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Research article

Evaluation of curcumin-based ophthalmic nano-emulsion on atropine-induced dry eye in mice

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ABSTRACT

Background: One of the most efficient treatments for dry eye syndrome (DES) is to use nano-carriers as a potential delivery system. We aim to evaluate curcumin in a nano emulsion formulation.
Methods: A new formulation containing 5.5% curcuminoid was used. DLS, Zeta potential, TEM, and HPLC tests were performed to determine the size and morphology. First, 30 mice were selected as atropine-induced dry eye models. Next, 25 mice in 5 groups were treated with the nano emulsion at different doses, and corneal tissues were separated for evaluation.
Results: The DLS test results were indicative of the particles' stability. Nano curcumin appeared to be thoroughly effective in all groups, with the highest dose showing the most similarity to the healthy control group.
Conclusions: Curcumin-based nano emulsion eye drop is a promising candidate for DES management. However, further investigation is required to evaluate the possible risks in humans.

1. Introduction

Vision impairment has a prevalence of at least 2.2 billion people globally, of which more than 1 billion have a preventable or potentially correctable source [1]. Dry eye syndrome (DES), also known as keratoconjunctivitis sicca (KCS), is a multifactorial disorder that is among the most common eye conditions and also one of the leading causes of patients visiting oculists. The prevalence is higher among elderly and postmenopausal women. DES is characterized by visual disturbance, blurred vision, tear film instability, and increased osmolality, which can result in ocular surface damage and often increase inflammation [2,3].

Abbreviations: DES, Dry eye syndrome; KCS, Keratoconjunctivitis sicca; BBB, Blood Brain Barrier; STT, Schirmer tear test; DLS, Dynamic light scattering; TEM, Transmission electron microscopy; HPLC, High-performance liquid chromatography.

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A dry eye can have a substantial burden and widely impacts the quality of life. Studies suggest that vision conditions like dry eye are associated with decreased ability to perform daily activities, social functioning as well as physical and emotional well-being. It is also accompanied by lower workplace productivity and increased healthcare costs to society. Risk factors for DES include advanced age, female sex, cataract surgery, vitamin A deficiency, contact lens wear, and frequent usage of visual display terminals such as smartphones and personal computers [4]. Nowadays, due to the increasing rate of electronic device utilization, it is essential to consider potential treatments as they may significantly increase patients' quality of life.

Different treatments have been recommended for this condition such as a short course of corticosteroids, which are not beneficial for long-term usage. Artificial tears, available as over-the-counter eye drops, are another way to go. Restasis®, a cyclosporine emulsion of 0.05%, is the popular treatment used for dry eye syndrome in the form of eye drops. Restasis® has shown increased natural tear production and reduced inflammation as its effect [5–8]. The majority of ocular diseases can be treated with topical drug administration due to their non-invasiveness and convenience. However, some challenges have been disclosed including poor ocular bioavailability, static and dynamic barriers like the conjunctiva tight junctions or rapid tear turnover, and also, the need for frequent administration which leads to increased side effects. This rather impenetrable surface clears 95% of the drugs before they can affect the intended target. Since DES involves constant long-term treatment, efforts have been made to enhance treatments utilizing different delivery platforms or novel formulations like polymeric micelles, hydrogels, lipid-based nanocarriers, and nanosuspensions [5,9].

Curcumin (diferuloylmethane), a yellow-colored bioactive, is a hydrophobic molecule derived from the root of the rhizome *Curcuma longa*L. Curcumin reveals a wide range of pharmacological effects like anticancer, anti-inflammatory, antioxidant, antiarthritic, anti-diabetic, antimutagenic and antimicrobial properties. It has been used in Ayurvedic medicine and traditional Chinese medicine as a curative and preventive agent for many disorders for thousands of years. Moreover, studies have uncovered its effectiveness for major ocular pathologies. Curcumin can suppress inflammation and oxidative stress, inhibit the multiplication of human lens epithelial cells, inhibit the expression of proinflammatory cytokines induced by ovalbumin, like IL-4 and IL-5, and protect retinal cells. Additionally, it protects the corneal epithelial cells from the IL-1β increase induced by hyperosmolality. Curcumin is also proven to be non-toxic to humans or animals even at high doses [5,9,10].

