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THE EFFICACY OF ORALLY ADMINISTERING LACTOBACILLUS CASEI SHIROTA IN ALLEVIATING SIGNS OF DRY EYES IN MICE MODELS

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Abstract

This study explores the efficacy of orally administering Lactobacillus casei Shirota (LCS) in alleviating the signs of dry eye in male ICR mice models. Using an experimental research design, twenty-six (26) male ICR mice were subjected to dry eye induction through the topical application of 0.2% benzalkonium chloride (BAC) twice daily for one week. The mice were then randomly assigned to a control group (n = 13), which received 300 µL of phosphate-buffered saline (PBS), and an experimental group (n = 13), which received 300 µL of PBS containing 1×10^9 colony-forming units (CFU) of Lactobacillus casei Shirota. Tear quantity and tear film quality were assessed using the Schirmer's test and the tear break-up time (TBUT) test, respectively. Topical application of 0.2% BAC successfully induced dry eye in both groups. Oral administration of PBS alone did not alleviate the signs of dry eye in the control group, whereas the experimental group that received LCS showed a statistically significant increase in both tear quantity and tear film quality ($p < 0.05$). These findings indicate that orally administering Lactobacillus casei Shirota can alleviate the signs of dry eye disease in mice models, as reflected in the improvement of tear quantity and quality, and suggest that this probiotic strain may hold potential as a therapeutic approach for dry eye disease.

Keywords: *Lactobacillus casei Shirota, dry eye disease, probiotics, tear quantity, tear quality, gut-eye axis*

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INTRODUCTION

The adage "we are what we eat," often attributed to the French gastronome Jean Anthelme Brillat-Savarin, has persisted throughout history as a reminder of how dietary choices shape overall health. Recent scientific investigations have lent renewed weight to this idea by examining the relationship between the gut microbiome and human physiological function (Shehata et al., 2022). Trillions of microorganisms, nearly equal in number to the body's own cells, reside primarily within the gut and play a critical role in regulating bodily functions through the signals they send (Sender et al., 2016). Diet has, in fact, been shown to influence mental health outcomes: individuals who consume more highly processed food are more likely to develop anxiety and depression (Martin et al., 2023).

The influence of gut microorganisms extends beyond behavior. Wierstema et al. (2021) demonstrated an interplay between the gut microbiome and the immune system, underscoring the role of nutrition in optimizing treatment strategies. A healthy gut microbiome can be cultivated through the use of probiotics—live microorganisms commonly found in fermented foods and supplements that help sustain the balance of beneficial gut bacteria, thereby supporting gastrointestinal and overall health (Wang et al., 2021).

As health continues to be understood as more than the sum of its individual systems (Robertson, 2023), researchers have increasingly examined the effects of probiotics on specific bodily systems, including the visual system—widely regarded as among the most valued of the human senses (Hutmacher, 2021). Vision underlies nearly every human activity, from basic tasks such as reading and safely navigating one's surroundings, to more meaningful experiences such as recognizing the faces of loved ones. Given this centrality, dry eye disease has drawn increasing attention because of its impact on vision-related quality of life.

Dry eye disease has a prevalence ranging from 5% to 50% depending on geographic location. It occurs when tear production is insufficient, tear evaporation occurs too rapidly, or tears are not properly distributed across the ocular surface. Common symptoms include a burning or stinging sensation, inflammation, light sensitivity, double vision, and blurred vision. If left untreated, dry eye disease can damage the cornea—the outer layer of the eye responsible for most of its refractive power—diminishing the patient's quality of life and productivity. Severe cases have also been linked to a greater risk of mental health problems (Wróbel-Dudzińska et al., 2023). Current treatment options for dry eye disease remain largely limited to topical gels, ointments, or drops.

The multifactorial nature of dry eye disease—its diverse causes, non-linear pathogenesis, and patient-specific treatment response—means that no single, permanent cure yet exists. Recent research, however, points to a possible connection between digestive health and ocular well-being, suggesting that the balance of gut bacteria may influence the frequency and severity of dry eye symptoms. Among available probiotic options, Yakult, a fermented milk drink widely consumed in Asia that contains Lactobacillus casei Shirota, has drawn attention for its potential health benefits (Mittal, 2022).

