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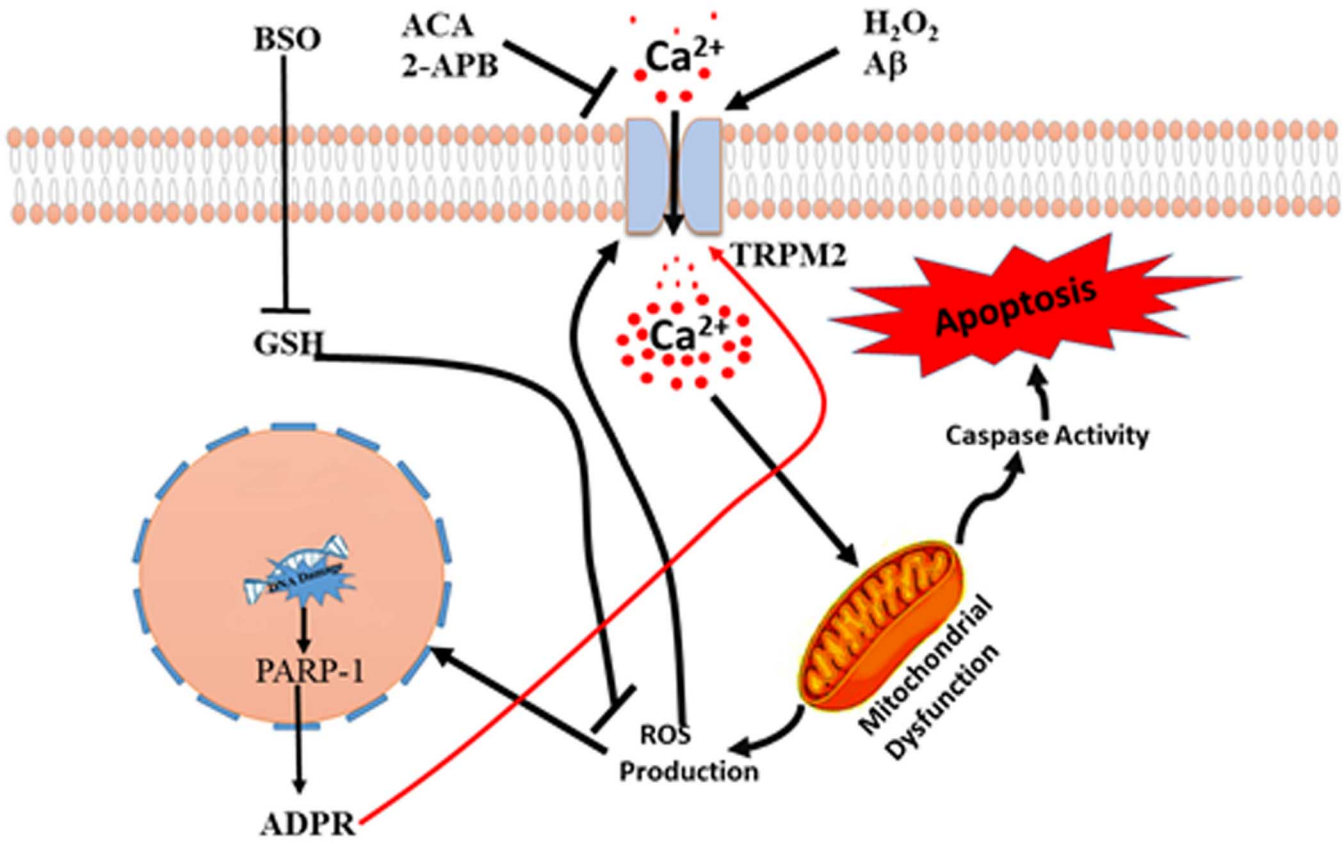

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AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate 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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Depletion of glutathione induced apoptosis and oxidative stress via the activation of TRPM2 channels in the microglia cells with Alzheimer' disease model

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List of Abbreviations;

ADPR, *ADP-ribose;* ***BSO,*** *buthionine sulphoximine;* ***Ca^{2+},*** *calcium ion;* ***Casps-3,*** *caspase -3;* ***Casps-9,*** *caspase -9;* ***cyROS,*** *cytosolic reactive oxygen species;* ***GSH,*** *glutathione;* ***ROS****, reactive oxygen species;* ***TRPM2,*** *transient receptor potential melastatin 2*

Abstract

Alzheimer's disease is a common neurodegenerative disease. Microglia induces oxidative stress in the brain for engulfing bacteria and viruses. The accumulating data indicate that oxidative stress and apoptosis are two main actors for the induction of microglia activation-induced Alzheimer's Disease. Oxidative stress is one of many triggers that activate the transient receptor potential melastatin 2 (TRPM2) channel. Glutathione (GSH) is a main cytosolic antioxidant in the mammalian cells. The GSH depletion via the activation of TRPM2 induces oxidative stress and apoptosis in neuronal cells. It has not yet been researched how GSH depletion via activation of TRPM2 affects oxidative stress and apoptosis in microglial cells with the Alzheimer's disease model. The BV2 cells divided into 5 groups as control, buthionine sulphoximine (BSO and 0.5 mM for 6h), amyloid beta (Aβ and 1 μM for 72h), Aβ+BSO, and Aβ+BSO+GSH (10 mM for 2h). In the BSO group, the levels of apoptosis, mitochondrial membrane potential, cytosolic free oxygen reactive species (cyROS), caspase (Casps) -3, Casps -8, and Casps -9 were

increased as compared to the control group, although cell viability level was decreased. The expression levels of TRPM2, Casps -3, Casps -9, Bax, Bcl-2, and PARP-1 were also increased in the BSO group. In addition, their levels were further increased in the Aβ and BSO+Aβ groups as compared to the BSO group. However, the changes were modulated in the BSO+Aβ+GSH group by the incubation of GSH. In conclusion, the depletion of GSH increased apoptosis and cyROS levels via activation of caspases and TRPM2 in the Aβ-induced microglia cells. The treatment of GSH may be a potential target on the apoptosis and oxidative stress in the Aβ-induced microglia cells.

