63)	0.225	> 0.05
Duration time (months)	10.79±3.15 (3 to 40)	11.01±3.66 (4 to 38)	0.753	> 0.05
Body mass index	22.36±4.98 (18.5-23.9)	23.14±4.86 (18.5-23.9)	0.817	> 0.05

Values are presented as the mean ± standard deviation with the range in parentheses (n=30 per group).

were stored in a 0.5-mL EP tube (Eppendorf, Hamburg, Germany) at -80°C. The total tear protein concentration was measured by the Bradford method with bovine serum albumin as the standard. Lactoferrin concentrations were measured by radioimmunoassay [13]. The lysozyme testing was conducted using a lysozyme test kit (Shanghai Xinyu Biological Technology Pharmaceutical Co., Ltd., Shanghai, China), following the manufacturer's protocol, with the preparation of a staining broth, lysozyme standards and a standard curve. The lipocalin concentrations were determined using a previously described protocol [14].

Corneal confocal microscopy

A Confoscan 4 slit-lamp scanning confocal microscope (Nidek Co., Ltd., Gamagori, Japan) was used for the analysis of the cornea, as previously described [15]. Following ocular surface anesthesia, the patient's head was fixed under the microscope with their eyes fixed, staring straight ahead. The examiner moved the lens to focus on the corneal epithelial cells and slowly pushed the lens forward. Full tomography was available from the central cornea. Valuable images and videos were selected and saved, and the density of the corneal epithelial basal cells and inflammatory cells was analyzed using the Navis software processing system (Nidek Co., Ltd.,).

Statistical analyses

Data were analyzed using the SPSS 19.0 software (IBM, Corp., Armonk, NY, USA) and the software program GraphPad Prism (version 6.02 for Windows; GraphPad Software, Inc., La Jolla, CA, USA). Values are expressed as the mean ± standard deviation. Analysis of the enumeration data was performed using a χ^2 test. A repeated measure ANOVA was used for all indexes with the subjective symptoms before and after the treatment comparisons; and Dunnett's test was applied for multiple comparisons. $P < 0.05$ was considered to indicate a significant difference.

Results

Clinical outcomes

The age, body mass index, spherical equivalent refractive errors, interval between onset of symptoms and treatment, and duration time exhibited no statistically significant differences between the two groups (all $P > 0.05$; **Table 1**).

Subjective symptoms of the ocular surface have been improved

As shown in **Table 2**, the subjective symptoms of dry eye presented no significant differences between the two groups prior to therapy (all $P > 0.05$). Compared with those pre-therapy, the symptoms of Group A exhibited no significant remission after 1 week (all $P > 0.05$), but were significantly ameliorated following 8 weeks of therapy (all $P < 0.05$). However, the symptoms of Group B did not significantly improve following 8 weeks of therapy compared with those pre-therapy, with the exception of photophobia and pain ($P < 0.05$).

Only a remission of the symptoms in Group A, but not those in Group B, was demonstrated in the results of the OSDI questionnaire. **Table 3** presents a summary of the mean total OSDI scores in the two groups. The results of the OSDI questionnaire revealed an improvement in the symptoms in the post-treatment patients treated with *Buddleja officinalis* eye drops and artificial tears (all $P < 0.05$), but not in the patients treated with artificial tears only. Following 8 weeks of therapy, the Mean Ocular Symptoms, Mean Vision Related Function and Mean Environmental OSDI subscores exhibited

Table 2. Comparison of the symptoms of the ocular surface in the two groups pre- and post-therapy

