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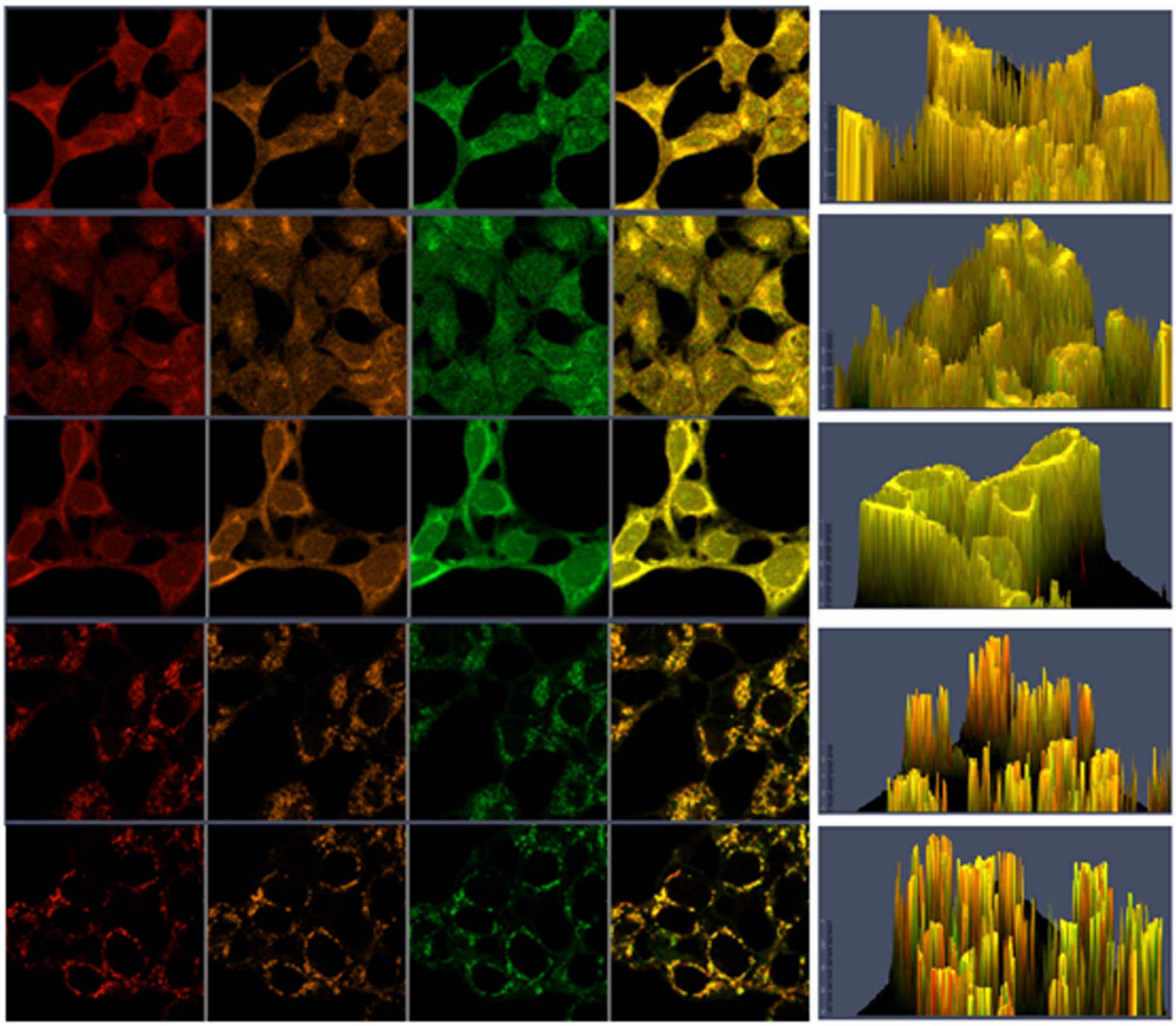

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AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate 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, Ca^{2+} 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)

READERSHIP

Biophysics	Biochemistry
Biology	Biomedical Engineering
Pharmacology	PhysiologyGenetics
Cardiology	Neurology
Oncology	Psychiatry
Neuroscience	Neuropharmacology

Keywords
Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide, ageing, antioxidants, neuropathy, traumatic brain injury, pain, spinal cord injury, Alzheimer's Disease, Parkinson's Disease.

RESEARCH ARTICLE

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Neuroprotective action of honey bee venom (melittin) against hypoxia-induced oxidative toxicity and cell death via inhibition of the TRPM2 channel

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List of Abbreviations;

ADPR, *ADP-ribose;* ***BF,*** *bright field;* ***Ca^{2+},*** *calcium ion;* ***CON,*** *control;* ***LSCM-800,*** *confocal laser scanning microscope;* ***DCFH-DA,*** *2',7'-dichlorodihydrofluorescein diacetate;* ***DCF,*** *2',7'-dichlorofluorescein;* ***$[Ca^{2+}]_i$,*** *intracellular free calcium;* ***HPO,*** *hypoxia;* ***iROS,*** *intracellular free reactive oxygen radicals;* ***ΔΨm,*** *mitochondrial membrane potential;* ***MEL,*** *melittin;* ***mROS,*** *mitochondrial free reactive oxygen radicals;* ***TRP,*** *transient receptor potential;* ***TRPM2,*** *transient receptor potential melastatin 2;* ***2APB,*** *2-aminoethoxydiphenyl borate.*

Abstract

One bioactive element of honeybee venom is melittin (MEL). MEL induced oxidant and apoptotic activities through the increase of mitochondrial Zn^{2+} and Ca^{2+} in tumor cells, but it also induced neuroprotective activity by inhibiting the cell death, intracellular reactive oxygen species (iROS), and mitochondrial ROS (mROS) productions in neurons. By stimulating the TRPM2 channel, hypoxia (HPO) enhances the effects of oxidative stress and neuronal death; however, its inhibition prevents the alterations. I studied the neuroprotective effect of MEL on HPO-mediated oxidative neurotoxicity and cell death in SH-SY5Y neuronal cells by altering the TRPM2 signaling pathways.

