

Original Article

Clinical findings associated with *Bidens bipinnata* L. eye drops on moderate and severe dry eye in postmenopausal women

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Abstract: Background: To investigate the therapeutic efficiency of *Bidens bipinnata* L. eye drops on moderate and severe dry eye in postmenopausal women. Methods: In this prospective random-controlled study, 60 postmenopausal patients diagnosed with dry eye in two hospitals from August 6th 2011 to August 6th 2013 were divided into two groups (30 cases in each group). One group (A) was treated with *Bidens bipinnata* L. forte eye drops and the other (B) was treated with artificial tears as a control. We compared these two groups by evaluating visual acuity, subjective symptoms of the ocular surface, Ocular Surface Disease Index (OSDI), tear film function tests, tear protein, and confocal scanning microscopy at before treatment and at 1, 2, 4, and 8 weeks after treatment. At 8 weeks after treatment, all patients were examined with systolic pressure, diastolic pressure, glutamic-pyruvic transaminase, glutamic oxaloacetic transaminase, urine creatinine, and blood urea nitrogen to determine the safety of the trial. Results: There were no significant differences for the symptoms of ocular surface, OSDI, tear protein, and tear film function between the two groups before the start of treatment ($P > 0.05$). After treatment, although all indices of patients in Group A were improved with the prolonged treatment time, the clinical curative effect in Group B was not significantly different. At 8 weeks after therapy, all indices of patients in Group A had improved, compared with pre-therapy ($P < 0.05$), while there was no improvement in Group B ($P > 0.05$). At postoperative week 8, all indices were significantly different between the two groups ($P < 0.05$). In addition, the mean number of corneal epithelium basal cells and inflammatory cells of Group A were greater than Group B ($P < 0.05$); the number in Group A were $3066 \pm 269 \text{ mm}^{-2}$ and $52 \pm 18 \text{ mm}^{-2}$, compared with Group B $4102 \pm 324 \text{ mm}^{-2}$ and $194 \pm 62 \text{ mm}^{-2}$, respectively. All safety values for all patients showed no change at 8 weeks after treatment. Conclusion: *Bidens bipinnata* L. eye drops can significantly alleviate the symptoms of moderate and severe dry eye in postmenopausal women.

Keywords: *Bidens bipinnata* L. eye drops, dry eye, postmenopausal women, treatment, androgen

Introduction

Dry eye can affect people's work and life by causing a notable decline in comfort and sight. Over the years, the prevalence of dry eye has increased and an epidemiological survey revealed that the prevalence is greater than 10% [1]. For postmenopausal women, dry eyes are primarily caused by decreased androgen levels [2]. This takes place over a prolonged period of time and symptoms may be severe, so this has become an important research topic.

In current therapies, androgen supplement is the only treatment for dry eye, but prolonged use of this therapy inevitably causes many side effects. *Bidens bipinnata* L. is a natural Chinese medicine for hepatitis and is often used to treat eye diseases due to its flavonoid content. Both as heterocyclic polyphenol compounds, androgens and flavonoids are similar in chemical structure and can be used to treat dry eye caused by gonadal hormone level imbalance. The aim of the present study was to investigate the therapeutic efficiency of *Bidens bipinnata*

L. eye drops in moderate and severe dry eye of postmenopausal women. From August 2011 to August 2014, 60 postmenopausal women diagnosed with moderate to severe dry eye in the ophthalmology department of our hospital were selected. The selected women were randomly divided into two groups: one group treated with a combination of *Bidens bipinnata* L. eye drops and artificial tears (Group A), and the other treated with artificial tears only (Group B). The results are given below.

Materials and methods

Preparation of the Bidens bipinnata L. eye drops

Bidens bipinnata L. dry leaves were purchased from a crude drug market in Nanchang, Jiangxi Province, China. The leaves were cut into small pieces and extracted twice in hot 80% alcohol, followed by centrifugal filtration; the filtrate passed through an HPD-100 macroporous resin column, and was eluted with 70% ethanol. Elution was collected, dried and crushed as the *Bidens bipinnata* L. extract. Columns: Phenomenex × Gemini C₁₈. Mobile phase: Methanol-0.1% phosphoric acid 55:45, (v/v); Rate: 0.8 mL/min; Detection wavelength: 326 nm; Column temperature: 300°C; Injection volume: 10 µL. Linarin standard solution was prepared as a control, using an external standard method for calculation. This control was prepared to establish the standard UV curve, and the total flavonoids in *Bidens bipinnata* L. were measured by UV spectrometry. *Bidens bipinnata* L. eye drops were made as previously described [3]. Briefly, extract of *Bidens bipinnata* L. was dissolved in distilled water (the quality ratio of water vs. extract was 1:0.1). The control solution was diluted in the eye lubricant carboxymethyl cellulose to the concentration of 1.5%, and further diluted to 0.1% or less with the supplement of potassium bicarbonate and potassium chloride buffer. Physical and chemical properties were adjusted to the following: pH value: 7.3-7.8; osmotic pressure: 311-350 mOsm; weight approximately equal to 1; refractive index: 1.336. Finally, 0.005% of the preservative benzalkonium bromide was added.

Study design

A prospective randomized control study was conducted. Important non-experimental factors, such as age, were allocated randomly to

minimize the unbalance index. Case history study and complete ocular surface examination were performed to determine participant eligibility. Female dry eye patients aged 50 to 56 years (average age 55.4±2.8 years) with moderate to severe symptoms were randomly divided into two groups. A combination of *Bidens bipinnata* L. eye drops and artificial tears-Hypromellose 2910, Dextran 70, and Glycerol Eye Drops (Alcon Corporation, USA) was used in Group A, whereas in Group B, only artificial tears were used. Both groups were treated 3 times per day for 8 weeks. Patients did not have other ocular medication histories, ophthalmology measurement histories, or histories of taking anti-hypertensive or anti-depressant medication. Furthermore, none of the patients were pregnant or lactating. Visual acuity, subjective symptom, OSDI, the tear film test with 4 terms, tear protein, and corneal confocal scanning were conducted at 1, 2, 4, and 8 weeks before and after treatment, respectively. Sample size was set to 30 based on the equation $n = 15.6R + 1.6$ under 80% confidence.

