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Clinical observation of esculin and digitalisglycosides eye drops with 0.3% sodium hyaluronate eye drops for dry eye disease: a randomized controlled trial

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Dry eye disease (DED) is a common ocular surface disorder. Esculin and digitalis possess anti-inflammatory and anti-oxidant properties, which may benefit patients with DED. This study aimed to assess the therapeutic efficacy of esculin and digitalis glycosides (EAD) eye drops, either alone or in combination with 0.3% sodium hyaluronate (SH) eye drops, in treating DED. In this randomized controlled trial, 78 participants with DED (78 eyes) were included and divided into three groups: Group A received 0.3% SH, Group B received EAD, and Group C received 0.3% SH combined with EAD eye drops for 4 weeks. The efficacy of the treatments was assessed at 2 and 4 weeks using the Ocular Surface Disease Index (OSDI), tear break-up time (TBUT), Schirmer I test (SIt), and corneal fluorescein staining (CFS) as primary evaluation metrics. After 4 weeks of treatment, Group A showed a decrease in OSDI and an increase in SIT ($p < 0.05$). Group B showed a decrease in OSDI score ($P < 0.05$) and a significant improvement in SIt ($P < 0.01$). Group C demonstrated a significant increase in both TBUT and SIt values at the 2-week mark. Improvements were noted across all parameters, including OSDI score, TBUT, SIt, and CFS score after 4 weeks of treatment ($P < 0.05$). The total effective rate for participants in Group C was 88.46%, significantly higher than Group A's rate of 65.38% ($P < 0.05$). In conclusion, the combination of EAD eye drops with 0.3% SH eye drops proved more effective than either treatment alone.

Keywords Dry eye disease, Esculin, Digitalisglycosides, 0.3% sodium hyaluronate

Dry eye disease (DED) is a chronic inflammatory ocular surface disease resulting in various symptoms, including eye redness, pain, foreign body sensation and epiphora[1]. Risk factors for DED include age, gender, lifestyle, thyroid disease, oral contraceptive therapy, etc[2]. The patient's symptoms, Ocular Surface Disease Index (OSDI) score, tear break-up time (TBUT) and Schirmer's I test (SIT) are the main diagnostic and classification criteria for DED[3].

The treatment goals of DED are improving the patient's ocular comfort and maintaining the ocular surface and tear film homeostasis[4]. As a chronic disease, pharmacological treatments such as artificial tears, glucocorticoids, immunosuppressants are the main options[5]. One of the commonly used artificial tears clinically is sodium hyaluronate (SH) eye drops. SH possesses remarkable viscoelastic and hydrating characteristics thereby protecting the ocular surface and reconstructing the tear film[6]. According to previous studies, the efficacy of 0.3% SH eye drops in relieving subjective symptoms and stabilizing tear film in participants with DED has been demonstrated, but without anti-inflammatory effects[7,8].

Esculin and digitalisglycosides (EAD) eye drops are a compound preparation. Each vial contains 0.040 mg of esculin and 0.006 mg of digitalis glycosides, with boric acid and purified water as excipients. Esculin is a coumarin derivative, present in trees of the Fraxinus or plants such as Artemisia capillaries and Citrus limonia[9].

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It works as an antibacterial, anti-inflammatory and anti-oxidant by activating different signaling pathways[10–12]. Digitalis is a drug that increases muscle excitability and improves contractility, and has been commonly used to treat heart failure for decades as well as in treating patients with cystic fibrosis who have normal hearts but need to reduce inflammation in the lungs[13].

In ophthalmic diseases, EAD eye drops are often used to treat visual fatigue and macular degeneration. Jiang et al. found that EAD eye drops can improve patients' accommodation ability and no patient appeared any adverse reaction in whole experiment[14–16]. In China, some studies using it to treat DED and achieved good results. However, there are no prospective randomized controlled studies.

The aim of the study was to assess the efficacy 3 treatment approaches for DED by evaluating changes in OSDI, TBUT, SIT, and corneal fluorescein staining (CFS) scores: 0.3% SH in combination with EAD, EAD, and 0.3% SH monotherapy.

Methods

Study design and subjects

This single-center, randomized, parallel-group, case-control trial was conducted from July 2024 to September 2024 at the Capital Medical University of Beijing Tongren Hospital, Beijing China. 78 eyes of 78 participants (12 male and 66 female) with DED were enrolled. The research was conducted in compliance with the Declaration of Helsinki, the Good Clinical Practices Standards. It was approved by the Medical Ethics Committee of Beijing Tongren Hospital, Capital Medical University and was registered with the Chinese Clinical Trial Registry (ethics number: TREC2024-KY177). Before the study began, informed consent was obtained from all participants. This study was conducted following the CONSORT guidelines.

