Research Paper

Clinical study on Mulberry water decoction in treatment of xerophthalmia of menopausal females

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ABSTRACT

Objective: To investigate the clinical effect of Mulberry water decoction on xerophthalmia of menopausal females. Method: This study was a prospective random controlled trial. Forty-eight menopausal females diagnosed with xerophthalmia (aged from 45 to 55) were randomly divided into in two groups: group A the control group (vitamin c tablets) (n = 24) and group B the Mulberry water decoction group (n = 24). Both groups were treated with Forte Eye Drops. All patients were detected at 7d, 1month, 2month, before and after treatment to evaluate OSDI, four tear film indicators and tear protein. Variance analysis and differential analysis on sample average or median were made on both groups before and after treatment, observe the status under confocal microscope before and after treatment under. Result: There were no significant difference in detection indexs between both groups before treatment. Group A after treatment for 7 d, 1 month, OSDI has changed, there were statistical differences（all P＜0.05）. After treatment for 2 months, OSDI, BUT, SIT, tears river height and FL were no significant changes, there were no statistically significant differences (P > 0.05). At 7d, 1 month, 2 month after treatment, B group of patients OSDI, BUT, SIT, tears river height and FL were improved, there were statistical differences（all P＜0.05）. TP, LF, LZM, sIgA all have different degree of change in group A and B, but there were not statistically differences（all P＞0.05）. The results corneal confocal microscopy showed that after 2mo treatment of group A, the nuclei of squamous epithelial cell is light, the border is high reflection. After 2 months treatment of Group B, corneal epithelial layer of mild scale highly reflective of change. At 6 weeks after treatment, the mean number of corneal basal cells and inflammatory cells in group A were (4489 ± 215)mm-2 and (311 ± 102)mm-2，respectively，which in group B were (3315 ± 212) mm-2 and (39 ± 14)mm-2 , respectively，there were statistical differences between two groups (all P ＜0.05). After 6 weeks treatment, there were no change of corneal nerve bending and less

density in group A while the nerve fiber bending and density decreased significantly in group B ， there were statistical differences between two groups (all P ＜0.05) . Conclusion: Mulberry water decoction can effectively improve symposiums and signs of xerophthalmia among menopausal females and thus showing clinical significance to some extent.

INTRODUCTION

Dysfunctional tear secretion syndrome, or dry eye, is a potential damage to the ocular surface caused by discomfort, visual impairment, and tear film instability under different conditions Female[1]， Common dry eye symptoms include: eye discomfort, pain, foreign body sensation, visual fatigue, red and swollen eyes, itchy eyes, twitchy eyelids, etc。 Several large epidemiologic studies to date have reported an overall prevalence of dry eye ranging from 8.7% to 30.1%, with a higher prevalence in older women[2-4]。Risk factors for dry eye disease include age, postmenopausal women, arthritis, chronic caffeine use, thyroid disease, and gout[5] 。 In addition, dry eye can interfere with vision at different levels, reducing the quality of daily life, social activities, and psychological and psychiatric changes Risk factors for dry eye disease include age, postmenopausal women, arthritis, chronic use of caffeine, thyroid disorders, and gout[6] 。 Menopause is defined as the complete cessation of menstruation with elevated serum levels of follicle-stimulating hormone follicle-stimulating hormone, FSH） for at least one year[7]。 Hormonal changes lead to a decrease in tear production, which is one of the causes of dry eye, and about 60% of menopausal women have dry eye. Menopause is the complete cessation of menstruation accompanied by elevated serum levels of follicle-stimulating hormone (FSH) for at least one year[8] ， it is characterized by high morbidity and difficult treatment. Reduced androgen levels can cause dysfunction of the lacrimal glands, leading to decreased secretion of lacrimal lipids, excessive tear evaporation, and instability of the tear film. Androgens can act on the lacrimal gland, which is therefore one of their important target organs. Hormonal changes lead to a decrease in tear production, which is one of the causes of dry eye. About 60% of menopausal women have dry eye. Menopause is the period of time when menstruation stops completely and serum levels of follicle-stimulating hormone (FSH) increase for at least one year.The structure and function of the lacrimal gland, including its cellular structure, gene expression, and immunologic activity, can be considerably altered by androgens. Androgen deficiency may lead to lacrimal gland dysfunction or tear deficiency, thus causing dry eye. Clinical studies of hormone therapy have been conducted for patients with dry eyes due to altered androgen levels, but in order to minimize side effects, the current treatment is still based on local symptomatic therapy. Therefore, a better solution is to explore previously unused hormone replacement drugs. Sugars combine with most flavonoids to form glycosides, which are preserved in the roots, leaves and fruits of plants, while the rest of the flavonoids exist in a free form and have a variety of bioactivities such as anti-inflammatory, antioxidant, anti-immune, and anticancer effects. Bilberry pigment is one of them, which can be extracted from mulberry plants and can compensate for androgen deficiency to a certain extent by binding to androgen receptors 。 Hormone Replacement Therapy Widely Accepted to Improve Quality of Life for Postmenopausal Women[9]， However, the side effects of this treatment are high, so finding a more effective treatment for dry eyes is a pressing issue for researchers. In this paper, mulberry pigment decoction was used to treat dry eye in postmenopausal women on this basis with satisfactory results。

