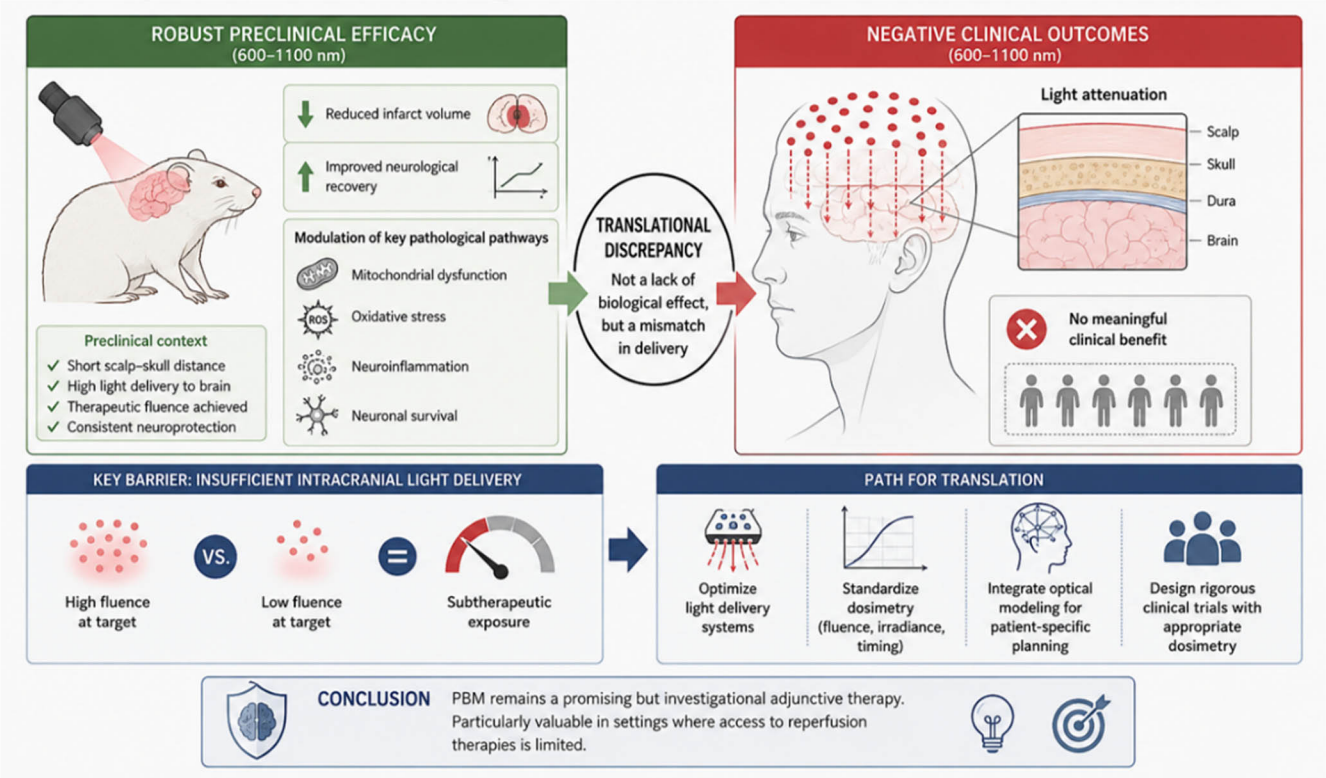
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[CONTENTS]

1315 Translational Failure of Photobiomodulation in Ischemic Stroke: A Systematic Review of Oxidative Stress and Dosimetric Limitations
Anang Maulana Yusuf, Prananda Surya Airlangga, Hamzah Hamzah

1328 The Venom of Tarantula Cubensis (Theranekron) Reduces Apoptosis and Mitochondrial Oxidative Toxicity Caused by $NaIO_3$–Induced Age-Related Macular Degeneration in Human Retinal Pigment Epithelium Cells
Mehmet Argun, Ömer Çelik

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Areas of particular interest are four topics. They are;

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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
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RESEARCH ARTICLE

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The Venom of Tarantula Cubensis (Theranekron) Reduces Apoptosis and Mitochondrial Oxidative Toxicity Caused by $NaIO_3$–Induced Age-Related Macular Degeneration in Human Retinal Pigment Epithelium Cells

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List of Abbreviations;