Inclusion criteria were the following: (1) aged ≥ 18 years; (2) having symptoms of dry eye and TBUT ≤ 5s or SIT ≤ 5 mm/5min; (3) having symptoms of dry eye and 5s < TBUT ≤ 10s or 5 mm/5min < SIT ≤ 10 mm/5min, and companied by positive corneal conjunctival fluorescein staining results. (1), (2) or (1), (3) were required.

Exclusion criteria were the following: (1) use of any systemic medication that may affect tear film or vision for < 3 months before screening; (2) ophthalmic surgery or trauma, which could affect corneal sensitivity and tear distribution within 6 months prior to screening; (3) current or previous use of topical ocular medication within 2 weeks of screening; (4) use of contact lens anticipated during the study or use ≤ 3 months before screening; (5) combined serious heart, kidney, and liver diseases; (6) history of allergy to the selected drugs for the experiment; (7) pregnant and lactating women.

Study treatment and procedures

The protocol was shown in Fig. 1. 78 participants were divided into 3 groups 1:1:1 based on the randomization numbers generated using Microsoft® Office Excel 2018. Group A: the participants were treated with 0.3% SH eye drops (Santen Pharmaceutical Co., Ltd.). Group B: the participants were treated with EAD (Pharma Stulln, GmbH) eye drops. Group C: the participants were treated with 0.3% SH in combination with EAD eye drops. Medications were dispensed open-label. Participants were treated with topical eye drops into the conjunctival sacs of both eyes 3 times a day for 4 weeks. Follow-ups were performed at week 2 and 4 after random assignment. The study's schedule at each visit included: OSDI, best correct visual acuity (BCVA), SIT, TBUT, CFS scores. All examinations were performed sequentially by the same physician. For statistical analysis, we randomly selected the right eye using a lottery method.

All patients were clocked in and recorded via an app after daily medication use and monitored by the researchers to ensure adherence. The investigational drug was discontinued if the principal investigator or patient judged that continuation of the study was not in the best interest of the latter, or if the female patient became pregnant.

Outcome measures

Subjective symptoms were graded using the OSDI score. The OSDI scale quantifies the impact of dry eye on a patient's ocular discomfort, ranging from 0 (indicating no disease) to 100 (indicating the most severe condition)[17]. BCVA was measured by Tumbling E Chart. SIT was used to measure tear volume, i.e., a piece of filter paper was inserted into the outer part of the inferior fornix without anesthetic drops, and the length of the filter paper wetting was measured after 5 min. The standard tear film TBUT was assessed by measuring the time interval between the last complete blink and the appearance of the first corneal black spot in the stained tear film, after 1% fluorescein dye was instilled into the conjunctival sac. Three times measurements were taken and the mean value was calculated. CFS was examined through slit-lamp evaluation with a yellow barrier filter and cobalt blue illumination. The fluorescein score was assessed with a 1% fluorescein solution using the 0 to 15 scoring system. Staining of the nasal and temporal cornea, the superior cornea, mid cornea, and inferior cornea was graded on a scale of 0 (no staining), 1 (staining 1 to 30 spots), 2 (Staining > 30 spots but no fusion), 3 (Staining fusion, filopodia, ulcers)[18].

Clinical efficacy evaluation criteria: Cured: all symptoms disappeared, SIT > 10 mm, monocular CFS score is 0; Significant: symptoms significantly reduced, 10 mm > SIT > 5 mm, monocular CFS score is 1; Effective: symptoms reduced, SIT < 5 mm, monocular CFS score is 2; Ineffective: no improvement in symptoms, SIT < 5 mm, monocular CFS score is 3. Total effective rate (number of cured cases + number of apparently effective cases + number of effective cases)/total number of participants × 100%[19].

Statistical analysis

Statistical analysis was performed using SPSS software (ver 26.0; SPSS Inc, Chicago, IL). Two-factor repeated-measures ANOVA was applied for comparison between the three groups according to time, and LSD test was