INFORMATION AND METHODS

1.General information

1.1 Inclusion criteria for menopausal women

After bilateral oophorectomy; aged 45-55 years old, menopause ≥ 12 months, not receiving tamoxifen, toremifene, chemotherapy or treatment to inhibit ovarian function, and FSH and estradiol levels should be in the postmenopausal range (basal FSH ＞ 40IU-L -1, estradiol: 40~100pmol-L -1); aged 45-55 years old, taking Toremifene or tamoxifen, FSH and estradiol levels should

be within the postmenopausal range (basal FSH>40IU-L-1, estradiol: 40~100pmol-L-1)[10]。

1.2 Inclusion criteria for dry eyes

(1) Main symptoms (required): subjective symptoms such as dryness, foreign body sensation, fatigue, burning sensation, and fluctuation of vision. (2) Tear film instability (required): through the tear film break-up test, the tear film break-up time (break up time, BUT) was measured as positive <10 s.(3) Tear secretion test (Schirmer's test, SIT): commonly used Schimer Ⅰ test, measuring the secretion function of the paracryocele, and >5mm/5min was considered as Normal. (4) Decrease in tear lactoferrin or increase in osmolality (to strengthen the diagnosis). (5) Ocular surface damage (enhanced diagnosis): corneal fluorescein staining (FL) to observe corneal epithelial defects and determine the height of the tear river, the normal height of the tear river is >0.3mm. when other causes are excluded, the corneal epithelial defects are characterized by (1) + (2) (≤ 5 s) or (1) + (3) (≤ 5mm/5min) or (1) + (5 s < (2) ≤ 10 s) + (3) + (5) can confirm the diagnosis. (4) (5) two for strengthening the diagnosis. The above diagnostic criteria are commonly used in China.[11]。

1.3 Exclusion criteria:

(1) patients who were allergic to the drugs in this study; (2) patients who suffered from eyelid diseases, other ocular surface diseases and intraocular diseases that had not yet been cured; (3) patients who underwent ocular surgeries within half a year; (4) patients who suffered from diabetes mellitus, thyroid diseases, gout and any gynecological diseases; (5) patients who had a combination of systemic immune disorders, such as rheumatoid arthritis, systemic lupus erythematosus and desiccation syndrome; (6) patients who had previously worn contact lenses diagnosed as dry eyes and patients who had received any medication. (6) Patients diagnosed with dry eyes from previous contact lens wear and patients who have received any medication. (7) People with gastrointestinal diseases or surgery .

2. Methods

20 g of mulberry pigment was used, cleaned and added to 200 ml of water, soaked in water for about 30 min in a casserole dish before decoction, and then boiled for 3 min in a direct-fire decoction method and then removed from the fire to form a mulberry pigment decoction. To ensure maximum comparability between the experimental and control groups in each experimental unit, we used a prospective randomized controlled study method. The influence of non-experimental factors, such as age and disease duration, was minimized, and 293 patients attending gynecological postmenopausal clinics at the First Affiliated Hospital of Nanchang University and the First Affiliated Hospital of Nanhua University from October 2021 to October 2022 were selected according to the principle of allocation with the smallest imbalance index. The case histories of the patients were clarified and they were subjected to complete ocular surface examination. Finally, 48 patients (60 eyes) who met the experimental criteria were selected and randomly divided into 2 groups: 24 patients in the control group (group A) were given placebo vitamin C tablets 50mg/times orally twice daily, and 24 patients in the control group (group B) were given mulberry pigment decoction of 350 ml/times orally twice daily for 2 months, with the addition of hydroxycitric acid drops in both groups. According to the formula n=15.6R+1.6, the number of cases in each group under 80% certainty, the sample size of the two groups in this study was determined to be 30 cases.

