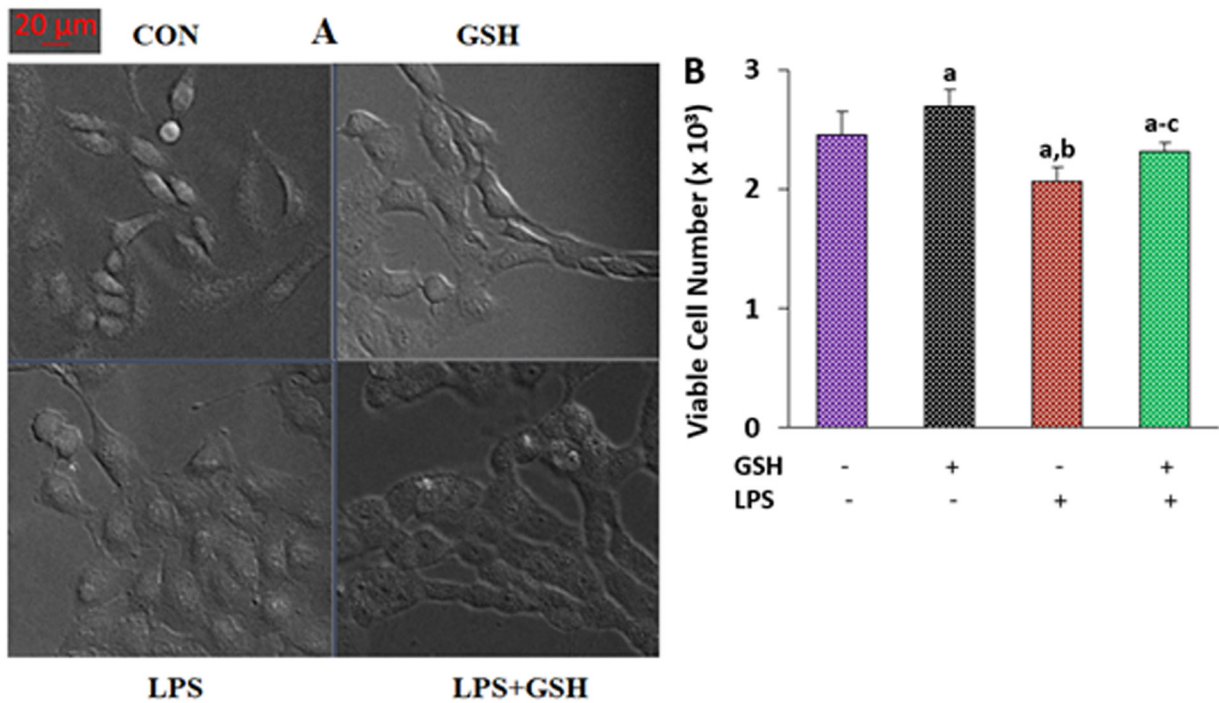


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Isparta, Türkiye.
Phone: +90 246 211 36 41
E-mail: mustafanaziroglu@sdu.edu.tr

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Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A-Ion Channels (Na⁺- K⁺ Channels, Cl⁻ channels, Ca²⁺ channels, ADP-Ribose and metabolism of NAD⁺, Patch-Clamp applications)

B-Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C-Interaction Between Oxidative Stress and Ion Channels in Neuroscience

(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD⁺ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

D-Gene and Oxidative Stress

(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

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Glutathione Protects Human Retinal Pigment Epithelial Cells from Lipopolysaccharide-Induced Apoptosis and Oxidative Stress by Inhibiting The TRPM2 Channel

 Anıl Selim Apa¹

¹Department of Ophthalmology, Isparta State City Hospital, Isparta, Türkiye

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*Address for correspondence:

Anıl Selim Apa

Department of Ophthalmology, Isparta State City Hospital, Isparta, Türkiye

Email: anilselimapa@gmail.com

List of Abbreviations;

iCa²⁺, intracellular free Ca²⁺; *ACA*, N-(p-amylicinnamoyl) anthranilic acid; *CON*, control; *DCF*, 2',7'-dichlorofluorescein; *H2DCFDA*, 2',7'-dichlorodihydrofluorescein diacetate; *GSH*, glutathione; *LPS*, lipopolysaccharides; *LSM/800*, laser scanning confocal microscope/800; *MiDis*, mitochondrial membrane dysfunction; *ROS*, reactive free radicals of oxygen; *RPE*, retinal pigment epithelium; *TRP*, transient receptor potential; *TRPM2*, transient receptor potential melastatin 2

Abstract

Gram-negative bacteria's outer membranes include lipopolysaccharides (LPS), which are a major contributor to inflammatory eye disorders. LPS cause apoptosis induction and produce reactive free radicals of oxygen (ROS). In microglia, LPS-induced ROS stimulates transient receptor melastatin 2 (TRPM2), whereas glutathione (GSH) and N-(p-amylicinnamoyl) anthranilic acid (ACA) decrease their activation. Anti-apoptotic and antioxidant properties of GSH through TRPM2 inhibition were not reported in human retinal pigment epithelial (ARPE-19) cells. Therefore, the modulator action of GSH

on the TRPM2-mediated molecular oxidant and apoptotic pathways in ARPE-19 was investigated.

Five main groups were generated in the ARPE-19: Control, GSH (10 mM for 2 h), LPS (1 µg/ml for 24 h), LPS + GSH, and LPS + ACA (25 µM for 30 min).