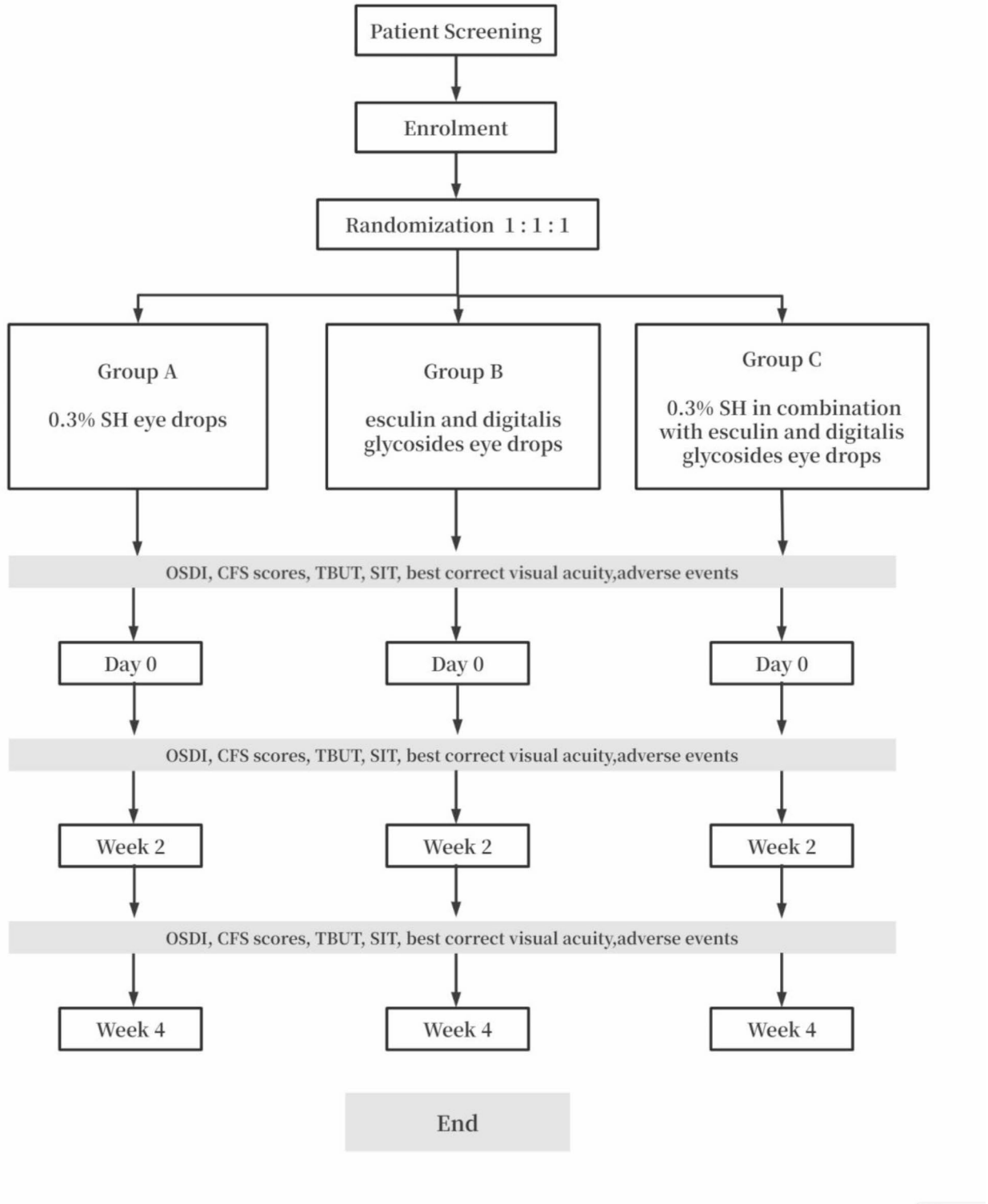

Fig. 1. Study design. We divided the study into 4 phases: pre-screening, treatment day 0, week 2 and week 4. Patient data were collected as planned.

Characteristics	Group A	Group B	Group C	*p*-value
Participant (n)	26	26	26	
Gender (male/female, n)	7/19	3/23	2/24	0.126
Age (years)	52.81 ± 5.99	47.58 ± 10.90	49.00 ± 12.48	0.166
OSDI score	30.71 ± 20.32	31.67 ± 19.11	32.46 ± 19.51	0.949
TBUT (s)	5.62 ± 2.47	5.04 ± 2.59	4.79 ± 0.99	0.367
SIT (mm/5min)	10.04 ± 6.48	11.19 ± 9.07	7.33 ± 5.84	0.151
CFS score	1.08 ± 1.03	1.15 ± 1.59	1.75 ± 1.61	0.188

Table 1. Patient demographic and baseline clinical characteristics. *OSDI* ocular surface disease index, *TBUT* tear film break-up time, *SIT* Schirmer's I test, *CFS* corneal fluorescein staining.

