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RESEARCH ARTICLE OPEN ACCESS

USE OF HOMEOPATHIC EYE DROPS CONTAINING EUPHRASIA OFFICINALIS TO TREAT EYE INFLAMMATIONS – IN VITRO PROOF OF CONCEPT

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ABSTRACT

Integrative medicine has increasingly embraced homeopathy due to its reported effectiveness against various diseases without the associated side effects commonly seen with allopathic medications. *Euphrasia officinalis* has been recognized for over 70 years for its beneficial properties in addressing eye disorders. This study's objective is to modulate the inflammatory process using *Euphrasia* 6CH eye drops in an *in vitro* setting. The medication was administered to the human mesenchymal stem cell culture after the induced cellular inflammation by LPS. The concentration of the product where no cytotoxic effects were observed (8 µL/mL) was assessed. Then, after culturing the cells, inflammation was induced using LPS, followed by treatment with the medication. The supernatant was collected, and the levels of cytokines associated with the inflammatory process were measured using the flow cytometry method. After the treatment, cytokines quantification revealed a statistically significant decrease in proinflammatory cytokine levels (IL-6 e IL-8). These results indicate that *Euphrasia* 6CH eye drops decreased proinflammatory cytokines. Consequently, further tests could explore diverse applications of this medication beyond its use in conditions like conjunctivitis.

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INTRODUCTION

Homeopathy originated in Germany over 200 years ago and revolves around the concept of using substances that, in their original form and dosage, could be toxic to patients. However, these substances are believed to retain beneficial effects without toxicity through multiple successive dilutions. This approach has gained traction in complementary medicine to treat various diseases, offering patients benefits while potentially minimizing side effects commonly associated with conventional medications[1]. Eye conditions like glaucoma, corneal ulcers, and keratoconjunctivitis [2,3] have been among the ailments treated with the assistance of homeopathy. These eye diseases trigger a significant inflammatory response, evident through clinical symptoms such as pain and redness. At a cellular level, they prompt the release of proinflammatory and anti-inflammatory cytokines, comprising the body's response to combat these conditions[4]. In this scenario, responses occur through innate and adaptive immunity, wherein cells collaborate to control inflammation. Heat shock-specific proteins (HSPs) function as self-antigens during glaucoma progression. They activate microglia, prompting the secretion of proinflammatory cytokines like IL-1β, IL-6, and TNF-α, thereby initiating innate immune responses through toll-like receptor 2 (TLR2) and TLR4 [5,6,7]. Another fairly common eye disorder is uveitis. This condition encompasses intraocular inflammation affecting the iris, ciliary body, and choroid. There are over 60 known causes, the most prevalent being infections triggered by bacteria, fungi, viruses, and protozoa [8]. Despite its multifactorial nature, investigating the cytokines involved in the inflammatory process can significantly assist in diagnosing and treating the disease [9]. Dry keratoconjunctivitis exhibits varying symptoms and signs, including dryness, pain, reduced visual clarity, decreased tear production, instability of the tear film, and inflammation of the eye's surface. This condition can impact anywhere from 5 to 50% of individuals, indicating a high incidence rate and numerous instances of delayed diagnosis [10]. Several treatments are currently accessible for this disorder, and ongoing studies assess the cytokines implicated in the inflammatory process to enhance therapeutic approaches [11]. Ultimately, diabetic retinopathy stands as one of the prevalent eye disorders and ranks as the primary cause of visual impairment among individuals with diabetes. Early detection and prompt treatment are essential in disease management, ensuring a better quality of life for the patient [12]. Given the array of eye diseases and the necessity for effective treatments, this study

aimed to assess the effectiveness of homeopathic eye drops containing *Euphrasia officinalis*. The evaluation was conducted through *in vitro* testing on mesenchymal stem cells, involving the quantification of cytokines to determine the product's impact in an inflammation scenario induced by LPS.

