Molecular Biology Reports (2025) 52:255
https://doi.org/10.1007/s11033-025-10345-1

ORIGINAL ARTICLE

Curcumin protects against MPP^+-induced neurotoxicity in SH-SY5Y cells by modulating the TRPV4 channel

Ramazan Çınar[1] · **Kenan Yıldızhan**[2]

Received: 4 December 2024 / Accepted: 6 February 2025

Abstract

Background It is well acknowledged that neuroinflammation, mitochondrial dysfunction, and oxidative stress (OS) play a role in the etiology of Parkinson's disease (PD). Curcumin (CUR) protect neuronal cells by interfering with the production of reactive oxygen species (ROS) in neuronal cells and suppressing OS. In this study, we investigated the role of the TRPV4 channel under CUR stimulation in the PD model induced by MPP^+ in SH-SY5Y cells.

Methods The cells were divided into four groups: control, CUR, MPP^+ and MPP^++CUR. In addition, incubations were performed with TRPV4 channel agonist GSK1016790A (GSK) and its antagonist Ruthenium red (Rr) to follow the Ca^{2+} current induced through the TRPV4 channel.

Results MPP^+ exposure increased mitochondrial and intracellular ROS production and mitochondrial membrane potential in the cell, while decreasing GSH levels. During CUR and Rr incubation, MPP^+ exposure and TRPV4 agonist GSK-induced TRPV4 overstimulation were down-regulated. The effects of MPP^+ on intracellular damage were changed by CUR treatment, as seen in changes in GSH levels, mROS, iROS, JC/1, apoptosis, and TRPV4 expression value compared to the MPP^+ group.

Conclusions The CUR treatment in the in vitro PD model created with MPP^+ reduced cellular damage by regulating mitochondrial dysfunction, OS and TRPV4 channel activation in MPP^+-induced neurotoxicity with the antioxidant properties of CUR.

Keywords Parkinson's disease · MPP^+ · Curcumin · SH-SY5Y cells · TRPV4 channel

Introduction

Neurodegeneration is a physiological process in which neurons lose their structure or functional abilities and finally die. Neurodegeneration plays a significant role in the etiology of neurodegenerative diseases, affecting approximately 15% of the population worldwide [1, 2]. One of the most important neurodegenerative disorders, Parkinson's disease (PD), is typified by tremors, muscular stiffness, poor movement, and issues with balance and coordination. The disease's main damaging process is yet unknown [3, 4].

Numerous studies have demonstrated the critical roles that neuroinflammation, oxidative stress (OS), accumulation of intracellular ions, and mitochondrial dysfunction display in the development of neurodegeneration in PD [5, 6]. 1-methyl-4-phenylpyridinium (MPP^+) is a neurotoxin that induces PD-associated changes in mitochondrial activity, such as inhibition of complex-1 and increases in reactive oxygen species (ROS) and is widely used in in vitro PD models [7, 8]. A study investigating the neuroprotective effect of insulin on neurotoxicity in MPP^+-induced SH-SY5Y cells showed that increasing MPP^+ concentrations caused a dose-dependent decrease in cell viability and increased intracellular ROS levels and Ca^{2+} accumulations [9]. Song et al., in their MPP^+/MPTP-induced in vitro and in vivo neurotoxicity model study, determined that there was an increase in MDA and ROS levels and a decrease in GSH levels in SH-SY5Y cells induced by MPP^+, and they suggested that MPP^+-induced neurotoxicity could be prevented if OS was suppressed [10]. Wang et al. reported that MPP^+-induced

✉ Kenan Yıldızhan
kenanyldzhan@gmail.com

[1] Department of Biophysics, Faculty of Medicine, Bilecik Seyh Edebali University, Bilecik, Turkey

[2] Department of Biophysics, Faculty of Medicine, Van Yuzuncu Yil University, Van TR-65090, Turkey

Published online: 20 February 2025

cells had decreased cell viability and increased intracellular ROS levels, caspase-3 activation, and mitochondrial dysfunction in the SH-SY5Y cells [8].

Mammalian transient receptor potential (TRP) channels are protein structures found in the membranes of different cell types. They can be thought of as "cellular entry points" for ions, responding to various physical and chemical stimuli [11, 12]. TRP vanilloid-4 (TRPV4), a member of the TRP channel family, is a non-selective cation channel that allows the influx of Ca^{2+}. TRPV4 is present in many excitable and non-excitable cells, such as the brain, kidney, bladder, and endothelium [13, 14]. A study examining biochemical, pharmacological and electrophysiological methods in SH-SY5Y cells incubated with paclitaxel reported overexpression of that TRPV4 channel in chemotherapy-induced peripheral neuropathy and an increase of Ca^{2+} influx toward the cytoplasm due to TRPV4 channel activation [15]. Also, it was shown that Ca^{2+} influx from TRPV4 plays an important role in cell loss through apoptosis, ferroptosis, necroptosis, pyroptosis and autophagy by mediating endoplasmic reticulum (ER) stress, OS, and inflammation [16, 17].

Curcumin (CUR) is a lipophilic substance with a polyphenol structure extracted from the rhizomes of a plant belonging to the ginger family, known as Indian saffron or turmeric (*Curcuma longa* L.) [18]. It has been used in traditional medicine since ancient times, especially in India and China. It has been determined in many studies that it has various properties that provide a great effect on a wide range of pharmacological developments, including neuroprotective, antioxidant, anti-inflammatory, antimicrobial and anticancer drugs, with the presence of hydroxyl and methoxy groups in its molecular structure [19–22]. In addition, CUR treatment has a high safety profile with minimal toxicity, as shown in experimental PD model studies. Therefore, a better understanding of the mechanisms by which CUR exerts its neuroprotective properties may further strengthen therapeutic approaches [22].

This study investigated the effect of CUR on OS, mitochondrial dysfunction, apoptosis and intracellular Ca^{2+} ($[Ca^{2+}]_i$) accumulation by stimulating the TRPV4 channel in SH-SY5Y cells treated with MPP^+.

Material and method

Cell culture and study groups

SH-SY5Y cells, a human neuroblastoma cancer cell line, were obtained from the Şap Institute (Ankara, Türkiye). The cells were grown in DMEM/F12 basal media mixture containing 1% penicillin-streptomycin and 10% fetal bovine serum (Sigma-Aldrich, USA) in a 5% CO_2 humidified incubator at 37 °C. The cells were seeded in four flasks at a density of 1×10^6 cells per flask for use in the plate reader. The cells were seeded in 35 mm^2 coverslips (Mattek Corporation Inc., USA) for confocal microscope (LSM800, Zeiss) analyses. The CUR (Cat_C1386) and MPP^+ (Cat_36913) were purchased from Sigma-Aldrich ((Sigma Aldrich, USA).

The SH-SY5Y cells were divided into four groups as follows;

Control (Cont) group: The cells in this group were not treated with any treatment.
CUR group: The cells in this group were incubated with CUR 5 µM for 24 h [23].
MPP+ group: The cells in this group were incubated with MPP^+ 0.5 mM for 24 h [6].
CUR + MPP+ group: The cells in this group were incubated with CUR (5 µM) and MPP^+ (0.5 mM) for 24 h.