2.1. four tear film BUT, FL, SIT and tear river height measurements. All the examinations in this experiment were conducted by the same person and at the same time, place, lighting brightness, temperature and humidity.

(1) Break-up time (BUT): A drop of 2% sodium fluorescein was placed in the conjunctival sac of the examinee, and the examinee was instructed to do a transient movement. After the fluorescein coated the cornea uniformly, the examinee's cornea was observed with a slit lamp drilled with a blue filter and the timer was started, and the time when the first black spot appeared in the tear film was recorded, and the

tear river was observed at the same time.

(2), fluorescein sodium staining (FL) is 1% fluorescein sodium solution drops in the patient's conjunctival sac, and then irradiated with cobalt blue light, mainly to check the condition of the cornea. Normal corneal epithelium does not stain when viewed under a slit lamp after staining with fluorescein sodium, and if the corneal epithelium is damaged then fluorescein sodium accumulates in the damaged area. After dividing the cornea into 4 quadrants, each quadrant staining was categorized as none, mild, moderate, and severe, corresponding to a score of 0-3, respectively, which resulted in a score of 0-12 for the entire corneal FL.

(3) Schirmer Test (SIT): Use a 5mm*35mm filter paper, bend one end by 5mm, place it in the conjunctival sac of the inner 1/3 of the lower lid of the examinee, and leave the rest of the paper hanging on the surface of the skin, ask the examinee to gently close his/her eyes, and measure the length of the paper wetted with tears after 5min (not including the back-folding), and record the data. If the length is less than 5mm, then dry eye is indicated.

(4), Lower Tear River Height (LTMH): Tear River Height is an index for preliminary judgment of tear secretion, which is a non-invasive examination. Tear River Height is measured by using a slit lamp to observe the level height of tears at the junction of the light band on the surface of the corneal and conjunctival membrane and the light band on the margin of the lower eyelid. The normal value is 0.3-0.5mm.Refer to the literature for specific methods[12]。

2.2 Determination of tear protein About 20 μL of non-irritating tear fluid was collected from the lower lacrimal canaliculi of the subjects in both groups using the capillary pipette method, and the collected tear fluid was immediately placed in a - 80 ℃ refrigerator for storage. The total protein content of tear fluid was determined by the Brandford method, and calf serum albumin was used as the standard.[13]。 For the determination of lactoferrin by radioimmunoassay, 10 μL of tear fluid sample was taken and diluted with saline 1:100, and then measured by radioimmunoassay kit with the same batch number, and a standard curve was made to find out the content of lactoferrin in the sample from the curve. Turbidimetric method for the determination of lysozyme content in test tubes, lysozyme test kit, preparation of stained bacterial solution, preparation of lysozyme standard solution and the drawing of the standard curve were carried out in accordance with the instructions; tear fluid and stained bacterial solution were mixed well, and then centrifuged to take the supernatant, and the difference in optical density between the measuring tube and the sample control tube was used to check the standard curve, which could be used to find out the lysozyme content in the samples[14]。

2.3 Ocular Surface Disease Index (OSDI):It can show the clinical ocular dryness of menopausal women individually, and menopausal women with dry eye symptoms have shorter tear film breakup time, less tear volume, and increased bulbar conjunctival congestion compared to those without dry eye symptoms. Therefore, the patients' OSDI, tear film four items, and tear protein were measured and evaluated before and after treatment at 7 d, 1 mo, and 2 mo, respectively, as well as between the two groups by analysis of variance (ANOVA) and analysis of sample mean or median difference (ANOVA), and the squamous cell layer changes in the epithelial cells were compared with those of the corneal epithelium under confocal microscopy.[15]。 The study was conducted in accordance with the principles of the Declaration of Helsinki, and the methodology and content of the study were thoroughly explained to each patient, and informed consent was obtained from the patients in accordance with the requirements of the ethics committees of both hospitals.

2.4 Confocal microscopy

The changes in corneal epithelium of the tested eyes in each group were examined by the same operator using a confocal microscope. The procedure was as follows: fixate the head, look straight ahead with both eyes, use 5 g-L-1 Elcaine eye drops to spot the eyes, scan the central cornea in full layer, save the clear and effective images, and use the computer software to calculate the densities of corneal activated mesenchymal stromal cells and inflammatory cells. Nerve density (mm / mm 2): the subcorneal nerve plexus was observed, and its observation depth was set at 35 to 50 μm. AUTOCAD software (Auto Desk Co., Ltd, CA, US) was applied to determine the length of the subcorneal nerve fibers. Each image was obtained at the rate of 0.16 mm 2 (400 × 400 μm)/frame of the actual corneal area, and the shape of the nerve fibers was depicted for individual images (Fig. 1).The total length of the folds was determined from the characteristics of the depicted

folds, and the resulting total length was divided by the area (0.16 mm 2) to obtain the nerve fiber density (mm / mm 2). Nerve fiber branching: for the total number of branches seen in each image. Curvature Score: The degree of nerve fiber curvature in the image is divided into 4 classes. The higher the score, the higher the nerve fiber curvature This study was conducted in accordance with the principles of the Declaration of Helsinki, and the methodology and content of the study were thoroughly explained to each patient and informed consent was obtained from the patients in accordance with the requirements of the ethics committees of the two hospitals[14]。