The amounts of ROS, mitochondrial dysfunction, apoptosis, caspases (caspase-3, -8, and -9), and cytosolic free Ca²⁺ were increased by the LPS incubation, while the incubations of GSH and ACA reduced their amounts. The viable cell number and viability percentage were decreased by LPS, but their viability and number were increased by the incubations of GSH and ACA.

In conclusion, GSH decreased the levels of LPS-caused oxidative stress and apoptosis via suppressing TRPM2 in the ARPE-19. One possible treatment agent for oxidative retinal injury and inflammatory eye diseases induced by LPS could be the GSH treatment.

Keywords: Apoptosis; ARPE-19; lipopolysaccharide; oxidative stress; TRPM2 channel.

Introduction

The light-sensitive photoreceptor outer segments and the blood supply of the retina via the choriocapillaris are separated in the outer retina by the retinal pigment epithelium (RPE) (Wildner et al., 2026). The RPE keeps

the neuronal portion of the outer retina isolated from the systemic bloodstream and supports the immunological privilege of the eye by inducing the outer blood-retina barrier (Strauss, 2005). Common inflammatory and oxidative stress eye conditions such as uveitis and age-related macular degeneration are induced by an increase in RPE inflammation (Chistyakov et al., 2024; Martins et al., 2025). Anti-inflammatory pharmaceuticals have recently treated severe eye inflammation that is unresponsive to immunomodulator medicines (Srejovic et al., 2024). The exact cause of inflammatory diseases is yet unknown. Finding an effective treatment is consequently a critical and pressing issue in the fight against inflammatory disorders. Human retinal pigment epithelial-19 (ARPE-19) cells are used in the studies of recent years for investigating the pathogenesis of several inflammatory ocular diseases (Özkaya et al., 2021; Daldal and Nazıroğlu 2022; Cao et al., 2024).

Outer membranes of Gram-negative bacteria contain significant amounts of lipopolysaccharides (LPS) (Wang et al., 2025). LPS-induced increases of inflammation and reactive free radicals of oxygen (ROS) are major contributors to the onset and progression of inflammatory ocular disorders (Ozal et al., 2018; Chistyakov et al., 2024; Shen and Xu, 2025). A full-blown inflammatory response is triggered by the buildup of ROS and oxidized lipoproteins in the retina, which encourages and intensifies persistent oxidative damage (Ana et al., 2023). In ARPE-19 cells, the accumulation of intracellular free Ca^{2+} (iCa^{2+}) in the mitochondria activates the transient receptor melastatin 2 (TRPM2) channel, resulting in reduced mitochondrial membrane depolarization and leading to apoptosis, ROS generation, and mitochondrial membrane malfunction (Meléndez García et al., 2016; Bardak et al., 2023; Ertuğrul et al., 2023). LPS-induced ROS promote TRPM2 activation and apoptosis, while N-(p-amylocinnamoyl) anthranilic acid (ACA) and glutathione (GSH) attenuate TRPM2 activation and apoptosis in microglial cells (Akpınar and Nazıroğlu, 2026). Although antioxidants like curcumin (Ertuğrul et al., 2023), selenium nanoparticles (Özkaya et al., 2021), and rituximab (Daldal and Nazıroğlu 2022) were able to control TRPM2-stimulation-mediated excessive increases of iCa^{2+} concentration in ARPE-19 cells, these features ultimately result in a decrease in mitochondrial membrane dysfunction (MiDis), which in turn induces a downregulation of ROS production and the induction of

apoptosis (Saddala et al., 2020; Özkaya et al., 2021; Daldal and Nazıroğlu 2022). Hence, the generation of mitochondrial ROS and the induction of apoptosis in ARPE-19 depend on the enhanced iCa^{2+} concentration induced by LPS (Özkaya et al., 2021; Daldal and Nazıroğlu 2022).

GSH, the most important intracellular thiol group antioxidant, by detoxifying ROS nonenzymatically, helps protect RPE cells from oxidative stress-caused apoptosis (Sun et al., 2018). Although GSH is highly concentrated in the retina and RPE (Huster et al., 1998), its depletion causes mitochondrial oxidative damage in ARPE-19 cells (Sun et al., 2018). In cultured human RPE and ARPE-19, GSH application protects against oxidative damage (Sternberg et al., 1993). In rats with cadmium-induced cardiotoxicity, a reduction in GSH triggered TRPM2 stimulation-mediated apoptosis and ROS generation (Yazğan et al., 2025). According to a recent study, GSH prevented microglia from undergoing mitochondrial ROS and apoptosis caused by LPS and TRPM2 channel stimulation (Akpınar and Nazıroğlu, 2026). GSH mediates a variety of cell-specific effects in the hippocampus of mouse, such as antioxidant, Ca^{2+} channel modulation, and anti-apoptotic activities through TRPM2 suppression (Çınar and Nazıroğlu, 2023). It is currently unclear how GSH modulates ARPE-19 cells through TRPM2 inhibition on antioxidant, Ca^{2+} channel modulation, and anti-apoptotic activities.

The purpose of this study was to look into how GSH incubation protects ARPE-19 cells from excessive Ca^{2+} influx and oxidative damage caused by TRPM2 stimulation. Additionally, I aimed to investigate the inhibition of apoptosis caused by TRPM2 stimulation after GSH administration.