ACA, *N-(p-amylcinnamoyl) anthranilic acid;* ***AMD****, age-related macular degeneration;* ***ARPE-19****, human retinal pigment epithelial cell-19;* ***CASP3****, caspase-3;* ***CASP8****, caspase-8;* ***CASP9****, caspase-9;* ***Cntr****, control;* ***DCF****, dichlorofluorescein;* ***H_2DCFDA****, 2',7'-dichlorodihydrofluorescein diacetate;* ***MiDi****s, mitochondrial membrane dysfunction;* ***$NaIO_3$****, sodium iodate;* ***OFR****, oxygen free radicals;* ***RPE****, retinal pigment epithelium;* ***TNE****, theranekron;* ***TRP****, transient receptor potential;* ***TRPM2****, transient receptor potential melastatin 2*

Abstract

Excessive generations of apoptosis and oxygen free radicals (OFRs) are two main common reasons of age-related macular degeneration (AMD). A homeopathic preparation of the Tarantula Cubensis spider venom is Theranekron (TNE). The antiapoptotic and antioxidant actions of TNE were indicated in different disease models except AMD. We investigated the protective actions of the TNE and TRPM2 antagonist (N-(p-amylcinnamoyl) anthranilic acid, ACA) on AMD (sodium iodate [$NaIO_3$])-mediated apoptosis and oxidative toxicity in ARPE-19 cells.

The ARPE-19 cells were incubated for 24 h in five primary groups: control, 108 ug/mL TNE, 10 mM $NaIO_3$, $NaIO_3$ + TNE, and $NaIO_3$ + 25 uM ACA (last one hour of $NaIO_3$ incubation).

The $NaIO_3$ treatment increased markers of apoptosis (caspase-3, caspase-8, and caspase-9) and oxidative stress (OFRs and mitochondrial membrane dysfunction), while diminishing cell viability. The treatments of TNE and ACA decreased the $NaIO_3$-induced increases of apoptotic and oxidant markers, although the treatments increased cell viability.

In conclusion, TNE and ACA treatments reduced $NaIO_3$-caused oxidative degeneration and apoptosis in the ARPE-19 cells. TNE through TRPM2 inhibition is a possible treatment for AMD-induced apoptosis and OFR production.

Keywords: Age-related macular degeneration; Apoptotic oxidative degeneration; Tarantula cubensis spider venom; TRPM2 channel antagonist.

Introduction

Aging is characterized by progressive cellular breakdown, including apoptosis, excessive generation of

OFRs, and mitochondrial dysfunction. The apoptosis and mitochondrial stability are recognized markers of cellular aging that result in cellular senescence and increased susceptibility to the retinal pigment epithelium (RPE) of AMD (Neupane et al., 2026). Age-related macular degeneration (AMD) is a major eye disease problem in the senior population, especially for those over 60. It is a major reason of irreversible eyesight loss worldwide (Fleckenstein et al. 2021). Numerous medicines that inhibit the function of vascular endothelial growth and oxidative degenerative factors have been used to treat AMD. However, these therapeutic interventions cannot restore eyesight that has already been irreversibly lost because oxygen free radicals (OFRs)-induced damage gets worse with age (De Silva and Xu, 2026). Therefore, research is essential to developing new anti-angiogenic and antioxidant treatments.

Photoreceptor survival and function depend on the RPE, which is the outermost layer of the retina. Early, intermediate, and severe stages of AMD can result from risk factors like oxidative stress, aging, lipofuscin buildup, and drusen (Neupane et al., 2026). These stress conditions raise the formation of OFRs, which can cause mitochondrial membrane dysfunction (MiDis) and encourage the death of RPE cells during retinal degeneration (Heo et al., 2026). Changes in the distribution of cytosolic free Ca^{2+} concentrations accurately control the oxidative stress signaling pathways of RPE activities (Li et al., 2015; Yang et al., 2024). In AMD-induced human RPE cell-19 (ARPE-19) cells, oxidative stress activates the cation channel of transient receptor potential (TRP) melatonin 2 (TRPM2), but antioxidants and other chemicals, such as N-(p-amylcinnamoyl) anthranilic acid (ACA), inhibit it (Argun and Nazıroğlu, 2026). Excessive TRPM2-stimulation driven Ca^{2+} influx and OFRs production by TRPM2 activation are intimately associated with cell death and oxidative degeneration caused by sodium iodate ($NaIO_3$) in the ARPE-19 (Argun and Nazıroğlu, 2026). Retinal injury-induced increases in Ca^{2+} build up in mitochondria in ARPE-19 cells, causing OFR productions and apoptosis through TRPM2 stimulation (Daldal and Nazıroğlu, 2022; Çiğ et al., 2026). Furthermore, the ACA incubation reduced MiDis, apoptosis, and apoptotic markers [caspase-3 (CASP3), caspase-8 (CASP8), and caspase-9 (CASP9)] produced by AMD ($NaIO_3$) in ARPE-19 cells (Argun and Nazıroğlu, 2026). The involvement of TRPM2 in the production of $NaIO_3$-mediated OFRs and apoptosis in ARPE-19 cells was not fully investigated.