Index	Group	Baseline	2w	4w
OSDI score	A	30.71 ± 20.32	26.63 ± 16.50	22.90 ± 16.23[a]
	B	31.67 ± 19.11	25.66 ± 14.51	22.50 ± 14.48[a]
	C	32.46 ± 19.51	27.61 ± 16.73[a]	22.72 ± 13.91[a]
TBUT (s)	A	5.62 ± 2.47	5.87 ± 2.48	5.37 ± 2.26
	B	5.04 ± 2.59	5.06 ± 2.08	5.77 ± 2.25
	C	4.79 ± 0.99	5.08 ± 2.08[a]	5.39 ± 1.63[a]
SIT (mm/5min)	A	10.04 ± 6.48	10.50 ± 6.52	11.83 ± 7.20[ab]
	B	11.19 ± 9.07	10.35 ± 8.44	15.48 ± 9.55[bc]
	C	7.33 ± 5.84	9.06 ± 6.33[a]	10.64 ± 7.63[bc]
CFS	A	1.08 ± 1.03	0.89 ± 0.98	1.00 ± 1.21
	B	1.15 ± 1.59	1.14 ± 1.43	0.67 ± 1.04
	C	1.75 ± 1.61	1.29 ± 1.09	0.98 ± 1.41[a]

Table 2. The indices of the three groups before and after treatment. *OSDI* ocular surface disease index, *TBUT* tear film break-up time, *SIT* Schirmer's I test, *CFS* corneal fluorescein staining [a]$P < 0.05$ vs. before treatment in the same group; [b]$P < 0.05$ vs. after 2 weeks of treatment in the same group; [c]$P < 0.01$ vs. before treatment in the same group.

used for post-test. one-way ANOVA was used to compare within-group categorical variable changes from the baseline value. A 2-sided test with $P < 0.05$ was considered to indicate statistical significance.

Results

General data

The demographic information of the participants is portrayed in Table 1. There were no differences between the three groups in the baseline test results including gender, age, OSDI, TBUT, SIT, CFS score.

Ocular surface disease index score

The OSDI scores declined in all 3 groups after 4 weeks of treatment compared to baseline (Group A: $P = 0.043$, Group B: $P = 0.044$, Group C: $P = 0.041$), with Group C showing an early reduction at 2 weeks of treatment ($P = 0.045$). However, when comparing the OSDI scores of participants in the 3 groups across all post-treatment time points, the differences were not statistically significant ($P > 0.05$). (Table 2; Fig. 2)

Tear film break-up time

In Group A, there was no significant improvement in TBUT before and after treatment, and the difference was not statistically significant ($P > 0.05$). After treatment in group B, there was a tendency for TBUT to increase with the prolongation of drug administration, but the difference was not statistically significant ($P > 0.05$). In Group C, TBUT showed a significant increase after both 2 weeks ($P = 0.023$) and 4 weeks ($P = 0.011$) of treatment, compared to baseline. There was no significant difference in TBUT between the 3 groups at 2 and 4 weeks after treatment ($P > 0.05$). (Table 2; Fig. 3)

Schirmer I test

Tear secretion increased in all groups after 4 weeks of treatment compared to baseline (A: $P = 0.012$; B: $P = 0.004$; C: $P = 0.002$), with significant enhancements observed in Groups B and C ($P < 0.01$). The SIT values were significantly elevated at 4 weeks compared to 2 weeks in all three groups (Group A: $P = 0.030$; Group B: $P = 0.000$; Group C: $P = 0.038$). There was no statistically significant difference between the 3 groups at each time point after treatment ($P > 0.05$) (Table 2; Fig. 4).

