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The protective effects of almond milk against hydrogen peroxide damage in Arpe-19 cell line and its biological effects

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Abstract

Background: Increasing evidence from basic and clinical studies indicates that oxidative stress plays a critical role in the pathogenesis of age-related macular degeneration (AMD). Therefore, natural agents with antioxidant properties may serve as promising therapeutic candidates. This study investigated the protective effects of almond milk against hydrogen peroxide (H_2O_2)-induced oxidative damage in retinal pigment epithelial (ARPE-19) cells and evaluated its biological activities.

Material & methods: Cytotoxicity of shelled and unshelled almond milk was assessed using the MTT assay. Antimicrobial activity was investigated via the disc diffusion method. Total antioxidant capacity and free radical–scavenging activities were determined spectrophotometrically. The proliferative effects of almond milk on *Lactobacillus rhamnosus* GAA6 and *Lactobacillus reuteri* ENA31 were evaluated in MRS medium.

Results: H_2O_2 reduced ARPE-19 cell viability in a concentration-dependent manner (25–500 µM), and the concentration causing approximately 50% cytotoxicity was determined as 50 µM. Almond milk significantly enhanced cell viability in ARPE-19 cells treated with H_2O_2, demonstrating a concentration-dependent protective effect. Total antioxidant capacity was measured as 3.52 mmol/L for shelled and 2.04 mmol/L for unshelled almond milk. Neither form exhibited antimicrobial activity against tested pathogens. Both almond milk types promoted the proliferation of probiotic *Lactobacillus* strains.

Conclusion: Almond milk provides notable protection against H_2O_2-induced oxidative damage in ARPE-19 cells and demonstrates prebiotic activity. Owing to its natural origin and biological properties, almond milk has potential as a therapeutic supplement and a functional protective food.

Keywords: Almond milk; ARPE-19; oxidative stress; hydrogen peroxide; cytotoxicity; antioxidant activity

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1. Introduction

Technological advancements and increasing consumer interest in plant-based nutrition have led to the widespread development of vegan milk alternatives. Plant-derived milk products can be produced from legumes (soybean), oil seeds (sunflower), nuts (almond, hazelnut, walnut, coconut), and cereals (oat, rice, sesame). (1,2) Growing concerns regarding lactose intolerance, milk allergies, and cholesterol content have boosted consumer preference for plant-based alternatives. Among these, almond milk is favored due to its low-fat content and lack of cholesterol. (3,4)

Almonds (*Prunus amygdalus*), members of the Rosaceae family, are rich in mono- and polyunsaturated fatty acids, vitamin E, magnesium, and copper, making them a valuable dietary component. (4–6) Oxidative stress occurs when reactive oxygen species (ROS) accumulate beyond the capacity of endogenous antioxidants, leading to cellular and tissue damage. (7) Milk of animal origin contains antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and vitamin constituents with antioxidative roles. (8) Antioxidant enzymes such as casein, milk proteins, peptides and amino acids, catalase, glutathione peroxidase, lactoperoxidase, superoxide dismutase, vitamin A, provitamins, carotenoids, vitamins C and E in milk of animal origin have an antioxidant effect. There are phenolic compounds, flavonoids, and vitamins A, E, and C that provide activity. (9,10)

Physiologically and pathologically, ROS contribute to various disease processes. Oxidative stress-induced injury to the retinal pigment epithelium (RPE) is well recognized. As the outermost retinal layer, the RPE is responsible for maintaining photoreceptor homeostasis, forming the blood–retina barrier, absorbing stray light, and regulating nutrient transport. (11)

Given the role of oxidative stress in retinal degeneration and the biological properties of almonds, this study aimed to evaluate the protective effects of shelled and unshelled almond milk on ARPE-19 cells exposed to H_2O_2-induced oxidative damage and to characterize its antimicrobial, antioxidant, and prebiotic properties.