Keywords: Alzheimer's disease, Neurodegenerative Disease, TRPM2 Channels, Apoptosis, Glutathione, Oxidative Stress

Introduction

Alzheimer's disease is a common neurodegenerative disease. In the USA, about 6 million people are suffering from the Alzheimer's disease (Pandey et al. 2020). The etiology of Alzheimer's disease has not been fully discovered. However, accumulating data indicate that oxidative stress and excessive Ca^{2+} influx are two main possible actors for the induction of Alzheimer's Disease (Övey and Nazıroğlu 2021).

Cytosolic reactive oxygen species (cyROS) generally consist of superoxide anion, H_2O_2, and single oxygen (Butterfield 2003). The species produced from the free oxygen species fight the bacteria and viruses in phagocytic cells, including microglia (Staerck et al. 2017). Hence, the invasion of bacteria and viruses in the brain are inhibited by the phagocytic activity of microglia (Simpson and Oliver 2020). Thus, cyROS are an essential part of the arsenal employed by microglia in the brain defense. Moreover, there is evidence that cyROS has a serious role in the pathogenesis of many diseases (especially in the brain system and neurons) and in neurological diseases that are vulnerable to oxidative stress (Halliwell 2006; Nazıroğlu 2007a). The purpose of cyROS is to keep unsaturated fatty acids in neurons and the brain, making these organs insensitive to oxidative damage. Increasing evidence of these physiological conditions suggests that cyROS regulate neuronal signals in both the central nervous system and the peripheral nervous system (Halliwell 2006). However, this peroxidative damage is protected by antioxidants in neurological cells (Butterfield 2003; Halliwell 2006; Nazıroğlu 2007a; Naziroğlu 2007b; Bond and greenfield 2007). The antioxidant defense mechanism consists of enzymatic and non-enzymatic systems (Dringen 2005; Naziroğlu 2007a). Enzymatic defense mechanisms include glutathione peroxidase, although non-enzymatic antioxidant defense system includes glutathione (GSH). Glutathione peroxidase enzyme uses GSH as substrate, and it reduces H_2O_2 to water and can also generate natural hydroperoxides. Low glutathione peroxidase activity and GSH levels were reported in the plasma of patients with Alzheimer's disease (Jiang et al. 2021).

Excessive proliferation of extracellular amyloid-beta (1-42) (Aβ) plaques and neurofibrillary tangles in the cytosol of hippocampus play an important role in the cellular pathogenesis of Alzheimer's disease. Previous scientific studies have determined that Aβ disrupts the cell membrane, permeability, and membrane integrity. In addition, it negatively affects the function of the ion channels in the membrane (Sciacca el al. 2012; Mucke and Selkoe 2012). The disruption of the cell membrane structure causes an excessive flow of calcium ion (Ca^{2+}) to the cytosol via the activation of transient receptor potential melastatin 2 (TRPM2) and causes the production of cyROS in neuron cells, including microglia and hippocampus (Çınar and Nazıroğlu 2022). Excessive cytosolic free Ca^{2+} ($cyCa^{2+}$) density causes separation of the ionic content of the intermembrane space in the mitochondria. The Dysfunction of mitochondria via excessive Ca^{2+} entry into the cytosol stimulates the generation of endogenous cyROS, apoptosis, and caspase activities (Singh et al. 2011; Zhang et al. 2015). Considering both cyROS overproduction and Ca^{2+} over accumulation in neurons, it has been revealed that it plays an important role in the pathogenesis of neurodegenerative diseases. Antioxidants and Ca^{2+} channel blockers can modulate dysfunction and aid normal cellular function (Wang et al. 2004; Hool 2008).

GSH is a main antioxidant in the cytosol of mammalian cells. The interaction between TRPM2 activation GSH depletion was recently reported in several neurons, including microglia (Yıldızhan and Nazıroğlu 2020; Övey and Nazıroğlu 2021). In the microglia activation, the involvement of GSH depletion via the activation of TRPM2 was well documented in the literature. However, the subject has not been clarified in the microglia of experimental Alzheimer disease model.

In the current study, I aimed to investigate GSH depletion-induced apoptosis and oxidative stress via the activation of TRPM2 channels in the Alzheimer' disease model of microglia cells

Materials and Methods

Cell culture

BV2 glia cells (ATCC, Manassas, Virjinya USA) were grown in Dulbecco's Modified Eagle's Medium (DMEM) culture containing 10% fetal bovine serum and 1% penicillin/streptomycin solution in an incubator providing 37 C° and 5% CO_2 living conditions.

Study groups

***Control group**:*

The microglia cells in the group were kept in the incubator without treatments of BSO, GSH and Aβ.

Buthionine sulphoximine (BSO) group:

BV2 cells in the group were incubated with 0.5 mM BSO (Cat No: 83730-53-4 Calbiochem, St. Louis, Missouri, ABD) for 6 hours (Yıldızhan and Nazıroğlu 2020; Övey and Naziroğlu 2015)

Aβ group:

BV2 cells in the group were incubated with Aβ 1 μM Aβ (1-42) (Cat No: RP10017-1. Genscript Inc, Galaxis West Lobby, Singapore) for 72 hours (Abe and Misawa 2003).

***Aβ +BSO group**:*

BV2 cells in the group were incubated with 1 μM Aβ (1-42) for 72 hours, then 0.5 mM BSO was added, incubated for 6 hours (Abe and Misawa 2003; Yıldızhan and Nazıroğlu 2020; Övey and Naziroğlu 2015)

Aβ +BSO+GSH group:

BV2 cells in the group were incubated with the Aβ (1-42) and BSO combination, and then they were additionally incubated with 10 mM GSH for 2 h.

Analysis of cell viability

The MTT test is a colorimetric assay used to determine cell metabolic activity and to demonstrate mitochondrial activity. MTT is a yellow dye that turns into purple product formazan by cellular enzymes. BV2 glia cells were incubated with MTT dye (0.5 mg/ml dose) at 37°C in the dark for 1.30 hours. Then the supernatant was removed, and DMSO was added to separate the formazan crystals. MTT testing was performed using the same absorbance wavelengths (490 and 650 nm) for each well using a multi-well plate reader (Infinite pro200; Tecan, GmbH, Groedig, Austria) (Güzel et al. 2021).