Measurement of miROS, JC/1 and iROS levels in the SH-SY5Y cells

MitoSOX Red (Cat_M36008, ThermoFisher) is a superoxide indicator, a fluorogenic dye specifically targeted to mitochondria in living cells. It was used to determine mitochondria ROS (miROS) formation in mitochondria in 35 mm dishes. MitoSOX (2 µM) was added to the treated cells and incubated for 30 min at 37 °C in the dark. Three rounds of ice-cold PBS washing were performed on the treated cells. Following analysis of the produced samples, flow cytometry assessed the fluorescence intensity. A 20x objective lens was used to capture the images [24]. The argon laser's stimulation wavelength stayed at 561 nm in the red MitoSOX recordings. The excitation and emission wavelengths, on the other hand, stayed at 578 and 598 nm. DCFH-DA (Cat_ ab113851, Abcam) dye was used to detect intracellular free oxygen radicals (iROS) in the confocal microscope. DCFH-DA (2 µM) was added to the treated cells and incubated for 30 min at 37 °C in the dark. Three rounds of ice-cold PBS washing were performed on the treated cells. Following analysis of the produced samples, flow cytometry assessed the fluorescence intensity. A 20x objective lens was used to capture the images. In green DCFH-DA recordings, the argon laser excitation wavelength was kept at 488 nm, while the excitation and emission wavelengths were kept at 504 and 525 nm, respectively [25]. JC/1 (Cat_T3168, Thermo Fisher) dye was used to detect mitochondrial membrane potential in the confocal microscope. JC/1 (3 µM) was added to the treated cells and incubated for 30 min at 37 °C in the dark. Three rounds of ice-cold PBS washing were performed on the treated cells. Following analysis of the produced samples, flow cytometry

was used to assess the fluorescence intensity. A 20x objective lens was used to capture the images. The fluorescence intensity of JC/1 aggregates was detected at 590 nm after the cells were excited (488 nm) [26]. The obtained miROS (red), JC/1 (orange) and iROS (green) changes of the images in fluorescence intensity were measured as arbitrary units (a.u.) using the ZEN program.

Measurement of cytosolic GSH levels in the SH-SY5Y cells

To measure the amount of GSH in SH-SY5Y cells, a commercial probe called ThiolTracker Violet (Cat_T10095, Thermo Fisher Sci., Turkey) was employed. The excitation and emission wavelengths were maintained at 404 and 526 nm, respectively, to calculate the GSH level. At 405 nm, laser excitation was carried out. The ZEN software calculated the intensity of GSH fluorescence in each confocal microscope cell. A.u. was used to represent the mean GSH (green) concentration.

Measurement of intracellular calcium $[Ca^{2+}]_i$ concentration

Fluo/3-AM is a single-wavelength excitation and emission dye excited by an argon laser with a wavelength of 488 nm under a confocal microscope. In this study, which investigated the effect of CUR on MPP-induced intracellular calcium increase in SHSY5Y cells via TRPV4, incubation with Fluo/3-AM (1 µM for 45 min) fluorescent dye (Calbiochem, Germany) was used to detect intracellular changes in $[Ca^{2+}]_i$ concentration in SH-SY5Y. In addition, cells were stimulated with GSK1016790A (GSK) (100 nM), which is a TRPV4 agonist, and Ruthenium red (Rr) (1 µM), which is a TRPV4 antagonist, to compare channel activation between groups during this period [14]. Cells were examined at 515 nm under the confocal microscope. The mean fluorescence intensity in an a.u. per cell was used to express Fluo/3 results in 15 mm^2 of cytosol.

Measurements of cell viability, apoptosis, and caspase levels

Cell viability was assessed using the MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) technique. The method's main objective is to assess the colour shift that occurs when tetrazolium (MTT: yellow) is used to produce formazan (purple) dye by determining the absorbance of the growing mitochondrial dehydrogenase enzyme activity of proliferating cells. In 96-well plates, SH-SY5Y cells were pre-incubated at a density of 1×10^6 cells/mL. When the incubation period was over, the cells were removed from the flasks. They underwent centrifugation. PBS was used to dilute the pellet. Following pipetting, 15 µl of MTT dye (5 mg/ml) was added to each Eppendorf tube along with 950 µl of 1x PBS. After adding MTT dye, it was placed in a shaking water bath at 37 ºC and let to shake gradually for 90 min. All Eppendorf was shaken and then centrifuged for five minutes at 500 *g*. Following centrifugation, 400 µl of dimethyl sulfoxide (DMSO) was added to each Eppendorf tube, and the supernatant was disposed away. After resuspending the pellet in DMSO, 250 µl of the sample was applied to every well. MTT absorbance changes were measured at 490 and 650 nm using an automated microplate reader (Infinite PRO 200; Tecan Austria GmbH, Groedig, Austria). An APOPercentage commercial kit (Biocolor Ltd., Co Antrim, UK) was used to measure the apoptosis of SH-SY5Y cells. The SH-SY5Y cells were pre-incubated in 96-well plates at a density of 1×10^6 cells/mL. During the experiment, the transmembrane transport cycle of phosphatidylserine caused the apoptotic cells to acquire a pink-coloured dye. Before the onset of blebbing, this intake happened all at once. The automated pale reader (Infinite PRO200) captured the colour variations at 550 nm. The SH-SY5Y cells were pre-incubated in 96-well plates at a density of 1×10^6 cells/mL. After being incubated with apoptotic cell lysates on black plates, active Casp-3, Casp-8, and Casp-9 mostly cleaved fluorogenic substrates (Ac-DEVD-AMC, Ac-IETD-AFC, and Ac-LEHD-AFC) (Bachem AG., Bubendorf, Switzerland) to the AMC and AFCs, respectively. The microplate reader (Infinite 200 PRO) was used to measure the cleavage levels of free AMC and AFCs at the excitation (360–400 nm) and emission (460–505 nm) wavelengths. Fluorescence units per milligram of protein were computed after the Biuret method measured the cells' total protein levels. Experiments were repeated at least 3 times. Cell viability, apoptosis, Casp-3, Casp-8, and Casp-9 treatment outcomes increased above the control value.

Western blotting

The SH-SY5Y cells were subjected to conventional protocols for Western blotting protein analysis of expression [27]. The SH-SY5Y proteins were separated on SDS-PAGE gels and then put onto PVDF membranes. Using primary anti-TRPV4 (Cat_A37574, AFG Scientific, and 1: 500), the membranes were incubated for an entire night at 4 °C before being displayed using the Gel Imagination System (G: Box, Syngene, UK). ImageJ software was utilised for the test of TRPV4 and β-actin signal intensities. The relative density of the signal activities was used.

Statistical analyses

Data were expressed as the mean ± standard deviation. A one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was conducted using SPSS software to assess statistical significance among the various groups. Experiments were repeated at least 3 times. A significance level of $P < 0.05$ was considered statistically significant.