Materials and Methods

Cell Lines

Human ARPE-19 cells were mostly used in the studies of retinal disease, oxidative stress, and LPS (Sun et al., 2018; Wildner et al., 2026). The native TRPM2 channel was also expressed by the cells (Meláez García et al., 2016; Özkaya et al., 2021; Daldal and Nazıroğlu, 2022). For two reasons, the ARPE-19 cells preferred (a gift from Professor Suat Erdoğan, Trakya University, Edirne, Türkiye). The mixture of Dulbecco's Modified Eagle's Medium (DMEM)/Ham's F12 medium with low glucose was used to cultivate ARPE-19 cells. Antibiotics (100

$\mu\text{g/ml}$ streptomycin + 100 units/ml penicillin) and fetal bovine (10%) were added to the medium mixture (Daldal and Nazırođlu 2022). The ARPE-19 cells were carefully kept in a humidified incubator at 37°C with 5% CO₂ under carefully regulated conditions.

Study Groups

Five main groups were established using the ARPE-19 (2 x 10⁶) cells: Control (CON), GSH (10 mM for two hours), LPS (1 $\mu\text{g/ml}$ for twenty-four hours), LPS + GSH, and LPS + TRPM2 blocker (25 μM ACA for thirty minutes). The cells from the CON were incubated in the incubator for a full day. The ARPE-19 of the second group was exposed to GSH (10 mM) for two hours (Akpınar and Nazırođlu, 2026). The cells in the third group were exposed to LPS from *Escherichia coli* O55:B5 (1 $\mu\text{g/ml}$) (Cat #L2880-100MG, Sigma-Aldrich) for a whole day (Daldal and Nazırođlu, 2022; Ertuđrul, 2024). The fourth group's cells were treated with LPS for twenty-four hours and GSH for the final two hours. ACA is a TRPM2 blocker (Kraft et al., 2006). The cells in the fifth group (LPS + TRPM2 blocker) were exposed to 25 μM ACA for thirty minutes and 1 $\mu\text{g/ml}$ LPS for twenty-four hours.

The Detection of iCa²⁺ Concentration

The 1 mM Fluo 3/AM (Cat #1242, Invitrogen by Thermo Fischer Scientific, Waltham, MA, USA) was used to stain the cells in 35-mm plates with bottom glasses for 50–60 minutes. The stained cells were washed and then imaged using a laser scan confocal microscopy (LSM/800) (Zeiss, Oberkochen, Germany) attached with a 20 x 0.8 objective. The TRPM2 channel in the cells was inhibited by the addition of ACA (25 μM), while it was activated by H₂O₂ (1 mM). The results of fluorescence intensity were presented as arbitrary units (a.u.).

The Analyses of Apoptosis and Cell Viability

To determine if LPS has an apoptotic effect on ARPE-19, staining with APOPercentage dye (Cat #A1000, Biocolor, Belfast, Northern Ireland), a particular marker for apoptosis that stains externalized phosphatidylserines, was utilized. Briefly, 96-white well plates containing ARPE-19 cells cultivated in 25 cm² flasks were stained for one hour using the APOPercentage dye. After carefully aspirating the medium, PBS was used twice to wash the cells. A Spectrophotometer Plus plate reader (Infinite 200 PRO, Tecan Group Ltd., Salzburg, Austria) was used to

measure the amount of apoptosis in the ARPE-19 cells at 550 nm (Li et al., 2010; Ertuđrul, 2024).

Cells of five groups were placed in a 96-well white plate, and the medium was changed out for new media that included the test chemical and 0.5 mg/ml MTT. The cells were then incubated for a further two hours. After aspirating the solution and lysing the cells with 200 μl DMSO, the absorbance was measured at 540 nm using a Spectrofluorometer Plus plate reader (Infinite 200 PRO) (Li et al., 2010).

The MTT and apoptosis results were displayed as a percentage of CON after the optic densities were recorded.

Assays for Caspase Activities

The substrates for caspase-3 (Ac-DEVD-AFC), -8 (Ac-IETD-AFC), and -9 (Ac-LEHD-AFC) (Bachem AG, Bubendorf, Switzerland) were utilized to detect caspase activity in ARPE-19 whole cell lysates (1 x 10⁴ per ml) on 96 black well plates (Ertuđrul, 2024). The fluorescence alterations were constantly monitored for 30 minutes using the microplate reader (Infinite 200 PRO) with an excitation (405 nm) and emission (505 nm) wavelengths. Following the measurement of the total protein concentrations, the variations in substrate fluorescence intensity were computed to produce CON findings. For caspases-3, -8, and -9 activity, the findings are shown as a percentage of CON change in fluorescence.

Viable Cell Counts

The numbers of viable cells were diluted in CASY tone solution (Roche Innovatis AG, Routlingen, Germany), and they were counted using the TT Model CASY Cell Counter. The number of viable ARPE-19 was expressed as 1 x 10³ cells.

The ROS Measurement

After being subjected to 20 mM non-fluorescence reagent 2',7'-dichlorodihydrofluorescein (DCF) diacetate (H₂DCFDA) (Cat # D399, Thermo Fisher Scientific) for 20 to 30 minutes at dark room temperature, the cells in the 96 black well plates were again rinsed with PBS (Ertuđrul, 2024). When H₂DCFDA is oxidized in the cytosol, the fluorescent DCF form is produced (Joshi and Bakowska, 2011). The plate reader (Infinite 200 PRO) with excitation (485 nm) and emission (530 nm) wavelengths was used to measure the fluorescence intensity. The ROS (DCF) statistics are displayed as a percentage of CON.