Assays of apoptosis level, caspase-3 caspase- 8, and caspase- 9 activities

Apoptosis analysis was performed using the appropriate APOPercentage apoptosis kit (Biocolor Ltd., Northern Ireland) with the optimal protocol (Kahya et al. 2017). The protocols of caspase -3 (Casps-3), caspase -8 (Casps-8), and caspase -9 (Casps-9) activities were assayed according to the substrate application. Casps-3 substrate (AC-DEVD-AMC), Casps-8 (Ac-IETD-AFC) and Casps-9 (AC-LEHD-AMC) substrate cleavages were measured with a multi-well plate reader (Infinite pro200) with an excitation wavelength of 380 nm and an emission wavelength at 460 nm (Kahya et al. 2017).

The calculation of mitochondrial membrane potential

Mitochondrial activity is an important precursor in the early stages of apoptosis. For this aim, BV2 glia cells are incubated with 1 μM JC-1 for 15 min at 37 °C. JC-1 as a cationic dye provides potential dependent accumulation in mitochondria (Uğuz et al. 2015). The decrease in red-green fluorescence intensity demonstrates the depolarization of mitochondria (Santo-Domingo and Demaurex 2010). The green JC-1 signal was measured at excitation 485 nm and emission at 535 nm, while the JC-1 red signal excitation was measured at 540 nm and emission at 590 nm. Changes in fluorescence intensity were measured using a multi-well reader (Infinite pro200) (Yildizhan et al. 2022)

The measurement of cyROS production

cyROS are reactive molecules containing oxygen. If the excessive increase of cyROS, which is a metabolic product in the cell, cannot be reduced to the normal level in the cell, it causes lipid peroxidation and DNA damage, leading to cell damage. Analysis of cyROS was made with a multi-well reader (Infinite pro200). Non-fluorescent DHR 123, which can easily penetrate the cell membrane, is an uncharged dye. After getting into the cell, DHR 123 becomes a fluorescent dye upon oxidation and transforms into rhodamine 123 (Rh 123). The fluorescence intensity is proportional to the production of cyROS. During this oxidation, the amount of free oxygen radicals in the cell formed by the oxidation of fats can be calculated. The more fluorescence disseminated show the presence of free oxygen radicals (Uğuz et al. 2016). The fluorescence