Results

CUR treatment attenuated MPP^+-induced miROS, JC/1 and iROS in the SH-SY5Y cells

Increased ROS in mitochondria is important for redox balance, while excessive ROS increase can trigger many damage mechanisms such as cascade activity in the cell, especially mitochondrial dysfunction [28]. In Fig. 1a, when miROS levels were examined between the groups, it was

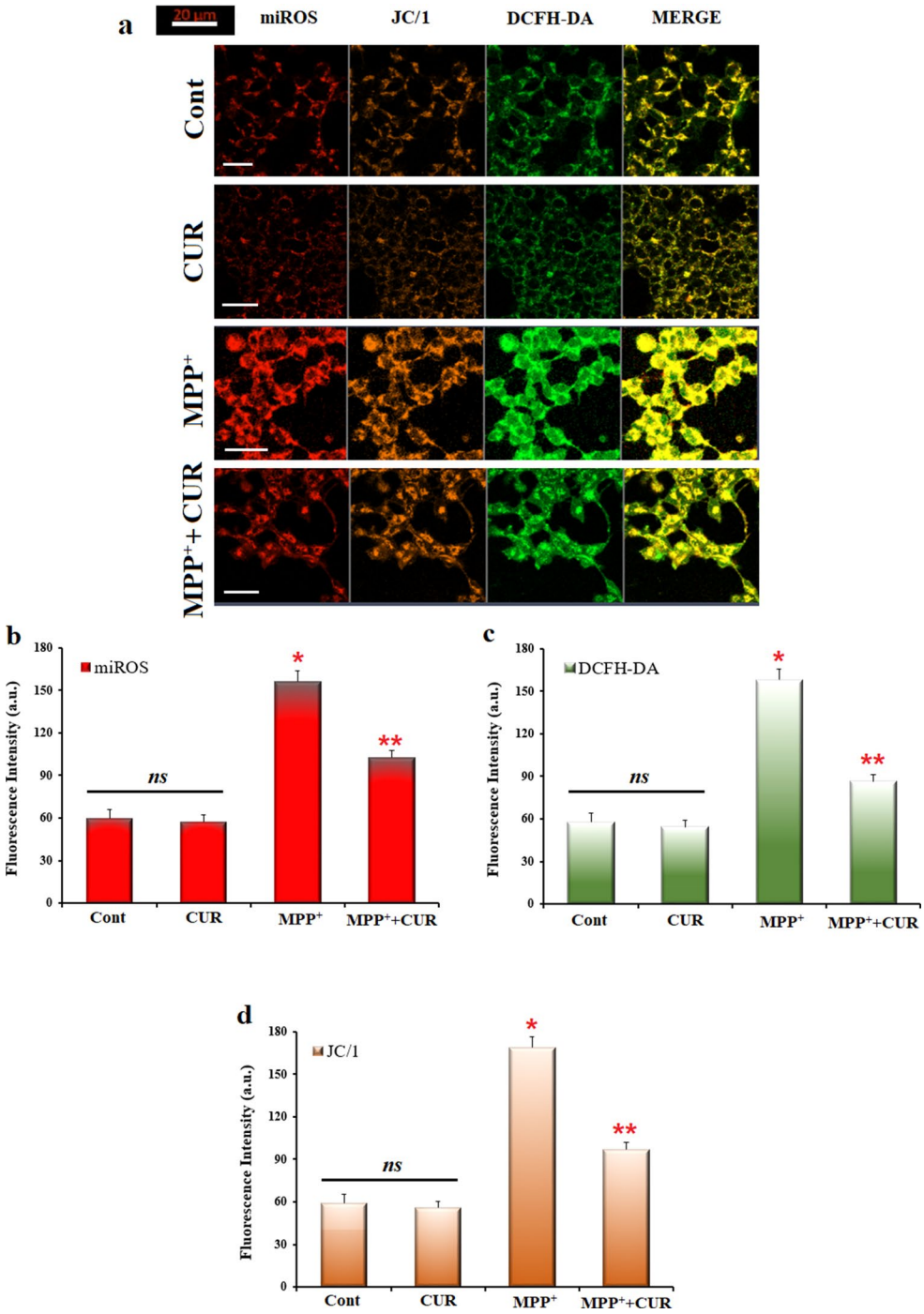

Fig. 1 The treatment of the CUR (5µM) attenuated MPP^+(0.5 mM)-induced miROS, JC/1 and DCFH-DA in the SH-SY5Y cells. (Mean ± SD). The images were captured in the LSM800 confocal microscope under an objective (20x, Bar: 20 µm) (**a**), and coloured pictures of the stains of miROS (red), JC-1 (orange), DCFH-DA (green), and overlay were captured. The miROS (**b**), JC/1 (**c**), and DCFH-DA (**d**) mean fluorescence intensity variations expressed in arbitrary units (a.u.). (non-significant (*ns*): $p > 0.05$, $^*p < 0.001$ vs. MPP^+, and $^{**}p < 0.01$ vs. MPP^++CUR)

determined that the highest miROS level in the cells was after MPP^+ application ($p<0.001$). CUR treatment with MPP^+ (MPP^++CUR) significantly reduced the miROS level compared to the MPP^+ group ($p<0.01$). There was no statistically significant difference between the control and CUR groups ($p>0.05$, Fig. 1b). The JC/1 stain is an important marker for examining mitochondrial membrane potential and determining mitochondrial health and activity [29]. When JC/1 levels were examined, it was seen that the JC/1 level of the MPP^+ group was the highest compared to the other groups ($p<0.001$). The JC/1 level of the MPP^++CUR group was significantly lower compared to the MPP^+ group ($p<0.01$, Fig. 1d). The DCFH-DA has no fluorescence. However, it can freely penetrate the cell membrane. Once inside the cell, DCFH-DA is hydrolyzed by intracellular esterase to produce DCFH. ROS then oxidizes DCFH to produce fluorescent 2',7'-dichlorofluorescein (DCF). The fluorescence intensity of DCF is used to measure the level of intracellular ROS [30]. When intracellular ROS levels were examined, it was determined that the iROS level was the highest in the MPP^+ group with MPP^+ application compared to the other groups ($p<0.001$). In the CUR + MPP^+ group, it was reported that CUR treatment significantly reduced the increased iROS level caused by MPP^+ compared to the MPP^+ group ($p<0.01$, Fig. 1c).

MPP^+-induced decreased intracellular GSH level was modulated by CUR treatment

GSH constitutes the main intracellular antioxidant defence against ROS and OS. Also, the fact that GSH depletion occurs at early stages during the cell death program during apoptosis indicates that it is a good marker for studies investigating cell damage mechanisms [31]. When GSH density was examined between the groups in the study (Fig. 2a), it was seen that MPP^+ reduced the GSH level in SH-SY5Y cells to the lowest level compared to the other groups ($p<0.001$). It was determined that CUR treatment and MPP^+ increased the GSH level compared to the MPP^+ group ($p<0.01$, Fig. 2b).

