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Analysis of the potential of the homeopathic eye drops *Marine Cineraria 4CH* to induce cell migration

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ABSTRACT

The ocular surface comprises diverse components that collaborate to maintain homeostasis, and any change can be termed an ocular surface disorder. One frequently observed change is the development of corneal ulcers, a primary contributor to visual impairment. Cineraria maritima is a botanical species renowned for its therapeutic application in managing diagnosed cataracts and other eye-related ailments. This study evaluated the effect of the homeopathic eye drops containing Cineraria maritima using cell viability assessments, a scratch experiment, and the quantification of IL-8 levels. The in vitro tests revealed the absence of any cytotoxic effects associated with the eye drops, thus demonstrating their safety for use. Furthermore, these eye drops exhibited the capacity to enhance the percentage of scratch closure. The treated group had an average closed area percentage of 57%, while the control group exhibited only 41% area closure, indicating the potential efficacy of eye drops in managing ocular disorders. Finally, IL-8 levels were measured following the induction of an inflammatory response by LPS and subsequent treatment with the eye drops. The outcomes indicate a decrease in the levels of this cytokine in the treated group. Such findings assure the safety and effectiveness of Cineraria maritima 4CH homeopathic eye drops, suggesting a potential avenue for their application to treat corneal ulcers.

Introduction

The ocular surface encompasses the anatomical structures covering the eye globe, including the eyelids and the tear film. All these components function in harmony to uphold homeostasis on the ocular surface. Any change in this environment is termed as an ocular surface disorder [1]. Ulcerative keratitis is one of the conditions within this category of diseases.

Corneal ulcers can arise from various causative factors, including pathogenic infections (bacterial, fungal, or viral), traumatic injuries, and other etiological agents. In addition, this disease can be one of the leading causes of visual impairment and blindness worldwide. Symptoms of the condition may include conjunctival hyperemia, pain, increased tear secretion, and photophobia. Risk factors may encompass contact lens usage or dry eye conditions [2].

Corneal ulcers result from the disruption of the corneal epithelium. Timely diagnosis during the initial stages is crucial to determine the appropriate treatment approach, which may involve administering eye drops to promote epithelial regeneration, averting infection, and mitigating inflammation. Surgical intervention may be necessary in more advanced cases of the disease [3].

Cineraria maritima is a plant native to the Mediterranean region. In India, this exotic plant is cultivated by the Central Research Council on Homeopathy in the Nilgiri hills. It has been used within the homeopathic system for a long time to address conditions like cataracts, conjunctivitis, and opacity, which are associated with severe eye-related afflictions [4].

In the context outlined, this study evaluated the *in vitro* effect of the homeopathic *Cineraria maritima* 4CH eye drops to treat keratinocytes (HaCaT) lesions, simulating the reparative process relevant to corneal ulcer management. The objective was to determine whether these homeopathic eye drops might also find application in such cases.

Method

Cell viability

Human keratinocytes (HaCaT) sourced from a commercial bank were cultivated in 75 cm² flasks with High Glucose medium supplemented with 10% Fetal Bovine Serum (FBS). Cultivation was done within a cell culture incubator at 37 ºC with a 5% CO_2 environment until reaching an approximate confluence of 80%. Subsequently, these cells were trypsinized and plated in 96-well plates at 10,000 cells per well. The plates were incubated in an oven for 24h, after which the eye drops were added at a concentration of 8 µL/mL and incubated for an additional 48h. The control group comprised wells containing culture medium without the addition of the eye drops. After this period, MTT was added and incubated for 4 hours, and cell viability was subsequently analyzed in a spectrophotometer.