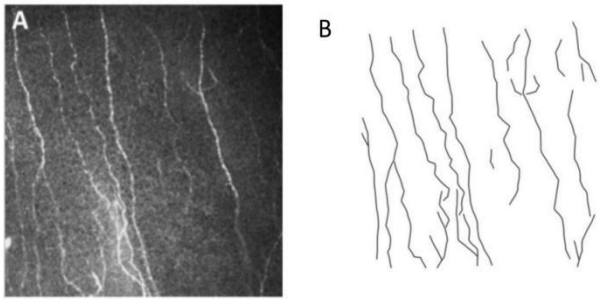

Fig. 1.A:Nerve fibers in confocal microscopy image obtained by CAD software.B:Folded lines depicted according to the nerve fibers

2.5 Adverse reactions and treatments during the experimental process :Adverse reactions that may occur during the experimental process include gastrointestinal discomfort, allergic reaction, pyrogenic reaction, or even cause shock and death. The main possible treatments involved: once the above adverse drug reactions occur, immediately stop drinking and withdraw from the study, according to the specific circumstances of anti-allergic or other treatments.

3.Statistical methods

All data were processed using SPSS19.0 statistical software, and the mean (X) ± standard deviation (s) was taken for the symptom scores of each subgroup to describe the centralized location and degree of dispersion of statistical data; and the median (M) ± quartile spacing (Q) was taken for the physical signs data to describe the centralized location and degree of dispersion of statistical data. To determine the difference in efficacy before and after drug treatment and between the two groups. The gender, age, and disease duration of each group were analyzed for comparability before treatment, and the count data were tested by $\chi 2$ test, and the measurement data were tested by t test. Comparison of indicators before and after treatment and determination of efficacy within the group were tested by rank-sum test, and $P<0.05$ was regarded as significant difference, and $P<0.01$ was regarded as highly significant difference。

RESULTS

1. Comparison of ocular surface symptom scores and tear film conditions between the two groups before and after treatment

Compared with group A, there were no significant changes in OSDI, BUT, SIT, tear river height, and FL in group B patients before treatment, and the differences were not statistically significant (tOSDI =0.577, tBUT=0.922, tTear River Height=0.563, tSIT =0.871, tFL =0.509). , P value all > 0. 05); 1 week and 1 month after treatment, compared with group A, patients in group B had a

significant increase in SIT and tear river height, and a significant decrease in FL, the difference was statistically significant (tSIT =5.532,tTear River Height=6.318, tFL =7.313, P value all < 0. 05), compared with group A, patients in group B had a slight decrease in OSDI, a slight increase in BUT, the The differences were not statistically significant (tOSDI =0.812,tBUT=0.674, P-value>0. 05); at 2 months after treatment, compared with group A, patients in group B had a significant increase in BUT, SIT and height of the tear river, and a significant decrease in OSDI and FL, and the differences were statistically significant (tOSDI =8.612, tBUT=6.324, tTear river height= 7.753, tSIT =8.109, tFL =7.137, p-value <0. 05). Compared with pre-treatment, OSDI decreased significantly in patients of group A at 1 week and 1 month after treatment, and the difference was statistically significant (tAOSDI = 8.642; tAOSDI = 10.532, both P-values <0.05). By the 2nd month after treatment, there were no significant changes in patients' OSDI, BUT, SIT, tear-river height, and FL, and the difference was not statistically significant (tOSDI = 0.932,tBUT=0.754, tTear River Height=0.432, tSIT =0.814, tFL =0.512,P value >0.05). Compared with pre-treatment, patients in group B, 1 week and 1 month after surgery, OSDI decreased significantly, BUT, SIT, and tear river height increased significantly, and the differences were statistically significant (tBOSDI = 6.163, tBBUT = 5.543, tB tear river height = 7.871, tBSIT = 5.5653; tBOSDI = 8.722, tB tear river height = 6.121, tBSIT = 5.652, P value <0.05), by 2 months after treatment, the patients' BUT, SIT, and tear river height increased significantly, and OSDI and FL decreased significantly, the differences were statistically significant (tBOSDI = 9.911, tBBUT = 10.223, tB tear river height = 13.9392, tBSIT = 12.763, tBFL =10.143,P value <0.05); the changes in the ocular surface before and after treatment of the two groups are detailed in Table 1.In addition, all the patients in this experiment had no significant ocular adverse reactions and systemic adverse (mainly gastrointestinal) reactions.