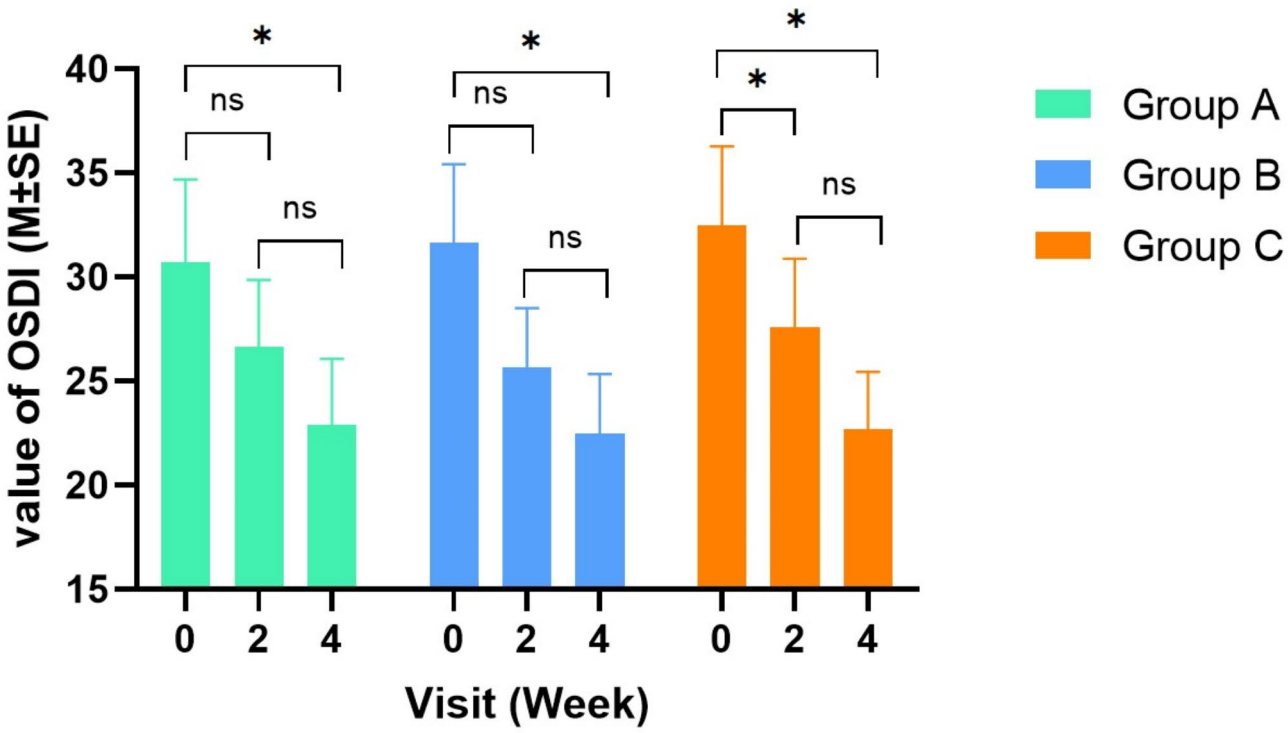

Fig. 2. OSDI score change from the baseline. Time course of OSDI. The OSDI score decreased after 4 weeks of treatment in all three groups, with Group C having decreased at 2 weeks of treatment. ns: no significance. * Significant difference of $P < 0.05$.

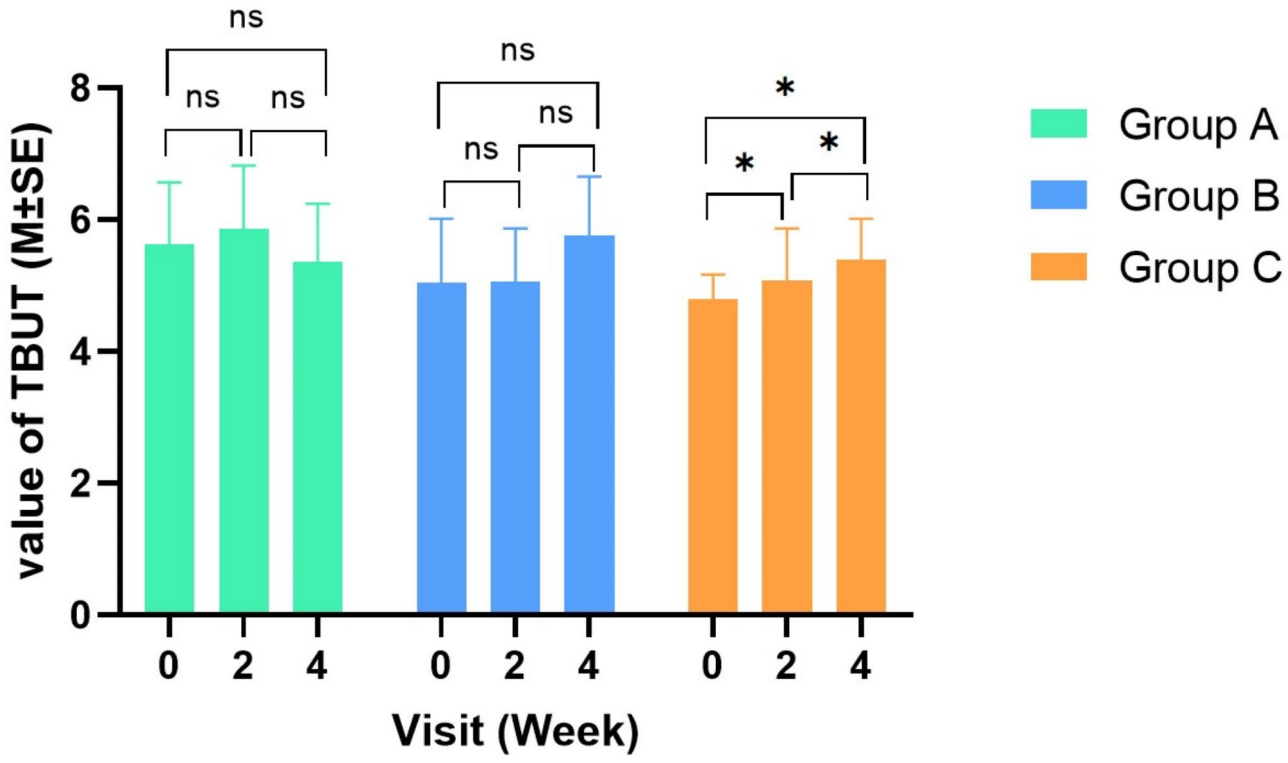

Fig. 3. TBUT change from the baseline. Time course of TBUT. There was no significant improvement in TBUT after treatment in groups A and B. After 2 and 4 weeks of treatment in group C, TBUT increased significantly compared with that before treatment. ns: no significance. * Significant difference of $P < 0.05$.