Scratch test

In addition, scratch testing was performed to evaluate cell migration following the simulation of a wound. Cells were cultured under the conditions described above, and 12-well plates containing 500,000 cells per well were opened. Each well was subjected to scratch creation 24 hours after the initial cell plating, and the eye drops were administered in triplicate. Images were captured when the eye drops were administered for subsequent comparison. Cells were treated with the *Cineraria maritima* 4CH eye drops at 8 µL/mL. The control group comprised wells containing culture medium without the addition of the eye drops. After 48 hours of incubation with the product, new images were captured, and analysis of the photos was performed using the ImageJ software to define the % of the healed area.

IL-8 dosage

Human mesenchymal stem cells were also cultured in 75 cm² flasks with DMEM medium supplemented with 10% FBS. Cultivation was done within a cell culture incubator at 37 ºC with a 5% CO_2 environment until reaching an approximate confluence of 80%. Subsequently, these cells were trypsinized and plated in 96-well plates at 10,000 cells per well. Control and groups were laid out in quadruplicate with a final volume of 100 µL per well.

After 24h incubation in an oven, LPS (Lipopolysaccharides obtained from *E. coli*; L2880, Sigma Aldrich®) was introduced at a final 200 µg/mL concentration. Subsequently, the plate was incubated in an oven for an additional 24 hours.

Once the incubation time elapsed, the wells were washed with saline solution (PBS), and the eye drops were added at a final concentration of 8 µL/mL. The control group comprised wells containing culture medium without the addition of the eye drops. A further incubation of 48h was carried out to allow the action of the product, after which the supernatant was collected for cytokine analysis.

Cytokine quantification was done by the BD™ Cytometric Bead Array (CBA) flow cytometry method, and samples were subjected to analysis following the manufacturer's recommended protocol.

Statistical analysis

Statistical analysis was performed using GraphPrisma Version 9.5.0. Data were analyzed for normality by the Shapiro-Wilk test. Afterward, the Student's T-test was performed to compare the means.

Results

The HaCat cell viability assessment after treatment with the *Cineraria maritima* homeopathic eye drops indicated that the medication did not exhibit cytotoxicity towards the cells, thereby affirming its safety for utilization under the evaluated conditions (Figure 1).

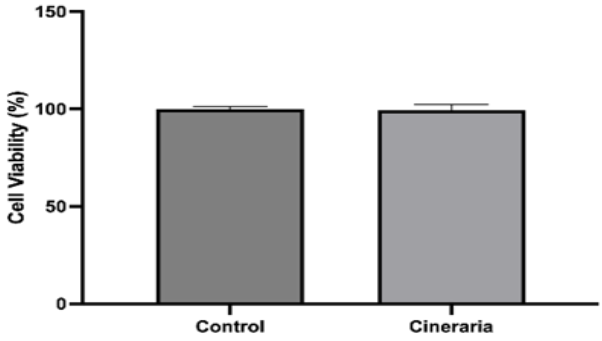

Figure 1. Assessment of HaCat cell viability after a 48h-treatment with the *Cineraria maritima* eye drops. The control group involved the culture medium without the inclusion of the medication.

Keratinocytes (HaCat) were cultured until a high confluence was achieved in the scratch test. Subsequently, a scratch was created in the cells to simulate an ocular lesion. The employed methodology effectively facilitated the creation of scratches in the cells, as illustrated in Figure 2.

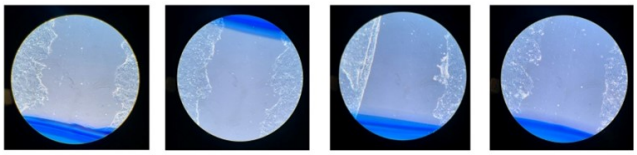

Figure 2. Scratch lesion in HaCaT cells cultivated in 12-well plates with 500,000 cells per well. Wells on the moment 0, when the scratch was performed.

After this process, the eye drops were added to the cells to treat the lesion, and the outcomes in terms of area closure percentage were compared with the control group (Figure 3).