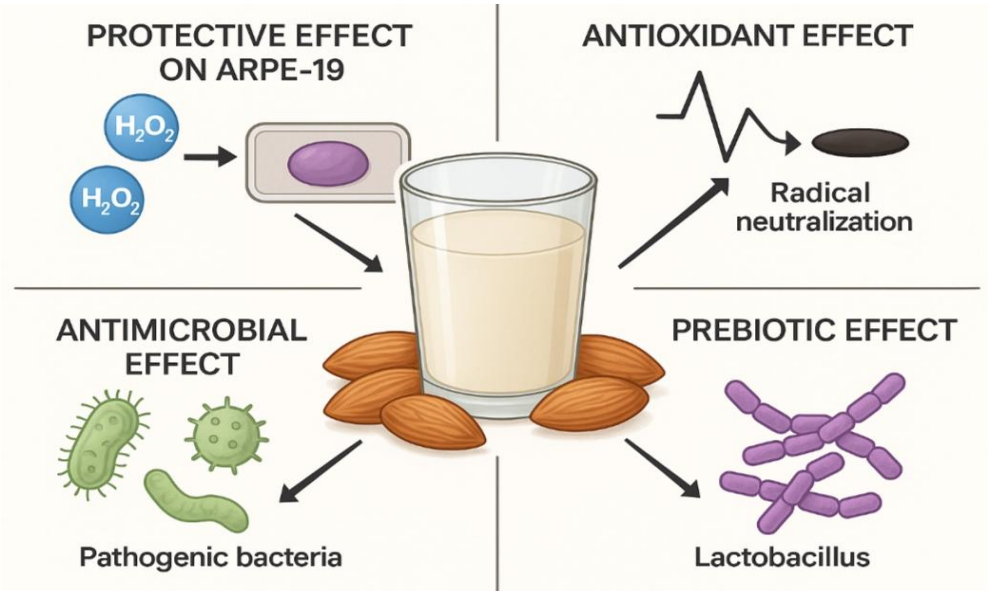

Figure 1. Protective effects of almond milk

2. Materials and methods

2.1 Cell Culture

ARPE-19 cells (ATCC CRL-2302) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and penicillin–streptomycin at 37 °C in 5% CO_2. Cells were seeded at 10,000 cells/100 µL per well in 96-well plates.

2.2 H_2O_2-Induced Oxidative Damage

Cells were exposed to H_2O_2 at concentrations of 25, 50, 100, 250, and 500 µM for 4 hours. Cell viability was quantified using the MTT assay. Untreated cells served as the control group (100% viability).

2.3 Preparation of Almond Milk

Commercial shelled and unshelled almonds (100 g) were soaked in distilled water (1:9 w/w) for 16 hours. After grinding for 10 minutes, the mixture was incubated at 100°C with stirring, filtered, cooled to 4 °C, and sterilized through a 0.22 µm filter. Almond milk preparations were used within 24 hours.

2.4 Cytotoxicity of Almond Milk

Cells were treated with almond milk (7.81–500 µM) for 18 hours, and viability was assessed by MTT assay.

2.5 Protective Effect Against Oxidative Damage

Cells were pretreated with almond milk for 18 hours, followed by 50 µM H_2O_2 for 4 hours. Viability was assessed via MTT.

2.6 Antimicrobial Activity

Antimicrobial effects were evaluated using the disc diffusion method against six pathogens: *S. aureus, E. faecalis, B. subtilis, P. aeruginosa, E. coli,* and *C. albicans.*

2.7 Antioxidant and Free Radical–Scavenging Activity

Total antioxidant status (TAS) was measured using the Rel Assay Diagnostics kit. Free radical scavenging was determined using the DPPH assay at 517 nm.

2.8 Prebiotic Activity

Lactobacillus rhamnosus GAA6 and *Lactobacillus reuteri* ENA31 were cultured in MRS medium containing 25-50-100 µl/ml almond milk. Proliferation was quantified spectrophotometrically at 600 nm after 24 hours.

2.9 Statistical analysis

IBM SPSS version 22 (IBM SPSS for Windows version 22, IBM Corporation, Armonk, New York, United States) package program and GraphPad Prism 10.1.1 were used to evaluate the data and in creating graphics. The conformity of the variables to the normal distribution was evaluated with the Shapiro-Wilk test. The One-Way ANOVA (one-way analysis of variance) method was used to examine the difference in cell viability between groups. Tukey HSD was used for multiple comparisons. Statistical significance was accepted as 0.05. Parameters were expressed as Mean±SD.

3. Results

Hydrogen peroxides (H_2O_2) exposure caused a significant, concentration-dependent decrease in ARPE-19 cell viability. The IC_{50} concentration was identified as 50 µM. A strong negative correlation between H_2O_2 concentration and cell survival was observed ($r = -0.980$, $p < 0.01$). (Figure 2 and Figure 3)

Both almond milk types demonstrated dose-dependent effects on ARPE-19 cells. Pretreatment significantly attenuated H_2O_2-induced cytotoxicity, with increased viability observed across concentrations of 7.81–250 µM (Figure 4).