Tarantula cubensis venom is used to make the homeopathic drug Theranekron (TNE), based on alcohol. Several animal models have demonstrated the antioxidant and antiapoptotic properties of TNE. TNE therapy reduced the amount of CASP3 expression and apoptosis caused by colitis (acetic acid)-induced rats (Savran et al., 2022). Similar protective effects were seen in the brains of rats given lipopolysaccharide, and TNE reduced the levels of total oxidant status through upregulation of total antioxidant status as well as the decreases of caspase-1 and CASP3 expressions (Oguzoglu et al., 2026). Additionally, TNE protected alkaline-induced corneal injury in rats (Karakuyu et al., 2025). Therefore, antioxidant and antiapoptotic TNE therapy of ARPE-19 cells may modify AMD ($NaIO_3$)-induced apoptosis and OFRs by suppressing the TRPM2 channel.

It is still unknown how TNE therapy and TRPM2 suppression protect AMD-induced ARPE-19 cells from oxidative degeneration and apoptosis. We investigated how TNE protects against oxidative degeneration and apoptosis through TRPM2 inhibition. According to our findings, TNE therapy increases cell viability in ARPE-19 cells by blocking TRPM2, which lowers the levels of apoptosis, caspases, and OFRs. In ARPE-19 cell culture and rodent models, $NaIO_3$ injection or incubation successfully causes retinal degeneration, which is typified by localized RPE loss (De Silva and Xu, 2026). Many of the oxidant and apoptotic features linked to dry AMD are well replicated by $NaIO_3$ (Yang et al., 2024; Heo et al., 2026). Thus, in the current study, AMD was induced by using $NaIO_3$.

Materials and Methods

Cell Culture

We used ARPE-19 cell lines (ATCC) in this investigation. Some tests of the cells such as authentication and mycoplasma testing were performed by the ATCC. The cells were grown in an incubator with a 5% CO_2/95% air ratio and 60-80% humidity using a low-glucose (1 g/L) cell culture medium equal mixture of Ham's F-12/Dulbecco's Modified Eagle's Media (DMEM) with L-glutamine, 1% antibiotics, and 10% fetal bovine serum (Diagvonum, Ebsdorfergrund, Germany).

The Determination of Non-Toxic Dose of TNE

In non-tumoral embryonic kidney 293 and Caco-2 human colorectal adenocarcinoma cells, toxic doses of TNE were recently tested using varying incubation times (24 h and 48 h) and doses (1–1000 µL/mL) (Kuccukturk et al., 2026). However, the dose and incubation time have not been tested in ARPE-19 cells. After incubating the cells with TNE at concentrations of 27, 54, 108, and 162 ug/mL for 24 hours, we found that 108 ug/mL was the non-toxic dose (Figure 1). Therefore, the current study used the incubation duration and dose.

Experimental Groups

Five primary groups were induced in the ARPE-19: control (Cntr), TNE (108 ug/ mL for 24 h) (Richter Pharma AG, Weis, Austria), $NaIO_3$ (10 mM for 24 h), $NaIO_3$ + TNE, and $NaIO_3$ + 25 uM ACA (last one hour of $NaIO_3$ incubation) (Argun and Nazıroğlu, 2026). The 25 cm^2 flasks were used for culturing the cells,

Cell Viability Percentage

The assays of cell viability were carried out keeping 100 microliters of MTT for 3–4 hours at 37 °C. The dimethyl sulfoxide (500 mL) used for solving the formazan crystals. An Infinite M200 Pro model automatic microplate reader of Tecan Inc. (Männedorf, Switzerland) was used to measure the absorbance at wavelengths of 490 and 650 nm. The results (n = 3) of MTT were presented as a percentage of Cntr.