Ethical considerations

The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol and procedure was fully explained to each patient, and consent was obtained according to the Ethical Committee of our hospital.

Patients

In order to be selected for this study, the postmenopausal women had to meet the following criteria: (1) had undergone bilateral oophorectomy; or (2) 50-56 years old, had been menopausal for more than 12 months, no chemotherapy, no tamoxifen or toremifene treatments, no any other treatments that sought to suppress ovarian function, and the FSH and estradiol levels were within the postmenopausal range (basic FSH > 40 IU/L, estradiol: 40~100 pmol/1); or (3) 50-56 years old, had tamoxifen or toremifene treatments, and the FSH and estradiol levels were within the postmenopausal range (basic FSH > 40 IU/L, estradiol: 40~100 pmol/1).

The dry eye recruitment criteria [4]

In this study, the postmenopausal women had to meet the following criteria: (1) chronic symp-

toms (> 1 item): visual tiredness, dry and unsmooth sensation, foreign body sensation, burning sensation, photophobia, pain, red eye, and tears; (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 fluorescent pigmentation/staining would have a score of 0-12 points; (3) tear film break-up time (TFBUT): positive < 10 s; and (4) Schirmer I test (SIT): SIT values \leq 10 mm/5 min. Patients with positive dry eye symptoms and any two positive results from criteria (2), (3), and (4) were diagnosed as dry eye. Patients who had only one positive result from criteria (2), (3), and (4) were considered for a subsequent check of tear lactoferrin concentration. Patients with positive dry eye symptoms and tear lactoferrin < 100 mg% were diagnosed as dry eye.

The dry eye exclusion criteria

In this study, postmenopausal women meet the following criteria were excluded from the study: (1) no obvious symptoms; (2) cornea fluorescent pigmentation/staining was 0 points; (3) BUT \leq 1 s or > 10 s; and (4) SIT \leq 1 mm/5 min or > 10 mm/5 min. For each patient, the eye with less severe symptoms was chosen as the control eye. Patients were evaluated at 1 week, 1 month, and 2 months before and after the administration of drugs. For classifying moderate to severe dry eye, dry eye patients were stratified according to the evaluation and classification standard of dry eye that was proposed by Jacobi. Mild dry eye was classified as follows: the dry eye symptom score was 0-2 points (0 and 2 were both included), the cornea fluorescent pigmentation/staining score was 0-4 points, BUT was 5-10 s, and SIT value was 5-10 mm/5 min. Moderate to severe dry eye was classified as follows: dry eye symptom score of 2-3 points (2 and 3 were both included), cornea fluorescent pigmentation/staining was 5-12 points, BUT < 5 s, SIT value < 5 mm/5 min. All measurements were conducted at 9:00-11:00 h on the test-day.

Efficacy evaluation

Subjective symptom score of the ocular surface: The patients were asked whether they had discomfort from dryness, abnormal sensation, or asthenia: 0 points represented asymp-

tomatic, 0.5 points represented occasional symptoms, 1 point represented intermittent mild symptoms, and 2 points represented sustained obvious symptoms. To assess symptoms, patients also completed the Chinese version of the Ocular Surface Disease Index (OSDI) questionnaire [5, 6]. This 12-item, self-administered questionnaire assessed ocular symptoms (photophobia; gritty sensation; eye pain; blurred vision; poor vision; difficulty in reading, driving at night, working with a computer or automatic teller machine, or watching television; discomfort under windy conditions, in places with low humidity, or in air-conditioned areas) during the 2- to 4-week period before the examination. Scores range from 1 to 100, with higher scores representing greater disability. OSDI questions included: 1. Have you experienced the following conditions in the last week: eyes sensitive to light? 2. 12. Have your eyes felt uncomfortable in the following situations in the last week: in an air-conditioned place? In the scoring, 4 points represented always, 3 points represented most of the time, 2 points represented half of the time, 1 point represented sometimes, and 0 points represented seldom. Patients were not required to answer questions if they were uncertain. OSDI = sum of the scores of all questions/number of answers \times 25.

Tear film test with four terms

For the corneal fluorescein staining procedure, the patients had fluorescent strips inserted into the fornix conjunctivae inferior, followed by blinking. Then, a 0 to 12 point scale was used to record staining. The cornea was divided into four quadrants, and 0-3 points were scored according to the degree and area of the staining of each quadrant. The ratings were then classified into four levels based on the National Ophthalmology Research Clinical Dry Eye Score [7]: 0: No staining; 1: Few disseminated stains; 2: moderate stains (between class 1 and class 3); and 3: severely fused stains. The height of the tear meniscus in the central lower eyelid was measured by a ruler under a slit-lamp microscope. Tear stability was assessed by performing a TFBUT evaluation. This measures the time interval between a blink and the appearance of a break in the tear film. A fluorescein strip was placed in the fornix of the lower eye lid and then removed. The patient was instructed to blink three times and then to look straight ahead without blinking. The tear film was observed under a slit-lamp microscope with a

Table 1. Participant characteristics

Variables	A	B	t (χ^2)	P
Age (range, years)	55.98±7.29 (50-56)	54.96±8.91 (50-56)	0.421	> 0.05
Spherical equivalent refractive error (diopters)				
Mean ± SD (range)	-1.28±2.25 (-3.5-3.75)	-1.14±1.96 (-3.75-3.75)	0.126	> 0.05
Interval between onset of symptoms and treatment (weeks)				
Mean ± SD (range)	-45.69±21.32 (27-64)	-42.94±23.79 (28-62)	0.225	> 0.05
Duration time (months)	10.56±3.34 (3-40)	11.19±3.48 (4-36)	0.916	> 0.05
BMI	23.19±4.76	23.62±4.97	0.822	> 0.05

cobalt-blue filter. Three measurements were taken in each eye, and the overall average of each eye was used for analysis. In the Schirmertest1, a thin strip of filter paper (35 × 5 mm) was used to quantify tear production over a period of 5 min. The strip was placed at the junction of the middle and lateral thirds of the lower eye lid. The patient was instructed to look forward and blink normally during the test.