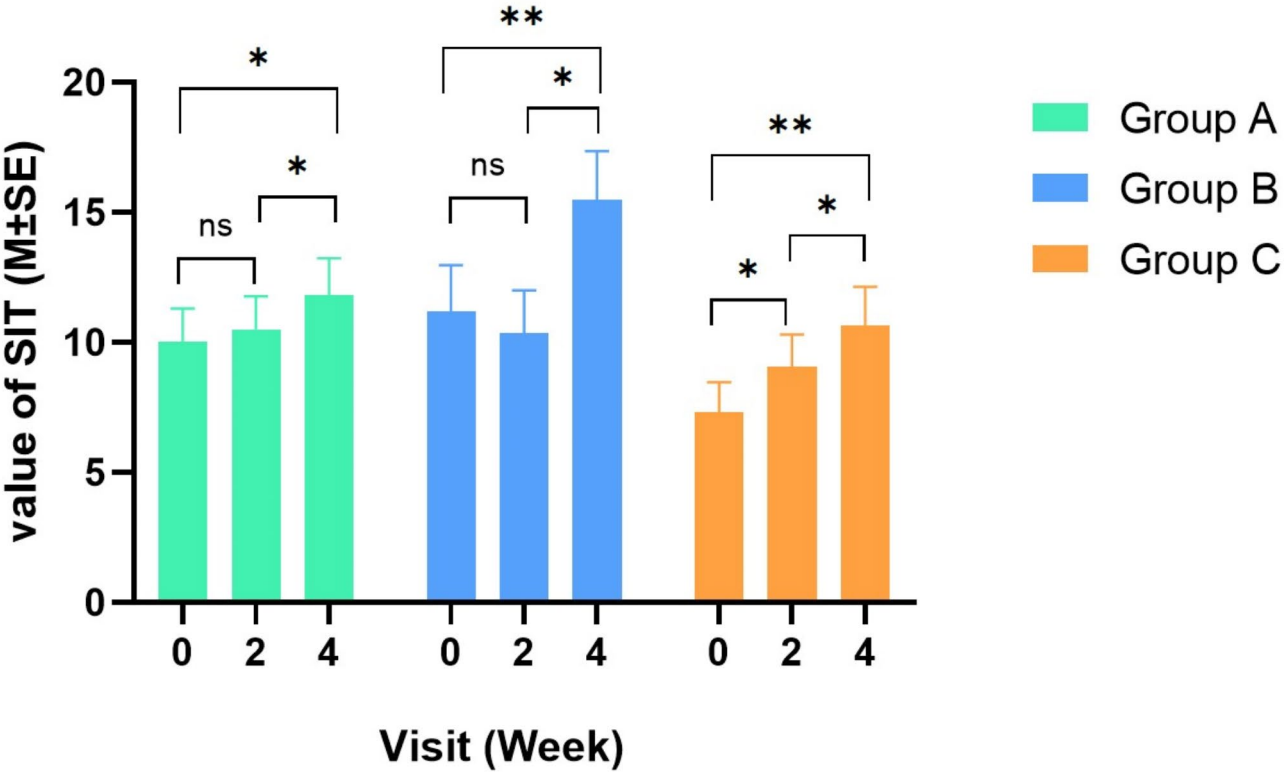

Fig. 4. SIT change from the baseline. Time course of SIT. Tear production increased in all groups after 4 weeks of treatment. SIt values for all three groups were significantly higher at 4 weeks than at 2 weeks. ns: no significance. *Significant difference of $P < 0.05$. ** Significant difference of $P < 0.01$.

Corneal fluorescein staining score

No significant improvement in CFS scores was observed in Group A before and after treatment ($P > 0.05$). CFS scores in group B tended to decrease as treatment progressed, but the difference was not statistically significant ($P > 0.05$). Group C exhibited significantly reduced CFS scores after 4 weeks of treatment, compared to pre-treatment levels ($P = 0.043$). There was no statistically significant difference between the 3 groups at each time point after treatment ($P > 0.05$). (Table 2; Fig. 5)

Comparison of clinical efficacy

The total effective rate of participants in Group C was 88.46%, which was significantly higher than that of Group A (65.38%) ($c2 = 3.900$, $P = 0.048$). the total effective rate of Group C was higher than that of Group B, but the difference was not statistically significant ($P > 0.05$). the total effective rate of Group B was higher than that of Group A, but the difference was not statistically significant ($P > 0.05$).