Curcumin has been used to treat DES in different forms such as oral capsules [11,12]. However, particular drawbacks have made it limited to utilize curcumin in Ophthalmology such as poor bioavailability and absorption, low stability, and rapid metabolism. Thus, research is necessitated to improve its biological and pharmaceutical function. One of the most efficient ways suggested is to use a potential delivery system as nanocarriers. Different types of nanocarriers have been explored such as liposomes, nano emulsions, phospholipids, nano micelles, and nanoparticles to decrease hydrophobicity and increase the solubility of curcumin [13,14].

In the nano emulsions system, droplets on the nanometer scale (0.1–500 nm) are produced with water or oil which are dispersed in the opposite phase. Nano emulsions can be either oil in water (O/W) or water in oil (W/O), with the help of a suitable surfactant. The combination is generally within 5–20% w/w. Nano emulsions are transparent, thermodynamically stable, can cross barriers very well, and have a sustained effect on the targeted area due to their nanometer scale. They can also increase solubility and be very effective in crossing the Blood blood-brain barrier (BBB) to deliver drugs [15–17].

Treatments using nanoparticles have gained tremendous attention over the past few years and studies have suggested different drugs in the form of nano emulsions such as Dorzolamide hydrochloride which is a potential antiglaucoma drug [18], ion-sensitive in situ gels to deliver acetazolamide and also Brimonidine tartrate for dry eye disease [19]. To stabilize the nano formulation, large amounts of surfactant and co-surfactant are required which can cause irritation in the eye. Using bio-compatible, low-cost, and easily accessible materials including Alpha-tocopherol acetate in the formulation can lead to a simple synthesis process that results in a novel enhancement [20–22].

In this study, attempts were made to first, produce nano emulsion formulation of curcumin and then, to evaluate the therapeutic effect of topical nano emulsion curcumin on atropine-induced dry eye in Syrian mice [23–25]. The outcome was measured through indicators of tear production and the assessment of possible corneal injuries via histopathology analysis.

2. Material and methods

2.1. Preparation of curcumin nano emulsion

In this study, a new formulation of curcumin Nano emulsion containing 5.5% curcuminoid, polyethylene glycol 400, polysorbate and Ascorbic acid, Vegetable oil, and Alpha-tocopherol acetate was used. Nano emulsion was prepared by solving curcuminoid in Polyethylene glycol 400 and polysorbate 80, Then, the other ingredients. The obtained mixture was heated and stirred for 2 h. For a better result, the mentioned solution was placed in an ultrasonic bath for 1 h. To obtain Nano emulsion, deionized water was gradually added to the oil phase while stirring. Nano emulsion was slowly prepared through the self-nano emulsification method. To achieve sterile formulations, the nano emulsions were filtered through a 0.22 m filter [26,27].

2.1.1. Nano emulsion characterization

Dynamic Light Scattering (DLS) (Qudix, scatterscope 1, South Korea) and a Malvern Nano Zetasizer (Malvern Instruments, UK) were used to determine the mean size and zeta potential of the prepared curcumin nano emulsion, respectively. Before examination, the samples were diluted with distilled water. The shape samples were investigated by transmission electron microscopy (TEM, Philips, Netherlands). The suspension of samples was diluted with distilled water and sonicated for 10 min. One drop of suspension was transferred on a holey formvar grid, and after dehydration, it was observed by TEM at 120 kV.

In addition, High-Performance Liquid Chromatography (HPLC) (Kenaver, Germany) was applied to identify and quantify curcumin

components in the formulation [17]. In brief, the mobile phase of HPLC consisted of 35% methanol, 25% acetonitrile, and 40% phosphate buffer solution. The flow rate was 1 ml/min and the UV detector was set at 420 nm.

As the pH value of tear fluid is 7.4–7.6, the pH value of the ophthalmic formulations should at least be in the ocular range of 6.6–7.8. Therefore, the pH of nano solutions was adjusted.

2.2. Animals

In this experiment, adult male Balb/c mice weighing 20–25 g, were purchased from the Pasteur Institute, Tehran, Iran. All mice were housed under standard laboratory conditions, each group in one appropriate cage (temperature of 22° ± 2 °C, relative humidity 60% ± 5%, and 12h light-dark cycles) and were provided with adequate water and food (standard mouse food provided from Pastor Institute). All procedures were performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and compliance with the guidelines for the care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 2010). The study protocol was approved by the Research Ethics Committees of Tehran Islamic Azad University of Medical Sciences, Iran (Ethic Code: IR.IAU.TMU.REC.1400.119) and was performed according to strict governmental and international guidelines on animal experimentation.