Tear protein measurements: All tear samples were collected from 9:00 to 11:00 am. About 20 µL of non-irritating tears were collected from the patients' tear meniscus by a capillary pipette and were then stored in a 0.5 ml EP tube (Eppendorf, Fremont, CA) at -80°C. The total tear protein concentration was measured by the Bradford method using bovine serum albumin as the standard. Lactoferrin concentrations were measured by radioimmunoassay. First, a 10 µL tear sample was collected and then diluted 100 times with saline. Subsequently, the lactoferrin concentration was calculated in accordance with the standard curve. Finally, the lysozyme concentration was measured with a turbidimeter. Following the manufacturer's instructions in the lysozyme test kit, the staining broth, lysozyme standards, and standard curve were all prepared. The protocol for Lipocalin concentrations was carried out as previously described [8].

Corneal confocal microscopy: Confoscan 4 slit-lamp scanning confocal microscopy (Nidek Cor. Ltd, Japan) was used as described [9]. After ocular surface anesthesia, the patient's eyes were fixed staring straight ahead under the microscope. The examiner slowly pushed the objective forward to focus on the corneal epithelial cells. Full-tomography was obtained 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 counted.

Safety evaluation: All adverse events that occurred during the study and details of their nature, occurrence, duration, severity, significance, and the relationship to the study drug were recorded. Safety indicators included: systolic pressure (SDP), diastolic pressure (DBP), glutamic-pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), urine creatinine (UCr), blood urea nitrogen (BUN).

Statistical analyses

All values are expressed as means ± standard deviation (SD). ANOVA was used for all indexes in subjective symptoms before and after treatment comparisons; Dunnett's-test was applied for multiple comparisons. Differences between two groups in subjective symptoms, age, spherical equivalent refractive error, interval between onset of symptoms and treatment, duration time, BMI, SDP, DBP, GPT, GOT, UCr, BUN were performed using the paired t-test. A value of $P < 0.05$ was considered statistically significant. Calculations and statistical analyses were performed using the 19.0 software package for Windows (SPSS, China).

Results

Clinical outcomes

There was no significant difference in age, BMI, spherical equivalent refractive errors, interval between the onset of symptoms and treatment, and duration time between the two groups ($P > 0.05$). The details of these results are presented in **Table 1**.

Visual acuity

As shown in **Figure 1**, there were no significant differences in dry eye visual acuity between the two groups before therapy ($P > 0.05$). After 8 weeks of treatment, visual acuity improved from baseline by an average of 1.3 lines in Group A and 1.1 lines in Group B (**Table 2**). The

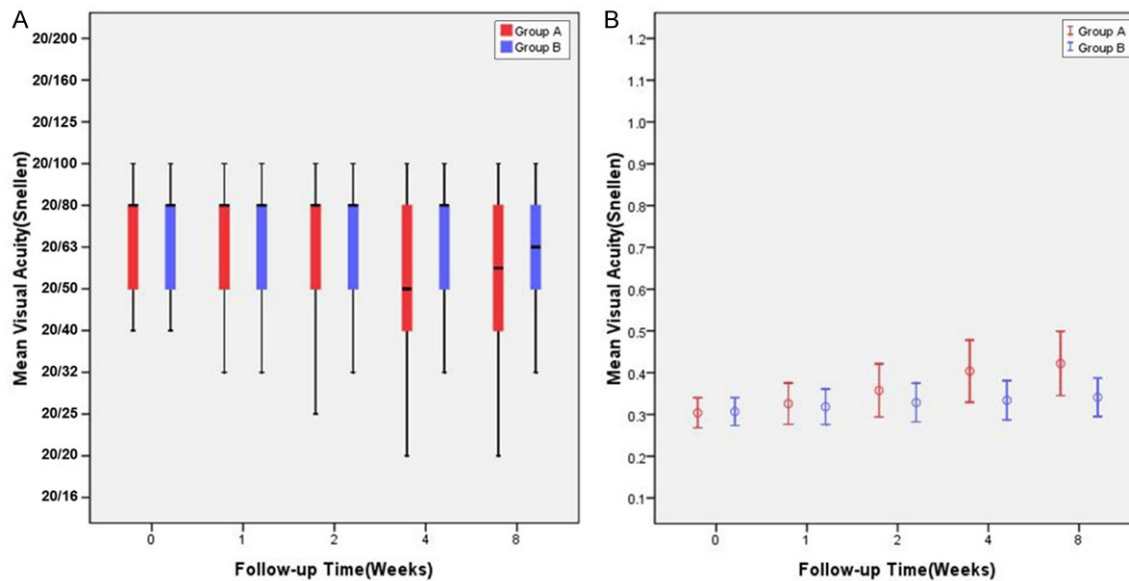


Figure 1. Mean visual acuity in dry eyes from baseline to 8 weeks at each time point. The box on the left demonstrates the distribution of dry eye visual acuity for Group A and the one on the right represents the data for Group B. The top and bottom of each box represent the 25th and 75th percentiles of the data and the lines in the box show the medians. The treatment group means are represented by a dot (Group A) or a square (Group B) and are connected across the visits with lines. The bars extending above and below each box represented 1.5 times the interquartile range (difference between the 25th and 75th percentiles), and the open circles are outlier values.