Effect of CUR treatment on MPP^+-induced $[Ca^{2+}]_i$ concentration change in SH-SY5Y cells: the role of TRPV4 channel

Cytosolic calcium is necessary for many physiological processes in the cell, such as growth, migration, and cell death. Disruption of intracellular calcium homeostasis can disrupt these physiological processes and damage the cell. TRPV4 is a cation channel permeable to the Ca^{2+} [32]. In Fig. 3a, we investigated how the free $[Ca^{2+}]_i$ fluorescence intensity in SH-SY5Y cells was affected by TRPV4 antagonist (Rr) and agonist (GSK). It was determined that without agonist and antagonist stimulation, the $[Ca^{2+}]_i$ intensity of the MPP^+ group was significantly higher than the other groups ($p<0.05$, Fig. 3b). The $[Ca^{2+}]_i$ intensity of the MPP^++CUR group was significantly lower than the MPP^+ group. After GSK stimulation, the $[Ca^{2+}]_i$ intensity of the MPP^+ group was significantly higher. At the same time, it was significantly lower in the groups with Rr incubation ($p<0.05$, Fig. 3c). It was determined that the $[Ca^{2+}]_i$ intensity of the MPP^++CUR group was lower than the MPP^+ group during the specified stimulations ($p<0.05$, Fig. 3b and c).

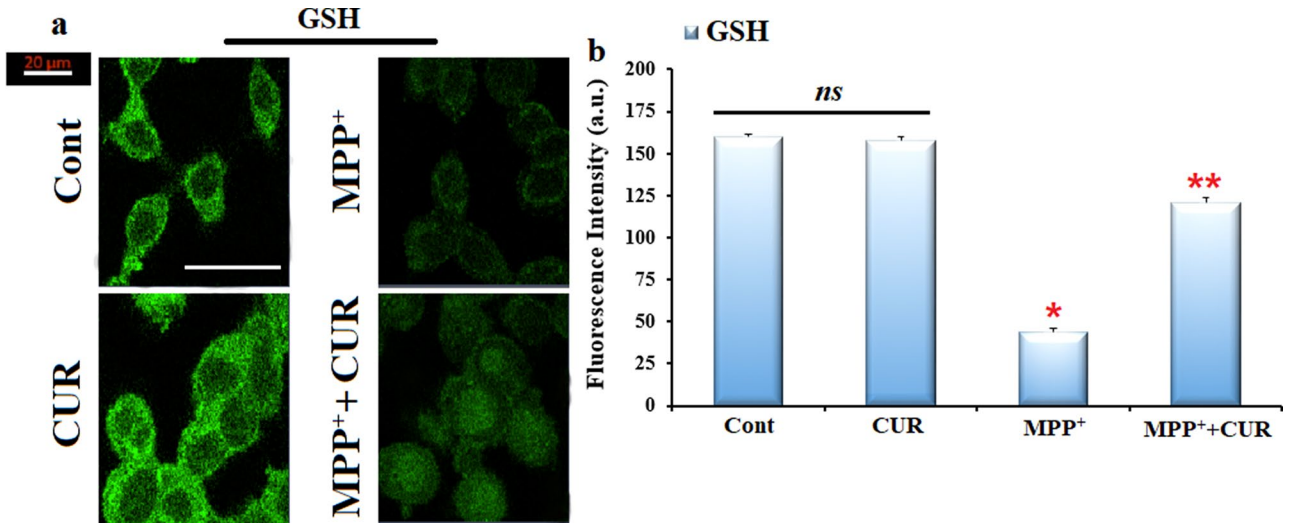

Fig. 2 MPP^+(0.5 mM) induced decreased intracellular GSH level was modulated by CUR (5µM) treatment in the SH-SY5Y cells. (Mean ± SD). To determine the quantity of GSH, the SH-SY5Y cells were stained with a 2 µM ThiolTracker Violet probe for 30 min. The image was captured in the LSM800 confocal microscope under an objective (20x, Bar: 20 µm) (**a**). The ThiolTracker Violet means a.u. (non-significant (*ns*): $p>0.05$, *$p<0.001$ vs. MPP^+, and **$p<0.001$ vs. MPP^++CUR)