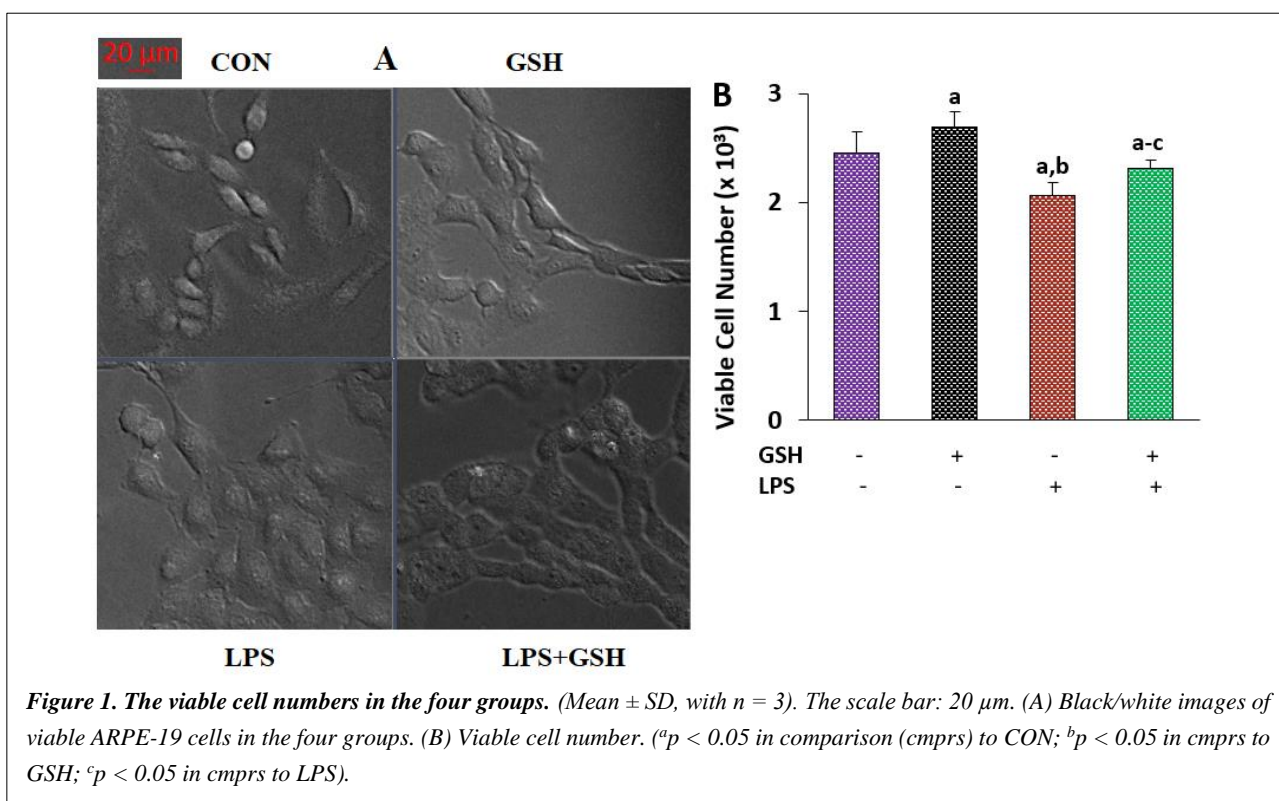
Evaluation of Mitochondrial Membrane Dysfunction (MiDis) Level

The amount of mitochondrial depolarization changes in the ARPE-19 was measured using the 2 μ M JC-1 probe (Cat # T3168, Thermo Fisher Scientific) (Joshi and Bakowska, 2011; Ertuğrul, 2024). After dye washing, the MiDis concentrations were detected using the Infinite 200 PRO. Green (monomer form) and red (JC-1 aggregates-dimer form) fluorescence emission wavelengths were determined to be 525/605 nm and 485/535 nm, respectively. The MiDis in ARPE-19 cells was computed using the green to red fluorescence ratio.

counter was used to count the number of viable cells (Fig. 1B). While the number of viable cells was reduced in the LPS as compared to the groups of CON and GSH, it was higher ($p < 0.05$) in the GSH group than in the CON. The LPS + GSH group had a higher viable cell count ($p < 0.05$).

GSH Decreased the iCa^{2+} Concentration and TRPM2 Activation Caused by LPS

Fig. 2A showed the Fluo 3/AM green fluorescence dye images of iCa^{2+} concentration indicators in four groups (CON, GSH, LPS, and LPS + GSH), while Fig. 2B showed the mean fluorescence intensity values by columns after ACA block and H_2O_2 stimulation. The LPS groups showed



Statistical Assessment

The mean \pm standard deviation (SD) was used to depict the current data. Student's t-test was used to determine whether statistical significance ($p < 0.05$) was present.

Black/White Images and Numbers of Viable ARPE-19 Cells

Fig. 1A displayed black/white images of ARPE-19 cells from each of the four groups. The Axiocam 702 camera was used to take the images. The CASY cell

greater iCa^{2+} increases ($p < 0.05$) than the CON and GSH. There were no increases in iCa^{2+} concentration in the GSH + H_2O_2 and LPS + GSH + H_2O_2 groups following the H_2O_2 stimulation, and the differences were less noticeable in the LPS + GSH and LPS + ACA groups versus to the LPS group ($p < 0.05$). The cells of the CON + H_2O_2 and LPS + H_2O_2 groups had the greatest iCa^{2+} concentrations. Consequently, I found that the GSH incubation reduced the rise in iCa^{2+} concentrations caused by LPS via blocking TRPM2 in the cells.