METHODS

The medication was obtained in the Injectcenter laboratory in Brazil. The Mother Tincture was used as the initial substance to prepare the tested solution (*Euphrasia 6CH*). The study employed the Hahnemannian Decimal Method, following the guidelines outlined in the Brazilian Homeopathic Pharmacopoeia. The process involved mixing one part of the active ingredient with nine parts of an inert ingredient, using a sterile isotonic solution. This mixture was succussed 100 times, yielding *Euphrasia* D1 (1×10^{-1}). Then, one part of *Euphrasia* D1 was mixed with nine parts of the inert ingredient and succussed 100 times, yielding *Euphrasia* D2 (1×10^{-2}). The successive dilution continued till *Euphrasia* 6CH was obtained.

Cell culture: Human mesenchymal stem cells (MSC) were cultured in 75 cm² culture flasks using Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum (FBS). The culture flasks were placed in an incubator at 37 ºC with 5% CO_2, and the culture medium was refreshed every 48 hours until the cells reached a confluence ranging between 60-80%.

Product cytotoxicity assessment: Before dosing with cytokines, cell viability was evaluated using MTT (3-4,5-dimethyl-thiazol-2-yl-2,5-diphenyltetrazolium bromide) to ensure that the chosen concentration would not induce cell death. In order to conduct this assessment, human MTC cells were seeded in 96-well plates (10,000 cells/well), and various concentrations of the eye drops were tested. Following 48 hours of treatment, MTT was introduced, and a spectrophotometer was used to obtain readings.

Induction of inflammation by LPS: Upon reaching cell confluence, the cultured cells underwent trypsinization and were then seeded into 96-well plates at a concentration of 10,000 cells per well. Following 24 hours of incubation at 37°C with 5% CO_2, the culture medium was removed, and the wells were washed with PBS. Subsequently, 200 µg/mL of LPS (*Lipopolysaccharides from Escherichia coli O55:B5 – Sigma Aldrich*), diluted in an antibiotic-free medium, was added to the wells, followed by another 24 hours of incubation. LPS was omitted in one of the control groups for comparative analysis. Following the inflammation induction with LPS, the medium was removed from the plate, and the wells were washed with PBS. Subsequently, the product was added at a final concentration of 8 µL/mL. The plate was incubated for another 24 hours in an oven under the conditions previously described. Once the treatment period had elapsed, the supernatant was withdrawn, and a serum-free culture medium was introduced for an additional 24 hours. Subsequently, the supernatant was collected for the analysis of the released cytokines.
For comparative purposes, the assay was conducted using one control group with LPS added and another without the addition of LPS.

Cytokine Quantification: Cytokine quantification was carried out using the BD™ Cytometric Bead Array (CBA) flow cytometry method, enabling the simultaneous measurement of multiple proteins. The CBA is designed for multiplex analysis, i.e., it performs multiple analyses on a single sample, maximizing the number of proteins that can be analyzed. The samples were subjected to analysis following the protocol recommended by the manufacturer. The cytokines dosed were IL-1β, IL6, IL8, IL12p70, and TNF-α.

Statistical analysis

Statistical analysis was performed using the GraphPrism Version 9.5.0. The data underwent normality analysis using the Shapiro-Wilk test. Subsequently, ANOVA was conducted, followed by post-hoc Tukey tests for multiple comparisons.

RESULTS

In order to evaluate the effect of Euphrasia 6CH homeopathic eye drops, human mesenchymal stem cells were cultured and subjected to *in vitro* testing to determine both safety and efficacy following treatment with the product. The cytotoxicity of the product was assessed using the MTT test, revealing that at a concentration of 8 µL/mL, cell viability remained similar to the control group (those that did not receive the treatment). This result suggests that this concentration would be safe for further utilization of the product in subsequent tests (Figure 1).

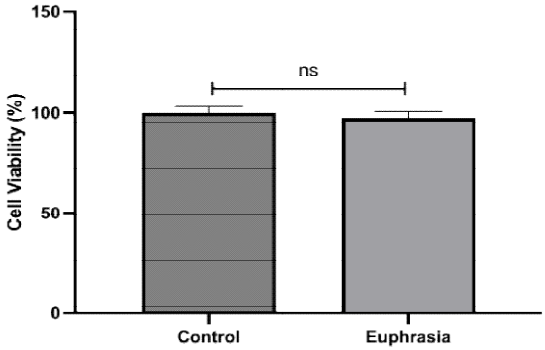

Figure 1. Cell viability of human mesenchymal stem cells determined via MTT assay following treatment with *Euphrasia* 6CH eye drops at the final concentration of 8 µL/mL.