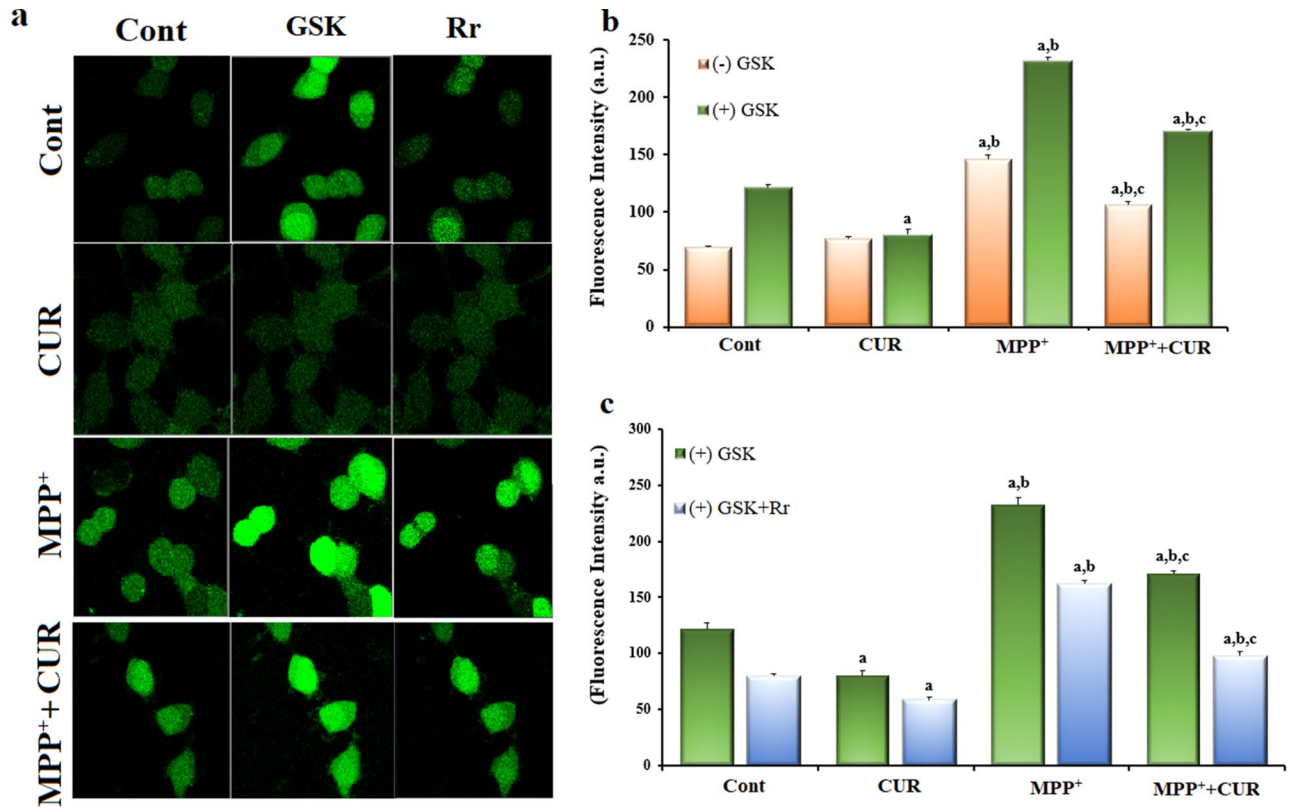

Fig. 3 Effect of CUR (5μM) treatment on MPP^+(0.5 mM)-induced $[Ca^{2+}]_i$ concentration change in SH-SY5Y cells and the role of TRPV4 channel in this mechanism. (Mean ± SD). The cells were stained with Fluo 3/AM (1 μM for 30 min) in dark and room temperature (**a**). TRPV4 in the SH-SY5Y cell was stimulated either by GSK (100 nM), although Ca^{2+} entry via the inhibition of TRPV4 was inhibited by Rr (1 μM). The green images of Fluo 3/AM in cells were recorded at 515 nm in the LSM800 confocal microscope attached with objective (×40 oil). The mean fluorescence intensity changes as an a.u. in the four groups after the MPP^+ and CUR treatments (**b** and **c**). ($^a p < 0.05$ vs. control group, $^b p < 0.05$ vs. CUR group, $^c p < 0.05$ vs. MPP^+ group)

CUR treatments altered the levels of MPP^+-induced cell viability, apoptosis, and caspases

Apoptosis, defined as programmed cell death, plays an important role in the processes that sustain the life of living organisms. Caspase activation plays a central role in the execution of apoptosis [33]. When apoptosis and caspase activity were examined among the groups in the study, it was determined that the apoptosis (Fig. 4a), Casp-3 (Fig. 4c), Casp-8 (Fig. 4d) and Casp-9 (Fig. 4e) levels of the MPP^+ group were significantly higher than the other groups and the cell viability rate (Fig. 4b) was lower ($p < 0.05$). On the other hand, with CUR treatment, it was observed that the apoptosis, Casp-3, Casp-8, and casp-9 levels of the MPP^+ group were significantly lower than the MPP^+ group, and the cell viability rate was higher ($p < 0.05$). There was no statistically significant difference between the apoptosis, Casp-3, Casp-8 and Casp-9 levels of the control and CUR groups ($p > 0.05$).

The expression levels of TRPV4 channel on MPP^+-induced neurotoxicity in SH-SY5Y cells

The TRPV4 expression level was measured by the Western blot method in all groups (Fig. 5a, Supplementary Fig. 5). The TRPV4 expression level of the MPP^+ group was at the highest level compared to the control, CUR and MPP^++CUR groups ($p < 0.05$). The TRPV4 expression level of the MPP^++CUR group was significantly lower compared to the MPP^+ group ($p < 0.05$, Fig. 5b).

Discussion

Parkinson's disease is a common neurodegenerative disease characterized by motor and non-motor symptoms [3, 4]. One of the most used cell lines in in vitro PD model studies is the SH-SY5Y cell line. Additionally, it was determined that the MPP^+ agent, which disrupts the electron transport chain in the cell and causes cell death by free radical formation, was used to create a PD model in the SH-SY5Y cell line [5, 6, 8, 9]. This study investigated the effect of CUR

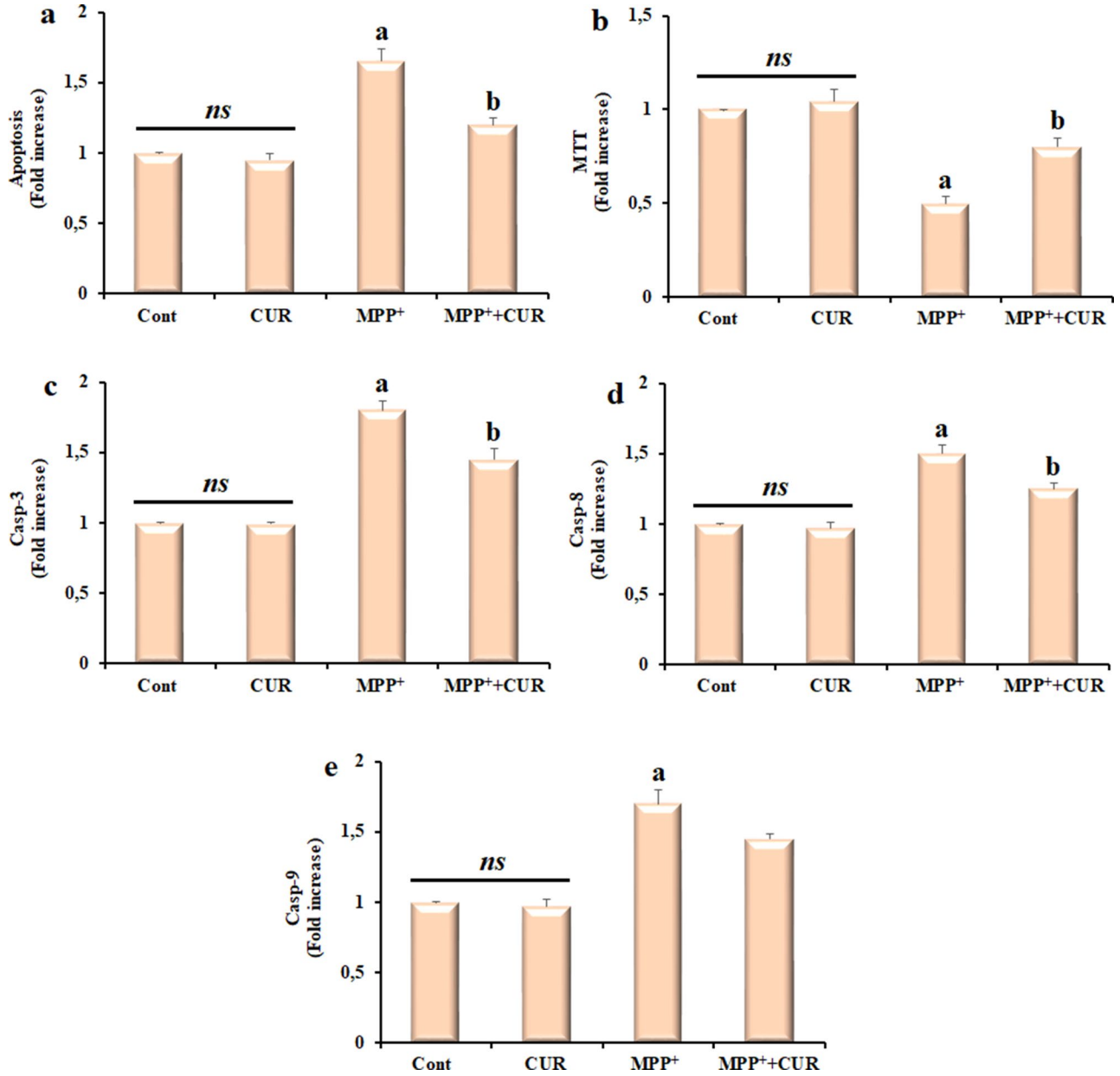

Fig. 4 MPP^+ (0.5 mM for 24 h)-induced increased apoptosis and caspase activities in SH-SY5Y cells were decreased by CUR treatment (5µM for 24 h). (Mean±SD, $n=3$). APOpercantage assay kit (**a**) and MTT test (**b**) were used in the plate reader (Infinite) analysis to execute cell viability and apoptosis in the cells, respectively. The active Casp-3 (**c**), Casp-8 (**d**), and Casp-9 (**e**) studies were carried out using the caspase fluorogenic substrates. (non-significant (*ns*): $p>0.05$, $^{a}p < 0.05$ vs. control group, $^{b}p < 0.05$ vs. CUR group, $^{c}p < 0.05$ vs. MPP^+ group)

on OS, mitochondrial dysfunction, apoptosis and $[Ca^{2+}]_i$ accumulation through stimulation of the TRPV4 channel in MPP^+-induced neuronal damage in SH-SY5Y cells.