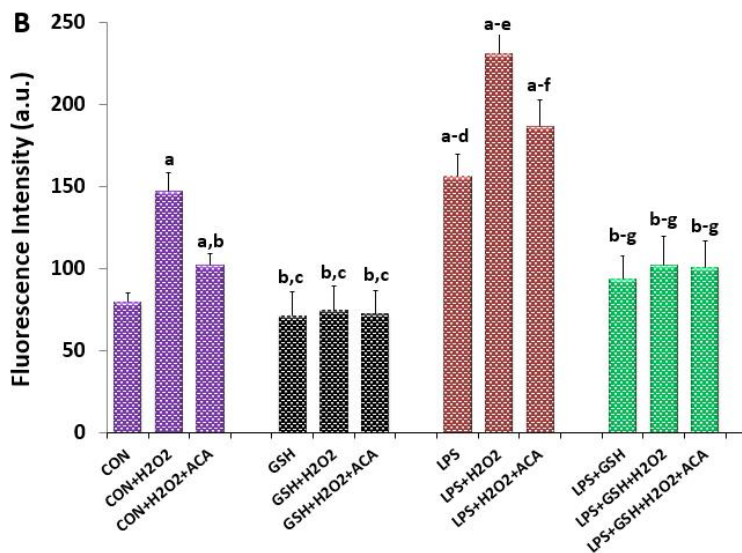
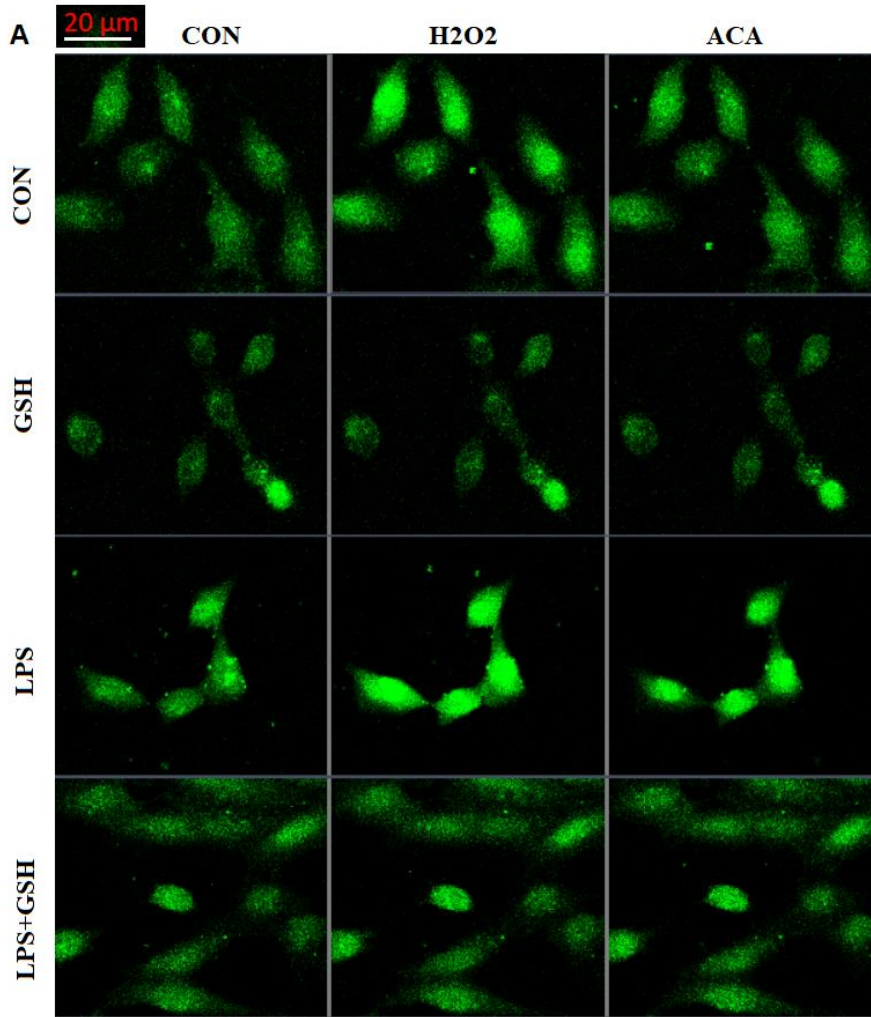


Figure 2. GSH decreased LPS- and TRPM2 stimulation-mediated Ca²⁺ fluorescence intensity in the ARPE-19. (Mean ± SD and n = 3). The cells were labeled with Fluo 3/AM (1 mM) for 50–60 minutes before receiving H₂O₂ (1 mM) to activate their TRPM2 channels. The channels were promptly blocked when 25 mM ACA was added (A). The mean fluorescence intensity changes (arbitrary unit, a.u.) of four groups (B). The scale bar: 20 μm. (^ap < 0.05 in comparison (cmprs) to CON; ^bp < 0.05 in cmprs to CON + H₂O₂; ^cp < 0.05 in cmprs to CON+H₂O₂+ACA; ^dp < 0.05 in cmprs to GSH, GSH + H₂O₂, and GSH + H₂O₂ + ACA; ^ep < 0.05 in cmprs to LPS; ^fp < 0.05 in cmprs to LPS + H₂O₂; ^gp < 0.05 in cmprs to LPS + H₂O₂ + ACA).