In the SH-SY5Y cells, five groups were induced as control, MEL (1 ug/ml for 24 hrs), HPO ($CoCl_2$ and 200 μM for 24 hrs), HPO + MEL, and HPO + TRPM2 antagonist (2-aminoethoxydiphenyl borate, 2APB) (100 mM for 2 hrs).

The amounts of cytosolic free Ca^{2+} were increased in the HPO group by the stimulation of hydrogen peroxide, although they were decreased in the cells by the treatment of 2APB and MEL. The amount of cytosolic free Ca^{2+} was higher in the HPO group than in the control group. The amounts of cell death (propidium iodide positive cell number), oxidants (mROS and iROS), mitochondrial membrane depolarization, and cytosolic free Zn^{2+} were higher in the HPO group than in the control and MEL groups, although their amounts were lower in the HPO + MEL and HPO + 2APB groups than in the HPO group only.

In conclusion, MEL therapy reduced the amount of HPO-induced oxidative stress and neuronal deaths in SH-SY5Y cells by inhibiting TRPM2. The MEL could be considered as a potential protective component against oxidative neuronal damage caused by HPO.

Keywords: Hypoxia; Melittin; Neuroprotective; Oxidative neurotoxicity; TRPM2 channel.

Introduction

Oxidative stress includes several intracellular (iROS) and mitochondrial (mROS) reactive oxygen species (Halliwell 1992). The brain and neuronal cells are very sensitive to the iROS and mROS because they have a high amount of oxygen consumption and polyunsaturated fatty acid contents but low amounts of antioxidant defense systems (Halliwell 2006). Accumulations of intracellular free zinc ($[Zn^{2+}]_i$) and calcium ($[Ca^{2+}]_i$) ions into mitochondria cause dysfunction of mitochondria through the increase of mitochondrial membrane depolarization (Granzotto and Sensi 2015; Kim et al. 2017). The malfunctioning of proteins, nucleic acids, and lipid membranes is consequently caused by a rise in iROS and mROS, which ultimately compromises the integrity and function of neurons (Halliwell 1992; Nazıroğlu 2007). Hypoxia (HPO) in the brain and neurons is a primary pathogenic effect of ischemia. Further damage also comes about by the ischemia-induced HPO after reperfusion, which is generated by an increased $[Ca^{2+}]_i$ amount and oxidative stress (Kumar et al. 2014).

Adults suffer from ischemia-induced hypoxic brain injury, which is primarily caused by problems during anesthesia and surgical procedures (Scolletta et al. 2007; Kounis et al. 2020). Within the restricted treatment time windows, controlling blood flow to the affected area is the primary goal of treating an ischemia injury. However, by stimulating reperfusion, the treatments exacerbate the injury (Scolletta et al. 2007). Melittin (MEL) is a honey bee venom polypeptide that may stimulate cell death, Zn^{2+}, Ca^{2+} influx, iROS, and mROS in tumor cells (Ertilav and Nazıroğlu 2023). However, the treatment of MEL induces neuroprotective actions in several diseases, including rotenone-induced cell death (Jung et al. 2015) and Alzheimer's disease (Nguyen and Lee 2021). Furthermore, it has been reported that MEL could exert protective action against cerebral ischemia-induced cytokine generations in the mouse neuron and murine BV-2 microglia cell lines by suppressing inflammation (Xing et al. 2024). In liver cancer cells, the treatment of MEL decreased HPO-mediated vascular and epithelial changes through suppression of the HIF-1α/Akt pathway (Chen et al. 2019). Neuroprotective effects of MEL on oxidative stress-induced cell death in SH-SY5Y neuronal cells were reported (Han et al. 2014). However, the neuroprotective action of MEL on HPO-induced cell death, iROS, and mROS in neuronal cells has not been clarified yet.

The Ca^{2+} permeable transient receptor potential (TRP) melastatin 2 (TRPM2) cation channel is a member of the TRP superfamily. It is activated by H_2O_2 and ADP-ribose (Hara et al., 2002; Nazıroğlu and Lückhoff, 2008), but antagonists, such as 2-aminoethoxydiphenyl borate (2APB) (Togashi et al. 2008), inhibit it. Because it stabilizes HPO-inducible factors 1α and 2α under normoxic conditions, cobalt chloride ($CoCl_2$)-induced chemical HPO is a useful model (Muñoz-Sánchez and Chánez-Cárdenas 2019). By activating TRPM2 through the excessive production of iROS and mROS, the induction of HPO promotes the Ca^{2+} influx processes (Akyuva and Nazıroğlu 2020; Armağan and Nazıroğlu 2021; Yıldızhan and Nazıroğlu 2023). The neuronal cell mitochondria endure an increase in $[Ca^{2+}]_i$ and $[Zn^{2+}]_i$ due to the stimulation of the TRPM2 channel. This results in an increase in the membrane potential of the mitochondria ($\Delta\Psi m$), mROS, iROS, and cell death (Akyuva et al. 2021; Osmanlıoğlu 2022; Yıldızhan and Nazıroğlu 2023). thereby suggests that one of the main causes of the formation of mROS and neuronal cell death is the raised TRPM2-mediated Ca^{2+} and Zn^{2+} influxes generated by $CoCl_2$-mediated HPO (Akyuva and Nazıroğlu 2020; Osmanlıoğlu 2022; Yıldızhan and Nazıroğlu 2023). While various antioxidants have been shown to modulate HPO-induced oxidative stress and neuronal cell death through

the suppression of TRPM2, the beneficial effect of antioxidant MEL on TRPM2-mediated oxidative death of neurons remains unclear.

As far as is currently known, no study has been done on the neuroprotective responses of MEL to HPO-induced oxidative neuronal death in SH-SY5Y cells via the blockage of the TRPM2 channel. My goal in this study was to find out the way MEL altered oxidative cell death of SH-SY5Y neuronal cells caused by HPO.