Adverse events

Only one patient in group B and one in group C had blurred vision. We performed an visual acuity test on the participants and found that the BCVA did not change before treatment.

Discussion

This study demonstrated that the OSDI scores and tear production of participants treated with 0.3% SH eye drops in Group A improved after 4 weeks of treatment, but there was no significant effect on the repair of ocular surface damage, which may be related to the short duration of the treatment period, suggesting that a longer intervention might be necessary to observe more pronounced healing effects. It may also be related to the lower CFS score of participants before treatment. In this study, Group B participants treated with EAD eye drops exhibited a notable increase in tear secretion at the end of the course of treatment, while Group C participants treated with 0.3% SH in combination with EAD eye drops displayed earlier symptomatic improvements, including signs and corneal staining, surpassing the other groups' timelines. Overall, the OSDI and SIT of participants in the three groups were improved after 4 weeks of treatment, and the TBUT and CFS of participants in group C were also improved. This indicates that both alone and in combination with SH are effective in dry eye, but the combined application is more effective.

EAD are the main components of this drug, both of which are not widely used in the treatment of DED. Drawing on the therapeutic mechanisms of esculin and digitalis in other conditions, we propose that their efficacy in DED is attributed to their anti-inflammatory and antioxidant capabilities. Mokdad-Bzeouich et al.

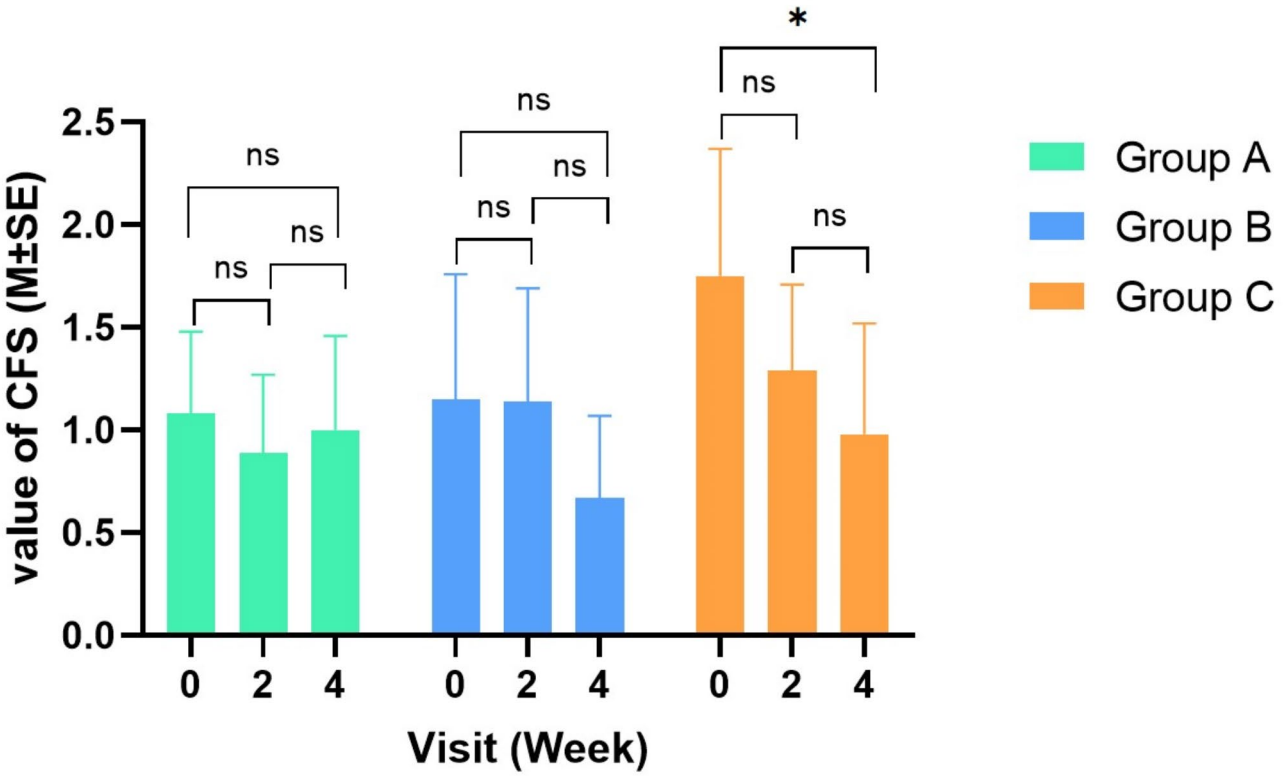

Fig. 5. CFS scores change from the baseline. Time course of CFS. There was no significant improvement in CFS scores before and after treatment in groups A and B. CFS scores in group C were significantly lower after 4 weeks of treatment compared to before treatment. ns: no significance. * Significant difference of $P < 0.05$.