Table 2. Mean treatment group improvement in visual acuity at follow-up visits (lines)

Group n	Before	1 wk (Lines)	2 w (Lines)	4 w (Lines)	8 w (Lines)
A 30	0.304±0.099	0.326±0.135 (1.1)	0.358±0.174 (1.1)	0.404±0.203 (1.3)	0.422±0.211 (1.3)
B 30	0.307±0.091	0.318±0.116 (1.0)	0.329±0.127 (1.0)	0.334±0.128 (1.1)	0.341±0.127 (1.1)

improvement level of visual acuity was no different between the groups ($P = 0.20$: **Figure 1A, 1B**).

Subjective symptoms of the ocular surface

As listed in **Table 3**, there was no significant difference for the subjective symptom of dry eye between the two groups before therapy ($P > 0.05$). After 1 week of treatment, Group A showed no significant improvement in their symptoms compared with pre-therapy ($P > 0.05$). The subjective symptoms of moderate and severe dry eye in Group A were improved at 8 weeks after therapy ($P < 0.05$). In Group B there was no significant improvement (except for photophobia and pain) at 8 weeks after therapy compared with pre-therapy ($P > 0.05$).

The results of the OSDI questionnaire showed an improvement of symptoms in Group A, but not in Group B ($P > 0.05$). A summary of mean total OSDI scores is presented in **Figure 2A**.

The results of the OSDI questionnaire showed an improvement of symptoms in patients treated with *Bidens bipinnata* L. eye drops and artificial tears but not with artificial tears only ($P < 0.05$). The Mean Ocular Symptoms OSDI SubScore, Mean Vision Related Function OSDI SubScore, and Mean Environmental OSDI SubScore showed no significant difference between the two groups at 8 weeks after therapy ($P < 0.05$, **Figure 2B**).

Tear film test

Figure 3 summarizes the results of tear film values in the 2 groups before therapy and at 1, 2, 4, and 8 weeks after therapy. No difference in SIT, BUT, FI, and HTM was detected at pre-therapy when comparing Group A with Group B, nor was a difference found at post-therapy week 1 ($P > 0.05$). Although SIT was increased in Group A, no difference was found at 2 and 4 weeks after therapy in both groups ($P > 0.05$,

Table 3. Comparison of the symptoms of the ocular surface in both groups before and after therapy

Group/subjective symptom	n (%)	pre-therapy	post-therapy				F1	F2	P
			1 w	2 w	4 w	8 w			
Group A									
Eye fatigue	30 (100)	2.82±0.76	2.72±0.64	2.48±0.57	1.45±0.41	1.34±0.28	12.6	2.16	< 0.05
Dryness	30 (100)	2.73±0.64	2.61±0.58	2.34±0.49	1.43±0.53	1.29±0.39	15.2	3.35	< 0.05
Abnormal sensation	30 (100)	2.36±0.99	2.21±0.73	2.06±0.56	1.36±0.59	1.27±0.42	18.8	3.51	< 0.05
Burning sensation	24 (80)	2.61±0.55	2.53±0.52	2.34±0.39	1.76±0.38	1.51±0.31	21.7	1.99	< 0.05
Photophobia	24 (80)	2.57±0.49	2.42±0.41	2.29±0.35	1.67±0.72	1.43±0.57	15.9	3.29	< 0.05
Pain	18 (60)	2.63±0.56	2.49±0.45	2.37±0.39	1.24±0.52	1.14±0.35	28.6	2.64	< 0.05
Redness	15 (50)	2.58±0.47	2.51±0.39	2.23±0.36	2.12±0.29	1.99±0.34	11.3	2.02	< 0.05
Tear	6 (20)	2.43±0.31	2.33±0.24	2.17±0.29	1.96±0.39	1.71±0.23	12.8	3.91	< 0.05
Group B									
Eye fatigue	30 (100)	2.87±0.69	2.78±0.76	2.61±0.61	2.56±0.53	2.53±0.45	0.98	0.13	> 0.05
Dryness	30 (100)	2.69±0.54	2.62±0.51	2.51±0.38	2.46±0.36	2.39±0.31	1.38	0.21	> 0.05
Abnormal sensation	27 (90)	2.39±0.96	2.32±0.53	2.25±0.49	2.17±0.57	2.12±0.45	1.06	0.09	> 0.05
Burning sensation	27 (90)	2.68±0.57	2.57±0.46	2.48±0.33	2.39±0.41	2.34±0.39	2.02	0.28	> 0.05
Photophobia	24 (80)	2.62±0.51	2.51±0.59	2.41±0.49	2.36±0.39	2.33±0.45	4.19	8.13	< 0.05
Pain	18 (60)	2.59±0.61	2.42±0.57	2.37±0.41	2.29±0.47	2.19±0.54	5.78	6.81	< 0.05
Redness	15 (50)	2.49±0.46	2.35±0.39	2.19±0.47	2.06±0.56	2.01±0.49	1.39	0.19	> 0.05
Tear	6 (20)	2.39±0.28	2.25±0.19	2.18±0.16	2.12±0.21	2.05±0.18	2.91	0.38	> 0.05

Note: F1, MD of the symptom of ocular surface pre-therapy; F2, MD of the symptom of ocular surface at 8 weeks post-therapy.