Material and Methods

Cell lines

In the neuroprotective studies of MEL, SH-SY5Y cells were primarily utilized (Han et al. 2016; Nguyen et al. 2021). Recent studies (Chen et al. 2013; Akyuva and Nazıroğlu 2020; Armağan and Nazıroğlu 2021) demonstrated the natural existence of TRPM2 in SH-SY5Y cells. These SH-SY5Y cells were chosen in the current investigation for two reasons. The cells (ATTC, Germany) were cultivated in a media combination consisting of 90% DMEM/Hams' F12 (1:1) mixture, 10% fetal bovine serum, and 1% penicillin/streptomycin and were maintained in a cell culture environment with 95% air and 5% CO_2 (Akyuva and Nazıroğlu 2020).

Experimental groups

Using the $1x10^6$ cells in 25T flasks, five major groups of SH-SY5Y cells were induced: control (CON), MEL, HPO, HPO + MEL, and HPO + 2APB. For a full day, the cells in the CON groups were maintained in the untreated cell culture conditions. For a duration of 24 hours, MEL (1 mg/ml) was added to the cells in the MEL and HPO + MEL groups (Han et al. 2014). $CoCl_2$ (200 mM) was applied to the cells in the HPO groups for a duration of 24 hours (Akyuva and Nazıroğlu 2020). Following the $CoCl_2$ (200 µM for 24 h) incubation, the cells in the HPO + MEL and HPO + 2APB groups were further incubated with MEL (1 mg/ml for 24 h) and 2APB (100 mM for 2 h), respectively.

The cells were cultivated in bottom-glass dishes (Mattek Corporation, Ashland, MA, USA) for laser confocal microscopy investigations (LSCM-800) and CCD Axiocam 702 camera using an Axio Observer.Z1/7 microscope and Plan-Apochromat 40x1.3 oil objective (Zeiss, Oberkochen, Germany).

The measurement of the $[Ca^{2+}]_i$ amount

I employed a fluorescent dye (Fluo 3-AM) under argon laser stimulation in the LSCM-800 at 488 nm to determine changes in the $[Ca^{2+}]_i$ amount carried on H_2O_2 in the SH-SY5Y cells (Osmanlıoğlu 2022). For 45 to 60 minutes, the cells in DMEM media in 35-mm dishes were incubated with 1 µM Fluo 3-AM (Cat # ab145254, Abcam, Istanbul, Türkiye) at 37 °C in the dark. H_2O_2 (1 mM) activated the TRPM2 channel in the cells, while 100 µM 2APB blocked it. The lazer stimulation of Fluo 3-AM was performed in LSCM-800 at 488 nm. The arbitrary unit (a.u.) was employed to describe the variations in fluorescence intensity.

Cell death analyses

The cell death percentage was measured using propidium iodide (PI, Cat # P1304MP, ThermoFisher Scientific) and Hoechst 33342 (Cat # H3570, ThermoFisher Scientific). The cells in the dish have been stained with 1.5 mM of PI and 8.1 mM of Hoechst 33342. Following a 25-minute incubation period and wash, images in the blue (Hoechst), red (PI), bright field (BF), and 2.5D formats were captured using the CCD Axiocam 702 camera. By manually counting each and every cell in the collected cells, the percentage of PI-positive cells in the CCD camera assessment was determined (Yıldızhan and Nazıroğlu 2023).

The assays for mROS, iROS, and mitochondrial membrane potential (ΔΨm) production

The production of mROS was measured using MitoSOX Red fluorogenic (MitoSOX) dye (Cat # M36008, Thermo Fisher Sci.), a superoxide indicator found in the mitochondria of living cells. The ΔΨm alterations in the cells were assessed using the JC-1 probe (Cat # sc-364116, Santa Cruz Chemical, Heidelberg, Germany). Using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) (Cat # D399, Thermo Fisher Sci.), the production of iROS in the cells was determined. For twenty-five minutes at 37°C in the dark, the cells were stained with 2 mM non-fluorescence MitoSOX, JC1, and DCFH-DA. Non-fluorescent DCFH-DA undergoes oxidation in the cytosol to produce fluorescent 2',7'-dichlorofluorescein (DCF) (Vaglienti et al. 2022). MitoSOX, JC-1, and DCF pictures were taken with the LSCM-800. DCF, MitoSOX, and JC-1 have excitation and

emission wavelengths of 504/525 nm, 576/598 nm, and 593/595 nm, respectively. ZEN software (blue edition 3.2) was used to calculate the fluorescence change of each treatment (n = 3 and 6-8 cells each) in the red (MitoSOX), orange (JC-1), and green (DCFH-DA) images that were obtained. The findings were displayed as a.u. (Yıldızhan and Nazıroğlu 2023).

Analysis of $[Zn^{2+}]_i$ amount

The amounts of $[Zn^{2+}]_i$ in the SH-SY5Y cells were stained using the FluoZin-3-AM (1 µM per ml) (Cat #: F24195, ThermoFisher Scientific), a fluorescent probe for Zn^{2+} labeling. Using the CCD Axiocam 702 camera, the green, bright field (BF), overlay, and 2.5D images were captured at 526/404 nm (Em/Ext). The intensities of fluorescence changes in the green-colored images were determined utilizing the ZEN program and findings were shown as a.u.