Table 1 Ocular surface symptom scores and tear film recovery before and after treatment in two groups of patients

groups (minute)	number of examples	OSDI	BUT(s)	SIT(mm)	Height of the River of Tears (mm)	2% FL
groupA						
pre-treatment	24	45.77±12.81	2.46±3.12	3.65±1.99	0.19±0.14	8.49±2.91
1 week of treatment		44.44±16.76#	2.59±2.97	3.85±2.85	0.25±0.16	8.46±2.25
1 month of treatment		39.83±16.14#	3.81±3.22	3.85±2.62	0.27±0.21	8.11±1.61
2 months of treatment		35.47±13.71	4.17±3.15	3.97±3.15	0.35±0.23	7.97±2.71
groupB						
pre-treatment	24	45.51±12.61	2.57±3.74	3.68±1.61	0.16±0.13	9.21±2.42
1 week of treatment		39.75±10.91$^{\#}$	3.91±2.93$^{\#}$	4.51±3.61$^{*\#}$	0.23±0.26*	8.81±2.03*
1 month of treatment		32.61±11.52$^{\#}$	7.12±3.71$^{\#}$	10.33±5.83$^{*\#}$	0.69±0.34*	3.71±2.72*
2 months of treatment		25.78±10.91$^{*\#}$	9.91±2.31$^{*\#}$	11.73±7.26$^{*\#}$	0.74±0.25$^{*\#}$	2.11±1.02$^{*\#}$

Note: * P < 0.05 vs Group A; #P < 0.05 vs pre-treatment

2. Comparison of tear proteins at various time points between the two groups before and after treatment

Before treatment, compared with group A, there was no significant change in tear TP, LF, LZM, sIgA in patients in group B, and the difference was not statistically significant (tTP = 0.571, tLZM = 0.889, tLF = 0.825, and tslgA = 0.915, P > 0.05). 1 week after treatment, compared with group A, patients in group B had different degrees of decrease in TP, LF, LZM, and sIgA compared with that before treatment, but the difference between the two groups at different time points was not statistically significant (P > 0.05). Patients' TP, LF, LZM, sIgA decreased to different degrees compared with that before treatment, but the difference between the two groups in different time periods was not statistically significant (P all >0.05); in January and February after treatment, compared with group A, patients' tear TP, LF, LZM, sIgA increased to different degrees in group B compared with that before treatment, and the difference was not statistically significant (P all >0.05), see Table 2 for details.

Table 2 Comparison of tear fluid proteins before and after treatment in two groups of patients

Groups	TP (ρ/g·L^{-1})	LZM (ρ/g·L^{-1})	LF (ρ/g·L^{-1})	sIgA (ρ/g·L^{-1})
Group A				
pre-treatment	11.66±2.94	1.15±0.96	1.36±0.65	132.85±13.94
1 week of treatment	11.82±1.92	1.43±0.93	1.32±0.21	132.81±21.62
2 months of treatment	13.63±2.12	1.73±1.09	1.47±0.56	135.27±19.21
1 month of treatment	14.95±2.92	2.12±1.63	1.62±0.44	139.32±20.26
Group B				
pre-treatment	10.51±2.04	1.22±0.75	1.21±0.75	134.05±12.71
1 week of treatment	10.12±2.82	1.32±0.83	1.32±0.66	135.37±19.82
2 months of treatment	12.11±1.72	1.68±1.09	1.46±0.33	138.16±16.77
1 month of treatment	14.29±2.74	1.84±1.51	1.59±0.32	141.55±17.53

Note：TP：total proteins；LZM：Lysozyme；LF：Lactoferrin sIgA：secretory immunoglobulins A

3. Corneal confocal microscopy results before and after treatment and between the two groups

Normal corneal squamous epithelial cell layer showed light and dark homogeneous cell bodies with clear cell borders (Figure 2A). Before treatment, the corneal squamous epithelial cell layer was seen to have unclear cell boundaries and a discontinuous distribution (Figure 2B).The squamous epithelial cell layer was seen to have bright nuclei and highly reflective boundaries at 2 months in Group A (Figure 2C).The corneal epithelial layer was

seen to be slightly squamous and highly reflective at 2 months in Group B (Figure 21D).