Assessment of The Apoptosis and Caspases

The manufacturer's instructions were adhered to for the apoptosis analyses (Biocolor Ltd., Carrickfergus, Ireland). Absorbance shift at 550 nm, an indication of cytosolic dye release, was measured in the Infinite PRO 200 (Daldal and Nazıroğlu, 2022).

The activity analyses of CASP3, CASP8, and CASP9 were detailly given in a recent study (Argun and Nazıroğlu, 2026). A typical reaction buffer was used to dissolve the peptide substrates of CASP3 (Ac-DEVD-AMC), CASP8 (Ac-LETD-AFC), and CASP9 (Ac-LEHD-AMC) (Bachem, Bubendorf, Switzerland). The substrate cleavage fluorescence change was assayed in the black 96-well microplate of the Infinite PRO 200. The excitation (380 nm) and emission (460 nm) wavelengths were used for the analysis.

The results of caspases and apoptosis were displayed as percentage changes from Cntr after the fluorescence units and total protein amount were established.

Cytosolic OFR Measurements

The cytosolic OFR productions were carried out using the fluorescent (2',7'-dichlorodihydrofluorescein, DCF) of non-fluorescent DCF diacetate (H_2DCFDA) probe (Vaglienti et al., 2022). The culture medium of the black 96-well microplate was then supplemented with 10 µM H_2DCFDA (Cat #: C6827, Thermo Fisher Scientific, Istanbul, Turkiye), and the reaction was allowed to continue for 25-30 minutes at 37 °C. After that, one PBS wash was performed on the cells. At emission wavelengths (488 nm) and excitation wavelengths (543 nm), the fluorescence variations caused by DCFs were measured. A value was established for the disparity. The DCF (n = 3) plate reader data were presented as a percentage change relative to the Cntr.

Measurement of MiDis

The ARPE-19 cells were incubated with 2 µg/mL JC-1 dye (Cat # T3168, Thermo Fisher Scientific) for 20–25 minutes in the dark at 37 °C for analyzing the MiDis. JC-1 has a red fluorescence when it accumulates in mitochondria in healthy organelles, but it turns green when depolarized. Green monomers and red J aggregates were measured twice at excitation (535/595 nm) and emission (485/535 nm) in the Infinite Pro 200. Green/red JC-1 fluorescence relative intensities were measured, and the results (n = 3) were displayed as a percentage of Cntr.

Statistical Analysis

The data is presented using a mean ± standard deviation (STD). To identify individual comparisons with $p < 0.05$, Tukey's post hoc tests (SPSS, 25.0) were employed after performing one-way ANOVA.

Results

The Non-Toxic TNE Dose Determination

The incubation dose of TNE in ARPE-19 cells over a 24-hour period was investigated in order to identify the TNE therapeutic dose. After evaluating TNE at doses of 0, 27, 54, 108, and 162 ug/mL for a whole day, we discovered that 108 ug/mL was a nontoxic amount for the cells (Figure 1). Thus, this dose was used in the research.

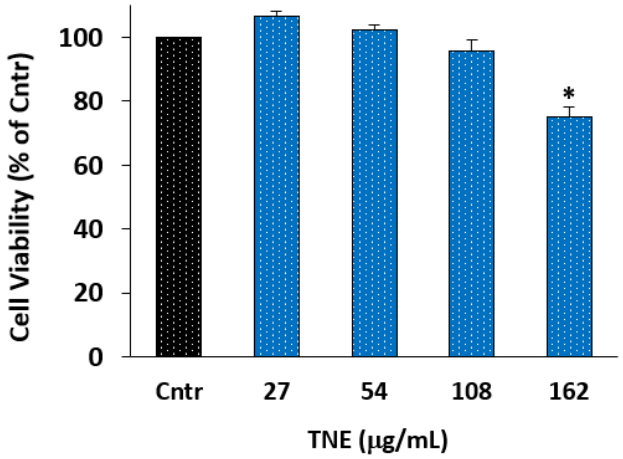


Figure 1. The impact of varying TNE dosages on ARPE-19 vitality. *(N = 3 and mean ± STD). The impacts of TNE at amounts of 0, 27, 54, 108, and 162 ug/mL for 24 h on the ARPE-19 cell viability were investigated, and a TNE amount of 108 ug/mL was found as a nontoxic dose. (*p < 0.05 vs. Cntr).*