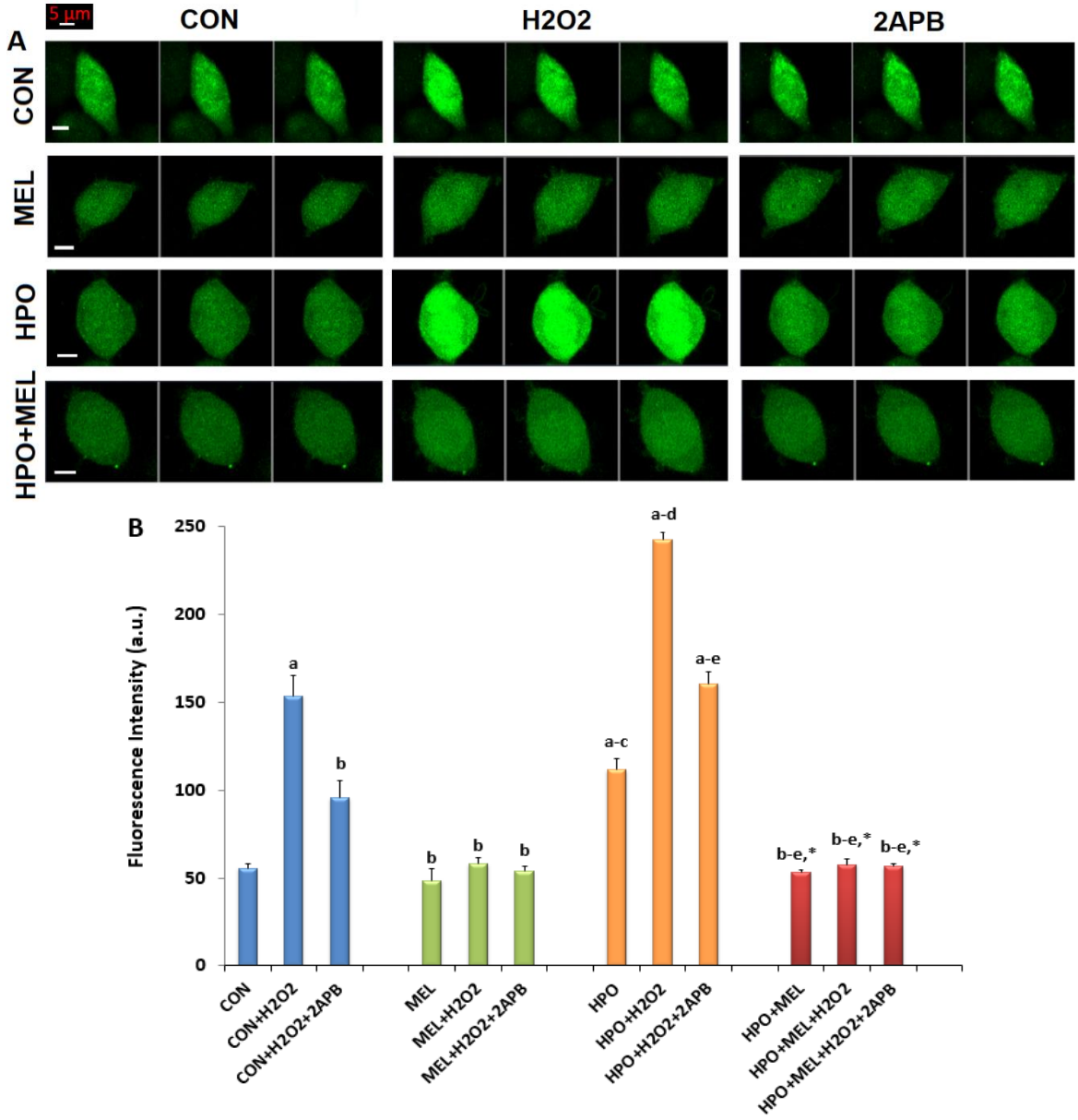


Figure 1. The HPO (200 µM $CoCl_2$)-induced rise in $[Ca^{2+}]i$ in the SH-SY5Y cells was decreased by the incubation of MEL at a rate of 1 µg/ml 24 hours. *(N=3 and 6–8 cells per. Mean ± SD). Fluo 3-AM (1 µM) was used to stain the SH-SY5Y cells for 45–60 minutes. The cells were washed with extracellular buffer, stimulated for five minutes with H_2O_2 (1 mM), and then inhibited for five minutes in the LSCM-800 with a 40x oil objective with 2APB (100 µM).* ***A.*** *The Fluo 3-AM representative pictures for each of the four groups—CON, MEL, HPO, and HPO + MEL.* ***B****. Following the H_2O_2 and 2APB treatments, the mean fluorescence intensity varies as an arbitrary unit (a.u.) in each of the four groups. In the pictures, the scale bar remained at 5 µm. (ap ≤ 0.05 vs CON. bp ≤ 0.05 vs CON + H_2O_2. cp ≤ 0.05 vs MEL, MEL + H_2O_2, and MEL + H_2O_2 + 2APB. dp ≤ 0.05 vs HPO. ep ≤ 0.05 vs HPO + H_2O_2. *p ≤ 0.05 vs HPO + H_2O_2 + 2APB).*

Statistical evaluations

The mean data in five groups were presented as mean ± standard deviation (SD). The one-way analysis of variance (ANOVA) was employed by the SPSS program (25.0) to ascertain the presence of statistical significance. The statistical significance was assessed using a p value ($p < 0.05$).

Results

Treatment with MEL reduced HPO-induced activation of TRPM2.

Figure 1A shows the green fluorescence dye (Fluo 3-AM) images for the groups of CON, MEL, HPO, and HPO + MEL. Figure 1B shows the mean fluorescence intensities as columns following H_2O_2 stimulation and 2APB