Excessive OS levels in cells are directly proportional to the increase in ROS, which can damage different biological targets such as proteins, lipids or DNA, leading to various pathologies. mirrors may have two roles: on the one hand, they maintain a proper redox balance. On the other hand, excessive production of miROS is known to be involved in cancer, hypertension, atherosclerosis, diabetes, autoimmune disorders, or age-related diseases [33]. MPP^+ binds to NADH dehydrogenase in the mitochondrion, stopping the electron transport chain and causing OS [34]. Yan et al. investigated the protective effect of artemisinin on OS, which plays an important role in PD pathogenesis. In this study, it was reported that iROS levels increased and antioxidant (SOD, GSH) levels decreased in SH-SY5Y cells after MPP^+ application. In addition, a significant increase was observed in the JC/1 dye density used for mitochondrial membrane potential in the MPP^+ treatment group compared

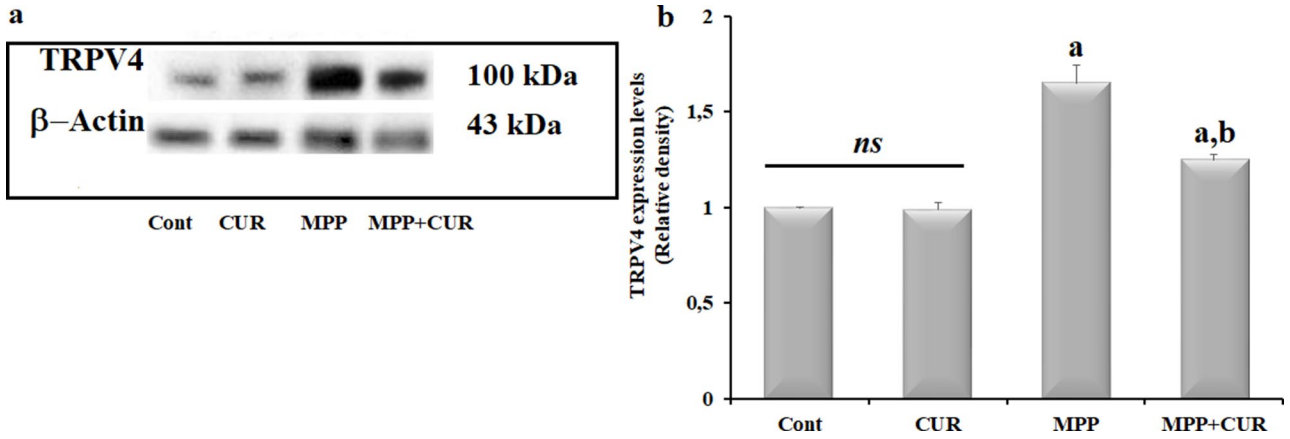

Fig. 5 MPP^+ (0.5 mM -induced increases of TRPV4 expression levels were decreased by CUR treatment (5µM). (Mean ± SD, $n = 3$). A. The protein band of TRPV4 channel (**a**). The TRPV4 (**b**) protein expression levels were calculated from the protein bands. β-Actin was used as load control. ((non-significant (*ns*): $p > 0.05$, $^a p < 0.05$ vs. control and CUR groups, $^b p < 0.05$ vs. MPP^+ group)

to the control group. In addition, an increase in the protein expression level of caspase-3 and cleaved caspase-3 was reported in the MPP^+ treatment group [35]. Liu et al. reported that MPP^+-induced ROS accumulation, accompanied by a depolarization of mitochondrial membrane potential and a decreased cell viability in PD model SH-SY5Y cells. Furthermore, it was stated that tyrosine hydroxylase, peroxiredoxin-2 and SIRT1 levels decrease, while Bax and Bcl-2 rates increased [36]. This study observed a significant increase in miROS, ROS and JC/1 levels in SH-SY5Y cells after MPP^+ application. In contrast, a significant decrease in miROS, ROS and JC/1 levels was reported in the MPP^++CUR group compared to the MPP^+ group. Accordingly, in the literature, we observed that CUR treatment may be an important therapeutic agent for the increased miROS, ROS and JC/1 levels with MPP^+ application (Fig. 1).

Wang et al. reported increased production of ROS, up-regulation of NADPH oxidase-2 expression, down-regulation of SOD activity, and decreased GSH levels in MPP-treated neuroblastoma cells [37]. Gong et al. studied the effect of tectorigenin on MPP^+-induced neurotoxicity in SH-SY5Y cells. In their study, treatment with MPP^+ caused a significant decrease in cell viability and increased apoptosis, as evidenced by the upregulation of apoptotic cells, caspase-3 activity and cytochrome c expression. In addition, they reported a significant decrease in the levels of superoxide dismutase, catalase and glutathione peroxidase [5]. Song et al. investigated the effect of baicalein, a bioactive flavone compound, against MPP^+-induced neurotoxicity, and they observed an increase in MDA and ROS levels and a significant decrease in GSH level in MPP^+-treated SH-SY5Y cells [10]. In our study, when GSH density was examined between the groups, it was noted that MPP^+ decreased the GSH level in SH-SY5Y cells to the lowest level compared to other groups. It was determined that applying CUR treatment together with MPP^+ increase the GSH level compared to the MPP^+ group (Fig. 2). Thus, we showed that CUR could modulate the MPP+-caused low level with its antioxidant properties.

TRPV4 is a non-voltage-gated tetrameric non-selective cation channel. Studies have emphasised that TRPV4 can trigger Ca^{2+} release from internal stores together with Ca^{2+} influx from outside the cell [32, 38]. Özşimşek and Naziroğlu emphasized that hypoxia-induced excessive miROS production and excessive Ca^{2+} entry into the cell via TRPV4 inhibition cause damage in SH-SY5Y neuronal cells. TRPV4 agonist (GSK) and antagonist (Rr) were used to understand the role of the TRPV4 channel in the hypoxia-induced damage mechanism, and it was reported that TRPV4 channel inactivation prevented excessive Ca^{2+} entry into the cell [39]. In another study, the role of the TRPV4 channel in MPP^+-induced endoplasmic reticulum stress in PC12 cells was examined, and it was emphasized that TRPV4 expression increased in MPP^+-induced neurotoxicity, leading to an increase the channel activation due to damage [40]. The current study demonstrates that excessive OS generation and overloaded Ca^{2+} influx in SH-SY5Y cells activated TRPV4. However, treatment with CUR reduces OS generation and increases GSK-induced TRPV4 current density, protein expression, and $[Ca^{2+}]_i$ concentration in the cells (Fig. 3).