AMD ($NaIO_3$) Exposure Increased Apoptosis Through the Diminish of Cell Viability (MTT) in the ARPE-19: The Modulator Role of TNE

The $NaIO_3$ + TNE group had higher cell viability percentages, while the group of $NaIO_3$ had low MTT values compared to the groups of Cntr, TNE, and $NaIO_3$ + ACA ($p < 0.05$), (Fig. 2A). Compared to the $NaIO_3$ + ACA group, the $NaIO_3$ + TNE group had greater MTT levels ($p < 0.05$).

While the $NaIO_3$ + TNE and $NaIO_3$ + ACA groups had lower percentages of apoptosis than the $NaIO_3$ alone, the $NaIO_3$ group exhibited higher levels of apoptosis as compared to the groups of Cntr and TNE ($p < 0.05$). Compared to the $NaIO_3$ + ACA, the $NaIO_3$ + TNE had reduced levels of apoptosis ($p < 0.05$).

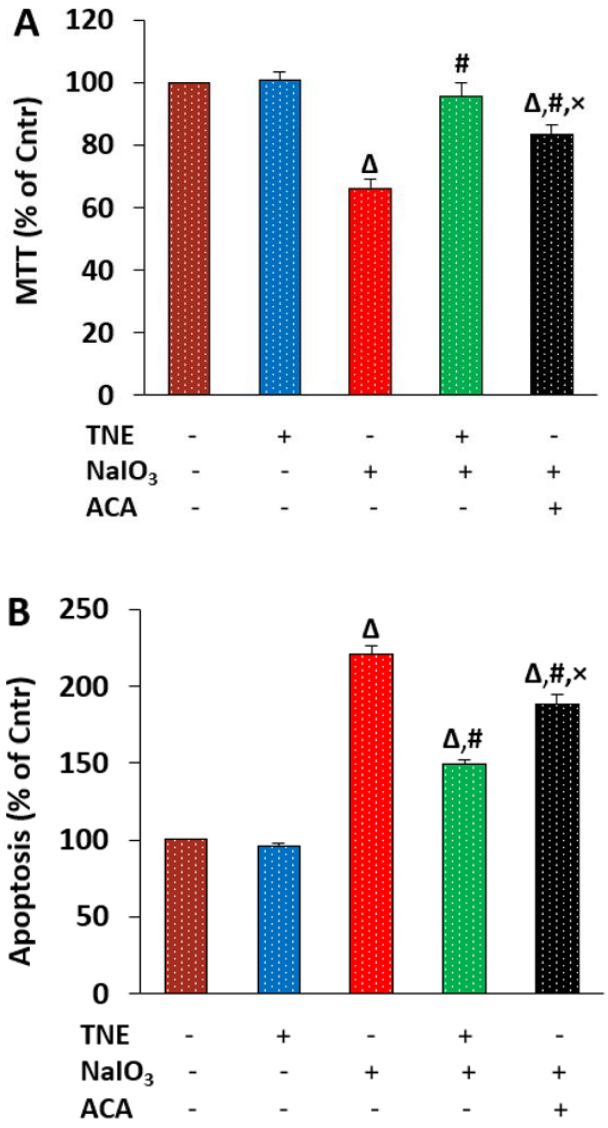


Figure 2. The $NaIO_3$-induced changes of apoptosis and cell viability were modulated by the incubation of TNE. *(N = 3 and mean ± STD).* ***A****. MTT.* ***B.*** *Apoptosis. (Δp < 0.05 vs. Cntr and TNE. #p <0.05 vs. $NaIO_3$. ×p <0.05 vs. $NaIO_3$ + TNE).*

TNE Decreased AMD ($NaIO_3$)-Induced Increases of CASP3, CASP8, and CASP9 in The ARPE-19 Cells

Using the plate reader, we investigated the actions of CASP3 (Fig. 3A), CASP8 (Fig. 3B), and CASP9 (Fig. 3C) in the cells based on changes in viable cell number and apoptosis. When compared to Cntr and TNE, the percentages of CASP3, CASP8, and CASP9 were raised by the incubation of $NaIO_3$ ($p < 0.05$). Following TNE and ACA therapy, the $NaIO_3$ + ACA and $NaIO_3$ + TNE groups had low levels of CASP3, CASP8, and CASP9. The caspase activities were lower ($p < 0.05$) in the $NaIO_3$ + TNE compared to the $NaIO_3$ + ACA.