2.3. Dry eye model

Atropine sulfate 1% eye drop solution (Sinadarou, Tehran, Iran), was used to induce a dry eye model. To estimate the exact onset of dry eye after atropine use, thirty mice were studied and followed in 9 days. As a control, five mice did not receive anything. In the experimental group, 25 mice received atropine sulfate 1% eye drops, in their left eyes. Schirmer tear test (STT) was applied every other day, 15 min before the next atropine administration. Five mice were sacrificed every other day on days 1, 3, 5, 7, 9. On due dates, corneas were removed and kept in formalin for histopathological evaluation.

2.3.1. Schirmer tear test (STT)

This test was applied without anesthesia by immobilizing the mice with a tightly wrapped towel around the body. The strips (Visio Aid manufactured by: SAVA VET) were presented to the left eye's inferior conjunctival fornix and the length of wetting was measured (in millimeters) and recorded after 15 s. Then the percentage of tear production was calculated in each group compared with their mean baseline.

2.3.2. Histopathology evaluations

In all due times, mice were anesthetized with appropriate doses of Ketamine 100 mg/kg/Xylazine 10 mg/kg and sacrificed. Their eyes were excised, corneas were removed, fixed in 10% formalin, and sent to the pathology lab. After fixation, tissue sections of 5 μm thickness were prepared for two kinds of staining. Hematoxylin and Eosin (H&E) and Masson's trichrome (Mt) staining were applied. Then microscopic evaluations with a microscope-equipped camera were performed by an expert to confirm the day of eye dryness and evaluate the histopathological injuries of the cornea and other parameters like vascular, epithelium, edema, and collagen.

2.4. Experimental procedure using nano curcumin drops

In this interventional study, 27 Syrian adult Balb/c male mice were assigned and were studied for 7 weeks. Except for the control group (n = 2) which did not receive any treatment, all groups including 5 mice each, received atropine 1% eye drops in the left eye,

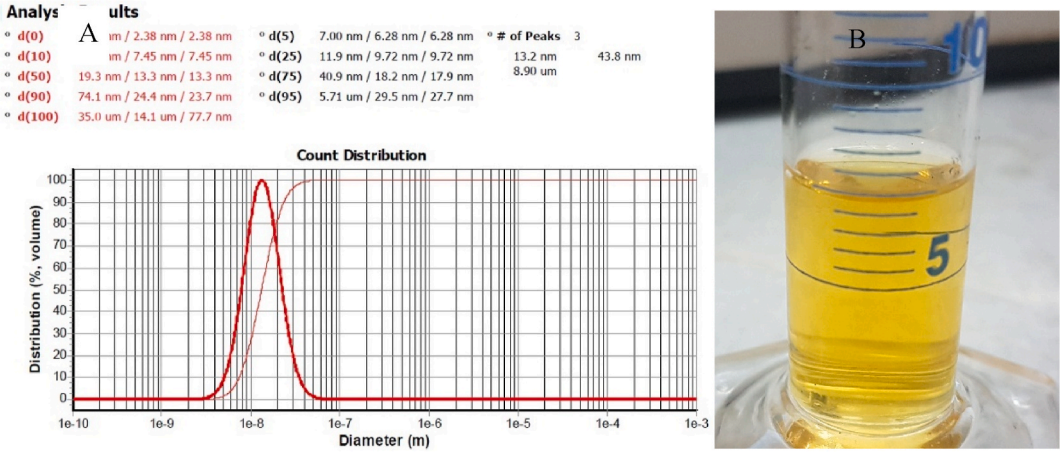

Fig. 1. A) DLS measurement of curcumin nano emulsion, B) The image of curcumin nano emulsion.

every day. The first group left without treatment. Group 2 received a nonsolvent teardrop. Groups 3 to 5 were treated with curcumin nano emulsion 0.025%, 0.05%, and 0.1%, respectively. All treatments were performed two times a week (on Sundays and Wednesdays) starting from the second week when eye dryness was confirmed. Also, there was a 15-min interval between atropine and nano curcumin utilization. STT was measured twice per week and histopathological analysis of corneas was performed at due time.

2.5. Statistical analysis

Data analyses were done by SPSS 18 to compare the tear production factors and Schirmer tear test results. The statistics were presented as mean ± standard error (SE). The significance level was considered $P < 0.05$. Repeated-measurements analysis of variance was performed via Tukey's procedure Regression analysis.