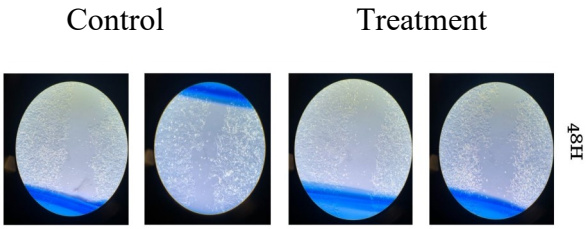

Figure 3. Comparison of the percentage of scratch closure between the group of cells that did not receive the eye drops (control) and the group that received the product for 48h (treatment).

During the normality analysis, the data were determined to be normally distributed, thus meeting the assumptions of parametric analysis. Statistical analysis showed that the *Cineraria maritima* 4CH eye drops could promote scratch closure compared to the control group (p=0.0201). The treated group achieved an average closed area percentage of 57%, whereas the control group closed only 41% of the injured area (Figure 4).

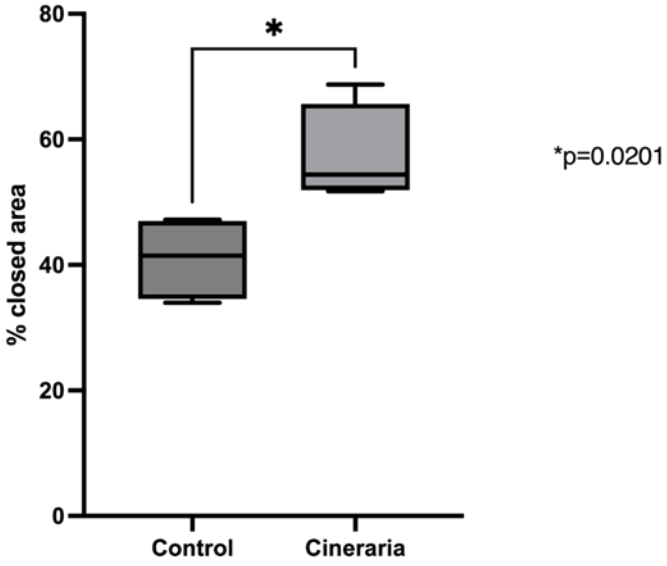

Figure 4. Analysis of the percentage of closed areas in Hacat cells treated with the Cineraria maritima 4CH eye drops and the control group that was not subjected to treatment.

Finally, a reduced release of IL-8 (p<0.0001) was recorded in the group that received treatment with the eye drops when compared to the control group (Figure 5).

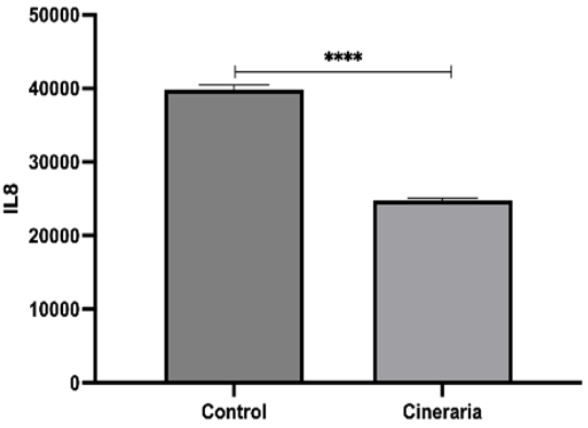

Figure 5.IL-8 dosing profile in human mesenchymal stem cells subject to treatment with eye drops compared to the control group.

Discussion

The cornea serves as the primary protective barrier of the eye. Therefore, cytotoxicity and other impacts of eye drops are assessed using cells that closely resemble the structural properties of the cornea. HaCaT cells are immortalized human keratinocytes that bear similarity to corneal cells and have been used in both 2D and 3D tests to evaluate eye-related products [6].

Following the cultivation of keratinocytes (HaCaT) and the execution of the scratch test using *Cineraria maritima* 4CH eye drops, it was evident that the medication exhibited no cytotoxic effects. Moreover, the group subjected to the treatment demonstrated a notably greater percentage of area closure in the scratch test compared to the control group. These findings suggest the product's effectiveness in facilitating scratch closure, indicating its potential as an adjuvant in treating corneal ulcers.