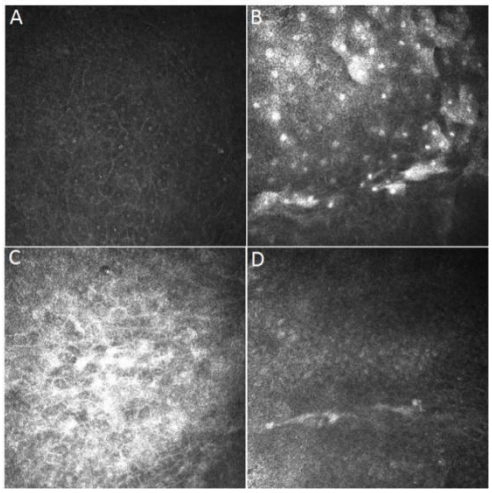

Figure 2: Altered squamous cell layer in epithelial cells. Figure 2A shows normal squamous epithelial layer; Figure 2B squamous epithelial layer with discontinuous distribution; Figure 2C squamous epithelial layer with bright nuclei and highly reflective borders; Figure 2D mildly squamous and highly reflective alterations in the epithelial layer

Corneal epithelial stromal cells with light and dark cell bodies were seen before the treatment and the cell borders were narrower (Figure 3A) . In group A, before treatment and after 2 months of treatment, the epithelial basal cells were seen to be bright light-like inflammatory cells with a significant increase in density (Fig. 3A, B); in group B, bright light-like inflammatory cells were seen before treatment, and after 2 months of treatment, the inflammatory cells were significantly reduced, but the density of the epithelial basal cells was slightly reduced compared with that of normal subjects (Fig. 3C). After 2 months of treatment, the densities of epithelial basal cells and inflammatory cells in group A were (4489 ± 215) mm-2 and (311 ± 102) mm-2, respectively, whereas the densities of epithelial basal cells and inflammatory cells in group B were (3315 ± 212) mm-2 and (39 ± 14) mm-2, respectively, and the differences between the two groups were statistically significant ($t = 5.567, 5.132$, both $P < 0.05$).

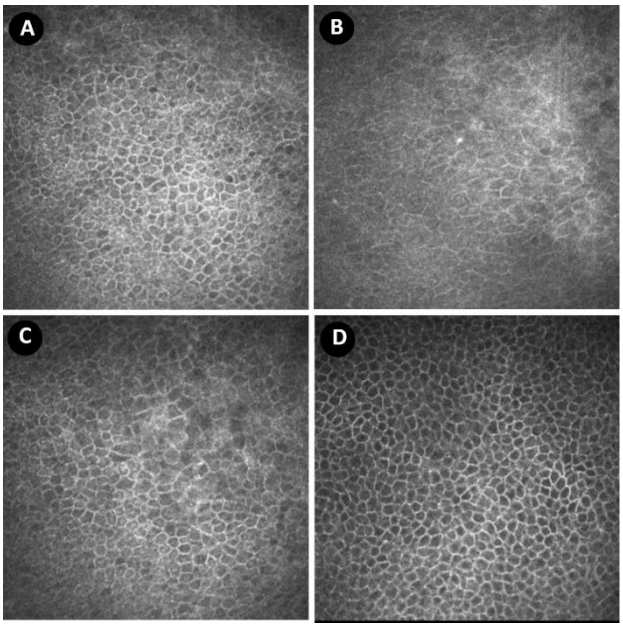

Figure 3 Corneal subepithelial basal cells in two groups.A: before treatment in group A; B: after 2 months of treatment in group A; C: before treatment in group B; D: after 2 months of treatment in group B.

The corneal subepithelial nerves in group A were straight and more numerous (Figure 4A), and after 6 weeks, the density of the corneal subepithelial nerves in group B was slightly reduced, and several nerve fibers were seen to show obvious curvature (Figure 4B).Obvious curvature and branching of the corneal subepithelial nerves were seen in group B (Figure 4C), and after 2 months of treatment, their density was slightly reduced, and curvature was obviously reduced (Figure 4D); before treatment, the density of corneal subepithelial nerves of the two groups was

compared to the density of corneal subepithelial nerves, branches or curvature were not significantly different, and the difference was not statistically significant (P > 0.05). After 2 months of treatment, the differences in density, branching or curvature of subepithelial corneal nerves in group A were significantly altered, and the differences were statistically significant (P<0.05); in group B, the density of nerve fibers was slightly reduced, the number of branches of nerve fibers was significantly reduced, and the curvature scores were lowered, and the differences were statistically significant (P<0.05), as shown in Table 3.