In the nervous system, programmed cell death is degenerative and genetically controlled in functional cells. Casp-3, 8 and 9 are proteases that play essential roles in apoptosis. Casp-8 initiates the extrinsic apoptosis pathway, activated by death signals outside the cell. In contrast, caspase-9 initiates the intrinsic pathway, activated by signals such as intracellular stress and DNA damage. Both paths lead to the activation of casp-3. Casp-3, as an effector caspase, cleaves intracellular proteins and ensures apoptosis occurs [41, 42]. Therefore significant progress in understanding

the structure and functional role of TRPV4, especially in neurological diseases, will be an important target for new therapeutic approaches [16]. Liu et al. reported increased expression of apoptosis-related proteins such as Bax/Bcl-2, GSK3β, Casp-3 and p53 nuclear ratio induced by MPP^+ in SH-SY5Y cells. In a study examining TRPM2 and TRPV4 activation in SH-SY5Y neuronal, BV-2 microglial and HEK293 cells, it was observed that OS/ADPR and GSK in cells caused an increase in TRPM2/TRPV4 current densities and an increase in mitochondrial membrane potential, iROS and miROS, leading to an overload of $[Ca^{2+}]_i$ flux. In parallel with the increase in $[Ca^{2+}]_i$ concentration, Casp-3 and Casp-9 levels were reported to increase [43]. In this study, when we examined apoptosis and caspase activity between the groups, we found that apoptosis, Casp-3, Casp-8, and Casp-9 levels of the MPP^+ group were significantly higher than other groups, and the cell viability rate was inversely proportional to these. On the other hand, with CUR treatment, the apoptosis, Casp-3, Casp-8, and Casp-9 levels of the MPP^++CUR group were significantly lower than the MPP^+ group, and the cell viability rate was higher (Fig. 4). Finally, our Western blot data shows a high level of TRPV4 expression in MPP^+ group compared to the control and CUR groups. TRPV4 expression levels of the MPP^++CUR group were significantly lower compared to the MPP^+ group (Fig. 5).

Conclusion

The study results showed that the increased MPP^+-mediated adverse effects such as mROS, iROS and antioxidant decrease in the PD in vitro model were modulated by the inhibition of TRPV4 and up-regulation of the GSH antioxidant system in cells. Moreover, CUR treatment in SH-SY5Y cells restored the decreases in MPP^+-induced antioxidant, apoptotic and mitochondrial oxidant effects by suppressing TRPV4 and increasing the glutathione redox system. Future studies will provide new therapeutic approaches for PD by preventing oxidative neurodegeneration by inhibiting TRPV4 in neuronal cells.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-025-10345-1.

Acknowledgements The authors thank BSN Health, Analyses, Innov., Consulting, Org., Agriculture., Trade Ltd., Isparta, Türkiye, for providing the necessary facilities to conduct this study.

Author contributions Study Design: RÇ and KY, Data collection: All authors. Statistical analysis: RÇ and KY, Data interpretation: RÇ and KY, Literature Search: RÇ and KY, Writing– review & editing: KY. All authors reviewed the manuscript. The authors declare that all data were generated in-house, and no paper mill was used.

Funding There is no financial disclosure of the current study.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

References

1. Chi H, Chang H-Y, Sang T-K (2018) Neuronal cell death mechanisms in major neurodegenerative diseases. Int J Mol Sci 19(10):3082
2. Van Schependom J, D'haeseleer M (2023) Advances in neurodegenerative diseases. MDPI, p 1709 https://doi.org/10.3390/jcm12051709
3. Zeng R, Wang J, Zheng C, Jiang R, Tong S, Wu H, Zhuo Z, Yang Q, Leung FW, Sha W (2023) Lack of causal associations of inflammatory bowel disease with Parkinson's disease and other neurodegenerative disorders. Mov Disord 38(6):1082–1088
4. Morris HR, Spillantini MG, Sue CM, Williams-Gray CH (2024) The pathogenesis of Parkinson's disease. Lancet 403(10423):293–304
5. Gong P, Deng F, Zhang W, Ji J, Liu J, Sun Y, Hu J (2017) Tectorigenin attenuates the MPP+–induced SH–SY5Y cell damage, indicating a potential beneficial role in Parkinson's disease by oxidative stress inhibition. Experimental Therapeutic Med 14(5):4431–4437
6. Yıldızhan K, Nazıroğlu M (2022) Protective role of selenium on MPP+and homocysteine-induced TRPM2 channel activation in SH-SY5Y cells. J Recept Signal Transduction 42(4):399–408
7. Zaim M, Kara I, Muduroglu A (2021) Black carrot anthocyanins exhibit neuroprotective effects against MPP+induced cell death and cytotoxicity via inhibition of oxidative stress mediated apoptosis. Cytotechnology 73(6):827–840
8. Wang Y, Gao J, Miao Y, Cui Q, Zhao W, Zhang J, Wang H (2014) Pinocembrin protects SH-SY5Y cells against MPP+-induced neurotoxicity through the mitochondrial apoptotic pathway. J Mol Neurosci 53:537–545
9. Ramalingam M, Kim SJ (2016) The neuroprotective role of insulin against MPP+-induced parkinson's disease in differentiated SH-SY5Y cells. J Cell Biochem 117(4):917–926
10. Song Q, Peng S, Zhu X (2021) Baicalein protects against MPP+/MPTP-induced neurotoxicity by ameliorating oxidative stress in SH-SY5Y cells and mouse model of Parkinson's disease. Neurotoxicology 87:188–194
11. Chaigne S, Barbeau S, Ducret T, Guinamard R, Benoist D (2023) Pathophysiological roles of the TRPV4 channel in the heart. Cells 12(12) https://doi.org/10.3390/cells12121654
12. Nazıroğlu M, Öz A, Yıldızhan K (2020) Selenium and neurological diseases: focus on peripheral pain and TRP channels. Curr Neuropharmacol 18(6):501–517
13. Rosenbaum T, Benítez-Angeles M, Sánchez-Hernández R, Morales-Lázaro SL, Hiriart M, Morales-Buenrostro LE, Torres-Quiroz F (2020) TRPV4: a physio and pathophysiologically significant ion channel. Int J Mol Sci 21(11):3837
14. Osmanlıoğlu HÖ, Nazıroğlu M (2024) Resveratrol modulates diabetes-induced neuropathic pain, apoptosis, and oxidative neurotoxicity in mice through TRPV4 channel inhibition. Mol Neurobiol 61(9):7269–7286
15. Sánchez JC, Muñoz LV, Ehrlich BE (2020) Modulating TRPV4 channels with paclitaxel and lithium. Cell Calcium 91:102266