	Eye fatigue	Dryness	Abnormal sensation	Burning sensation	Photophobia	Pain	Redness	Tear
Group A								
Pre-therapy	2.85±0.71 (30 to 100)	2.78±0.61 (30 to 100)	2.32±0.96 (30 to 100)	2.69±0.59 (24 to 80)	2.56±0.56 (24 to 80)	2.61±0.54 (18 to 60)	2.59±0.46 (15 to 50)	2.44±0.41 (6 to 20)
1 week	2.74±0.62 (30 to 100)	2.62±0.52 (30 to 100)	2.18±0.75 (30 to 100)	2.54±0.51 (24 to 80)	2.41±0.53 (24 to 80)	2.47±0.47 (18 to 60)	2.52±0.31 (15 to 50)	2.32±0.29 (6 to 20)
2 weeks	2.42±0.55 (30 to 100)	2.32±0.46 (30 to 100)	2.02±0.51 (30 to 100)	2.37±0.32 (24 to 80)	2.23±0.65 (24 to 80)	2.34±0.33 (18 to 60)	2.27±0.39 (15 to 50)	2.16±0.28 (6 to 20)
4 weeks	1.41±0.42 (30 to 100)	1.39±0.41 (30 to 100)	1.34±0.57 (30 to 100)	1.71±0.44 (24 to 80)	1.58±0.78 (24 to 80)	1.22±0.56 (18 to 60)	2.14±0.22 (15 to 50)	1.91±0.36 (6 to 20)
8 weeks	1.31±0.26 (30 to 100)	1.21±0.31 (30 to 100)	1.23±0.46 (30 to 100)	1.53±0.36 (24 to 80)	1.41±0.51 (24 to 80)	1.11±0.37 (18 to 60)	1.96±0.35 (15 to 50)	1.76±0.24 (6 to 20)
Group B								
Pre-therapy	2.85±0.63 (30 to 100)	2.56±0.47 (30 to 100)	2.31±0.96 (27 to 90)	2.58±0.54 (27 to 90)	2.61±0.56 (24 to 80)	2.69±0.62 (18 to 60)	2.49±0.41 (15 to 50)	2.32±0.26 (6 to 20)
1 week	2.79±0.67 (30 to 100)	2.62±0.54 (30 to 100)	2.31±0.58 (27 to 90)	2.55±0.41 (27 to 90)	2.56±0.57 (24 to 80)	2.46±0.53 (18 to 60)	2.33±0.34 (15 to 50)	2.29±0.24 (6 to 20)
2 weeks	2.66±0.58 (30 to 100)	2.54±0.35 (30 to 100)	2.24±0.49 (27 to 90)	2.43±0.35 (27 to 90)	2.40±0.41 (24 to 80)	2.39±0.45 (18 to 60)	2.15±0.46 (15 to 50)	2.11±0.19 (6 to 20)
4 weeks	2.58±0.55 (30 to 100)	2.43±0.32 (30 to 100)	2.12±0.57 (27 to 90)	2.38±0.44 (27 to 90)	2.33±0.35 (24 to 80)	2.23±0.42 (18 to 60)	2.08±0.59 (15 to 50)	2.08±0.26 (6 to 20)
8 weeks	2.52±0.42 (30 to 100)	2.36±0.33 (30 to 100)	2.08±0.45 (27 to 90)	2.33±0.32 (27 to 90)	2.29±0.42 (24 to 80)	2.12±0.46 (18 to 60)	2.04±0.35 (15 to 50)	2.01±0.17 (6 to 20)
t	4.242	3.791	3.926	3.105	2.983	4.619	1.526	1.387
P	0.012	0.025	0.021	0.029	0.031	0.018	0.041	0.046

t/P, Group A *vs* Group B in 8 week. Values are presented as the mean ± standard deviation (n=30 per group), t, the mean difference of the ocular surface symptom at 8 weeks post-therapy.

Table 3. Subjective symptom score of the ocular surface prior to and following treatment

	OS-OSDI	VRF-OSDI	E-OSDI
Group A			
Pre-therapy	41.24±6.65	43.56±4.19	57.11±9.02
1 week	38.47±5.28	40.29±4.06	43.45±8.19
2 weeks	31.43±5.78	35.45±3.14	47.93±6.12
4 weeks	25.18±4.56	27.69±3.81	38.52±5.54
8 week	16.29±3.49	18.44±2.65	30.78±5.41
Group B			
Pre-therapy	41.85±2.14	39.65±3.05	56.16±8.43
1 week	38.74±2.09	35.98±2.94	53.55±7.18
2 weeks	36.85±1.95	34.75±2.16	49.41±6.17
4 weeks	35.43±1.69	33.12±2.59	44.19±6.06
8 weeks	33.57±1.74	29.17±2.73	40.26±6.53
t	7.436	5.759	4.241
P	0.013	0.017	0.021

t/P, Group A *vs* Group B in the 8th week. Group A, postmenopausal females with moderate to severe dry eye treated with *Buddleja officinalis* eye drops and artificial tears; Group B, postmenopausal females with moderate to severe dry eye treated with artificial tears; OS-OSDI, the Mean Ocular Symptoms, VRF-OSDI, Mean Vision Related Function and E-OSDI, Mean Environmental OSDI.