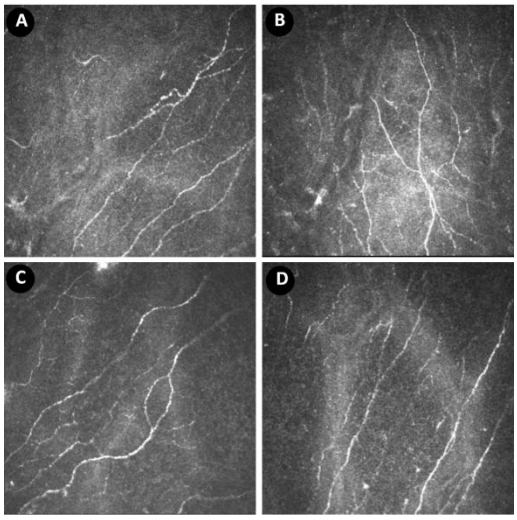

Figure 4 Morphology of corneal subepithelial nerves in the two groups.A: before treatment in group A; B: after 2 months of treatment in group A; C: before treatment in group B; D: after 2 months of treatment in group B.

Table 3 Comparison of corneal subepithelial nerves after treatment in the two groups (x±s).

Clusters	Group A			GroupB		
	Nerve fiber density Nerve (mm/mm2)	Fiber branching Curvature score (number of branches/plot)	Curvature score	Nerve fiber density Nerve (mm/mm2)	Fiber branching Curvature score (number of branches/plot)	Curvature score
Before treatment	16.02±2.16	7.34±1.16	3.24±0.32	15.23±2.12	7.31±1.12	3.36±0.41
1 week of treatment	14.23±2.06△	8.61±1.45△	3.42±0.33△	16.31±2.13 *	7.16±0.91 *	3.24±0.42 * △
1 week of treatment	13.11±2.04△	9.21±1.54△	3.63±0.41△	17.51±2.11 * △	7.11±1.12 * △	2.91±0.24 * △
2 months of treatment	12.51±2.11△	10.57±1.71△	3.57±0.31△	18.39±2.82 * △	7.05±0.89 * △	2.71±0.42 * △

Note: *P<0.05 vs group A; △P<0.05 vs before treatment Figure 4 Morphology of corneal subepithelial nerves in two groups of patients.A: before treatment in group A; B: after 2 months of treatment in group A; C: before treatment in group B; D: after 2 months of treatment in group B.

Discussion

The prevalence of dry eye is 5-35% worldwide, and in China, the prevalence of dry eye remains high and even increases year by year. The risk factors for dry eye are also a hot topic of research nowadays.As reported in the literature[14,15], The extensive use of video terminals for long periods of time and insufficient sleep and rest are the culprits in the development of dry eye. Studies have also shown that sex hormone imbalance is one of the main pathogenic mechanisms of dry eye. In addition to regulating the development of the body's sexual characteristics, androgens also regulate the body and local immune function. Androgens can regulate the morphology, development, differentiation and secretion of the lacrimal and levator glands, and have an important role in regulating tear stability, inflammatory response and apoptosis in the eye.Stern [16] believed that if the body's androgen is too low, it will not be enough to maintain the stability of the tear fluid on the ocular surface, resulting in dryness of the ocular surface, which will stimulate the lacrimal nerves, triggering an inflammatory response, which in turn will exacerbate the degree of dryness of the ocular surface, resulting in a vicious circle. It is thus clear that androgen levels play an important role in the maintenance of the normal state of the ocular surface. In clinical practice, systemic changes such as aging and autoimmune diseases often lead to a decrease in androgen levels, which makes androgen-induced dry eye increasingly common.

Dry eye symptoms may appear suddenly or slowly, lasting for hours or days, and may resolve spontaneously in some patients. In clinical practice, dry eye is recognized as one of the most common causes of chronic ocular surface disease and may result in damage to the ocular surface, a number of associated ocular discomforts and impaired visual quality. Symptoms, etiology, and treatment options vary greatly from patient to patient. Current treatment is mostly based on topical symptomatic therapy, including artificial tears, ophthalmic solutions such as glucocorticoids and immunosuppressants, and lachrymal embolization.