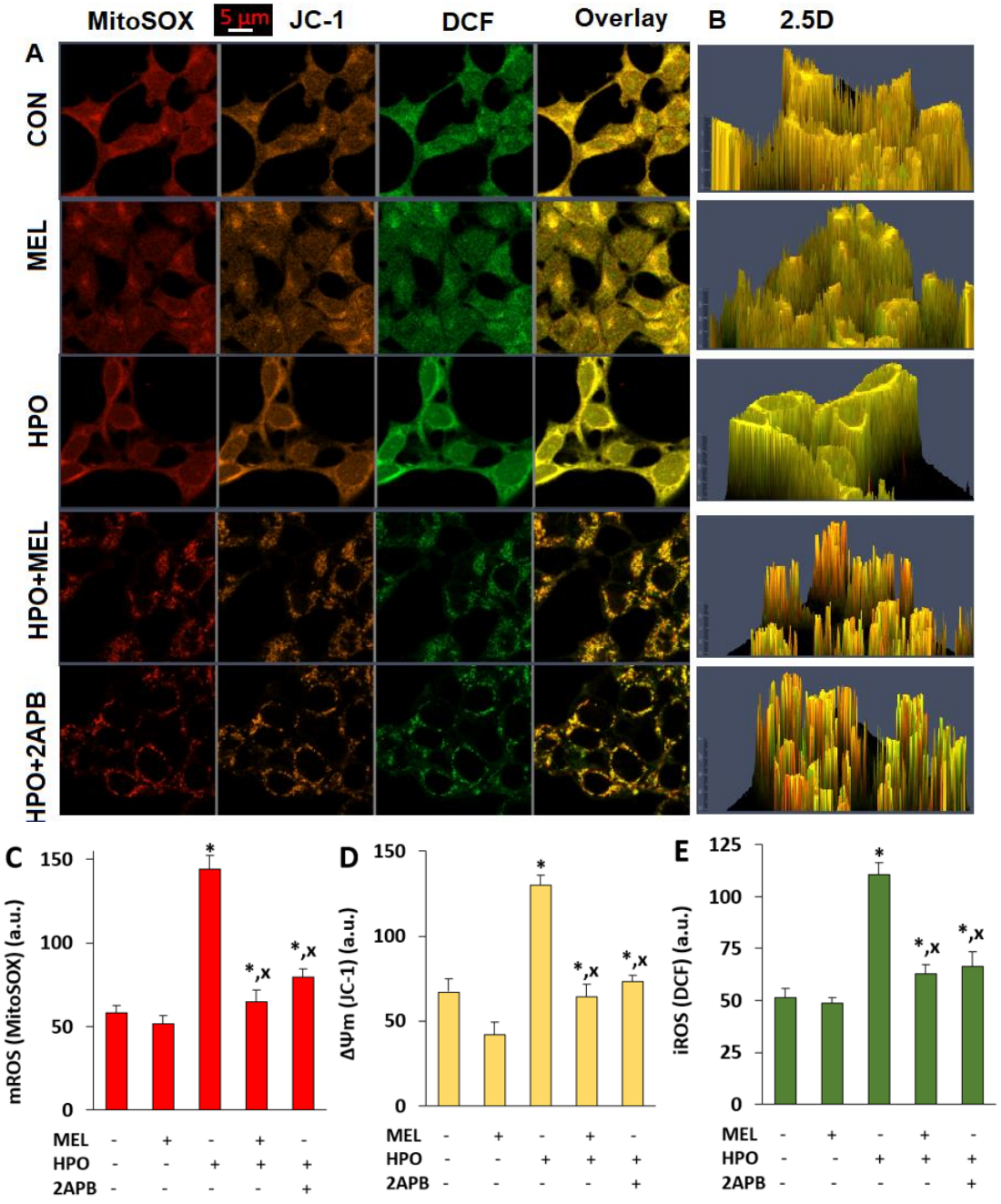


Figure 2. MEL of (1 μg /ml for 24h) modulated HPO (200 μM $CoCl_2$ for 24h)-mediated the increase of mitochondrial membrane potential (ΔΨm) in the SH-SY5Y cells*. (Mean ± SD. N=3 and 6-8 cell each). The SH-SY5Y cells were stained with MitoSOX, JC-1, and DCFH-DA (5 μM for 25 min). After washing the cells with extracellular buffer, their red (MitoSOX), orange (JC-1), and green (DCF, a fluorescent form of DCFH-DA) images were captured in the LSCM-800 with 40x oil objective.* ***A.*** *The MitoSOX, JC-1, and DCF, and their overlay images in the five groups [control (CON), MEL, HPO, HPO + MEL, and MEL + 2APB).* ***B.*** *2.5D images of MitoSOX, JC-1, and DCF.* ***C.*** *The mean values of MitoSOX fluorescence intensity.* ***D.*** *The mean values of JC-1 fluorescence intensity.* ***E.*** *The mean values of DCF fluorescence intensity. a.u. arbitrary unit. The scale bar: 5 μm. (*p ≤ 0.05 vs the groups of CON and MEL. xp ≤ 0.05 vs the group of HPO).*

inhibition. In comparison to the CON and MEL groups, the Fluo 3-AM intensity changes were greater in the HPO groups ($p \leq 0.05$) (Figure 1B). When comparing the HPO + MEL group to the HPO group alone, the effects were, however, less pronounced ($p < 0.05$). Thus, I found that the incubation of MEL in SH-SY5Y reduced the HPO-mediated elevation of $[Ca^{2+}]_i$ through TRPM2 activation.

The incubations of MEL and TRPM2 antagonist (2APB) reduced the HPO-caused generates of mROS, ΔΨm, and iROS in the cells.

The images that were taken with the LSCM-800 include the overlay, red mROS (MitoSOX), orange ΔΨm (JC-1), green iROS (DCF) (Figure 2A), and 2.5D (Figure 2B). The HPO treatment resulted in an upregulation of the mean fluorescence intensity changes of MitoSOX (Fig. 2C), JC-1 (Figure 2D), and iROS (Figure 2E) in the cells ($p \leq 0.05$). Nevertheless, incubations with MEL and TRPM2 channel blocker (2APB) decreased the adverse effects of HPO by inhibiting the formation of mROS, ΔΨm, and iROS in the SH-SY5Y ($p < 0.05$).