Table 4. Tear film test results prior to and following treatment

	SIT	BUT	FL	HTM (mm)
Group A				
Pre-therapy	2.19±0.22	1.81±0.42	9.52±1.36	0.16±0.05
1 week	3.12±0.75	4.09±0.39	7.31±1.68	0.34±0.09
2 weeks	5.29±0.52	6.07±0.21	6.14±1.42	0.49±0.12
4 weeks	8.83±1.09	9.49±0.55	4.23±0.52	0.57±0.16
8 weeks	10.37±2.76	10.84±1.92	3.41±0.61	0.78±0.13
Group B				
Pre-therapy	2.39±0.22	1.96±0.37	9.53±1.29	0.16±0.05
1 week	3.26±1.15	2.16±0.38	9.36±1.69	0.13±0.04
2 weeks	3.87±1.13	2.54±0.21	9.42±1.46	0.16±0.07
4 weeks	4.22±1.25	2.15±0.28	8.31±1.32	0.19±0.07
8 weeks	4.17±1.27	2.47±0.29	8.35±1.79	0.22±0.08
t	4.357	5.291	3.574	2.985
P	0.012	0.007	0.023	0.031

t/P, Group A *vs* Group B at 8 weeks. Group A, postmenopausal females with moderate to severe dry eye treated with *Buddleja officinalis* eye drops and artificial tears; Group B, postmenopausal females with moderate to severe dry eye treated with artificial tears; SIT, Schirmer I test; BUT, break-up time; FL, fluorescein staining; HTM, height of tear meniscus; CI, confidence interval.

a statistically significant difference between the two groups (all P < 0.05).

Improved tear film stability

The results of the tear film tests in the two groups prior to therapy and at 1, 2, 4, and 8 weeks after the initiation of therapy are summarized in **Table 4**. No difference in SIT, BUT, FI or HTM was detected pre-therapy between Group A and Group B; nor was a difference detected at post-therapy week 1 (all P > 0.05). Although the SIT value increased following treatment in Group A, the results following 1 and 2 weeks of therapy did not differ significantly between the two groups (P > 0.05). However, the SIT value of Group A was significantly greater than the value of Group B after 8 weeks of treatment (P < 0.05). The FL score of Group A was significantly decreased following 4 and 8 weeks of therapy (all P < 0.05); by contrast, the FL score of Group B exhibited no significant change following therapy compared with pre-therapy (all P > 0.05). The BUT and HTM results of Group A were significantly increased following 4 and 8 weeks of therapy (all P < 0.05), while no significant changes in these results prior to and following therapy were observed for Group B (all P > 0.05).

Tear proteins were increased

Table 5 shows the tear protein levels in the two groups pre-therapy and at 1, 2, 4, and 8 weeks after the initiation of therapy. Prior to the treatment, no significant differences were detected in the total tear protein, lactoferrin, lysozyme or lipocalin levels of the two groups (all P > 0.05). However, total tear proteins, and the lactoferrin, lysozyme, and lipocalin levels in Group A were significantly different from those in Group B after 8 weeks of treatment (P < 0.05). The total tear protein, lactoferrin, and lysozyme levels of Group A were significantly increased from 4 weeks of therapy onward (P < 0.05). The lipocalin level in Group A