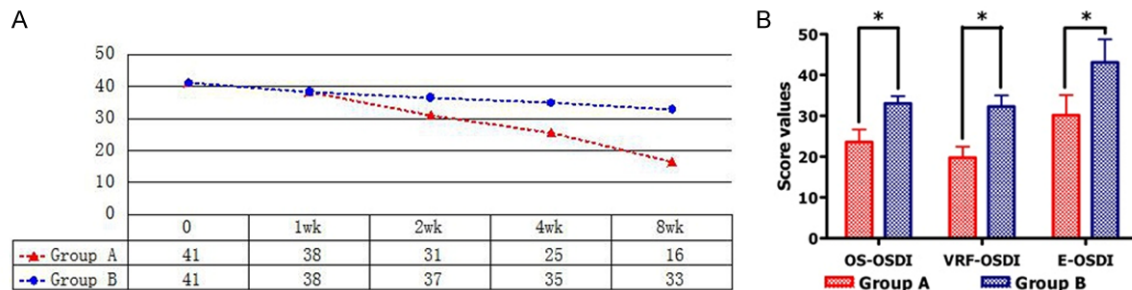


Figure 2. A. Time course of the Mean Total OSDI scores of eyes of postmenopausal patients who underwent treatment with *Bidens bipinnata* L. eye drops with artificial tears or with artificial tears only. The results showed an improvement in the symptoms of the ocular surface in patients treated with *Bidens bipinnata* L. eye drops and artificial tears, but not with artificial tears only ($P < 0.05$). B. The Mean Ocular Symptoms OSDI SubScore, Mean Vision Related Function OSDI SubScore, and Mean Environmental OSDI SubScore surface between the two groups at 8 weeks after therapy. Data are mean \pm SD of values from all eyes per group. * $P < 0.05$ (Dunnett's test). OS-OSDI, Mean Ocular Symptoms OSDI SubScore, VRF-OSDI, Mean Vision Related Function OSDI SubScore, E-OSDI, Mean Environmental OSDI SubScore, * $P < 0.05$.

Figure 3A). The FL of Group A decreased at 4 weeks and 8 weeks after therapy ($P < 0.05$), while for Group B there was no significant change after therapy compared with pre-therapy ($P > 0.05$, **Figure 3C**). The BUT and TMH of Group A were improved at 4 weeks and 8 weeks after therapy ($P < 0.05$), while there was no significant change at the time after therapy com-

pared with those of pre-therapy ($P > 0.05$, **Figure 3B, 3D**).

Tear proteins

Figure 4 shows tear proteins in the 2 groups at pre-therapy and 1, 2, 4, and 8 weeks after therapy. Before treatment, there was no significant

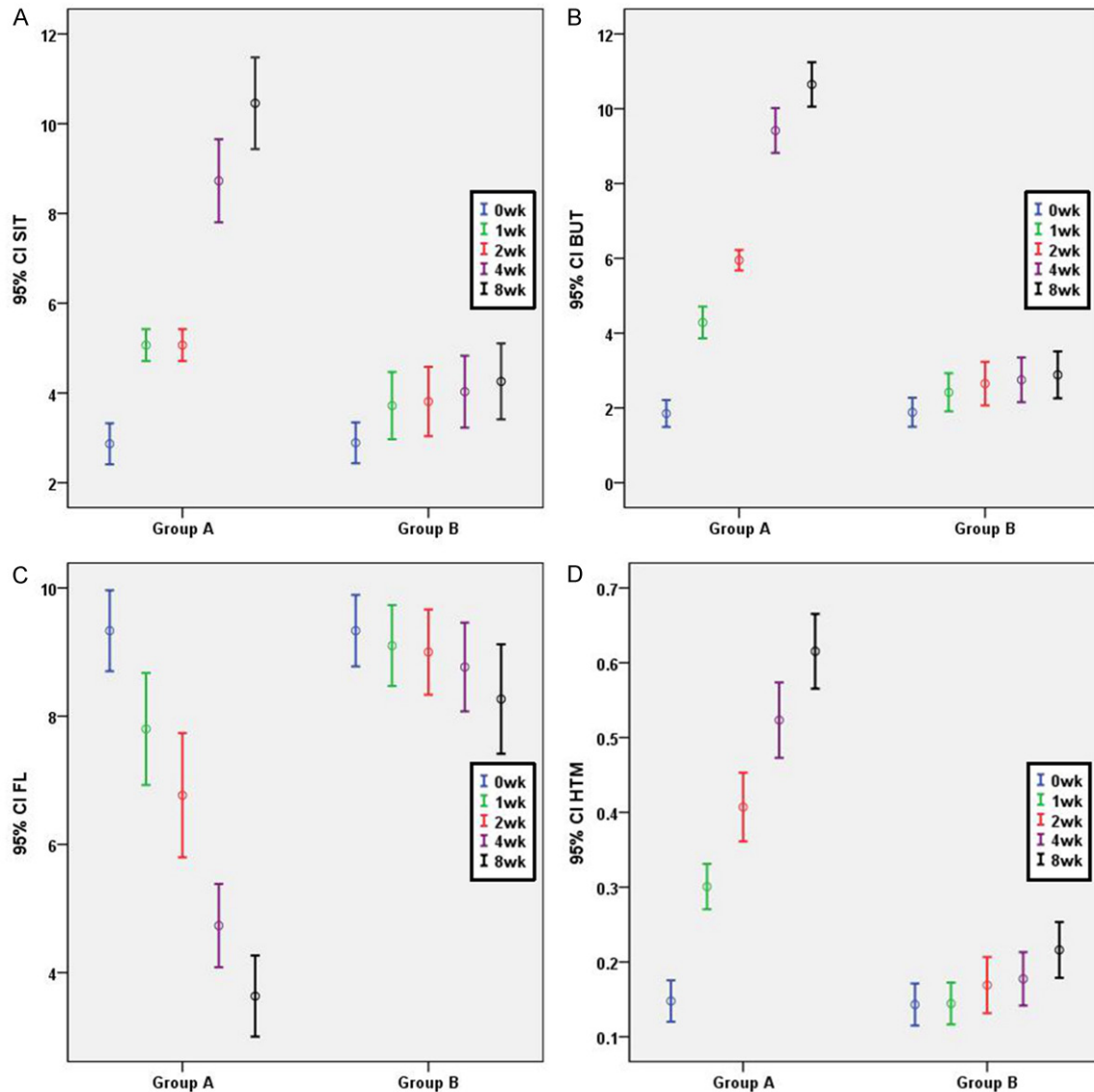


Figure 3. The trend of schirmer I test, tear film break-up time, fluoresce in staining and height of Tear meniscus before and after treatment (A-D). The SIT showed no significant difference between Groups A and B until 8 weeks of treatment ($P > 0.05$, respectively, A). The SIT in Group A was significantly greater than that of Group B after 8 weeks of treatment ($P < 0.05$). The BUT and HTM of Group A were greater than those of Group B at 2, 4, and 8 weeks, while there was no significant difference at 1 week after therapy ($P < 0.05$, respectively, B, D). The FL of Group A was decreased below that of Group B at 2, 4, and 8 weeks, while there was no significant difference at 1 week after therapy ($P < 0.05$, respectively, FC). Thirty cases were tested in each study group.