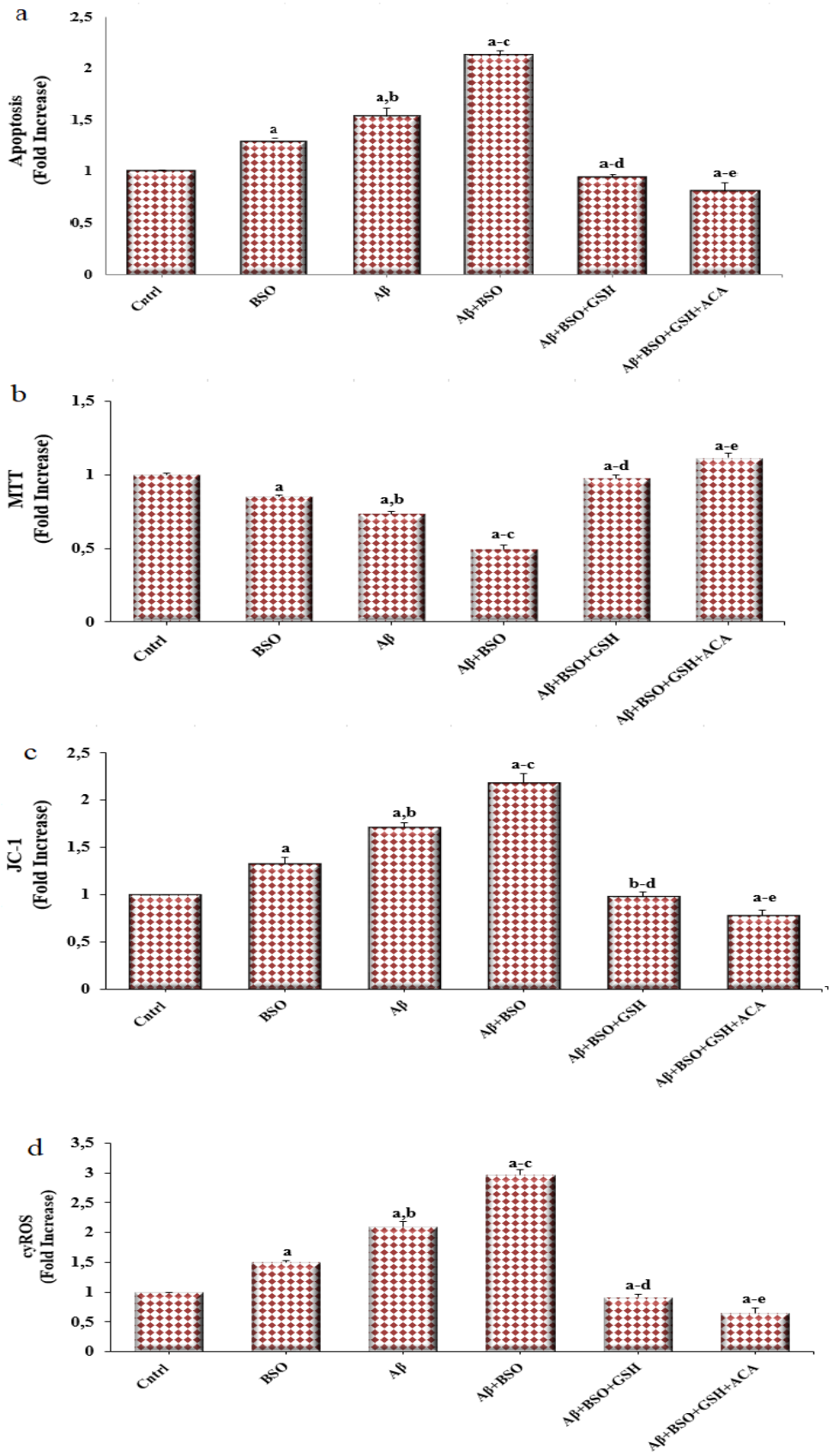

Fig 1: Induction of Aβ (1 μM and 72 hours) and BSO (0.5 mM and 6 hours) in BV2 microglia cells increased the levels of apoptosis, mitochondrial membrane depolarization (JC-1), ROS, while these levels decreased with GSH treatment*. The red bars represent apoptosis (**a**), cell viability (**b**), JC-1 (**c**), and cyROS (**d**) in five groups. The research was done using a microplate reader device. ([a]p ≤ 0.01 vs Cntrl group, [b]p ≤ 0.01 vs BSO group, [c]p ≤ 0.01 vs Aβ group, [d]p ≤ 0.01 vs Aβ+BSO group, [e]p ≤ 0.01 vs Aβ+BSO+GSH group).*

intensity of Rh 123 was analyzed in a multi-well reader with a wavelength of excitation 488 nm and emission 543 nm (Öz and Çelik 2022).

Western blot analyses through TRPM2 activation in BV2 glia cells

Standard procedures were used in the Western blot analyses of BV2 microglia cells (Akyuva et al. 2021). In the analyses, Casps-9 (p35/p10 Polyclonal Antibody), Casps-3 (p17-specific Polyclonal Antibody), beta-actin (polyclonal antibody), Bax (polyclonal antibody), Bcl2 (polyclonal antibody), TRPM2 (Polyclonal Antibody) and Poly-ADPR polymerase 1 (PARP-1) (polyclonal antibody) were procured from Proteintech (USA) while secondary antibodies (Rabbit IgG, HRP-linked whole anti-Aβ, from donkey) were procured from GE Healthcare (Amersham, UK). Rabbit anti-β-actin (1:2000) was used as an internal control for the concentration of proteins loaded. The data were expressed as relative density over the control level (Yildizhan et al 2022).

Statistical analyses

All data were given as mean ± standard deviation (SD). The differences between the arithmetic mean values of the scientific values studied in BV2 microglia cells were compared statistically using SPSS, a Windows 17.0 licensed package computer program. The presence of statistical significance in the groups was evaluated with the ANOVA test. The significance level was accepted as $p<0.05$ in all statistical comparisons.

Results

The results of apoptosis, caspases and cell viability in the plate reader analyses

When the levels of mitochondrial membrane depolarization, cyROS, Apoptosis, Casps-3, Casps-8 and Casps-9 were examined in BV2 cells with Aβ and BSO incubation (Fig 1 a-d and Fig 2 a-c), it was observed that the cells in the Aβ+BSO group increased significantly compared to the Control, Aβ and BSO groups. ($p<0.05$) MTT level decreased. When the Aβ and BSO groups were compared, it increased in the Aβ group, and the MTT level of these two groups decreased significantly compared to the Control group ($p<0.05$). Mitochondrial membrane depolarization, cyROS, Apoptosis, Casps-3, Casps-8 and Casps-9 levels were found to decrease with the application of GSH treatment in the Aβ +BSO+GSH group when compared to the Aβ, BSO and Aβ+BSO groups, while MTT levels increased.

The results of Western blot analyses

When the TRPM2, PARP-1, Bax, Casps-3, and Casps-9 expression levels of the groups were examined, it was observed that there was a significant increase in the TRPM2, PARP-1, Bax, Casps-3, and Casps-9 expressions of the other groups except for the control group and the GSH added group. It was observed that the Bcl2 expression level decreased (Figure 3 b-g) ($p<0.05$). In addition, TRPM2, PARP-1, Bax, Casps-3, and Casps-9 expressions were observed to be the highest in the Aβ+BSO group compared to the other groups, while the Bcl2 expression level was observed to be the lowest ($p<0.05$), in the group incubated with GSH. On the other hand, it was observed that the expression level decreased significantly compared to the other groups except for the control group ($p<0.05$). When Aβ and BSO groups were compared in TRPM2, PARP-1, BAX, Casps-3, and Casps-9 expression levels, it was determined that Aβ increased while BCL2 expression level decreased ($p<0.05$).

Discussion

It is widely known that during the increased neuronal damage linked to Alzheimer's disease, microglia constitute a persistent source of inflammation and ROS (Zhao et al. 2017). Normally, microglia carry out crucial tasks such as clearing away cellular waste, providing support for neurons, and immune defense (Carvalho da Fonseca et al. 2014; Buelow et al. 2008). Consequently, it is harmful when the normal microglial function is lost as a result of Alzheimer's disease induction and GSH depletion (Lee et al. 2012). For completing crucial duties, normal microglia function must be improved with GSH therapy. We, therefore, examined the protective effects of GSH therapy on microglia cell apoptosis, mortality, mitochondrial cyROS, and values utilizing TRPM2 activation. The findings of this study demonstrate that the TRPM2 channel mediates mitochondrial cyROS brought on by GSH depletion (BSO) and Alzheimer's disease (Aβ) and the anti-oxidant GSH reduced the harm Aβ and BSO caused to the microglia cells (Scheltens et al. 2021). TRPM2 (ACA and 2-APB) inhibitors greatly increased the protective function of GSH in the microglia cells (Abdul and