Table 5. Amount of tear proteins measured prior to and following treatment

	Total protein	Lysozyme	Lactoferrin	Lipocalin (mg/ml)
Group A				
Pre-therapy	9.63±0.21	1.19±0.22	1.11±0.15	0.22±0.17
1 week	10.24±0.14	1.64±0.41	1.29±0.11	0.28±0.12
2 weeks	10.46±0.25	1.67±0.39	1.39±0.27	0.29±0.12
4 weeks	11.21±0.29	1.69±0.37	1.48±0.29	0.38±0.11
8 weeks	12.87±0.31	1.89±0.39	1.46±0.31	0.45±0.16
Group B				
Pre-therapy	9.73±0.49	1.37±0.21	1.16±0.23	0.23±0.12
1 week	9.88±0.31	1.35±0.24	1.18±0.19	0.22±0.09
2 weeks	10.18±0.39	1.32±0.26	1.19±0.22	0.27±0.10
4 weeks	10.21±0.21	1.35±0.27	1.25±0.20	0.23±0.11
8 weeks	10.24±0.26	1.37±0.21	1.24±0.26	0.25±0.11
t	4.538	6.752	5.301	7.947
P	0.043	0.032	0.039	0.026

t/P, Group A *vs* Group B at 8 weeks. Group A, postmenopausal females with moderate to severe dry eye treated with *Buddleja officinalis* eye drops and artificial tears; Group B, postmenopausal females with moderate to severe dry eye treated with artificial tears.

was significantly greater than that of Group B at 8 weeks ($P < 0.05$), but not at 1, 2, or 4 weeks ($P > 0.05$).

Changes in corneal epithelial cells in patients with dry eye after intervention

The corneal epithelial basal cells of a healthy female appear dark with clear cell borders (**Figure 1A**). However, for postmenopausal patients with dry eye, the corneal epithelial basal cells are atrophied with infiltrated bright inflammatory cells (**Figure 1B**). Following 8 weeks of treatment, Group A exhibited only a few inflammatory cells infiltrating the epithelial basal layer, and the density of the corneal epithelial basal cells was slightly decreased compared with the density in the normal female eye (**Figure 1C**). By contrast, Group B exhibited numerous bright inflammatory cells in the epithelial basal layer, and the density was markedly increased (**Figure 1D**). At 8 weeks after the initiation of treatment, the average numbers of corneal epithelium basal cells and inflammatory cells were 3,218±312 and 56±22 cells/mm^2, respectively, in Group A, and 4,421±372 and 212±91 cells/mm^{-2}, respectively, in Group B. These values were significantly different between the two groups ($P < 0.05$; **Figure 1E** and **1F**). No significant difference was observed in the density of corneal epithelial basal cells and inflammatory cells between Group B and the untreated postmenopausal females ($P > 0.05$) (**Table 6**).

Discussion

The eye has been shown to be a target organ of hormones, and receptors for androgens, estrogens, progesterone, and prolactin have been confirmed to exist in the lacrimal gland, meibomian gland, cornea, and other ocular surfaces in humans, rabbits and mice [16]. Several factors contribute to declining androgen levels, including menopause, aging, autoimmune diseases, and anti-androgenic drug use. This reduction in androgens induces tear film instability, local inflammatory responses, and increased apoptosis of the glandular tissue, such as the lacrimal gland, which subsequently results in dry eye [17]. The long-term use of androgen therapy produces severe side effects in men and women, such as prostate enlargement, the development of cancer, and the masculinization of women, which is distressing to patients [18]. In consideration of this, and the lack of other effective treatments, the need to develop alternative drugs is evident.

Androgens and flavonoids are able to combine with the androgen receptor (AR); certain flavonoids have been demonstrated to act as agonists of membrane ARs [19]. Therefore, they have the potential to be used to provide androgen-like effects in the treatment of dry eye caused by decreased androgen levels. *Buddleja officinalis* is a traditional Chinese medicine that is widely used in the treatment of diseases including malaria, diarrhea, dysentery, hepatitis and acute nephritis. The effective components of *Buddleia officinalis* are flavonoids, and eight different flavonoids have been identified in its flowers: Acacetin, apigenin, luteolin, neobudofficide, linarin, luteolin-7-O-rutinoside, luteolin-7-O-glucosidase, and cosmoslin [20]. The flavonoids contained in *Buddleja officinalis* may also combine with AR and have androgenic effects, which may be useful in the treatment of diseases caused by reductions in androgen