difference in total tear proteins, lactoferrin, lysozyme, and lipocalin of both groups (respectively, $P > 0.05$). However, after 8 weeks of treatment, the total tear proteins, lactoferrin, lysozyme, and lipocalin from Group A were all significantly different from those of Group B (respectively, $P < 0.05$). The total tear proteins, lactoferrin, and lysozyme of Group A began to increase at 4 weeks after therapy ($P < 0.05$, respectively, **Figure 4A-C**). The lipocalin in Group A was significantly greater than that of

Group B at 8 weeks ($P < 0.05$, **Figure 4D**), while there were no significant changes at 1, 2, and 4 weeks between the two groups ($P > 0.05$, respectively, **Figure 4D**).

Corneal confocal microscopy

Normal female corneal epithelial basal cells are dark cells with clear cell borders (**Figure 5A**); however, in postmenopausal patients with dry eye, the corneal epithelial basal cells were

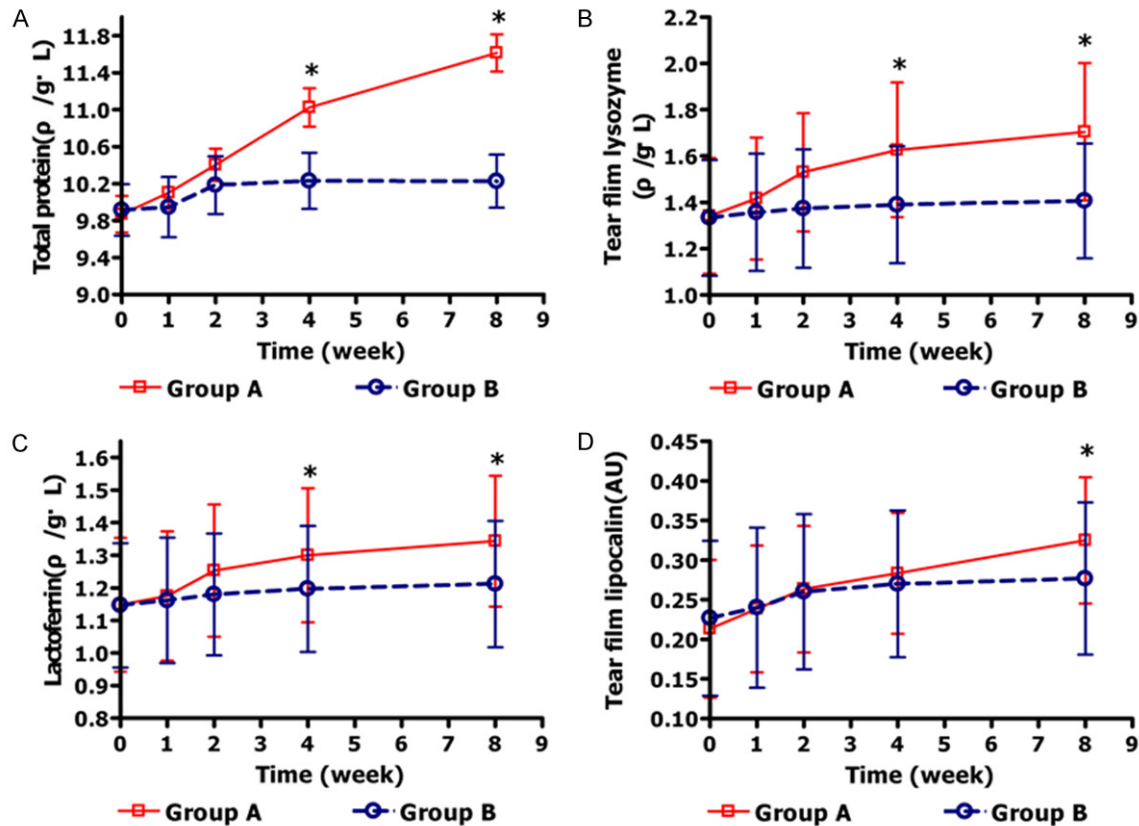


Figure 4. Total tear proteins, lactoferrin, and lysozyme of Group A were greater than those of Group B at 4 weeks and 8 weeks, while there was no significant difference at 1 week and 2 weeks after therapy ($P < 0.05$, respectively, A-C). The lipocalin in Group A was significantly greater than that of Group B at 8 weeks ($P < 0.05$, D), while there were no significant change at 1 week, 2, and 4 weeks between the two groups ($P > 0.05$, respectively, D). * $P < 0.05$ vs. group A. The sample size was 30 cases for Group A and 30 cases for Group B throughout the study.

shrunken and were infiltrated by bright inflammatory cells (Figure 5B). Subsequently, after two months of treatment, Group A only had a few inflammatory cells that infiltrated the epithelial basal layer; however, the density of corneal epithelial basal cells was slightly decreased compared to normal levels in women (Figure 5C). In contrast, in Group B, many bright inflammatory cells were found at the epithelial basal layer, and the density was significantly increased (Figure 5D). At 8 weeks after treatment, the mean number of corneal epithelium basal cells and inflammatory cells in Group A were $3066 \pm 269 \text{ mm}^2$ and $52 \pm 18 \text{ mm}^2$, respectively; in Group B the same figures were $4102 \pm 324 \text{ mm}^2$ and $194 \pm 62 \text{ mm}^2$, respectively; there were statistically significant differences between the two groups ($P < 0.05$, Figure 5E, 5F).