In addition, a decrease in IL-8 levels was observed after treatment with the medication. This cytokine is known for its role in the inflammatory process, where it serves to attract neutrophils, basophils, and T cells. It is released by various cell types to regulate and contain inflammation. It is also recognized for acting in angiogenesis, inflammation, chemotaxis, neutrophil degranulation, leukocyte activation, and regulation of calcium homeostasis [7,8]. The role of cytokines in skin ulcers has demonstrated that high levels of IL-8 and other cytokines are present in cases where healing is not expected. Conversely, in instances of healing, these cytokine levels diminish [9], as observed in this study through the cytokine quantification following treatment with the eye drops.

Limited knowledge exists regarding the potential toxicity of *Cineraria maritima* in homeopathic treatments for corneal ulcers. Therefore, this study represents a distinctive and possibly pioneering endeavor in this research domain, as it has enabled the examination of the medication's impact on the closure of induced scratches in human keratinocytes. In addition, safety and efficacy tests are important to define doses and certify that a product acts as expected. In the context of eye medications, *in vitro* tests are significant as they reduce animal use in research. These tests have gained prominence, particularly since the inception of the 3Rs (Reducing, Refining, and Replacing) framework [5].

Conclusion

The present study demonstrated that the homeopathic *Cineraria maritima* eye drops effectively facilitated the closure of a simulated scratch, representative of certain ocular disorders, including corneal ulcers. Therefore, the utilization of this medication can prove to be efficacious in contributing to the treatment of corneal ulcers, supported by scientific evidence.

References

1. Agarwal S, Srinivasan B, Harwani AA, Fogla R, Iyer G. Perioperative nuances of cataract surgery in ocular surface disorders. Indian J Ophthalmol. 2022;70(10):3455-3464. doi:10.4103/ijo.IJO_624_22
2. Chang YS, Tai MC, Ho CH, et al. Risk of Corneal Ulcer in Patients with Diabetes Mellitus: A Retrospective Large-Scale Cohort Study. Sci Rep. 2020;10(1):7388. Published 2020 Apr 30. doi:10.1038/s41598-020-64489-0
3. Stamate AC, Tătaru CP, Zemba M. Update on surgical management of corneal ulceration and perforation. Rom J Ophthalmol. 2019;63 (2):166-173.
4. Srivastava N & Bagchi G. Influence of micronutrient availability on biomass production in Cineraria maritima. Indian Journal of Pharmaceutical Sciences, 2006:68(2), 238. doi: https://doi.org/10.4103/0250-474X.25724
5. Stephens ML & Mak NS. History of the 3Rs in toxicity testing: From Russell and Burch to 21st century toxicology. Reducing, refining and replacing the use of animals in toxicity testing, 2013:19(1). doi:10.1039/9781849737920-00001
6. da Silva ACG, Chialchia AR, de Castro EG, et al. A new corneal epithelial biomimetic 3D model for in vitro eye toxicity assessment: Development, characterization and applicability. Toxicol In Vitro. 2020;62:104666. doi:10.1016/j.tiv.2019.104666

7. Brennan K, Zheng J. Interleukin 8. Xpharm: The comprehensive Pharmacology Reference. 2007;1-4. doi: 10.1016/B978-008055232-3.61916-6
8. Gonzalez-Aparicio M, Alfaro C. Statement of Retraction: Significance of the IL-8 pathway for immunotherapy. Hum Vaccin Immunother. 2020;16(10):2312-2317. doi:10.1080/21645515.2019.1696075
9. Burian EA, Sabah L, Karlsmark T, et al. Cytokines and Venous Leg Ulcer Healing-A Systematic Review. Int J Mol Sci. 2022;23(12):6526. Published 2022 Jun 10. doi:10.3390/ijms23126526.