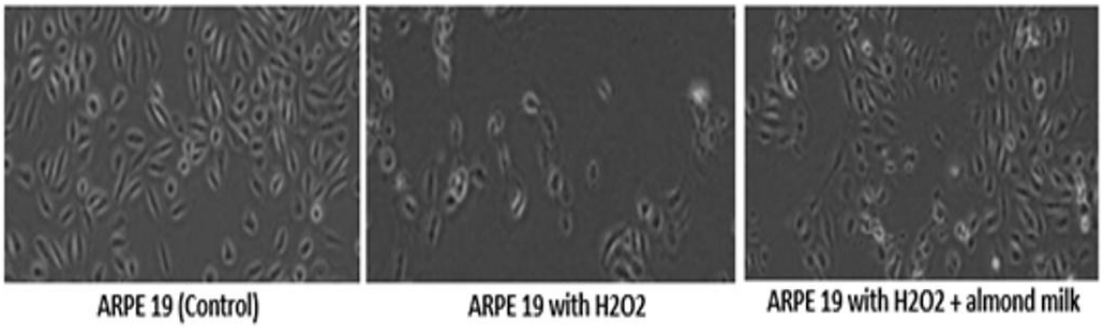

Figure 2. Photographs were taken in phase-contrast at a magnification of 100×

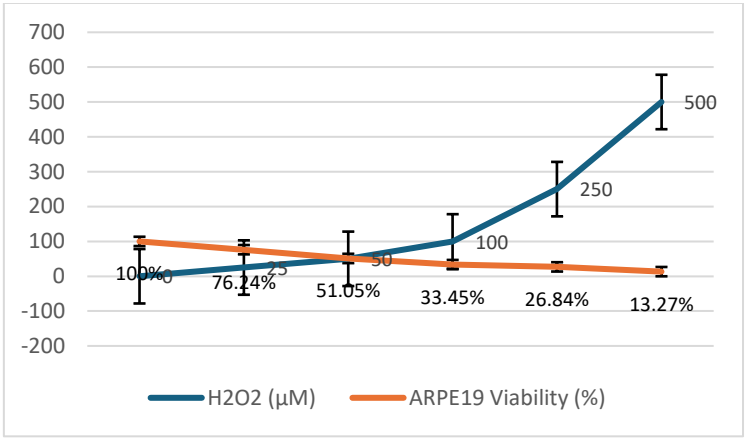

Figure 3. Damage model data generated with H_2O_2 on ARPE 19 cells.

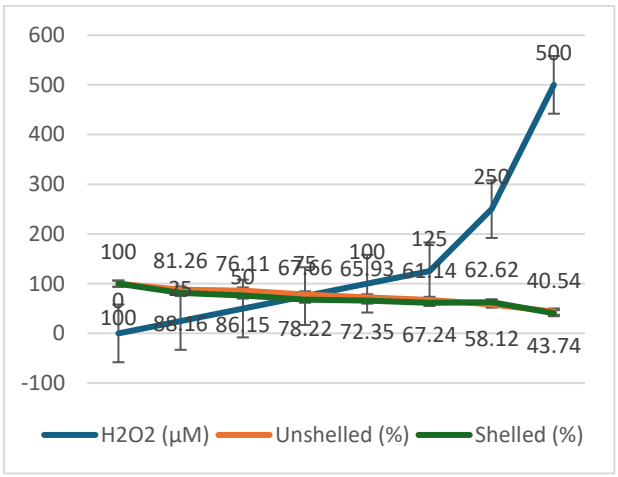

Figure 4. Effect of shelled or unshelled almond milk on ARPE-19 cell viability.