16. Ma Q, Wu J, Li H, Ma X, Yin R, Bai L, Tang H, Liu N (2024) The role of TRPV4 in programmed cell deaths. Mol Biol Rep 51(1):248
17. Sun X, Kong J, Dong S, Kato H, Sato H, Hirofuji Y, Ito Y, Wang L, Kato TA, Torio M (2023) TRPV4-mediated Ca2 + deregulation causes mitochondrial dysfunction via the AKT/α-synuclein pathway in dopaminergic neurons. FASEB BioAdvances 5(12):507
18. Razavi BM, Ghasemzadeh Rahbardar M, Hosseinzadeh H (2021) A review of therapeutic potentials of turmeric (Curcuma longa) and its active constituent, curcumin, on inflammatory disorders, pain, and their related patents. Phytother Res 35(12):6489–6513
19. Kaur K, Al-Khazaleh AK, Bhuyan DJ, Li F, Li CG (2024) A review of recent curcumin analogues and their antioxidant, anti-inflammatory, and anticancer activities. Antioxidants 13(9):1092
20. Panda AK, Chakraborty D, Sarkar I, Khan T, Sa G (2017) New insights into therapeutic activity and anticancer properties of curcumin. J Exp Pharmacol, 31–45
21. Huyut Z, Uçar B, Yıldızhan K, Altındağ F (2023) The protective effect of curcumin on cardiac markers and fibrosis in abemaciclib-induced cardiac damage in rats. J Biochem Mol Toxicol 37(1):e23226
22. Nebrisi EE (2021) Neuroprotective activities of curcumin in Parkinson's disease: a review of the literature. Int J Mol Sci 22(20):11248
23. Öz A, Çelik Ö (2016) Curcumin inhibits oxidative stress-induced TRPM2 channel activation, calcium ion entry and apoptosis values in SH-SY5Y neuroblastoma cells: involvement of transfection procedure. Mol Membr Biol 33(3–5):76–88
24. Lee TK, Ashok Kumar K, Huang CY, Liao PH, Ho TJ, Kuo WW, Liao SC, Hsieh DJY, Chiu PL, Chang YM (2023) Garcinol protects SH-SY5Y cells against MPP+-induced cell death by activating DJ-1/SIRT1 and PGC-1α mediated antioxidant pathway in sequential stimulation of p-AMPK mediated autophagy. Environ Toxicol 38(4):857–866
25. Yıldızhan K, Nazıroğlu M (2023) NMDA receptor activation stimulates hypoxia-induced TRPM2 channel activation, mitochondrial oxidative stress, and apoptosis in neuronal cell line: modular role of memantine. Brain Res 1803:148232
26. Osmanlıoğlu HÖ, Yıldırım MK, Akyuva Y, Yıldızhan K, Nazıroğlu M (2020) Morphine induces apoptosis, inflammation, and mitochondrial oxidative stress via activation of TRPM2 channel and nitric oxide signaling pathways in the hippocampus. Mol Neurobiol 57(8):3376–3389
27. Yıldızhan K, Nazıroğlu M (2020) Glutathione depletion and parkinsonian neurotoxin MPP+-induced TRPM2 channel activation play central roles in oxidative cytotoxicity and inflammation in microglia. Mol Neurobiol 57(8):3508–3525
28. Zorov DB, Juhaszova M, Sollott SJ (2014) Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev 94(3):909–950
29. Carrageta DF, Freire-Brito L, Oliveira PF, Alves MG (2022) Evaluation of human spermatozoa mitochondrial membrane potential using the JC-1 dye. Curr Protocols 2(9):e531
30. Yu D, Zha Y, Zhong Z, Ruan Y, Li Z, Sun L, Hou S (2021) Improved detection of reactive oxygen species by DCFH-DA: New insight into self-amplification of fluorescence signal by light irradiation. Sens Actuators B 339:129878
31. Franco R, Cidlowski J (2009) Apoptosis and glutathione: beyond an antioxidant. Cell Death Differ 16(10):1303–1314
32. Jiang D, Guo R, Dai R, Knoedler S, Tao J, Machens H-G, Rinkevich Y (2024) The multifaceted functions of TRPV4 and calcium oscillations in tissue repair. Int J Mol Sci 25(2):1179
33. Agod Z, Fekete T, Budai MM, Varga A, Szabo A, Moon H, Boldogh I, Biro T, Lanyi A, Bacsi A (2017) Regulation of type I interferon responses by mitochondria-derived reactive oxygen species in plasmacytoid dendritic cells. Redox Biol 13:633–645
34. Rubio-Osornio M, Orozco-Ibarra M, Díaz-Ruiz A, Brambila E, Boll M-C, Monroy-Noyola A, Guevara J, Montes S, Ríos C (2017) Copper sulfate pretreatment prevents mitochondrial electron transport chain damage and apoptosis against MPP+-induced neurotoxicity. Chemico-Biol Interact 271:1–8
35. Yan J, Ma H, Lai X, Wu J, Liu A, Huang J, Sun W, Shen M, Zhang Y (2021) Artemisinin attenuated oxidative stress and apoptosis by inhibiting autophagy in MPP+-treated SH-SY5Y cells. J Biol Research-Thessaloniki 28:1–10
36. Liu M, Zuo S, Guo X, Peng J, Xing Y, Guo Y, Li C, Xing H (2023) The study of overexpression of Peroxiredoxin-2 reduces MPP+-Induced toxicity in the cell model of Parkinson's Disease. Neurochem Res 48(7):2129–2137
37. Wang CY, Sun ZN, Wang MX, Zhang C (2018) SIRT1 mediates salidroside-elicited protective effects against MPP+-induced apoptosis and oxidative stress in SH-SY5Y cells: involvement in suppressing MAPK pathways. Cell Biol Int 42(1):84–94
38. Dunn KM, Hill-Eubanks DC, Liedtke WB, Nelson MT (2013) TRPV4 channels stimulate Ca2+-induced Ca2 + release in astrocytic endfeet and amplify neurovascular coupling responses. Proc Natl Acad Sci 110(15):6157–6162
39. Özşimşek A, Nazıroğlu M (2021) The involvement of TRPV4 on the hypoxia-induced oxidative neurotoxicity and apoptosis in a neuronal cell line: protective role of melatonin. Neurotoxicology 87:136–148
40. Liu N, Liu J, Wen X, Bai L, Shao R, Bai J (2020) TRPV4 contributes to ER stress: relation to apoptosis in the MPP+-induced cell model of Parkinson's disease. Life Sci 261:118461
41. Wu Y, Zhao D, Zhuang J, Zhang F, Xu C (2016) Caspase-8 and caspase-9 functioned differently at different stages of the cyclic stretch-induced apoptosis in human periodontal ligament cells. PLoS ONE 11(12):e0168268
42. Kashyap D, Garg VK, Goel N (2021) Intrinsic and extrinsic pathways of apoptosis: role in cancer development and prognosis. Adv Protein Chem Struct Biology 125:73–120
43. Nazıroğlu M (2022) A novel antagonist of TRPM2 and TRPV4 channels: Carvacrol. Metab Brain Dis 37(3):711–728