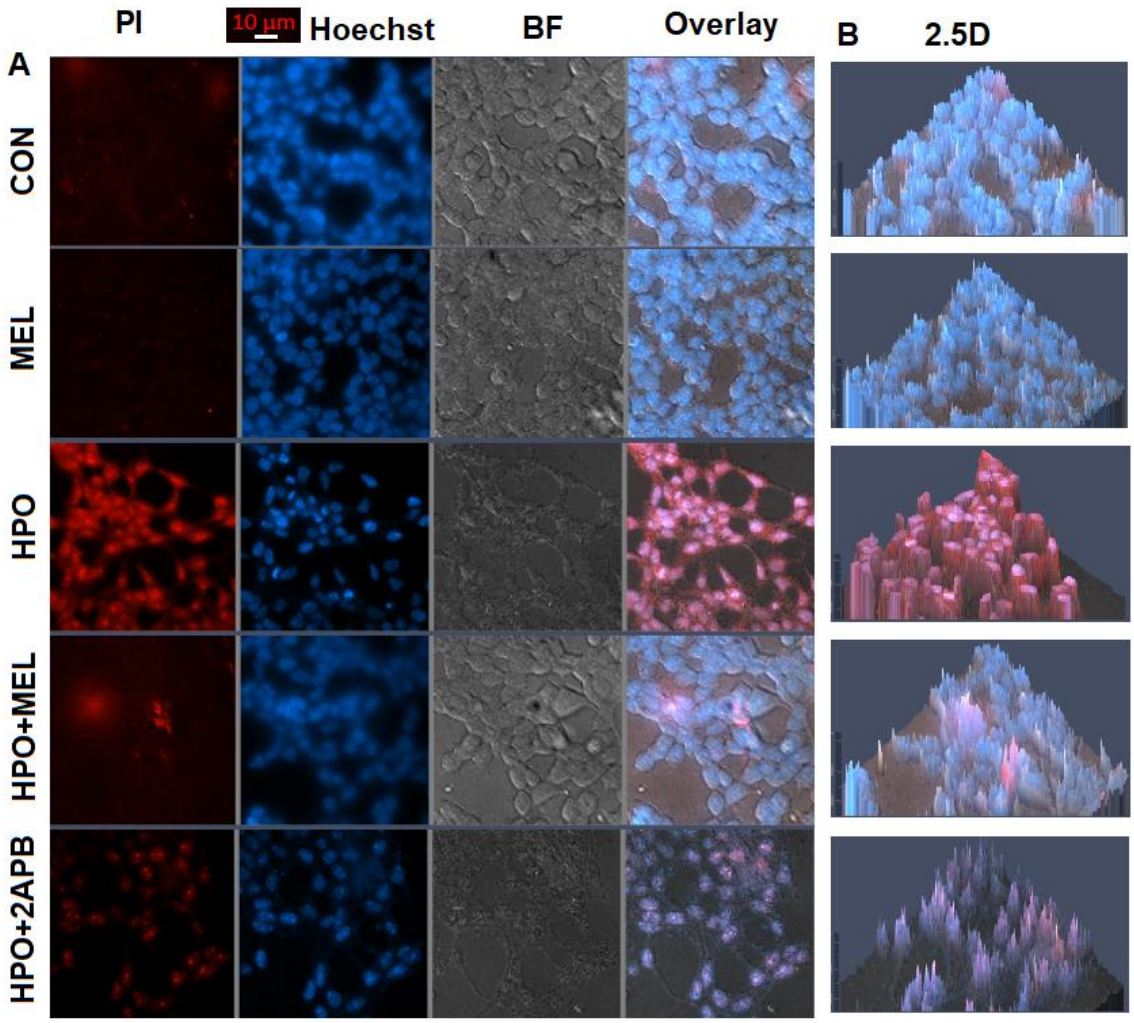
Even though the MEL and 2APB treatments downregulated the increases in mROS, ΔΨm, and iROS production, they continued to correlate with the rise in $[Ca^{2+}]_i$ following $CoCl_2$ incubation. Thus, the results of mROS, ΔΨm, and iROS further indicated how MEL and HPO affect oxidative stress produced by TRPM2 stimulation in SH-SY5Y.

The MEL treatments reduced the increase of HPO-induced cell death

The ZEN program was used to save the images of red (PI), blue (Hoechst), bright field (BF), their overlay (Figure 3A), and 2.5D (Figure 3B) in the LSCM-800. The PI positive cell numbers in the PI/Hoechst pictures were considerably ($p < 0.05$) lower in the HPO + MEL and HPO + 2APB groups than in the HPO only, while the numbers in the HPO group were higher ($p \leq 0.05$) than in the CON and MEL groups (Figure 3C). The present results confirmed HPO-induced SH-SY5Y cell death that is caused by TRPM2 activation. Nevertheless, the MEL incubation provided a protective action against the rise in cell death induced by HPO.

The rise in $[Zn^{2+}]_i$ caused by HPO was diminished by the MEL treatments.

The green (FluoZin-3), bright field (BF), overlay (Figure 4A), and 2.5D (Figure 4B) images in the CCD Axiocam 702 camera were saved using the ZEN application. While the numbers in the HPO group were higher ($p \leq 0.05$) than in the CON and MEL groups, the amount of $[Zn^{2+}]_i$ in the green photos was significantly ($p \leq 0.05$) lower in the HPO + MEL and HPO + 2APB groups than in the HPO alone (Figure 4C). The current findings validate the theory that TRPM2 activation generates the HPO-induced rise in $[Zn^{2+}]_i$. Nevertheless, the MEL incubation provided defense against the increase in $[Zn^{2+}]_i$ produced by HPO.



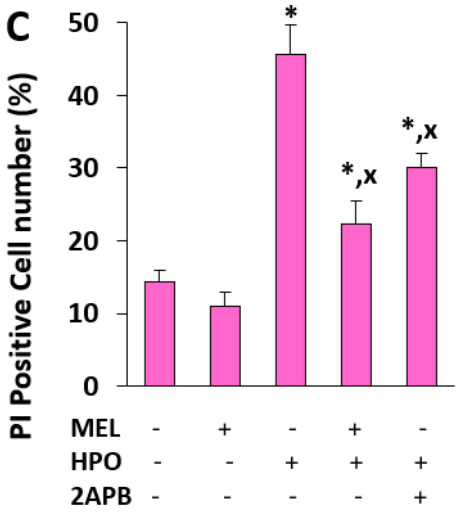


Figure 3. The HPO (200 µM $CoCl_2$)-induced increase of PI-positive SH-SY5Y cell number was decreased by MEL (1 µg/ml for 24 hours). *(Mean ± SD. N=3 and 6-8 cell each). Hoechst (8.1) and PI (1.5 mM) were used to stain the SH-SY5Y cells for a duration of 25 minutes each. Following the washing with extracellular buffer, the CCD Axiocam 702 camera and the Axio Observer.Z1/7 inverted fluorescent microscope were used to record the red (PI), blue (Hoechst), bright field (BF), merge (**A**), and 2.5D (**B**) images of the cells. **C.** The mean percent change of PI-positive cell counts in each of the five groups. (*p ≤ 0.05 vs. the groups of CON and MEL. xp ≤ 0.05 vs. the group of HPO).*