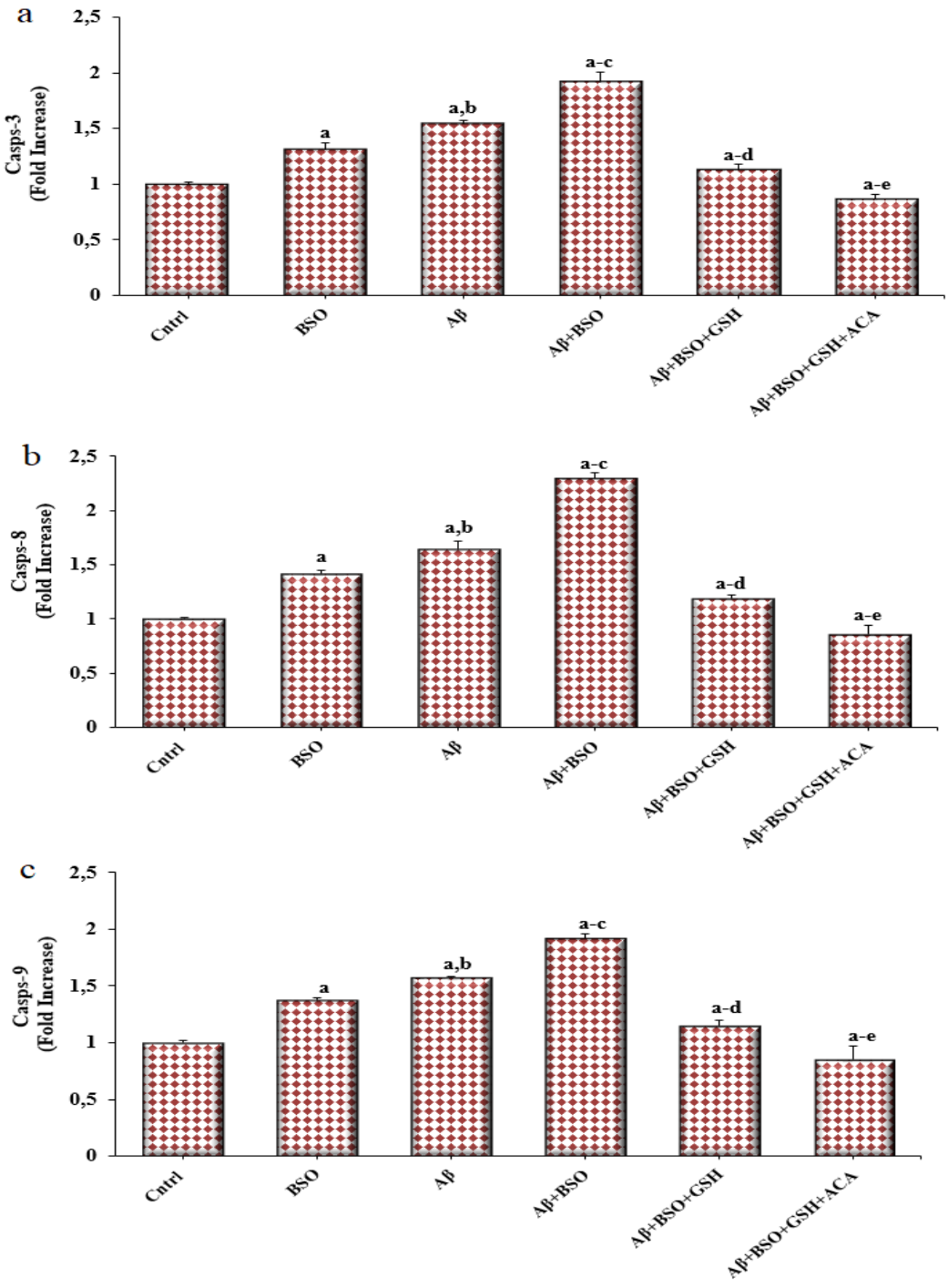

Fig 2: Induction of Aβ (1 μM and 72 hours) and BSO (0.5 mM and 6 hours) of BV2 microglia cells increased Casps-3, Casps-8, and Casps-9 levels, while these levels were decreased by GSH treatment. *The red bars represent Casps-3 (**a**), Casps-8 (**b**), and Casps-9 (**c**) levels in five groups. The research was done using a microplate reader device. ($^{a}p \leq 0.01$ vs Cntrl group, $^{b}p \leq 0.01$ vs BSO group, $^{c}p \leq 0.01$ vs Aβ group, $^{d}p \leq 0.01$ vs Aβ+BSO group, $^{e}p \leq 0.01$ vs Aβ+BSO+GSH group).*