This study aimed to investigate the relationship between gut health and vision by examining the effects of Lactobacillus casei Shirota in alleviating the signs of dry eye in mice models, contributing to the broader understanding of the connection between the gut microbiome and ocular health.

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METHODS

Research Design

This study employed an experimental research design, which allowed the researchers to control extraneous variables and ensure that the observed effects were attributable to the treatment applied rather than to other external factors.

Study Subjects and Ethical Considerations

Twenty-six (26) eight-week-old male ICR mice in good health were used as study subjects. All procedures adhered to established ethical guidelines and regulatory standards for the use of animals in biomedical research.

Induction of Dry Eye Disease

Dry eye disease was induced in all subjects through the topical application of 0.2% benzalkonium chloride (BAC), administered twice daily for seven consecutive days. This concentration and dosing schedule follow protocols established in prior dry eye models and were selected to disrupt the tear film and produce a uniform baseline of induced dry eye across subjects prior to treatment.

Grouping and Treatment

Following dry eye induction, the mice were randomly assigned to one of two groups of thirteen (13) mice each:

- Control Group (n = 13): Received 300 µL of phosphate-buffered saline (PBS) administered orally, without any probiotic supplementation.
- Experimental Group (n = 13): Received 300 µL of PBS containing 1×10^9 colony-forming units (CFU) of Lactobacillus casei Shirota, administered orally.

Tear Quantity and Quality Assessment

Tear production and tear film stability were assessed at baseline (prior to BAC induction), after dry eye induction, and following treatment. Tear quantity was measured using the Schirmer's test, and tear film quality was assessed using the tear break-up time (TBUT) test.

Statistical Treatment

Quantitative data were analyzed using appropriate statistical methods to determine significant differences between the control and experimental groups. Comparative analysis was used to confirm that observed improvements in the signs of dry eye were attributable to treatment rather than chance, with a p-value of less than 0.05 considered statistically significant.

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RESULTS

Tear Quantity and Quality Before and After Dry Eye Induction

Results of the Schirmer's test and TBUT test confirmed that seven days of topical 0.2% BAC application effectively induced dry eye disease (DED) in both the control and experimental groups, producing a statistically significant reduction in tear quantity and tear film quality relative to baseline. This confirmed the effectiveness of the induction protocol.

Effects of PBS on Tear Quantity and Quality in the Control Group

Oral administration of 300 µL of PBS alone did not produce a significant improvement in tear quantity in the control group. A slight further decline in tear quality was observed in this group over the treatment period.

Effects of PBS with Lactobacillus casei Shirota in the Experimental Group

The experimental group, which received PBS containing Lactobacillus casei Shirota, showed a statistically significant increase in both tear quantity and tear film stability following treatment.

Comparison Between Groups Post-Treatment

Post-treatment comparison between the two groups showed that oral administration of Lactobacillus casei Shirota produced significantly greater improvement in both tear quantity and tear quality than PBS alone ($p < 0.05$).

DISCUSSION

These findings confirm that topical 0.2% BAC is an effective and reliable method of inducing dry eye disease in mice models, consistent with previous research showing that benzalkonium chloride disrupts the lipid layer of the tear film, resulting in tear film instability (Carpena-Torres et al., 2020).

The absence of improvement—and, in fact, the slight further decline in tear quality—following oral administration of PBS alone in the control group suggests that phosphate-buffered saline does not, on its own, address tear film deficiencies. This finding strengthens the interpretation that the improvements observed in the experimental group can be attributed specifically to the Lactobacillus casei Shirota supplementation, rather than to the oral administration procedure itself.

In contrast, the statistically significant increase in tear quantity and quality observed in the experimental group suggests that Lactobacillus casei Shirota exerts a systemic effect on tear film physiology. This is consistent with existing evidence that LCS produces measurable immunomodulatory effects in mice (Chen, Pan, Huang, Chan, & Huang, 2021) and aligns with findings from other probiotic strains, such as Lactobacillus fermentum HY7302 (Lee et al., 2023) and the IRT5 probiotic combination (Choi et al., 2020), both of which have shown efficacy in alleviating dry eye signs in mouse models. Taken together, these results support the existence of a gut-eye axis, in which the composition of gut microbiota may influence ocular surface health, potentially through the modulation of systemic inflammatory and immune pathways.