Once the appropriate concentration was determined, a fresh culture was established under identical conditions. Subsequently, inflammation was induced by LPS to facilitate cytokine quantification. Following cytokine quantification, a statistically different IL-1β dosage was observed between the group treated with *Euphrasia* 6CH eye drops and the control group that received only LPS. However, no difference was observed between the group treated with *Euphrasia* 6CH eye drops and the group that did not receive LPS treatment. Regarding the levels of IL-6 and IL-8, statistical differences were observed between treatment and both controls (with LPS and without LPS) (Figures 2B and 2C). This result indicates that the product decreased the levels of these two proinflammatory cytokines. In the case of IL12p70 and TNF-α levels, no statistical difference was noted between the sample treated with the eye drops and the control groups (Figures 2D and 2E). Data were assessed for normality and determined to exhibit a normal distribution (parametric).

DISCUSSIONS

Interleukins hold a crucial role in inflammatory processes, and quantifying them facilitates comprehension of product actions within this context, as specific cytokines may exhibit varying levels of increase or decrease during inflammation. Its primary function is to modulate the growth, differentiation, and activation of different cells during inflammatory and immune responses [13]. In this study, the levels of cytokines TNF-α, IL-1β, IL-6, IL-8, IL-10, and IFN-γ were evaluated after inflammation induction by LPS and subsequent treatment with homeopathic eye drops. As presented, a noticeable reduction in IL-8 levels following treatment with *Euphrasia* 6CH eye drops suggests their potential to mitigate inflammation. Studies have shown that IL-8 has high levels in different cases of glaucoma and stimulates the recruitment of macrophages to regions of inflammation [14]. The role of IL-8 in ocular inflammations is linked to angiogenesis in the conjunctiva, cornea, iris, retina, and orbit, consequently triggering inflammation. In light of this information, there is a potential interest in utilizing immunotherapy to reduce IL-8 levels in targeted areas, aiming to enhance treatment efficacy in these cases [15].