3. Results

3.1. Nano curcumin formulation

A new formulation of curcumin Nano Emulsion containing 5.5% curcuminoid, polyethylene glycol400, polysorbate80, Ascorbic acid, vegetable oil, and alpha-tocopherol acetate was used in this study. After obtaining a stable formulation, particle size was determined by DLS which is indicated in Fig. 1. The size of this solution is around 19.3 nm with a zeta potential of −11.6 mV. pH of the solution was adjusted in the normal range of 7.2–7.4.

The morphology of the prepared curcumin Nano emulsion was assessed using TEM (Fig. 2). As shown the emulsions had spherical and homogenous shapes.

Curcuminoid has three components: curcumin, desmethoxycurcumin and bisdemethoxycurcumin. As shown in the HPLC chromatogram (Fig. 3) the major part of curcumin Nano emulsion is curcumin. Two minor peaks are detected desmethoxycurcumin and bisdemethoxycurcumin.

Using safe and antiallergic materials could accelerate the dry eye healing duration. Accordingly, the biocompatible nature composition of this nano emulsion could keep the eye surface moisture to prevent irritation and at the same time deliver curcumin as healing medicine. Besides, the sterilization of the prepared nano formulation by 0.22 m filter warranty the safety of the final curcumin nano emulsion that does not have bacterial activity. Moreover, this curcumin nano emulsion formulation exhibited satisfying stability over 50 days so neither phase separation and precipitation nor significant size changes (Fig. 4) were observed.

3.2. Dry eye induction

The results of dry eye induction using STT and histopathology evaluation are described.

3.2.1. Tear production measurement in STT

The recorded results of STT during the first nine days are presented (data not shown). A decrease in tear production was reported on the third day of atropine eye drop administration. A sudden drop in tear production was observed on day 5 and showed a downward trend till day 9. When compared to the control group, STT results in atropine atropine-treated group were significantly low. The difference was the most on day 9 with ***$p < 0.001$. The Healthy group maintained a relatively stable tear production throughout the study.

3.2.2. Histopathology evaluations

In histopathology evaluations, on the first day of the experiment, with both H&E and Mt staining, corneal layers were intact without any changes regarding parameters like vascular, epithelium, edema, and collagen (Fig. 5A and B). On day 3, microscopic

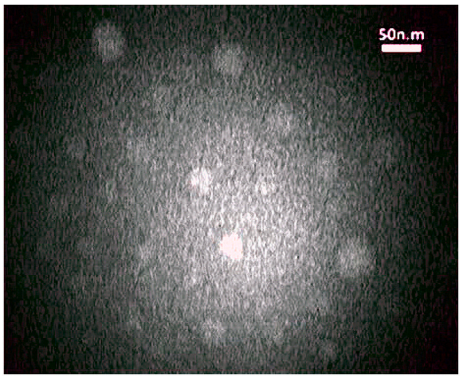

Fig. 2. TEM image of curcumin Nano emulsion.

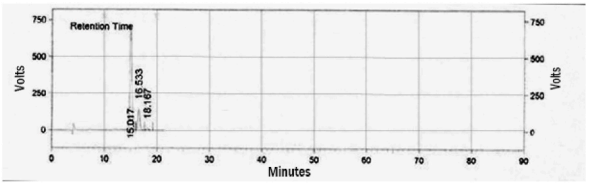

Fig. 3. HPLC chromatogram of curcumin nano emulsion.

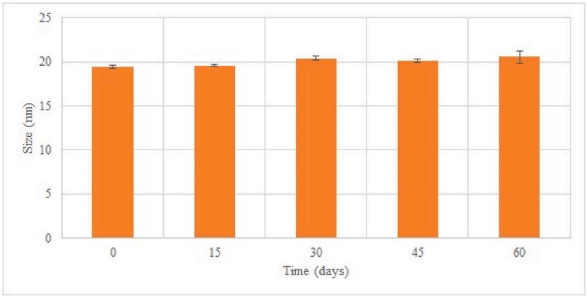

Fig. 4. The stability examination of curcumin nano emulsion over 50 days.

analysis of corneal tissue did not show any significant pathology changes except for some epithelium disturbance shown only in Mt staining (Fig. 5C and D). On day 5, corneal layer changes were more significant; irregular arrangement and discontinuity in collagen fibers were reported by both H&E and Mt staining (Fig. 5E and F). Furthermore, corneal rupture, severe epithelial separation, and Bowman's membrane tearing were reported after seven days (Fig. 5G and H).