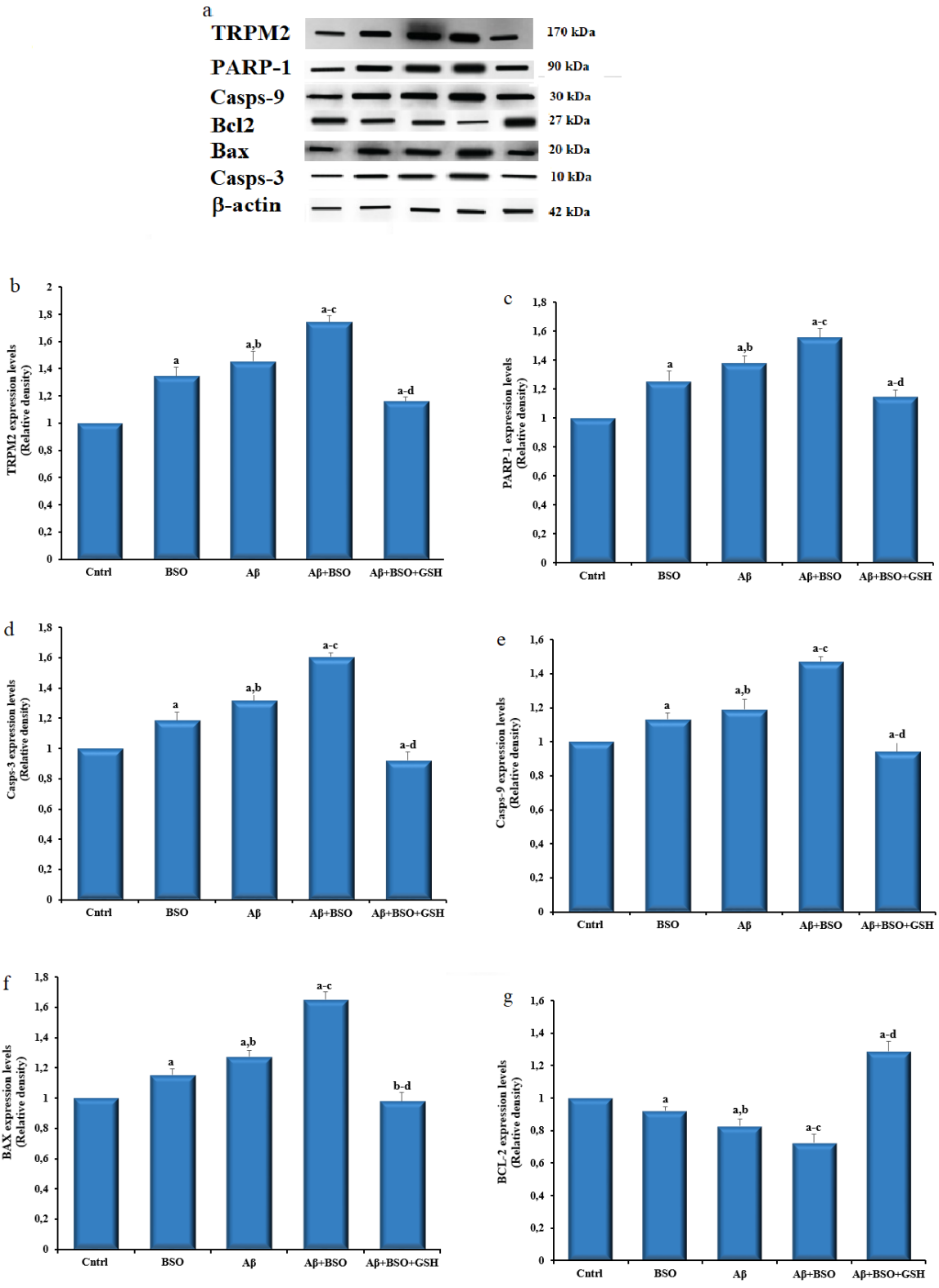

Fig 3: Effect of GSH on the PARP1, TRPM2 channel, Casps-3, Casps-9, Bax, and Bcl2 expression levels in Aβ and BSO induced BV2 microglia cells. *The BV2 cells in the Aβ and BSO groups were incubated with Aβ (1 μM and 72 hours) and BSO (0.5 mM and 6 hours). β-actin protein bands were used as controls for equal protein loading. Western blot analysis techniques and details are dedicated in detail in the "Materials and Methods" section.* ***(a)*** *Representative bands of TRPM2 channel* ***(b)****, PARP-1* ***(c)****, Casps-3* ***(d)****, Casps-9* ***(e)****, BAX* ***(f)****, and BCL2* ***(g)*** *expressions. ([a]p ≤ 0.01 vs Cntrl group, [b]p ≤ 0.01 vs BSO group, [c]p ≤ 0.01 vs Aβ group, [d]p ≤ 0.01 vs Aβ+BSO group).*

Butterfield 2007; Wang et al. 2020). Collectively, our results support the pathogenic involvement of TRPM2 in Alzheimer's disease-related microglia cells and GSH depletion-induced oxidative toxicity via increased Ca^{2+} influx to microglia cells. By increasing the formation of ROS (Figure 4).

Exposure to Aβ and GSH depletion, either separately or together, changed the morphological features of microglial cells. Exposure to Aβ and BSO produced ROS and decreased GSH levels. The activation of NADPH oxidase by AB results in the production of excessive ROS (Sun et al. 2019). Furthermore, mounting data indicate that complex I inhibition by excessive Ca^{2+} influx via activation of TRPM2 may cause ROS to be produced in large quantities by activating the mitochondrial permeability transition pores in neurons (Yazgan and Naziroğlu 2017; Ataizi et al. 2019; An et al. 2019; Ozkaya and Naziroglu 2020). Treatments with Aβ and BSO had a comparable impact on the microglia cells' suppression of complex I (Franco et al. 2008; Diaz-Hung et al. 2016). BSO and Ab therapy inside the mitochondria as well as excessive Ca^{2+} inflow are both known to increase ROS levels, further depolarize the mitochondrial membrane (JC-1), and produce an excessive amount of ROS. Depletion of GSH, a redox buffer against ROS that protects cells from apoptosis, is a characteristic of this type of cell death (Roychowdhury et al. 2002). However, in this work, treatment with Aβ and BSO caused alterations in the rates of JC-1, Casps-3, Casps-9, apoptosis, and cell viability along with a decrease in the cellular GSH pool and an increase in ROS generation in microglia cells. The activation of TRPM2 channels as a cellular defense mechanism against GSH disturbance by causing variations in the amounts of thiol group antioxidants, including GSH for ROS elimination, was probably a contributing factor. Different Aβ models' hippocampus, glia, and microglia have shown a regulatory response resembling GSH depletion (Murphy et al. 2003; Lee et al. 2010; Akhtar et al. 2017). Addition to worsening oxidative damage, ROS also functions as a secondary messenger by opening TRPM2 channels that are essential for microglia cells to die and undergo apoptosis (Mortadza et al. 2017; Ghoweri