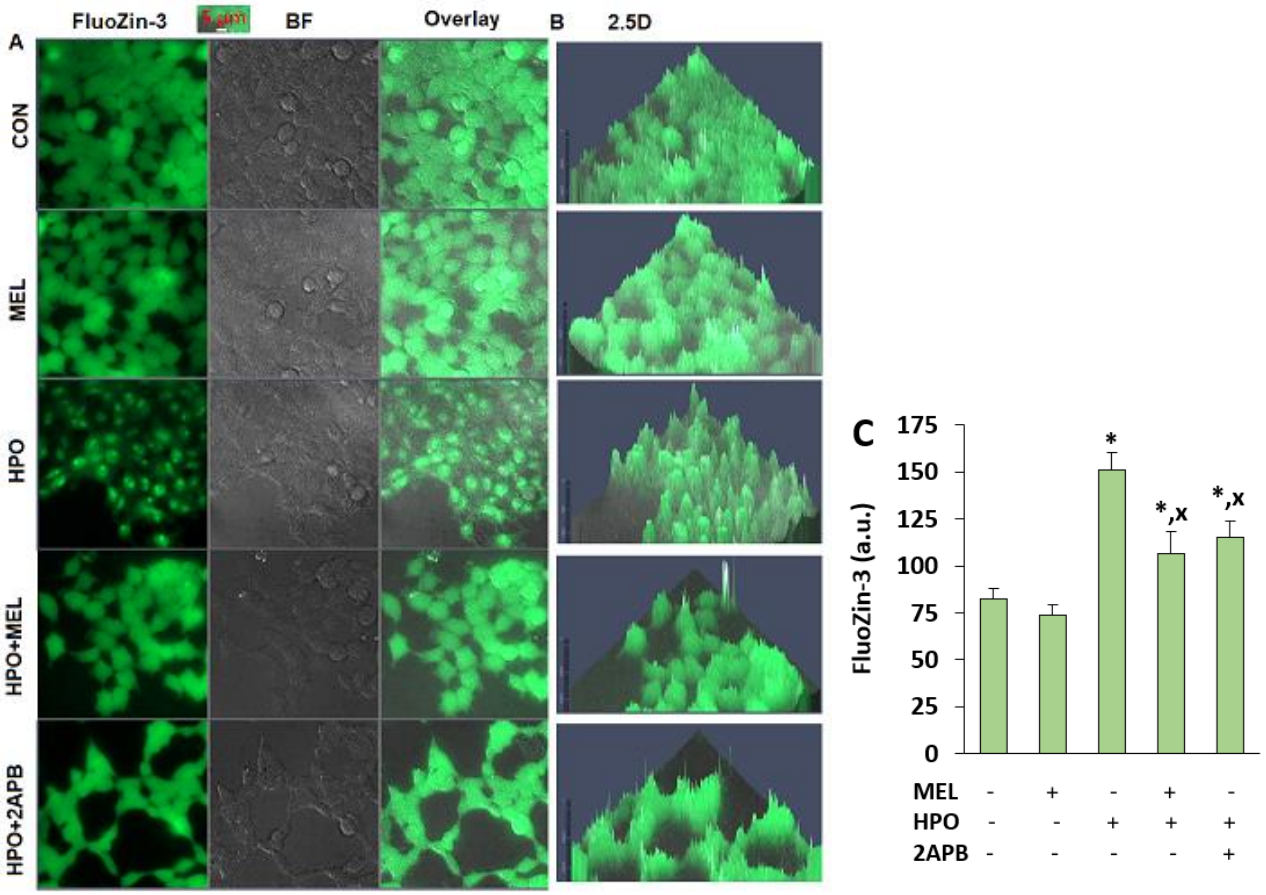


Figure 4. The incubation of MEL of (1 µg/ml for 24h) modulated HPO (200 µM $CoCl_2$ for 24h)-induced the increase of $[Zn^{2+}]_i$ amount. *(Mean ± SD). The SH-SY5Y cells were stained with FluoZin -3 (1 µM for 15-20 min). After washing the cells with extracellular buffer, green (FluoZin -3), bright filed (BF), overlay (**A**), and 2.5D (**B**) images were captured in the CCD Axiocam 702 camera and fluorescent microscope with 40x oil objective. **C**. The mean values of FluoZin-3 fluorescence intensity in the five groups. a.u. arbitrary unit. (*p ≤ 0.05 vs the groups of CON and MEL. xp ≤ 0.05 vs the group of HPO).*

Discussion

HPO generated by ischemia-reperfusion causes neuronal damage by activating many mechanisms, one of which is TRPM2 activation (Akyuva and Nazıroğlu 2020). Consequently, enhanced mitochondrial membrane depolarization caused by HPO-induced excessive Ca^{2+} influxes lead to increased production of mROS and iROS via TRPM2 stimulation. Thus, in HPO-mediated neuronal damage, the iROS and mROS production are also essential (Armağan and Nazıroğlu 2021; Osmanlıoğlu 2022). A production of mROS and iROS that is upregulated in HPO circumstances causes neuronal apoptosis and death via activating TRPM2 (Yıldızhan and Nazıroğlu 2023). In glioblastoma tumor cells, MEL incubation increased cell death, $[Ca^{2+}]_i$, $[Zn^{2+}]_i$, iROS, and mROS through the stimulation of TRPM2 channel in tumor cells (Ertilav and Nazıroğlu 2023). However, subtoxic concentrations of MEL induced neuroprotective action through the decrease of normal HT-22 hippocampal cell death, iROS, and mROS in Parkinson's and Alzheimer's disease (Nguyen and Lee 2021). The treatment of subtoxic MEL protected BV-2 microglia cells against the cerebral ischemia-induced cell death (Xing et al. 2024). Since oxidative stress and cell death within the ischemic focal area are part of the pathophysiology of ischemic stroke (Kumar et al. 2014; Silva et al. 2015; Carpena et al. 2020), my hypothesis is that MEL could reduce HPO-induced cell death, $Ca^{2+}]_i$, $[Zn^{2+}]_i$, iROS, and mROS by blocking the TRPM2 channel in neuronal cells. In the present study, I observed that the MEL induced neuroprotective action against HPO-mediated increase of oxidative neuronal injury via the inhibition of TRPM2 in the neuronal cells (Figure 5).