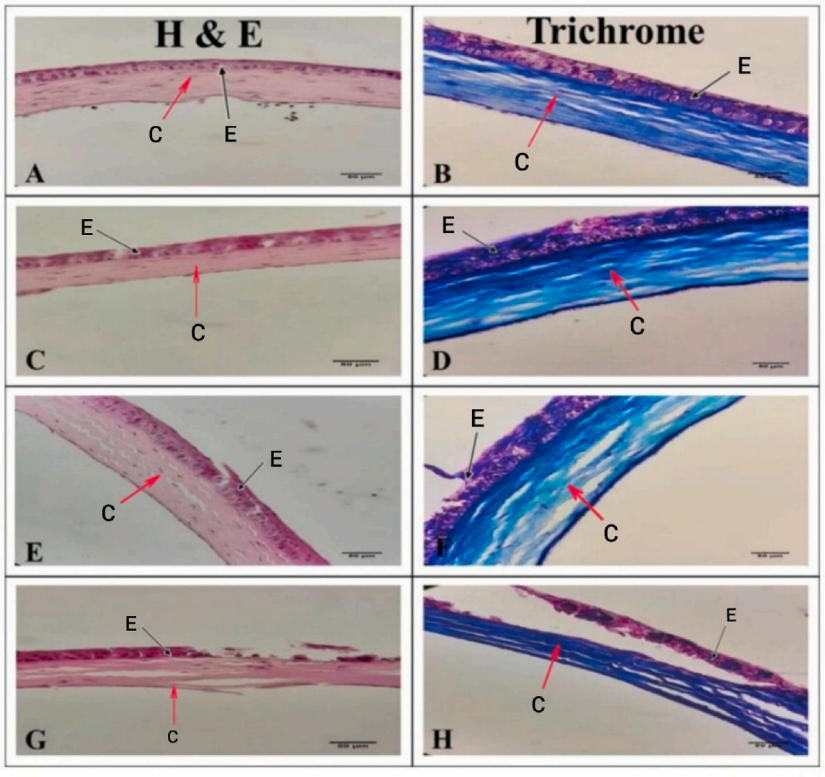

Fig. 5. (A–H). Histopathology analysis of ocular tissues of dry eye-induced mice: A, B) Day 1, C, D) Day 3, E, F) Day 5, G, H) Day 7, respectively with ×40 magnification. The red arrow (C) shows collagen and the black arrow (E) shows epithelium. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Overall, STT and pathology results showed destruction in the epithelium and Bowman's membrane after nine days, as well as collagen deposition. However, dry eye induction was confirmed from day five which was correlated to the STT results.

3.3. Nanocurcumin effects on tear production

3.3.1. STT results

STT results within seven weeks are shown in Fig. 6.

To evaluate the efficacy of the nano curcumin in dry eyes, tear production measurements were performed twice a week 15 min before the dosing of the various nano formulations on mice, and the simple mean at the end of each week was calculated and reported. All groups (except the healthy control group) underwent dry eye induction, which was reflected in the sudden drop in tear production on day 7 (Fig. 6). The healthy group maintained a relatively stable tear production throughout the study, except on week 3 (day 21).

The tear production for the Atropine group, At + nano solvent group, and At + nano curcumin 1 (0.025%) remained at or below half of the initial tear production rate throughout the study.

All the nano curcumin-treated groups were able to increase and maintain their tear production till the end of the study (day 49) compared to the Atropine group. For the At + nano curcumin 1 (0.025%) group, the increasing tear production was significant from week four post-treatment. For At + nano curcumin 2 (0.05%) and At + nano curcumin 3 (0.1%) groups, this increment was started obviously and significantly from week three, post-treatment. At the end of the first week administrations, (day 7) nano curcumin (0.05%) and nano curcumin (0.1%) ($p < 0.05$) had significant effects on dry eye, but they were only able to restore tear production up to about 50% of the initial rate (day 1). The maximum efficacy of nano curcumin was observed at a higher dose which is 0.1%. However, its tear production never reached the control group. It is noteworthy to mention that in all nano curcumin-treated groups, the tear production rate was preserved nearly constant with few variations.

3.3.2. Histopathology analysis

Pathology results demonstrate the effect of nano curcumin treatments on dry eyes (Fig. 7).

The ocular tissues of the mice in the control group showed all the signs of healthy eyes. The corneal epithelium displayed surface epithelium cells with normal morphology (Fig. 7A and B, H&E and MT staining, respectively).