discovered that esculin and its oligomer fractions inhibit cell adhesion, migration in U87 glioblastoma cells, and in vitro angiogenesis, thereby exhibiting their anti-neoplastic properties[20]. In the treatment of diabetic nephropathy, the esculin attenuated the development of renal injuries caused by hyperglycemia, proinflammatory and oxidative mechanisms mediated by P2×7 receptor[21]. Additionally, esculin has been demonstrated to offer protection against depressive dysfunction by inhibiting the TLR4/NF-κB signaling pathway, which is regulated by CCR5. Besides, esculin led to up-regulation of the CREB/BDNF neuroprotective pathway and suppression of inflammatory cytokines both in the central and peripheral system[22]. In addition to esculin, glycosides have been shown to have anti-inflammatory effects. glycosides were scientifically established as drugs against heart failure. It has been surprisingly discovered that glycosides have anti-inflammatory effects in vivo and in vitro, and mainly through inhibition of leukocyte proliferation and secretion of proinflammatory cytokines[13,23–25]. It was also found that glycosides exert anti-inflammatory effects not only by targeting Na^+-K^+-ATPase, but also through different systems, such as RORγt[26].

It is widely recognized that DED, a chronic inflammatory condition, involves significant activation of the immune cascade and inflammatory mediator release[27]. As mentioned above, TLR4/NF-κB signaling pathway, ATP/P2×7 pathways, RORγt all play important roles in the pathogenesis of dry eye. Activation of P2×7 can jeopardize the survival of goblet cells and CCR5 expression is increased in the ocular surface and tear film of patients with DED[28,29]. Thus, we speculate that the EAD eye drops exert their effect in dry eyes primarily through anti-inflammatory mechanisms, contrasting with the aqueous replenishment provided by SH eye drops. In our study, although indices such as OSDI, TBUT and SIT in the three groups of participants did not differ significantly between groups during the experimental period, intra-group changes were evident in Group C participants. Therefore, in the management of a chronic disease like DED, the combination of multiple drugs is more effective than monotherapy. Although there was no significant difference between groups A and B, we do not believe that there was no difference in the efficacy of these two eye drops, and the reason for this result may be due to the short observation period.

Only two participants in this study experienced blurred vision, but optometry results showed that the BCVA of the participants was no different from that before treatment. This may be related to the role of EAD in treating visual fatigue and improving accommodation ability. EAD has the effect of increasing the contractility of the ciliary muscle, which may cause the patient's pupil to dilate and experience blurred vision when looking at close objects[14,30].

The limitations of this study included the total amount of medication used in group C was inconsistent with groups A and B, therefore, the subjects in this study were unmasked. In addition, the follow-up period of this

study was short, and there is a need to extend the observation period, expand the sample size, and unify the number of medications administered between groups to confirm our findings.

Conclusions

The combination therapy of EAD with 0.3% SH eye drops significantly ameliorates OSDI scores, TBUT, SIT, and corneal fluorescein staining in participants with dry eye syndrome, outperforming the use of digitalisglycosides and preserved SH 0.3% eye drops alone. Among them, digitalisglycosides mainly work by inhibiting the inflammatory response of the ocular surface. However, a large multicenter trial with a prolonged follow-up is needed to determine this combination therapy's efficacy conclusively.

Data availability

The datasets generated and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Author contributions
YHW: Investigation, Methodology, Data Curation, Writing - Original Draft, Writing - Review & Editing. JHY: Conceptualisation, Formal Analysis, Writing - Original Draft, Writing - Review & Editing. YW: Investigation, Data Curation. SYL: Data Curation, Investigation. LT: Conceptualization, Funding Acquisition, Resources, Supervision, Writing - Review & Editing. YJ: Conceptualization, Funding Acquisition, Resources, Supervision, Writing - Review & Editing. All authors read and approved the final manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics statement
The research was conducted in compliance with the Declaration of Helsinki, the Good Clinical Practices Standards. It was approved by the Medical Ethics Committee of Beijing Tongren Hospital, Capital Medical University (ethics number: TREC2022-118) and was registered with the Chinese Clinical Trial Registry in 30/05/2023 (ChiCTR2300071953). Before the study began, informed consent was obtained from all participants.

Additional information
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