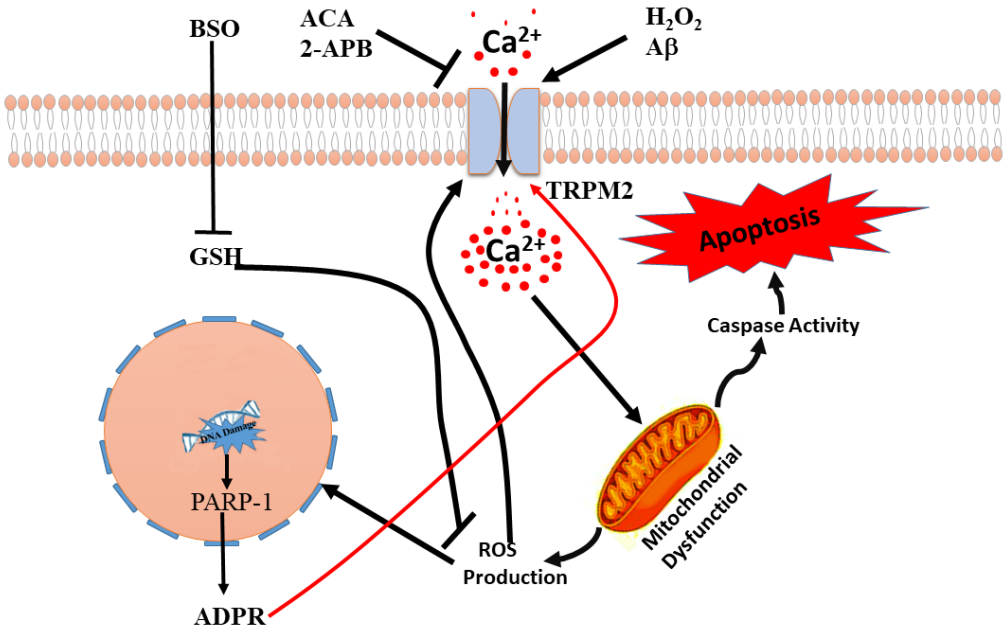

Figure 4. Possible pathways of cytosolic glutathione (GSH) depletion and TRPM2 activation on the experimental Alzheimer's disease model in the BV2 microglial cells*. The development of Alzheimer's disease (Aβ) and GSH depletion (BSO) can activate the microglia. By activating the PARP-1 enzyme and ROS cause DNA damage. ADPR-ribose (ADPR) synthesis in the nucleus. Excessive Ca^{2+} influx is stimulated by TRPM2 activation caused by ADPR and ROS. By increasing free cytosolic Ca^{2+} (through activation of TRPM2) after BSO therapy, ROSs are generated in the mitochondria; they are decreased by TRPM2 channel blockers (ACA and 2-APB). The primary mechanism by which BSO causes cell death is stimulated ROS-mediated activation of caspase-3 and caspase-9 via activation of TRPM2.*

et al. 2020). Similar to this, it was observed that GSH administration decreased increases in JC-1, ROS, Casps-3, Casps-9, and apoptosis that were brought on by BSO treatment in the DRG and hippocampi of rats (Ozgul and Nazıroglu 2012; Ovey and Nazıroglu 2015).

In conclusion, the current findings showed, for the first time, that the mechanisms of Aβ and BSO-induced oxidative neurotoxicity may be induced by stimulating TRPM2 in the microglia cells by generating mitochondrial ROS and cell death. However, the oxidative and apoptosis-inducing effects of Aβ and BSO were lessened by the GSH treatment, which also supported the microglia cells' thiol antioxidant redox system. In order to prevent the onset of Alzheimer's disease, as well as the oxidative neurotoxicity brought on by GSH depletion and the progression of Alzheimer's disease, it is, therefore, possible to explore the control of TRPM2 via GSH treatment in the experimental microglia cells Alzheimer's disease model.

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Declaration of Conflicting Interests The authors have declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Compliance with Ethical Standards This article does not contain any studies with human and animals performed by any of the authors.

References

Abdul H, M., Butterfield DA. (2007). Involvement of PI3K/PKG/ERK1/2 signaling pathways in cortical neurons to trigger protection by cotreatment of acetyl-L-carnitine and alpha-lipoic acid against HNE-mediated oxidative stress and neurotoxicity: implications for Alzheimer's disease. Free Radic Biol Med. 42(3):371-384. https://doi.org/10.1016/j.freeradbiomed.2006.11.006

Abe K, Misawa M. (2003). Amyloid beta protein enhances the clearance of extracellular L-glutamate by cultured rat cortical astrocytes. Neurosci Res. 45(1):25-31. https://doi.org/10.1016/s0168-0102(02)00190-6.

Akhtar F, Rouse CA, Catano G, et al. (2017). Acute maternal oxidant exposure causes susceptibility of the fetal brain to inflammation and oxidative stress. J Neuroinflammation 14(1):195. https://doi.org/10.1186/s12974-017-0965-8

Akyuva Y, Nazıroğlu M, Yıldızhan K. (2021). Selenium prevents interferon-gamma induced activation of TRPM2 channel and inhibits inflammation, mitochondrial oxidative stress, and apoptosis in microglia. Metab Brain Dis. 36(2):285-298. https://doi.org/10.1007/s11011-020-00624-0.

An X, Fu Z, Mai C, et al. (2019). Increasing the TRPM2 channel expression in human neuroblastoma SH-SY5Y cells augments the susceptibility to ROS-induced cell death. Cells. 8(1). https://doi.org/10.3390/cells8010028

Ataizi ZS, Ertilav K, Naziroglu M. (2019). Mitochondrial oxidative stress-induced brain and hippocampus apoptosis decrease through modulation of caspase activity, Ca(2+) influx and inflammatory cytokine molecular pathways in the docetaxel-treated mice by melatonin and selenium treatments. Metab Brain Dis 34(4):1077–1089. https://doi.org/10.1007/s11011-019-00428-x

Bond CE, Greenfield SA. (2007). Multiple cascade effects of oxidative stress on astroglia. Glia. 55(13):1348-1361. https://doi.org/10.1002/glia.20547.

Buelow B, Song Y, Scharenberg AM. (2008). The Poly(ADP-ribose) polymerase PARP-1 is required for oxidative stress-induced TRPM2 activation in lymphocytes. J Biol Chem. 283(36): 24571–24583. https://doi.org/10.1074/jbc.M802673200

Butterfield DA. (2003). Amyloid beta-peptide [1-42]-associated free radical-induced oxidative stress and neurodegeneration in Alzheimer's disease brain: mechanisms and consequences. Curr Med Chem. 10(24):2651-2659. https://doi.org/10.2174/0929867033456422.

Carvalho da Fonseca AC, Wang H, Fan H, et al. (2014). Increased expression of stress inducible protein 1 in glioma-associated microglia/macrophages. J Neuroimmunol, 274(1-2), 71–77. https://doi.org/10.1016/j.jneuroim.2014.06.021

Çınar R, Nazıroğlu M. (2022). TRPM2 Channel Inhibition Attenuates Amyloid β42-Induced Apoptosis and Oxidative Stress in the Hippocampus of Mice. Cell Mol Neurobiol. 2022 Jul 15. https://doi.org/10.1007/s10571-022-01253-0. Epub ahead of print.