No evidence of acute or chronic inflammation and edema was reported.

By contrast, the Atropine group showed epithelium and collagen changes consistent with disease (Fig. 7C–and D, for the Atropine group). There were mild to moderate levels of mixed inflammatory infiltrates.

The mice treated with At + nano curcumin 0.05%, showed conjunctiva with no signs of inflammatory infiltrates (Fig. 7E and F), displaying morphological features similar to those seen in the control mice group. Moreover, the surface epithelium demonstrated partial to complete recovery of epithelial injury. In terms of the vascular, epithelium, and collagen of the eye samples in this group, closely resembled the control group. Similarly, the mice treated with higher doses of nano curcumin (0.1%) showed no signs of inflammatory infiltrates and edema (Fig. 7G and H).

4. Discussion

One of the most efficient treatments for DES is to use nanocarriers as a potential delivery system. A new nano formulation containing 5.5% curcuminoid showed promising results when treating 25 dry eye-induced mice with atropine. The tear production rate

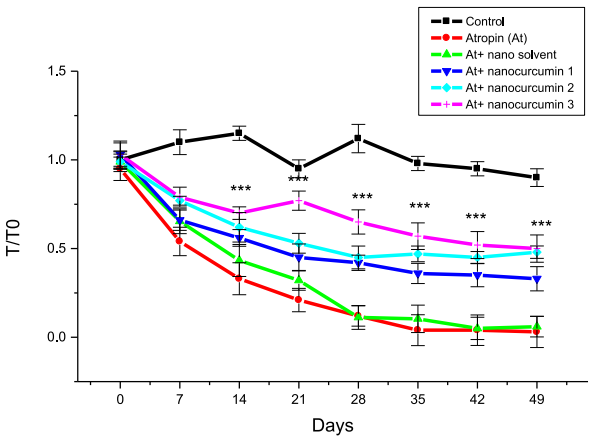

Fig. 6. Tear production measurement of 25 mice with different treatment groups (each containing 5 mice). The tear volumes (T) were normalized concerning their initial tear volume (T0). The twice-weekly dosing regimen of nano treatments; Control, Atropine (At), At + nano solvent, At + nano curcumin 1 (0.025%), At + nano curcumin 2 (0.05%), and At + nano curcumin 3 (0.1%) groups. *P < 0.05; **p < 0.01; ***p < 0.001 when compared At + nanocurcumin 3 (0.1%) with Atropine group.