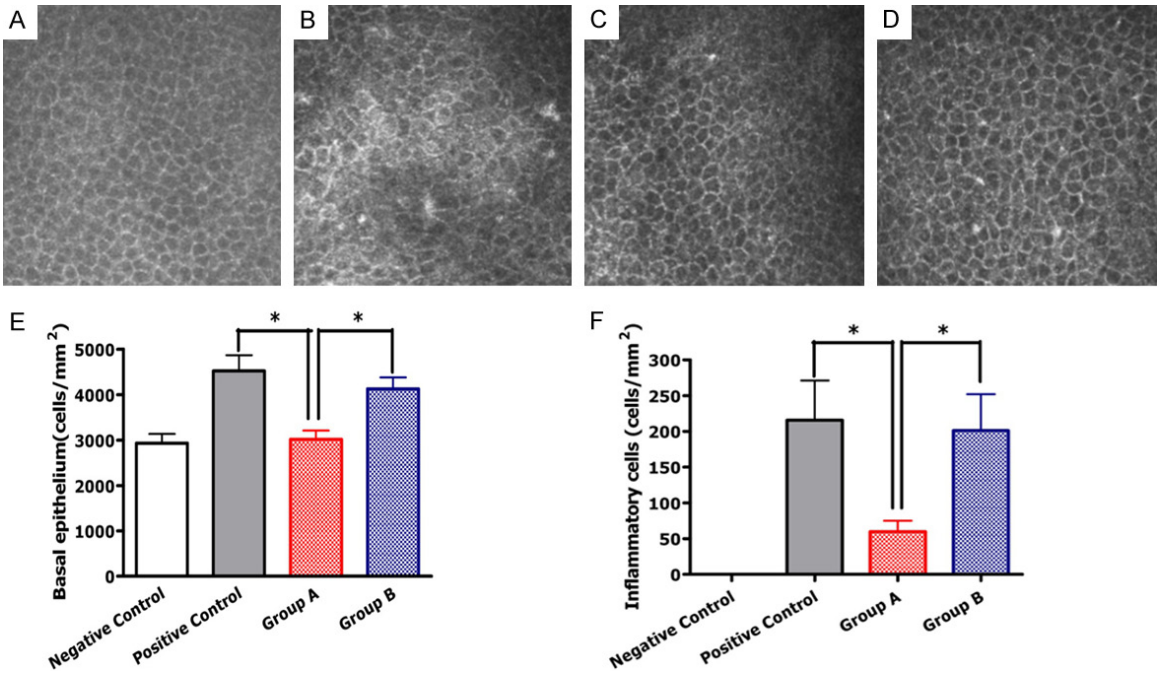

Figure 1. Effects of *Buddleja officinalis* eye drops and PBS on the morphology of the female corneal epithelial basal layer. Representative confocal microscopy images of the corneal epithelium in different patient groups. (A) Negative control, (B) Positive control, (C) Group A, which were treated with a combination of *Buddleja officinalis* eye drops plus artificial tears after 8 weeks, and (D) Group B, which were treated only with artificial tears. The average numbers of corneal epithelium basal cells (E) and inflammatory cells (F) in the two groups.

Table 6. The effect on the corneal epithelial basal layer after 8 weeks (cells/mm^2)

Group	Corneal epithelial basal cells	Inflammatory cells
Group A	3414±389	51±15
Group B	4796±292	196±81
t	5.134	3.657
P	0.026	0.007

Table 6 shows the mean corneal epithelial cell densities and mean inflammatory cell densities in the four groups. Data are presented as the mean ± standard deviation of all eyes per group. $^*P < 0.05$ vs. Group A. Negative control, healthy females; positive control, postmenopausal females with untreated moderate to severe dry eye; Group A, postmenopausal females with moderate to severe dry eye treated with *Buddleja officinalis* eye drops and artificial tears; Group B, postmenopausal females with moderate to severe dry eye treated with artificial tears.

levels, including dry eye syndrome. Therefore, in the present study, eye drops containing *Buddleja officinalis* Maxim. extract were prepared and their therapeutic effect in the treatment of postmenopausal females with dry eye was investigated.