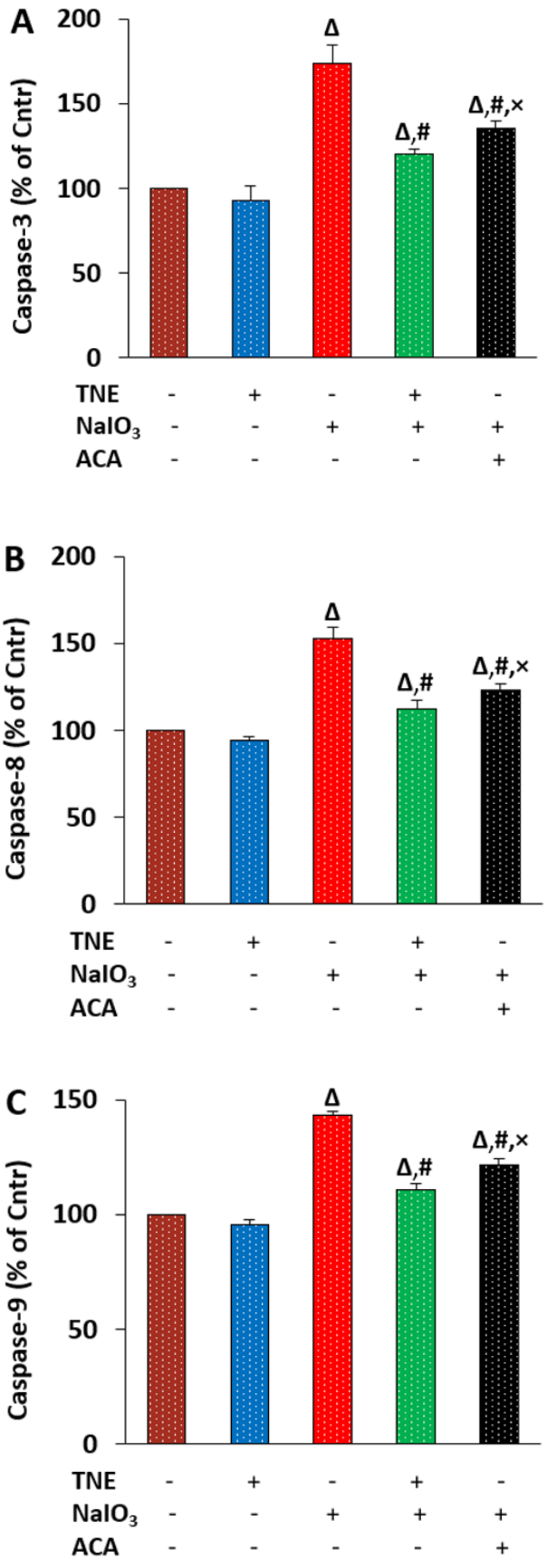


Figure 3. The $NaIO_3$-induced increases of caspases were diminished by the incubation of TNE. *(N = 3 and mean ± STD). **A**. Caspase-3. **B**. Caspase-8. **C**. Caspase-9. ([Δ]p < 0.05 vs. Cntr and TNE. [#]p <0.05 vs. $NaIO_3$. [×]p <0.05 vs. $NaIO_3$ + TNE).*

$NaIO_3$-Mediated Productions of Cytosolic OFRs and MiDis were Diminished by The Incubation of TNE

The $NaIO_3$ had higher levels of DCF (cytosolic OFRs) (Fig. 4A) and JC-1 (MiDis) (JC-1) than the groups of Cntr and TNE ($p < 0.05$). Following TNE and ACA treatments, these levels were low in the $NaIO_3$ + TNE and $NaIO_3$ + ACA ($p < 0.05$). DCF and JC-1 levels were lower ($p < 0.05$) in the $NaIO_3$ + TNE compared to the $NaIO_3$ + ACA.