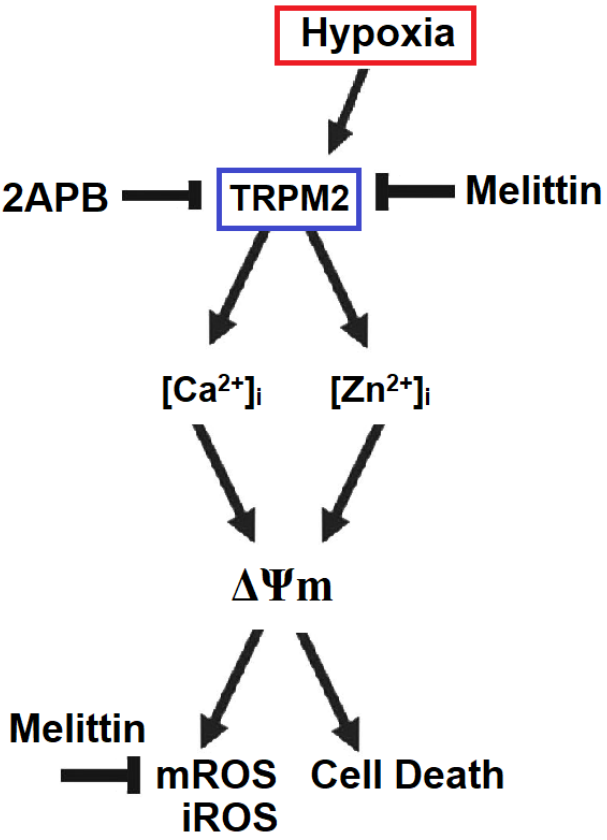


Figure 5. Possible neuroprotective action of melittin against hypoxia-induced cell death and oxidative stress through the inhibition of the TRPM2 channel. *Hypoxia-mediated increases of $[Ca^{2+}]_i$ and $[Zn^{2+}]_i$ amounts in the cytosol and mitochondria induce an increase in mitochondrial membrane depolarization (ΔΨm). In turn, its increase induces an increase in mitochondrial (mROS), intracellular (iROS) ROS, and neuronal death. The increases are decreased by the TRPM2 channel blocker (2APB) and melittin incubations.*

Oxidative stress stimulates the TRPM2 channel (Hara et al. 2002; Nazıroğlu and Lückhoff 2008). While MEL therapy diminishes HPO-induced oxidative stress, HPO is defined by a rise in iROS and mROS (Chen et al. 2019; Xing et al. 2024). MEL promotes oxidative stress and apoptosis in cancer cells by stimulating the TRPM2 and store-operated Ca^{2+} entry channels (Nakagawa et al. 2020; Ertilav and Nazıroğlu 2023). However, MEL exhibited antiapoptotic and beneficial antioxidant properties in SH-SY5Y neuronal cells (Han et al. 2014). MEL was discovered to produce neuroprotective activities in rats with traumatic brain damage by reducing iROS (Liang et al. 2018). Furthermore, MEL therapy reduced the HPO-induced apoptotic pathways in neurons (Xing et al. 2024). Nonetheless, there lack a lot of studies on the antioxidant impact of MEL on neural cells. According to my research, MEL reduced iROS, mROS, and HPO-induced Ca^{2+} influx via downregulating TRPM2 in SH-SY5Y.

Increases in mitochondrial Ca^{2+} during hypoxia promote the production of iROS and mROS in neuronal cells (Kumar et al. 2014; Wu et al. 2017). In the SH-SY5Y cells, the mitochondrial $[Ca^{2+}]_i$ and $[Zn^{2+}]_i$ accumulation of HPO was reported through TRPM2 stimulation (Akyuva et al. 2021; Nazıroğlu 2022; Yıldızhan and Nazıroğlu 2023). The activities then lead to an increase in ΔΨm, which in turn causes an elevation of cell death markers and oxidants (iROS and mROS) (Akyuva et al. 2021; Nazıroğlu 2022; Yıldızhan and Nazıroğlu 2023). However, in neuronal cells, including SH-SY5Y, suppression of TRPM2 (excessive Ca^{2+} influx) diminishes the formation of iROS and mROS as well as cell death (PI-positive cell number) (Akyuva and Nazıroğlu 2020; Sha'fie et al. 2022; Yıldızhan and Nazıroğlu 2023). Based on the available information, SH-SY5Y exhibited an elevation of ΔΨm due to HPO-induced TRPM2 activation (by excess Ca^{2+} inflow), which subsequently resulted in an upregulation of mROS, iROS, and cell death (PI positive cell number). In the same manner, MEL inhibited the oxidative stress signaling pathway to provide anti-apoptotic effects in bicuculline-induced seizures and hippocampus astrocyte activation (Soares-Silva et al. 2022). Furthermore, MEL prevented the SH-SY5Y cells from undergoing apoptosis caused by hydrogen peroxide (Han et al. 2014). In a rat model of acetic acid-induced colitis, MEL reduced apoptosis and oxidative stress through the upregulation of the antioxidant redox system (Ahmedy et al. 2020).

By incubating MEL through the attenuation of TRPM2, SH-SY5Y cells became protected against HPO-mediated cell death and oxidative mediators resulting from the downregulation of TRPM2 stimulation-induced damage to neurons. HPO-induced oxidative harm is thought to be caused by triggering TRPM2-mediated excessive Ca^{2+} influx, neuronal death, and oxidant mediators, even though MEL therapy diminishes HPO-induced iROS and mROS-mediated neuronal injury and death.

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Author contributions

KE conceived and designed the study, and he also prepared figures and images, and he revised the manuscript and her performed laser confocal microscope analyses.

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Declarations

Competing Interests

No relevant financial or non-financial interests to disclose.

Ethical Approve

No.

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