In menopausal women, the decline in androgen levels becomes the main reason for the development of dry eye. Since androgens interact with various sex hormones to influence endocrine processes, it is important to maintain androgens at optimal biological levels for tear secretion. Androgen replacement therapy is currently the main treatment for this type of dry eye. However, long-term androgen use can produce many serious side effects, such as acne, erythrocytosis, male prostate cancer, breast development, and female masculinization, which greatly affects the patient's quality of life. AlAwlaqi[17]Studies have found that long-term hormone therapy use seems to increase the risk of dry eye syndrome, and the search for androgen replacement drugs is imperative. In recent years, it has been shown that mimoso tea and onychomycetes not only can be used in animal experiments to treat dry eye models caused by decreased estrogen/androgen in rabbits [18], but also can significantly reduce the signs and symptoms of moderate to severe menopausal dry eye in women, which is of some clinical value. The structure of the flavonoids in it can be effective because of their

similarity to androgens [19]。However, Mimulus has a sweet taste and is not suitable for diabetic patients; Ghostwort is bitter and has a poor taste, and patient compliance is reduced.

Mulberry pigment comes from the bark of mulberry plants such as yellow mulberry wood and mulberry-orange tree and many herbs. As a flavonoid, it inhibits enzyme activity. Studies have elucidated the application of mulberry pigment in anticancer, antibacterial, anti-inflammatory, anti-atherosclerosis, antioxidant, blood sugar lowering and anti-stress. Mulberry pigment mainly contains flavonoids, and androgens similar to the structure of the cell surface androgen receptor binding to play a weak androgenic effect, but not like androgen-induced female masculinization, acne and other complications, with obvious advantages, and can effectively shorten the course of the disease, to achieve the purpose of curing the root cause[20]。

Androgen replacement therapy is also currently the only allopathic treatment for such dry eye patients. However, prostate enlargement and even tumors in male patients and feminization of the female sex are serious side effects. Flavonoids bind to the cell membrane androgen receptor, a cell membrane androgen receptor stimulator [21], and can be used as an alternative to androgen therapy. Morusin is clinically used against viral infections, as well as for the relief of headaches, treatment of gastric disorders and coronary heart disease. Similar to other flavonoids, mulberry pigment binds to the androgen receptor and exerts androgenic activity. The use of mulberry pigment eye drops can exert its androgenic effect, improve the secretion of the lacrimal gland and the lid gland, so as to reduce the systemic and local autoimmune reaction, inhibit the apoptosis of the lacrimal gland and the cornea, and ultimately achieve the purpose of the treatment of dry eye syndrome. The results of this study show that mulberry pigment decoction can improve dry eye symptoms in menopausal women. In group B after 2 months of treatment with mulberry pigment decoction, patients' OSDI, BUT, SIT, tear river height, FL, etc., improved, probably because the flavonoids in mulberry pigment decoction exerted androgen-mimetic effects, binding with androgen receptors in the ocular surface tissues, and promoting secretion of eyelid glands. However, it was not obvious for changing the tear protein composition, probably because of the short experimental time, which had not changed the ocular microenvironment, and the cells and glands secreting proteins and enzymes had not fully recovered their main functions.In group A patients, there was also a slight improvement in the OSDI at 1 week and 1 month after the treatment, which might be the natural process of the disease. In addition, we compared the corneal squamous cell layer changes under corneal confocal microscopy, and found that the corneal epithelial layer was mildly squamous and hyperreflective after 2 months in group B treated with mulberry pigment decoction, and gradually returned to the normal morphology of squamous epithelial layer, while the control group, group A, showed hyperreflective, and the disease was aggravated. There was no obvious ocular reaction after the treatment of dry eye with mulberry pigment decoction, but in the early pre-test, we found that the decoction itself would have a certain

effect on the patients with poor gastrointestinal function, so we will further explore its application in the clinical aspects of this part of the patients, and explore the effective treatment of dry eye in menopausal women to reduce gastrointestinal reaction, so as to solve the patient's problems.

In conclusion, for the dry eye caused by the decrease of sex hormone level during menopause, mulberry pigment decoction can improve the dry eye symptoms of menopausal women faster, it is a safer and more effective treatment for dry eye with simple route of administration, fewer side effects, and can be taken by the patients for a long period of time, and it has a wide range of clinical applications and popularization value.

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AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The study methods and protocols were approved by the Medical Ethics Committee of the First Affiliated Hospital of Nanchang University (Nanchang, China) and followed the principles of the Declaration of Helsinki. All subjects were notified of the objectives and content of the study and latent risks, and then provided written informed consent to participate.

PATIENT CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

This study did not receive any industrial support. The authors have no competing interests to declare regarding this study.

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