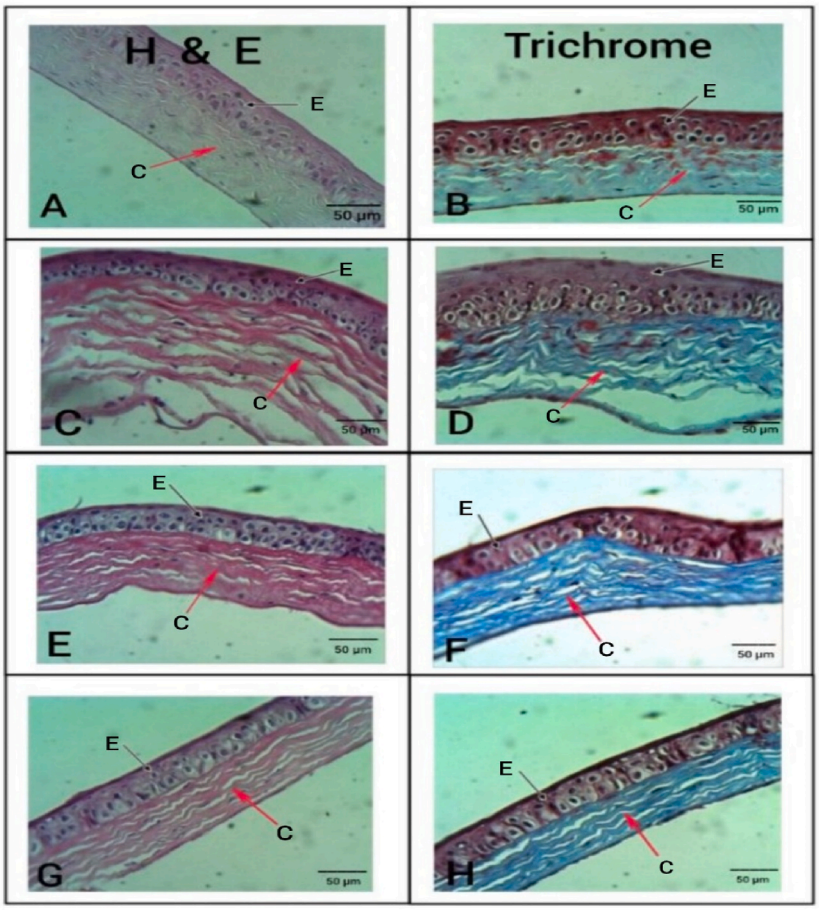

Fig. 7. (A–H). Histopathology analysis of ocular tissues of mice with different treatments after 7 weeks: A, B) Control, C, D) Atropine, E, F) At + nano curcumin 0.05%, G, H) At + nano curcumin 0.1% with H&E and MT staining, respectively with ×40 magnification. The red arrow (C) shows collagen and the black arrow (E) shows epithelium. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

was significantly increased after three weeks in all nano curcumin-treated groups, with the maximum efficacy at the highest dose (0.1%).

The effect of topical atropine administration on tear production (and potentially dry eye) was explored in dogs by Hollingsworth et al., using the Schirmer eye tear test. The results demonstrated tear decrease beginning with the first application, persistent several days after atropine treatment [28]. In the present experiment, a dry eye model of atropine in mice was induced successfully and was followed day by day and approved with both STT and histological studies.

Over the years, various treatments have been recommended for dry eye management, including anti-inflammatory ophthalmic drops. However, their side effects especially in long-term administration limited their usage. Additionally, aqueous-based tear substitutes have shown promising effects on mild to moderate symptoms [29]. In the present study, after the preparation of curcumin-based nano emulsion, its clinical efficacy was evaluated in an atropine-induced dry eye model. To the best of our knowledge, this study was the first to investigate the therapeutic potential of nano curcumin to treat dry eye disease in mice, especially in chronic treatment. The results demonstrated that the new ophthalmic drop formulation especially at higher concentrations when administered only twice per week, is significantly effective. Tear production reserves for 7 weeks of study constantly and no signs of eye irritation were observed. Besides pathological evaluations, parameters like vascular, epithelium, edema, collagen, and inflammatory cell status were improved after nano curcumin treatment.

Curcuminoid is a non-polar and lipophilic compound. Also, curcumin has low bioavailability and absorption is low in the human body despite its anti-inflammatory properties. One of the ways to increase curcumin absorption is to use nanocarriers. It seems that nanoparticles have high permeability, so curcumin nano emulsion has higher bioavailability and absorption compared to curcumin and has a better effect [30]. In this research, a novel curcumin nano emulsion was successfully manufactured. The results were aligned with other previous works. In one study, curcumin Nano emulsion was developed by Rachmawati et al. for transdermal delivery. They showed that it was more stable than the unencapsulated form, protecting curcumin from chemical degradation, and showing a significantly improved permeability [31]. The nano formulation is new and designed to apply to dry eyes. In comparison to other

curcumin nano formulations [22,30], not only the materials are low cost and available in the present study, but also their combination is biocompatible and non-irritating. In general, stabilization of the nano emulsion formulations needs large amounts of surfactant and co-surfactant that irritate [20]. However, in this study using Alpha-tocopherol acetate in the emulsion formulation prevents the side effect. Given that the zeta potential of a stable nano formulation should be between +30 mv and −30 mv, the prepared curcumin nano emulsion exhibited satisfying stability due to its zeta potential (−11.6 v). This result is in agreement with previous studies [32,33]. Thus, the repulsive force between emulsion drops was strong enough to hinder aggregation and phase separation. The presented formulation was found to be efficient in addressing the limitations of curcumin such as low solubility and rapid degradation while delivering curcumin and penetrating to the eye surface.

Recent research has explored different eye drop formulations for dry eye treatment. Tacrolimus in the form of propylene glycol nano-vesicles was investigated on rabbits as a potential therapy for dry eye syndrome by Mohammad et al. which proved to have a positive effect on epithelial and ocular damage. Dry eye was induced by atropine and benzalkonium chloride and the treatment was applied once daily for a week [34]. You et al., proposed lubricant eye drops of propylene glycol/hydroxypropyl-guar-based Nano emulsion (Systane® Complete) which were well tolerated and effective, instilled twice a day for 28 days and passed phase IV of a trial in DES participants [35]. Yu et al. suggested nanoparticles of glycol chitosan cerium oxide, administered twice a day for 7 days, as another potential treatment for dry eye disease which has higher solubility and improves tear film secretion [36]. Curcumin nano formulation was administered twice a week in this study; thus, a simpler treatment plan and fewer side effects can be expected.