Several limitations should be considered when interpreting these findings. First, as with any animal model, results obtained in mice may not directly translate to human physiology, and clinical trials would be needed to confirm efficacy in humans. Second, the study examined only a single dosage over a short treatment period, leaving questions about optimal dosage, administration frequency, and long-term efficacy unanswered. Third, the precise

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mechanism by which Lactobacillus casei Shirota influences tear film parameters was not elucidated in this study and warrants further mechanistic investigation.

Despite these limitations, the findings offer preliminary but promising evidence that oral probiotic supplementation may serve as a complementary, systemic approach to managing dry eye disease—one that could work alongside, rather than replace, existing topical treatments.

CONCLUSION

Based on the specific objectives and findings of this study, the following conclusions were drawn:

- Topical administration of 0.2% benzalkonium chloride effectively induces dry eye disease in mice models, supporting its continued use in future dry eye research.
- Oral administration of phosphate-buffered saline alone does not improve tear film parameters and may be associated with a further decline in tear film quality.
- Oral administration of Lactobacillus casei Shirota shows promising potential in alleviating the signs of dry eye disease.
- Oral administration of Lactobacillus casei Shirota significantly improves tear film parameters, specifically tear quantity and quality.

RECOMMENDATIONS

Based on the study's findings and conclusions, the researchers present the following recommendations:

Future Researchers

- Expand to Human Trials: Given the positive results in mice models, future studies should consider clinical trials in humans to further confirm the efficacy of Lactobacillus casei Shirota in alleviating signs of dry eye.
- Assess Long-Term Effects: Conduct long-term studies to evaluate the sustained efficacy of Lactobacillus casei Shirota over extended periods in alleviating signs of dry eye.
- Optimize Dosage: Determine the optimal dosage and administration frequency for maximum therapeutic benefit.
- Conduct Comparative Studies: Compare the effectiveness of Lactobacillus casei Shirota with other probiotics and conventional treatments for dry eye.
- Analyze Formulation Synergy: Evaluate Yakult as a complete formulation to explore potential synergistic effects among its composite ingredients and to better reflect real-world consumption scenarios.

Optometrists

- Advocate for Research: Support research studies that explore additional management options for dry eye, including the role of probiotics.

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- Strengthen Patient Management: Incorporate patient education on diet and gut health into dry eye patient care.
- Pursue Professional Development: Stay informed on the latest treatments and clinical applications for dry eye management.

Optometry Faculty and Clinical Instructors

- Encourage Research Opportunities: Support students in pursuing studies related to dry eye disease.
- Promote Interdisciplinary Collaboration: Work with other health professionals to explore complementary treatment approaches.

Nutritionists and Dietitians

- Provide Patient Guidance: Offer counseling on dietary sources that support gut and ocular health.
- Collaborate on Care: Partner with eye care providers to develop nutritional strategies for ocular health.

Microbiologists

- Pursue Mechanistic Studies: Investigate probiotic strains to better understand their specific roles in ocular health.
- Advance Microbiome Research: Study the interplay between probiotics, gut health, and their systemic effects on eye health.
- Develop Probiotic Formulations: Explore the development and optimization of probiotic formulations targeted specifically at alleviating dry eye signs.

Probiotic Drink Companies

- Invest in Clinical Trials: Support further clinical trials to confirm the efficacy of probiotics in alleviating signs of dry eye.
- Launch Educational Campaigns: Inform consumers about the link between gut health and different body systems, including ocular health.
- Form Partnerships: Collaborate with eye care providers to advocate for the potential benefits of probiotics for eye health.
- Support Continuous Research: Fund research exploring additional health benefits of probiotics beyond gut health.
- Innovate Delivery Methods: Consider alternative delivery methods, such as capsules, to improve the stability and viability of probiotic strains.

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