In a previous study, the present research team investigated the effectiveness of *Buddleja officinalis* water decoction in the treatment of dry eye disease [21]. The present study demonstrates that *Buddleja officinalis* eye drops are also able to significantly improve the symptoms of dry eye. The present study also reveals that a combination of *Buddleja officinalis* with artificial tear eye drops significantly improves the OSDI scores and symptoms of moderate to severe dry eye following 8 weeks of treatment, whereas artificial tears alone did not. This result may be associated with the antibacterial function of *Buddleja officinalis* against multiple ocular microorganisms and its high content of choline, which may induce tears.

The tear film is a dynamic entity and is critical for ocular surface function. The SIT is used to for the clinical detection of tear secretion and reflects the reflex secretion function of the lachrymal gland. For patients with dry eye, the SIT results exhibit improved repeatability and consistency as the severity of the disease increases [22]. Clinically, morphological examination of the tear film and BUT have served major

roles in the evaluation of the tear film stability of patients [23]. When the BUT is short, the tear film stability is poor, and the eye does not receive sufficient lubrication, which results in eye puffiness or pain. Fluorescent staining of the ocular surface is frequently used to detect epithelium damage of the ocular surface in patients with dry eye. Fluorescein has little toxicity and is used to perform corneal and conjunctival staining and classification with observation under blue light.

Lactoferrin is a type of glycoprotein with a molecular weight of 70-80 kD that mainly exists in milk and other bodily fluids, including tears, semen, synovial fluid and neutrophilic leukocyte particles. In addition, this glycoprotein exerts a sterilization effect with lysozyme by combining with iron in bacteria to prevent bacterial growth [24]. In a previous study, Ohashi *et al.* [25] demonstrated that the tears of patients with dry eyes exhibit significant changes in lactoferrin content. Apolipoprotein, the main protein of plasma lipoprotein, carries out functions including lipid transport, protein structure stabilization and metabolic stimulation. Furthermore, apolipoprotein also serves a role in the activation of lipoprotein metabolic enzymes and recognition receptors [26]. A study of dry eye in mice conducted by Nyunt *et al.* [27] revealed that the corneal immunofluorescence staining score was decreased and corneal epithelium thickness increased following exogenous apolipoprotein A-I (APOA-I) treatment, suggesting that APOA-I might be effective for the treatment of dry eye in humans [27].

BUT and SIT results are reported to have a strong association with age [28, 29]. In the present study, *Buddleja officinalis* drops notably improved the SIT of menopausal dry eye and modified the composition of tear proteins. The SIT reflects the basic amount of tear secretion, and the tear protein levels reflect the tear composition, which are important factors in dry eye. The mechanism underlying these effects may be that *Buddleja officinalis* acts as a quasi-androgen to stimulate secretion by the eyelid glands (lipids from the meibomian gland and tear secretion from the lacrimal gland), which significantly improves the SIT and tear protein results. Corneal topography (surface regularity index), wavefront aberration analysis and visual sensitivity measurement methods are able to detect abnormalities of the corneal surface caused by epithelial cell dryness and reveal instability in patients with dry eyes [30, 31]. In the present study, *Buddleja officinalis* effectively reduced inflammatory cell infiltration and the shrinkage of corneal basal cells in dry eye disease due to androgenic abnormality. This could be associated with the ability of *Buddleja officinalis* to improve secretion by the lacrimal and meibomian glands, and its ability to attenuate apoptosis of the cornea.

On the basis of the results of the present study, it is suggested that eye drops containing *Buddleja officinalis* extract may be useful as an alternative treatment for dry eye with improved safety and therapeutic potential; this is supported by preliminary studies using *Buddleja officinalis* extract. The underlying mechanism may involve an androgen-like effect of *Buddleja officinalis* flavonoids; however, the mechanism requires investigation in a further study. The authors consider that the symptoms of dry eye in several patients of group A did not improve due to poor sight at this age. Therefore, these drops may still be valuable for clinical application. In the future, clinical trials with large samples are required, and studies on the intervention mechanism should clarify their possible ocular anti-inflammatory effect and benefit to neovascular diseases and may be useful for elucidating novel therapeutic strategies for ocular degenerative diseases.

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Disclosure of conflict of interest

None.

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