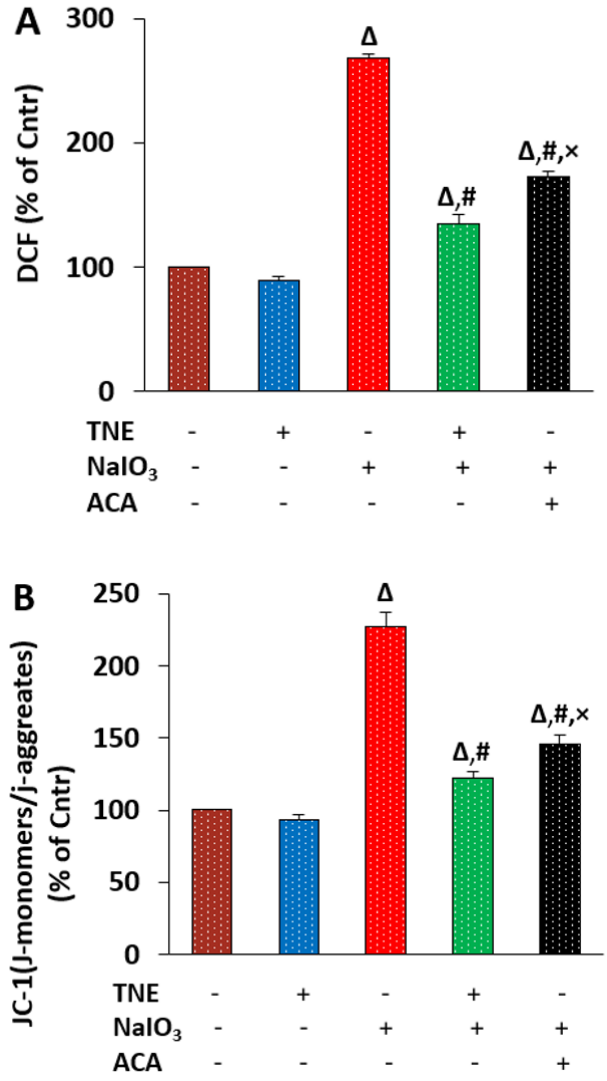


Figure 4. The $NaIO_3$-induced increases of cytosolic OFR and MiDis were diminished by the incubation of TNE. *(N = 3 and mean ± STD). **A**. DCF (OFR). **B**. JC-1 (MiDis). ([Δ]p < 0.05 vs. Cntr and TNE. [#]p <0.05 vs. $NaIO_3$. [×]p <0.05 vs. $NaIO_3$ + TNE).*

Discussion

In retinal disease, aberrant neovascularization and retinal AMD are closely associated processes, and they are

the primary causes of visual loss in neovascular AMD (Fleckenstein and Keenan, 2021). Ca^{2+} channels, such as TRPM2 stimulation overload-induced OFRs and apoptosis, are potential pathways in the etiology of AMD (Li et al., 2015; Argun and Nazıroğlu, 2026). An increase in Ca^{2+} causes the inner mitochondrial membrane to depolarize, which causes retinal cell death (Neupane et al., 2026). In order to cure retinal damage, novel approaches that aid in preventing Ca^{2+} excess during AMD are desperately needed. In mice, TNE therapy protects via lowering caspases and overall oxidant status (Savran et al., 2022; Oguzoglu et al., 2026). The effects of TNE and TRPM2 inhibition on $NaIO_3$ (AMD)-induced OFRs and apoptosis by employing ARPE-19 are not known. First, we evaluated apoptosis, caspases, MiDis, and OFRs to determine how TRPM2 contributed to $NaIO_3$-induced degeneration in ARPE-19. Second, we investigated the possible modulator action of TNE on apoptosis, caspases, MiDis, and OFRs in ARPE-19 cells using TRPM2 inhibition.

Our findings show that $NaIO_3$ reduced cell viability while increasing oxidants (OFRs and MiDis), apoptosis, and caspase activity (CASP3, CASP8, and CASP9). TNE incubation reduced these alterations. The use of the TRPM2 blocker (ACA) provided more evidence that TRPM2 was involved in the alterations. In ARPE-19 cells, TNE treatments also influenced these alterations.