Cyclosporine A (CsA), commercially available as Restasis®, is another common treatment that has been explored throughout the years. Kim et al. suggested that Clacier™ (CsA 0.05% Nano emulsion), applied twice daily for 12 weeks, improved the quality of life and alleviated clinical symptoms in DES patients, in comparison with Restasis® [37]. In a study by Duall et al., a cationic emulsion of Cyclosporin A (CsA) was proven to be very effective in severe dry eye mouse models and performed better than a glucocorticosteroid (methylprednisolone). It was suggested that a combination of CsA (0.1%) and tear film-oriented therapy can be a new promising treatment [29]. Liu et al. significantly showed the efficacy of nanoparticle cyclosporine A (NP-CsA) administration, in comparison with the ophthalmic emulsion form (Restasis®). The mucoadhesive nanoparticle both eliminated the inflammation signs and demonstrated recovery of goblet cells in a month while Restasis® only exhibited the former. In addition, it was suggested that nanoparticle formulation could prolong the dosing interval which increases compliance with the result [5]. A recent clinical trial conducted by Choi et al. compared the therapeutic effect of Nano emulsion and emulsion CsA in patients with dry eye, after short-term treatment with unpreserved fluorometholone [38]. As previously shown, nano formulations appear to have a significant effect and improve both stability and bioavailability. Therefore, like new formulations, CsA is being investigated in nano emulsion forms, too. However, there are also conflicting results. Park et al. compared cyclosporin 0.05% in emulsion and Nano emulsion form and interestingly, they were equivalently efficient. However, the nano emulsion group showed a lower conjunctival staining score [39].

Li et al. developed a topical Nano micelle curcumin formulation which proved to exhibit significantly improved higher solubility, anti-inflammatory efficacy, antioxidant activity, and chemical stability. This formulation resulted in enhanced in vivo corneal permeation. This study focused on dry eye treatment involving different tests and histopathologic assessments, while the former research was more focused on establishing the best method to facilitate curcumin encapsulation, solubilization hydrolytic stability in the polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol [9]. The utilized materials in this study are easily accessible and have a low cost which provides the potential for production scale-up of this nano formulation.

Taken together, based on our results nano emulsion curcumin-based eye drops seem to be a promising candidate for the management of dry eye disease. The anti-inflammatory effects have been observed significantly, However, its exact mechanism is not clear, whether it's due to suppressing interleukin production or other inflammatory mediators or due to its antioxidant properties remains to be investigated. There is a lack of literature on curcumin nano emulsion application in humans and like any novel method, further investigation is required to evaluate different aspects and the existing challenges. A major problem is our lack of knowledge of curcumin nano formulation risks in the human eye. Generally, using nano emulsions in pharmaceutical formulations is common for different types of delivery such as ocular, topical, parenteral, etc. So, this nano emulsion can also be used in a clinical setting. In this regard, further assessments are required, such as bioavailability, in vivo drug release, pharmacokinetics, biodistribution, toxicity, and residence of nano emulsion on the ocular surface. Thus, clinical trials must be conducted to assess the safety of curcumin nano formulation and the appropriate dosage.

5. Ethics statement

The study protocol was approved by the Research Ethics Committees of Tehran Islamic Azad University of Medical Sciences, Iran (Ethic Code: IR.IAU.TMU.REC.1400.119) and was performed according to strict governmental and international guidelines on animal experimentation.

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The work was self fund.

CRediT authorship contribution statement

Mahsa Hadipour Jahromy: Writing – review & editing, Validation, Software, Resources, Project administration, Formal analysis, Conceptualization. **Mahnaz Qomi:** Writing – original draft, Methodology, Conceptualization. **Simin Fazelipour:** Writing – original

draft, Formal analysis, Data curation. **Nafiseh Sami:** Investigation, Writing – original draft, Writing – review & editing. **Farzaneh Faali:** Investigation, Writing – original draft, Writing – review & editing. **Mehrnaz Karimi:** Data curation, Methodology, Writing – original draft. **Farhad Adhami Moghadam:** Writing – review & editing, Validation, Supervision, Resources. All authors contribute equally in this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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