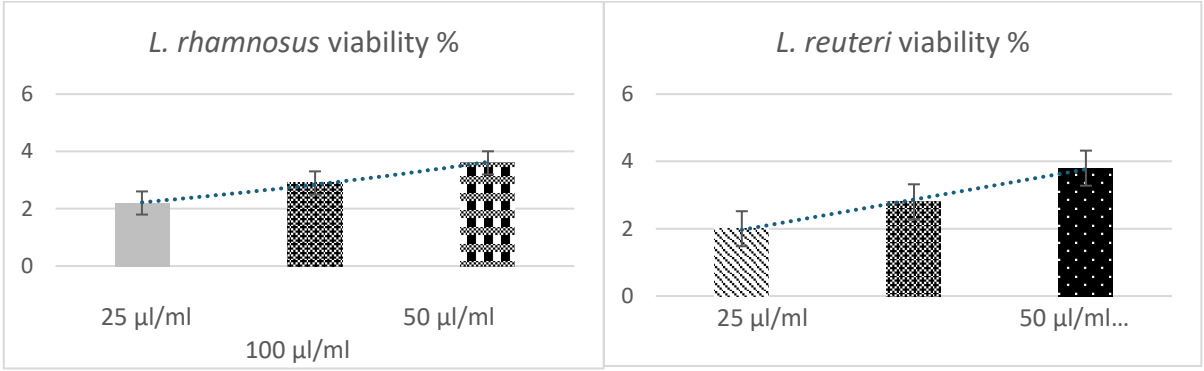

Figure 5. The proliferative effect of almond milk on *L. rhamnosus* and *L. reuteri*.

Disc diffusion testing revealed no antimicrobial activity against the selected microorganisms.

Total antioxidant capacity was 3.52 mmol/L for shelled and 2.04 mmol/L for unshelled almond milk. DPPH assay results supported these findings.

Both almond milk preparations enhanced the proliferation of *L. rhamnosus* and *L. reuteri* compared with controls, demonstrating prebiotic potential. (Figure 5)

4. Discussion

Almonds are recognized by the FDA as nutrient-dense foods due to their high vitamin, mineral, and monounsaturated fatty acid content. (4,12,13) Their antioxidant potential, attributed to α-tocopherol and phenolic compounds, supports their use in disease prevention. (14) Recently, the use of vegan milk such as soy, oat, and almond milk has increased. Among these milk, almond milk stands out due to its high nutritional value, low saturated fat content, and lactose-free. (13) In this study, almond milk exerted significant protective effects against oxidative stress in the ARPE-19 cell line, consistent with the known antioxidative characteristics of almonds. And it evaluated the antimicrobial effect, prebiotic effect, and antioxidant effects of almond milk.

Oxidative stress is a major contributor to retinal degenerative diseases, including age-related macular degeneration. (14) While some tissues can tolerate hypoxia/ischemic conditions for a long time, others can suffer severe damage from low oxygen levels. (15) Hypoxia is known to be associated with various diseases, including the growth of solid tumors, due to vascular insufficiency, blood vessel damage, and tissue edema. (16) The retina, which is one of the most metabolically active tissues, consumes oxygen rapidly due to double blood circulation. Therefore, hypoxia induced by decreased oxygen levels in these oxygen-sensitive tissues is the most common pathophysiology in various retinal diseases such as pathological neovascularization, diabetic retinopathy, retinal vein occlusion and age-related macular degeneration. (16,17) Recently, there has been increasing interest in seeking to prevent and treat many diseases with compounds derived from natural products and plants. (18) Studies on almonds have reported that this nutrient reduces the risk of cardiovascular diseases and anemia and has a protective effect against free radicals. (8,19) The health benefits of almond shells containing phenolic compounds are known. (20,21) Almond milk is used as an alternative for people with lactose intolerance, pregnant women, and celiac patients due to its high calcium, phosphorus, and potassium levels. (22) Almonds have high antioxidant activity thanks to α-tocopherol and polyphenolic components. (23) Our findings demonstrate that almond milk reduces H_2O_2-induced cytotoxicity, supporting its potential role as a natural retinal protective agent. It was determined that the application of almond milk to ARPE-19 cells alone had a concentration-dependent effect on cell viability. In ARPE-19 cells, administration of low concentrations of almond milk before H_2O_2 statistically significantly reduced the effect of H_2O_2 on cell viability ($p<0.001$). Even at the highest concentration, cell viability was found to be over 40%. On the other hand, a significant protective effect of almond milk on cell viability was observed when applied to ARPE-19 cells 4 hours before oxidative damage was caused by H_2O_2. While almond milk exhibited no antimicrobial effects, it promoted the proliferation of probiotic bacteria, indicating prebiotic functionality - an effect previously noted for plant-derived substrates.

5. Conclusion

Almond milk, prepared from both shelled and unshelled almonds, demonstrates protective effects against oxidative stress in ARPE-19 cells and exhibits notable prebiotic activity. These findings suggest that almond milk may serve as a valuable natural supplement with therapeutic potential in conditions involving oxidative damage.

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Conflict of Interest

he author declares that there is no conflict of interest regarding the publication of this article.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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