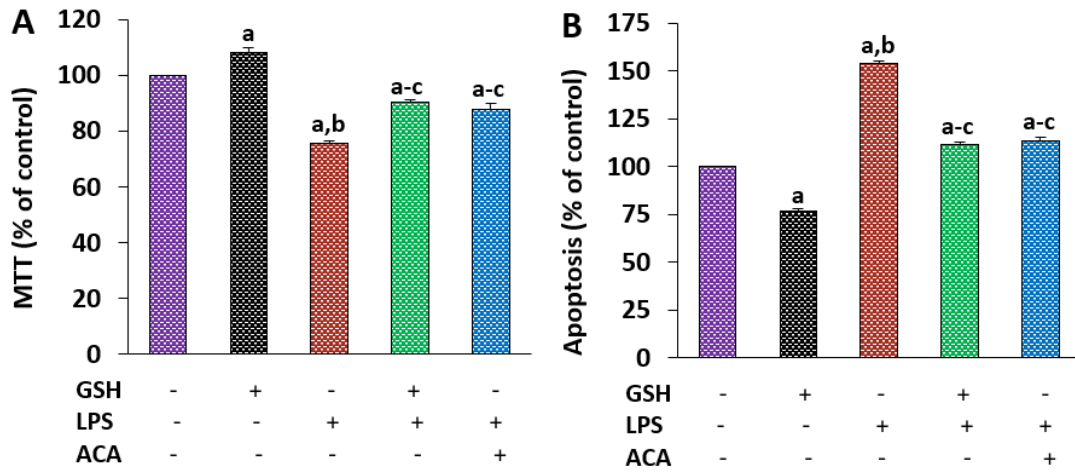


Figure 3. GSH enhanced LPS-induced decreases in cell viability by reducing LPS-induced increases in apoptosis in ARPE-19 cells. (Mean ± SD and n = 3). The MTT (A) and apoptosis (B) analyses were performed in the five groups (CON, GSH, LPS, LPS + GSH, and LPS + ACA). (^ap < 0.05 in comparison (cmprs) to CON; ^bp < 0.05 in cmprs to GSH; ^cp < 0.05 in cmprs to LPS).

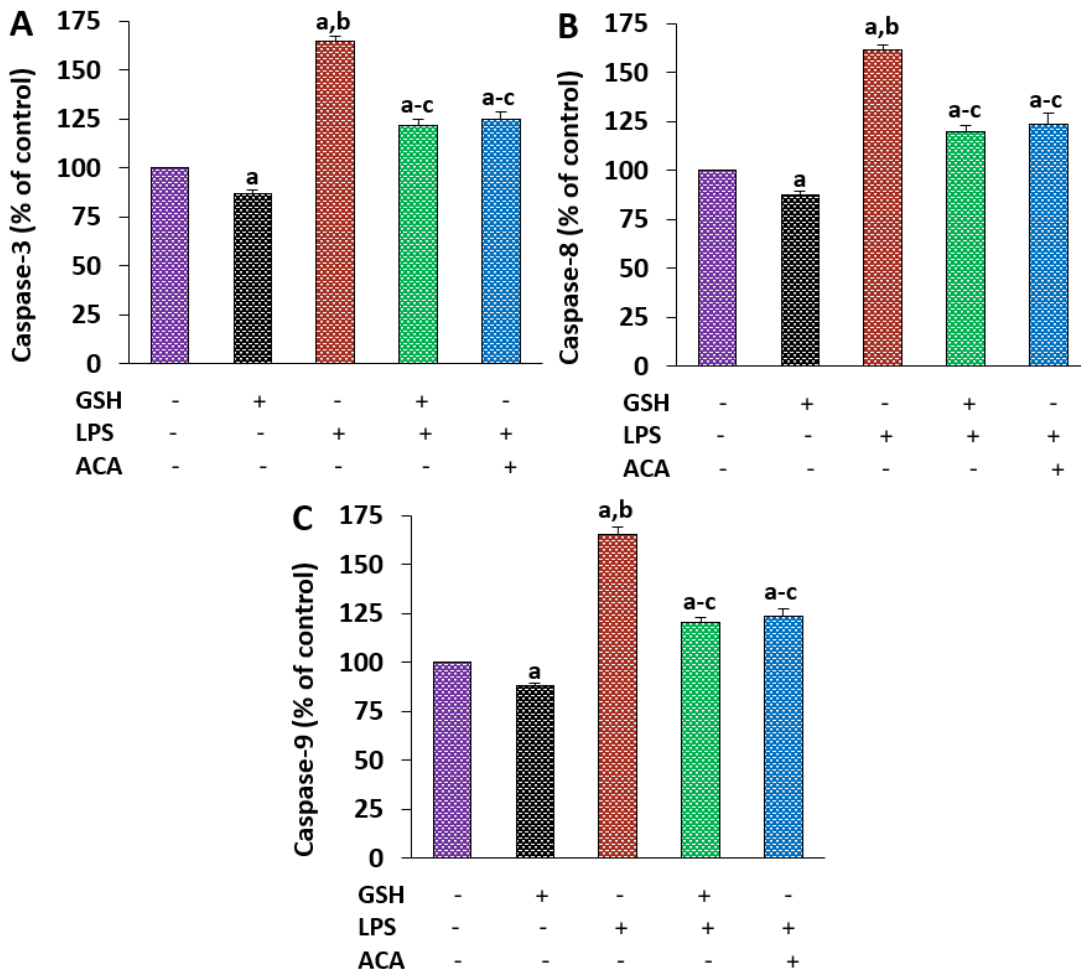


Figure 4. GSH treatments reduced the increases in caspases caused by LPS by blocking the TRPM2 channel. (Mean ± SD and n = 3). The activities of caspase-3 (A), caspase-8 (B), and caspase-9 (C) were determined in the five groups (CON, GSH, LPS, LPS + GSH, and LPS + ACA). (^ap < 0.05 in comparison (cmprs) to CON; ^bp < 0.05 in cmprs to GSH; ^cp < 0.05 in cmprs to LPS).