Clinical safety

Compared with before therapy, all items (SDP, DBP, GPT, GOT, UCr, BUN) showed no obvious change at 8 weeks after therapy in Group A ($P > 0.05$, Table 4).

Discussion

Bidens bipinnata L. is a well known traditional Chinese medicine [10], and it is widely used in the treatment of malaria, diarrhea, dysentery, hepatitis, acute nephritis, and other diseases. We previously reported that *Bidens bipinnata* L. water-extract liquid can be used to treat dry eye of menopausal women [11]. In this study, we investigated the effects of *Bidens bipinnata* L. eye drops on moderate and severe dry eye of postmenopausal women.

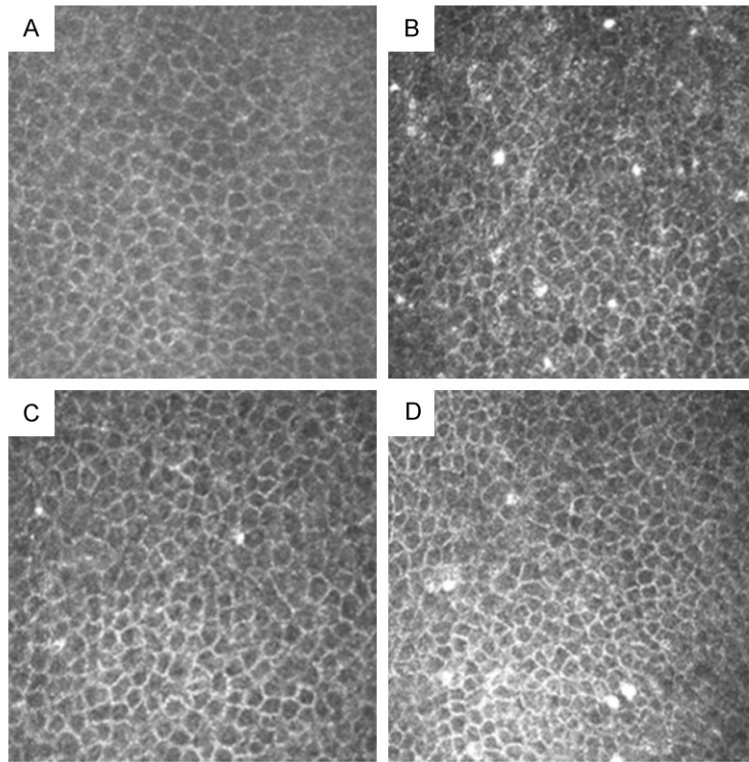
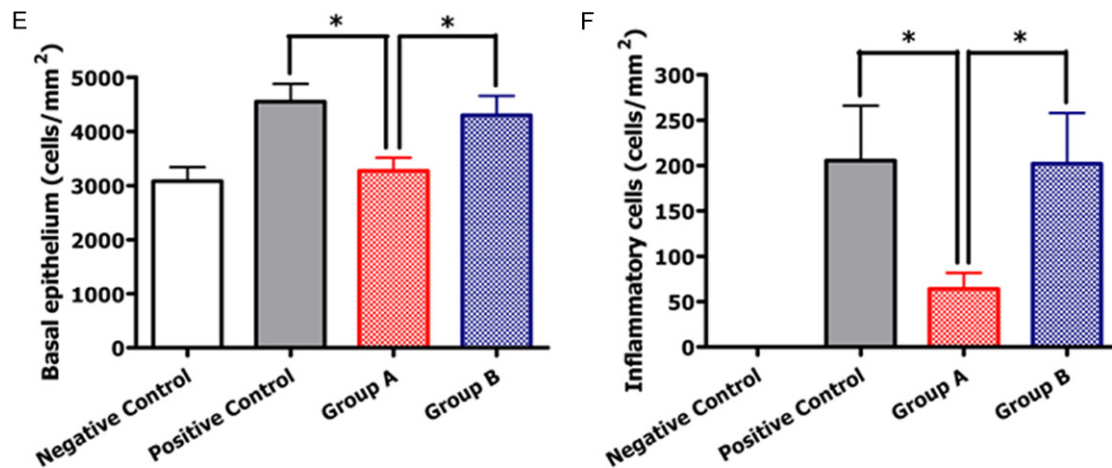


Figure 5. The effects of *Bidens bipinnata* L. eye drops and PBS on the morphology of female corneal epithelial basal layers. Representative *in vivo* confocal images of the corneal epithelium in different groups. A. Normal females. B. Moderate and severe dry eye of the postmenopausal females. C. Moderate and severe dry eye of postmenopausal females treated with *Bidens bipinnata* L. eye drops and artificial tears. D. Moderate and severe dry eye of the postmenopausal females treated with artificial tears. E. The mean corneal basal cell densities in four groups. Data are mean \pm SD of values from all eyes per group. * $P < 0.05$ (Dunnett's test). F. The mean inflammatory cell densities in the four groups. Data are mean \pm SD of values from all eyes per group. * $P < 0.05$ (Dunnett's test).