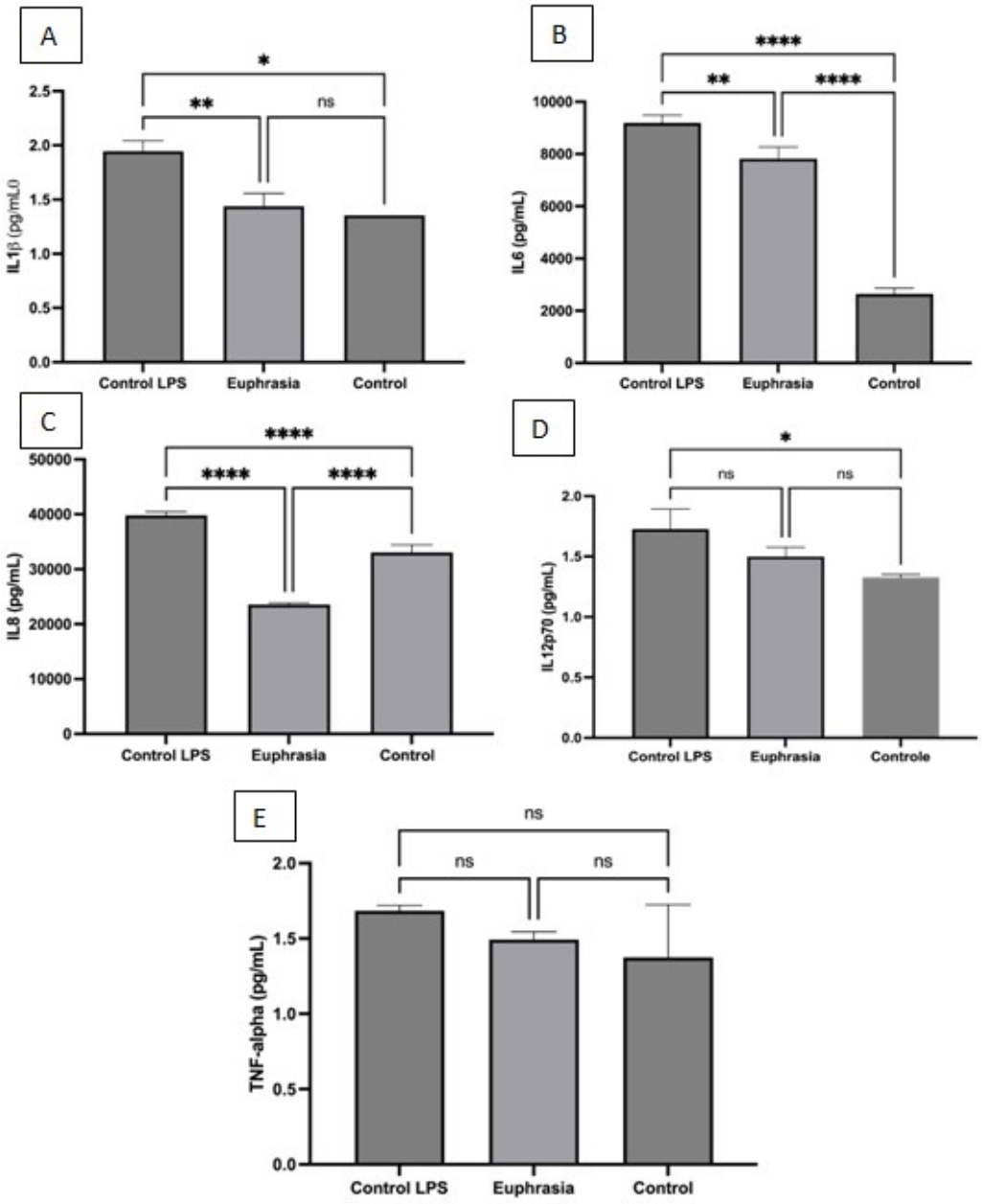

Figure 2. Dosing profiles of cytokines IL-1β, IL6, IL8, IL12p70, and TNF-α in human mesenchymal stem cells following LPS stimulation of inflammatory process

IL-6, another interleukin found in ocular inflammations, exhibited a decrease post-treatment. Elevated levels of IL-6 are often observed in individuals with uveitis, and its suppression has been linked to the reduction of the TH17-type immune response, thereby ameliorating chronic inflammation. The production of this interleukin at sites of inflammation increases the vascular endothelial growth factor (FEVG) expression and, consequently, increases angiogenesis and vascular permeability [16]. Elevated IL12p70 levels are considered potential biomarkers for diagnosing glaucoma [17]. However, there was no statistically significant difference in the dosage of this cytokine compared to the control in this study. Tumor necrosis factor (TNF-α) is produced by macrophages in response to infections and inflammatory irritation. This cytokine is present in high levels in cases of uveitis; therefore, anti-TNF-α factors have been increasingly studied to treat this ocular disorder [11]. Nevertheless, this study did not observe a decrease in this cytokine following treatment with eye drops. Apart from its role in uveitis, studies have examined the elevated levels of TNF-α, IL-1β, IL-6, IL-8, IL-10, and IFN-γ concerning dry keratoconjunctivitis and patients with diabetic retinopathy [18,19]. *Euphrasia officinalis* has been a part of alternative medicine for over 70 years [19], particularly in addressing ophthalmic issues like allergies and conjunctivitis.

Reported successes include cases of conjunctivitis where over 95% of patients experienced the complete disappearance of symptoms within 3 to 17 days of using these eye drops [20]. The observed reduction in inflammatory cytokines IL-6 and IL-8 during *in vitro* tests implies that *Euphrasia officinalis* could potentially treat ocular inflammations beyond conjunctivitis.

CONCLUSION

The *Euphrasia* 6CH homeopathic eye drops reduced proinflammatory cytokine levels, suggesting its potential application in reducing ocular inflammation. Indeed, further tests are imperative to validate this action, especially concerning its efficacy in distinct ocular diseases.

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