GSH Modulated the LPS-Induced Changes in Apoptotic and Cell Viability Indicators

The GSH group had more ARPE-19 viability (MTT) than the CON group ($p < 0.05$) (Fig. 3A). As compared to the LPS group, MTT was higher in the CON and GSH group. The MTT was higher ($p < 0.05$) in the LPS + GSH and LPS + ACA groups. The GSH group had reduced levels of apoptosis (Fig. 3B), caspase-3 (Fig. 4A), caspase-8 (Fig. 4B), and caspase-9 (Fig. 4C) activity versus the CON group ($p < 0.05$). Versus to the CON and GSH groups, the LPS groups displayed greater values of apoptosis, caspase-3, -8, and -9 activity. The GSH and ACA incubations decreased apoptosis and caspase activity in the LPS + ACA and LPS + GSH groups ($p < 0.05$).

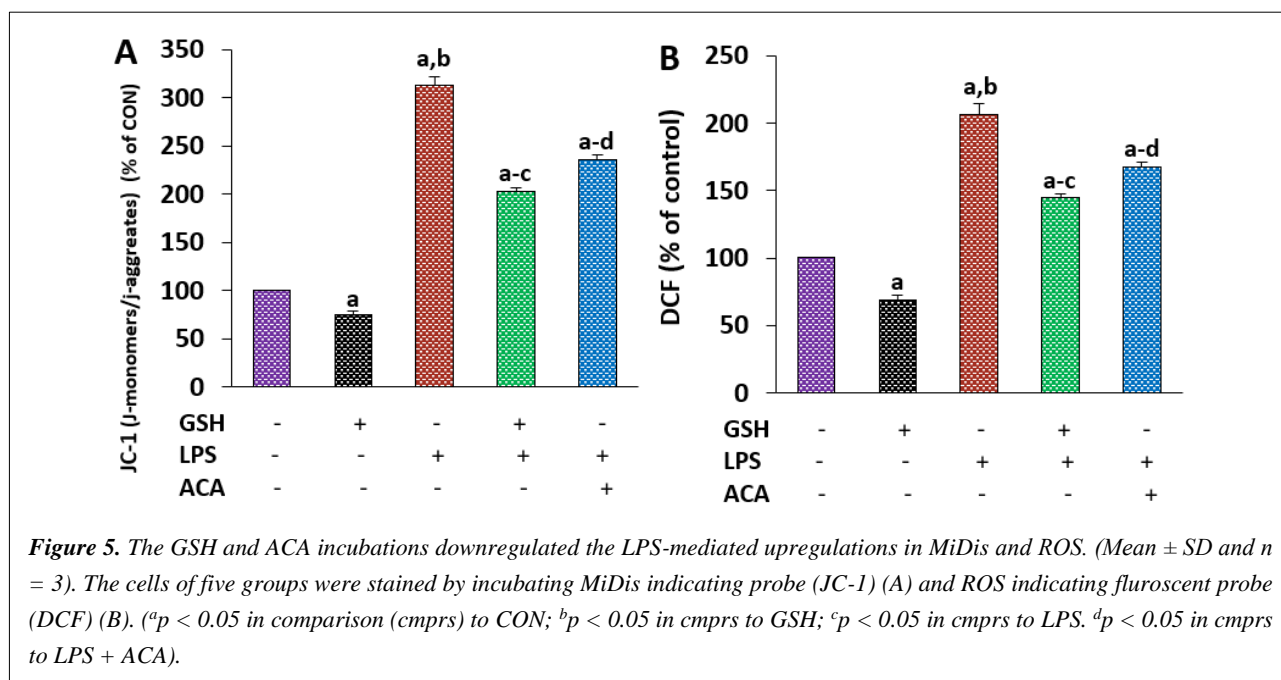
GSH Reduced LPS-Induced ROS and MiDis Increases

The findings of the investigation demonstrate that LPS incubation in the cells increased the ratios of JC-1 (green J-monomers/red J-aggregates) (Fig. 5A) and levels of ROS (Fig. 5B), suggesting an increase in mitochondrial membrane polarization and mitochondrial damage. On the other hand, cells treated with LPS + GSH and LPS + ACA

group.

Discussion

The retina is particularly vulnerable to oxidative injury since it is the most metabolically active tissue in the human body and uses more oxygen than other tissues (Böhm et al., 2023). The excessive Ca^{2+} influx-mediated redox equilibrium disturbances during retinal inflammation lead to elevated amounts of ROS, MiDis, and apoptosis. LPS-induced TRPM2 activation stimulates ROS generation in ARPE-19 cells by causing oxidative stress and apoptosis (Saddala et al., 2020; Özkaya et al., 2021; Daldal and Nazıroğlu 2022). In the present investigation, LPS-induced TRPM2 stimulation led to increases in iCa^{2+} concentration, ROS, MiDis, apoptosis, and caspase mediators. The levels of mediators were significantly decreased after pretreatment with GSH and ACA, increasing the viability percentage and number of ARPE-19 (Fig. 6). This study emphasizes the protective function of GSH in reducing oxidative, apoptotic, and TRPM2 activation mediators caused by LPS.



had reduced levels of JC-1 (MiDis) and DCF (ROS) ($p < 0.05$). There were lower ($p < 0.05$) alterations of MiDis and ROS in the LPS + GSH and LPS + ACA. However, the LPS + GSH group showed significantly ($p < 0.05$) lower MiDis and ROS changes as compared to the LPS + ACA

It has been demonstrated that blocking the generation of ROS or the activation of ROS-dependent signaling pathways is an effective therapeutic strategy to protect cells from LPS-induced increases in iCa^{2+} concentration and ARPE-19 apoptosis (Daldal and Nazıroğlu 2022). When