Despite dry eye being one of the most common eye diseases, effective treatments are lacking [12]. Sex hormones regulate the body and local immune function, morphology, development, differentiation, and secretion of the lacrimal and meibomian glands. Moreover, they also regulate the tear stability, inflammation, and apoptosis. The post menopausal decline in androgen levels leads to tear film instability, local inflammatory responses, and increasing apoptosis of glandular tissue, such as the lacrimal gland, which subsequently results in a high incidence of dry eye [13]. Currently, close rela-

tionship between androgens and dry eye has been widely acknowledged. It has been reported that androgens, estrogens, progesterone, and prolactin receptors are widely present in the lacrimal gland, meibomian gland, cornea, and other ocular tissues of humans, rabbits, and rats, with which the biological effects of androgen replacement drugs are associated. However, long-term usage of androgens produces severe side effects in both men and women, such as male prostate enlargement, development of cancer, and masculinized women, which causes obvious distress to

Table 4. The clinical safety surface in Group A before and after therapy

	SDP (P/kPa)	DBP (P/kPa)	GPT ($\mu\text{mol}\cdot\text{L}^{-1}$)	GOT ($\mu\text{mol}\cdot\text{L}^{-1}$)	UCr ($\mu\text{mol}\cdot\text{L}^{-1}$)	BUN ($\mu\text{mol}\cdot\text{L}^{-1}$)
Before therapy	132.6 \pm 10.7	86.2 \pm 8.6	22.48 \pm 6.69	29.14 \pm 6.45	61.36 \pm 15.29	3.38 \pm 0.58
After therapy	133.8 \pm 11.6	88.7 \pm 9.4	23.66 \pm 7.08	30.72 \pm 6.92	59.94 \pm 16.76	3.29 \pm 0.79

patients. Given the aforementioned information in conjunction with the lack of effective treatments, the need to develop effective male hormone replacement drugs becomes obvious.

Bidens bipinnata L. is a natural Chinese medicine that has been used for the extraction of luteolin, quercetin, isoquercitrin, hyperoside, and eight other types of flavonoids. It contains 4.035% flavonoids that have a similar chemical structure to androgens [14, 15], so they can bind to androgen receptors on membranes to function as quasi-androgens.

Results from the current study showed that vision acuity improved in Group A over Group B and confirmed the effects of *Bidens bipinnata* L. eye drops. Previous research has found that flavonoids are stimulants of membrane androgen receptors by combining with cell-membrane androgen receptors [16]. Mrukwa et al. detected the expression of estrogen receptor in the lacrimal gland and meibomian, suggesting that estrogen may directly affect their function [17]. Recently, some studies have found that many plants containing high levels of isoflavones including genistein [18], epimedium [19], mignonette [20], apigenin [21] and butterfly bush flower [22] have estrogenic effects, and these can interact with estrogen receptors. We previously discussed the role of *Bidens bipinnata* L. water decoction in dry eye disease, and this study shows that *Bidens bipinnata* L. drops can also significantly improve the symptoms of dry eye. Moreover, it also reduces the systemic side effects of decoction for gastrointestinal malabsorption patients. The OSDI questionnaire has 12 questions including ocular symptoms, vision-related function, and environmental stimuli. The score ranges from 0 to 100 points with a good correlation with the severity according to the patient's subjective feeling. Our experiment showed that a combination of *Bidens bipinnata* L. with artificial tear eye drops can significantly improve the OSDI and symptoms of moderate to severe dry eye (eye fatigue, dryness, abnormal sensation, burning sensation, and redness) after two months of treat-

ment, whereas artificial tears alone did not show the improvement. This result may be related to the antibacterial function of *Bidens bipinnata* L. against multiple ocular microorganisms, which secrete a large number of choline to cause tears.

SIT values were significantly lower in patients with dry eye [23]. *Bidens bipinnata* L. drops significantly improved the SIT of menopausal dry eye and modified the composition of tear proteins. Because the SIT reflects the basic amount of tear secretion and the tear protein reflects tear composition, this result was much more significant than BUT. The main mechanism of this result is likely due to the ability of *Bidens bipinnata* L. to act as a quasi-androgen to stimulate eyelid gland (lipid layer composition from the meibomian gland and tears secretion from lacrimal gland) secretion, which can significantly improve the results of SIT and partial tear proteins.

The methods of corneal topography (surface regularity index), wave front aberration analysis, and visual sensitivity measurement can reveal abnormal corneal surfaces caused by dry and unstable epithelial cells in patients with dry eyes [24]. In our study, *Bidens bipinnata* L. effectively reduced inflammatory cell infiltration and the shrinkage of the corneal basal cells of dry eye disease caused by the reduction in male hormone levels. This could be related to the ability of *Bidens bipinnata* L. to improve the secretion of the lacrimal gland and meibomian gland as well as its ability to partially inhibit the apoptosis of the cornea. Unlike other eye drops, there were no differences between the two treatments compliance and the adverse effects on subscales were not seen at 8 weeks after treatment.

Limitations

Bidens bipinnata L. eye drops produce fewer side effects, which encourages patients to continue the treatment for an extended period of time. Therefore, these drops are valuable in terms of clinical application and popularization. However, *Bidens bipinnata* L. eye drop treat-

ment did not meet our pre-specified vision criterion to local artificial tears when initiating treatment of moderate and severe dry eye. We believe that failure to meet the pre-specified criterion was largely influenced by several subjects in Group A, who showed no improvement during the study; this was likely due to poor sight at this age. Otherwise, there were some limitations to our study, such as the relatively small sample size, the classification as a single-center study, and the lack of comparison between patients before and after treatment.

Conclusions

In summary, for postmenopausal dry eye caused by a decline in hormone levels, *Bidens bipinnata* L. eye drops can quickly and significantly improve symptoms and maintain tear protein composition in situations where local artificial tears, glucocorticoid eye drops, and lacrimal duct embolization treatments show poor effects. Because the average difference in visual acuity improvement between the two groups was less than half a line, and there was less burden of treatment on the patients and family, *Bidens bipinnata* L. eye drop treatment is a reasonable option to consider when initiating treatment of moderate and severe dry eye. In the future, clinical trials should be conducted with larger samples, and studies on the intervention mechanism would provide a new means of exploring possible ocular anti-inflammation and its benefit on neovascular diseases. Further investigation will elucidate novel therapeutic strategies for ocular degenerative diseases.

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Disclosure of conflict of interest

None.

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