One of the essential pathogenic reasons of AMD is oxidative degeneration. In RPE cells, aging causes elevated levels of OFR generations (Neupane et al., 2026). The retina experiences severe RPE cell degeneration due to aging-related reductions in antioxidant potential and the ability to scavenge OFRs (Fleckenstein et al., 2021). Because $NaIO_3$ causes ARPE-19 cells to produce excessive amounts of OFRs, prior research has demonstrated that it can be utilized to investigate the pathogenesis of AMD (Yang et al., 2024; Heo et al., 2026). In order to generate cytosolic OFRs, which are linked to the aetiology of AMD, we cultured cells using $NaIO_3$. In line with earlier research (Savran et al., 2022; Oguzoglu et al., 2026), we found that the $NaIO_3$ group had higher levels of cytosolic OFRs than the Cntr and TNE groups, while the $NaIO_3$ + TNE group had lower levels than the $NaIO_3$ group.

The transition pore of mitochondrial permeability also plays a action in the physiological control of $NaIO_3$-induced mitochondrial Ca^{2+} overload (Neupane et al., 2026). By expanding the mitochondrial permeability transition pore, Ca^{2+} can more readily move from the cytosol to the mitochondria in $NaIO_3$-induced AMD (Ren et al., 2024; Son et al., 2026). According to Li et al. (2015) and Argun and Nazıroğlu (2026), an increase in MiDis leads to the production of excessive OFRs and the triggering of apoptosis. $NaIO_3$ treatment enhances apoptosis and OFR production in ARPE-19 by activating TRPM2 and MiDis (Argun and Nazıroğlu, 2026). Antioxidant treatments stop these aberrant cellular functions (Ren et al., 2024; Argun and Nazıroğlu, 2026; Son et al., 2026). The current evidences showed that $NaIO_3$ increased the generations of apoptosis, caspases (CASP3, CASP8, and CASP9), MiDis, and OFRs but decreased ARPE-19 cell viability. On the other hand, treatment with TNE and ACA reduced the excessive productions of these markers. An increase in TRPM2 stimulation-caused Ca^{2+} influx and their accumulation in mitochondria appears to be the source of the excessive generation of apoptosis, caspases (CASP3, CASP8, and CASP9), MiDis, and OFRs. Our theory is that TRPM2 modulator effects of TNE can prevent these aberrant activities. Although the antioxidant activities of TNE against these markers have been documented, no prior research has investigated the antioxidant effects of TNE on oxidative degeneration and cell death markers through TRPM2 suppression in the cells. According to these findings, TNE reduced oxidative stress and apoptosis in human embryonic kidney cells (HEK293) (Kuccukturk et al., 2026). The apoptosis and CASP3 expression were decreased in colitis-induced rats by TNE treatment (Savran et al., 2022). In the brain of lipopolysaccharide-administrated rats, similar protective effects were observed, and TNE decreased the levels of total oxidant status by upregulating total antioxidant status and decreasing the expressions of caspase-1 and CASP3 (Oguzoglu et al., 2026). Furthermore, TNE protected the corneas of rats from alkaline-induced damage (Karakuyu et al., 2025). A lizard's amino acid is called exendin-4. Heloderma spectrum venom reduced Ca^{2+} influx in the RPE cells of AMD-induced mice (Shu et al., 2026).

The current study has a few limitations. Future research should use a multiple cell line analysis and TRPM2 knockout animals to validate the results. Second, we did not look into whether TNE inhibits TRPM2 channels in the ARPE-19 cells. Third, pharmacokinetic data and in vivo validation are absent from this study. Fourth, the current study's low budget prevented us from using high n numbers and conducting some analyses,

including calcium signaling and imaging analyses. These restrictions should be addressed in future studies.

In summary, TNE therapy enhanced ARPE-19 cell viability while reducing AMD ($NaIO_3$)-induced increases in apoptosis, caspases (CASP3, CASP8, and CASP9), MiDis, and OFRs. The cells treated with TRPM2 antagonist (ACA) showed comparable protective effects. TNE treatment may improve ARPE-19 defenses against AMD-caused oxidative degeneration and apoptosis caused blocking TRPM2. All things considered; these results point to TRPM2 activation as a possible treatment approach to stop oxidative retinal damage caused by AMD.

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Authors' Contribution
Mehmet Argun: conceptualization, data curation, formal analysis, writing – original draft, writing – review and editing, **Ömer Çelik**: conceptualization, data curation, writing – original draft, writing – review and editing.

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Availability of Data and Materials
The data are available upon request from MA.

Ethics Approval
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Declaration of Conflicting Interests
None.

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