GSH or TRPM2 antagonist (ACA) and LPS were co-incubated, ARPE-19 cell viability was increased and LPS-induced increases of iCa^{2+} concentration were reduced. GSH is a crucial cytosolic thiol antioxidant that promotes immunity and protects against conditions affecting the retina (Sun et al., 2018). I, therefore, postulated that GSH incubations could reduce iCa^{2+} levels and oxidative stress in LPS-treated cells. In this study, GSH is sufficient to significantly lower H_2O_2 -mediated TRPM2 activity. Therefore, reducing iCa^{2+} levels by TRPM2 inhibition may be the primary antioxidant approach against LPS-induced retinal damage. According to the findings, GSH reduced LPS-induced elevations in iCa^{2+} concentration in microglia cells by inhibiting TRPM2 (Akpınar and Nazıroğlu, 2026).

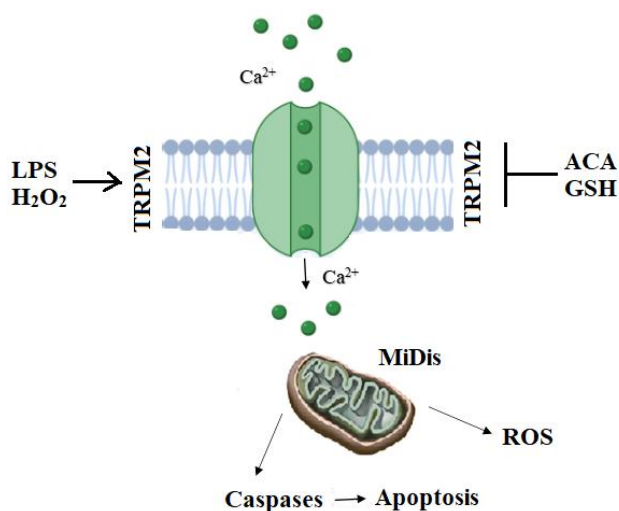


Figure 6. GSH protects ARPE-19 cells from oxidative stress and apoptosis caused by LPS. The LPS and the TRPM2 agonist (H_2O_2) cause TRPM2 activation, which raises reactive free radicals of oxygen (ROS) and mitochondrial membrane dysfunction (MiDis) by accumulating free Ca^{2+} in mitochondria. By activating caspases (caspases-3, -8, and -9), these modifications encourage apoptosis. GSH and the TRPM2 antagonist (ACA) prevent oxidative and apoptotic actions of LPS in the ARPE-19 cells.

As the main source of ROS in mammalian cells, mitochondria are essential to oxidative stress. According to previous studies, the mitochondrial apoptotic (caspase) pathway, which is characterized by enhanced ROS production and MiDis activation, is connected to excessive iCa^{2+} -induced ROS in ARPE-19 cells (Zhu et al., 2018; Panvini et al., 2022). This process includes the accumulation of LPS- and TRPM2-stimulation-mediated Ca^{2+} from the cytosol to mitochondria, thereby activating

caspase-3, caspase-8, and caspase-9 (Ertuğrul et al., 2023; Akpınar and Nazıroğlu, 2026). The caspases, MiDis, ROS, and apoptosis results of the current study demonstrated that exposure to LPS induced the production of MiDis and ROS. Additionally, LPS and TRPM2 stimulation increased the activity of apoptotic markers (caspase-3, caspase-8, and caspase-9). It's interesting to note that GSH and the TRPM2 channel blocker (ACA) successfully increased MTT and viable cell number while suppressing LPS-induced ROS production and MiDis generation in mitochondria. Additionally, ACA and GSH treatments restored the altered caspases and apoptosis after being exposed to LPS, suggesting that they may prevent ARPE-19 cells from maintaining mitochondrial function and reducing apoptosis by lowering ROS, which may be connected to TRPM2 signaling pathways. These results imply that GSH has potential as a therapeutic agent to treat oxidative retinal damage by increasing TRPM2 channel function. Similar results of TRPM2 inhibition on LPS-mediated TRPM2 activation in different cells have been reported. Accordingly, TRPM2 silencing extended the longevity of type 2 cardiorenal syndrome cell lines by lowering oxidative stress, Ca^{2+} influx, and apoptosis (Li and Wang, 2025). TRPM2 deletion (TRPM2-KO mice) reduced LPS-caused mice hippocampus damage by reducing the apoptosis-related proteins (Zhu et al., 2019). GSH incubations significantly decreased LPS-induced microglia cell death, ROS formation, MiDis, and apoptosis induction in microglia cells (Akpınar and Nazıroğlu, 2026).

In conclusion, LPS increases TRPM2 activity, produces excessive ROS and mitochondrial dysfunction, and induces apoptosis in cultivated ARPE-19 cells, resulting in severe oxidative damage. Notably, ROS, apoptosis, caspase, MiDis, and TRPM2-mediated excessive Ca^{2+} influx levels could all be reduced by GSH treatment, which also increased cell viability and viable cell number by lowering TRPM2 activity. The mechanism of LPS-induced oxidative toxicity on ARPE-19 cells is clarified by this work, which may also highlight the protective role of GSH in inflammatory diseases related to RPE.

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(Figure 6), a program (www.biorender.com) was used.

Author's contribution

ASA organized, arranged, and designed the study. Analyses of the present study were conducted in BSN Health, Analyses, Innov., Consult., Org., Agricul., Trade Ltd., Isparta, Türkiye.

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Availability of data

ASA will supply the row data upon request.

Declarations

Ethical approve No human and animal samples.

Conflict of interest None.

Competing interest None.

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