Diaz-Hung ML, Yglesias-Rivera A, Hernandez-Zimbron LF, et al (2016). Transient glutathione depletion in the substantia nigra compacta is associated with neuroinflammation in rats. Neuroscience 335:207–220. https://doi.org/10.1016/j.neuroscience.2016.08.023

Dringen R. (2005). Oxidative and antioxidative potential of brain microglial cells. Antioxid Redox Signal. 7(9-10):1223-33. https://doi.org/10.1089/ars.2005.7.1223.

Franco R, DeHaven WI, Sifre MI, Bortner CD, Cidlowski JA. (2008). Glutathione depletion and disruption of intracellular ionic homeostasis regulate lymphoid cell apoptosis. J Biol Chem. 283(52):36071–36087. https://doi.org/10.1074/jbc.M807061200

Ghoweri AO, Gagolewicz P, Frazier HN, et al. (2020). Neuronal Calcium Imaging, Excitability, and Plasticity Changes in the Aldh2-/- Mouse Model of Sporadic Alzheimer's Disease. J. Alzheimer's Dis. 77(4):1623–1637. https://doi.org/10.3233/JAD-200617

Güzel M, Nazıroğlu M, Akpınar O, Çınar R. (2021). Interferon Gamma-Mediated Oxidative Stress Induces Apoptosis, Neuroinflammation, Zinc Ion Influx, and TRPM2 Channel Activation in Neuronal Cell Line: Modulator Role of Curcumin. Inflammation. 44(5):1878-1894. https://doi.org/10.1007/s10753-021-01465-4.

Halliwell B. (2006). Oxidative stress and neurodegeneration: where are we now? J Neurochem. 97(6):1634-1658. https://doi.org/10.1111/j.1471-4159.2006.03907.x.

Hool LC. (2008) Evidence for the regulation of L-type Ca2+ channels in

the heart by reactive oxygen species: mechanism for mediating pathology. Clin Exp Pharmacol Physiol. 35(2):229-34. https://doi.org/10.1111/j.1440-1681.2007.04727.x.

Jiang C, Zhang C, Song J, Ji X, Wang W. (2021). Cytidine-gold nanoclusters as peroxidase mimetic for colorimetric detection of glutathione (GSH), glutathione disulfide (GSSG) and glutathione reductase (GR). Spectrochim Acta A Mol Biomol Spectrosc. 250:119316. https://doi.org/10.1016/j.saa.2020.119316.

Kahya MC, Nazıroğlu M, Övey İS. (2017). Modulation of Diabetes-Induced Oxidative Stress, Apoptosis, and Ca2+ Entry Through TRPM2 and TRPV1 Channels in Dorsal Root Ganglion and Hippocampus of Diabetic Rats by Melatonin and Selenium. Mol Neurobiol. 54(3):2345-2360. https://doi.org/10.1007/s12035-016-9727-3.

Lee M, Cho T, Jantaratnotai N, Wang YT, McGeer E, McGeer PL (2010). Depletion of GSH in glial cells induces neurotoxicity: relevance to aging and degenerative neurological diseases. FASEB J 24(7):2533–2545. https://doi.org/10.1096/fj.09-149997

Lee M, Kwon BM, Suk K, McGeer E, McGeer PL. (2012). Effects of obovatol on GSH depleted glia-mediated neurotoxicity and oxidative damage. J Neuroimmune Pharmacol. 7(1):173-186. https://doi.org/10.1007/s11481-011-9300-9

Mortadza SS, Sim JA, Stacey M, Jiang LH. (2017). Signalling mechanisms mediating Zn(2+)-induced TRPM2 channel activation and cell death in microglial cells. Sci Rep 7:45032. https://doi.org/10.1038/srep45032

Mucke L, Selkoe DJ. (2012) Neurotoxicity of amyloid β-protein: synaptic and network dysfunction. Cold Spring Harb Perspect Med. Jul;2(7):a006338. https://doi.org/10.1101/cshperspect.a006338.

Murphy A, Sunohara JR, Sundaram M, et al. (2003). Induction of protein kinase C substrates, Myristoylated alanine-rich C kinase substrate (MARCKS) and MARCKS-related protein (MRP), by amyloid beta-protein in mouse BV-2 microglial cells. Neurosci Letters, 347(1):9-12. https://doi.org/10.1016/s0304-3940(03)00648-7

Nazıroğlu M, Özgül C, Çiğ B, Doğan S, Uğuz AC. (2011). Glutathione modulates Ca(2+) influx and oxidative toxicity through TRPM2 channel in rat dorsal root ganglion neurons. J Membr Biol. 242(3):109-118. https://doi.org/10.1007/s00232-011-9382-6.

Nazıroğlu M. (2007a). New molecular mechanisms on the activation of TRPM2 channels by oxidative stress and ADP-ribose. Neurochem Res. 32(11):1990-2001. https://doi.org/10.1007/s11064-007-9386-x.

Nazıroğlu M. (2007b). Molecular Mechanisms of vitamin E on intracellular signaling pathways in brain. In Reactive oxygen species and diseases. Ed.Laszlo; Goth, Kerala, India: Research Signpost Press: pp 239–256.

Ovey IS, Naziroglu M. (2015). Homocysteine and cytosolic GSH depletion induce apoptosis and oxidative toxicity through cytosolic calcium overload in the hippocampus of aged mice: involvement of TRPM2 and TRPV1 channels. Neuroscience 284:225–233. https://doi.org/10.1016/j.neuroscience.2014.09.078

Övey İS, Naziroğlu M. (2015). Homocysteine and cytosolic GSH depletion induce apoptosis and oxidative toxicity through cytosolic calcium overload in the hippocampus of aged mice: involvement of TRPM2 and TRPV1 channels. Neuroscience. 284:225-233. https://doi.org/10.1016/j.neuroscience.2014.09.078.

Övey İS, Nazıroğlu M. (2021). Effects of homocysteine and memantine on oxidative stress related TRP cation channels in in-vitro model of Alzheimer's disease. J Recept Signal Transduct Res. 41(3):273-283. https://doi.org/10.1080/10799893.2020.1806321.

Ozgul C, Naziroglu M (2012) TRPM2 channel protective properties of N-acetylcysteine on cytosolic glutathione depletion dependent oxidative stress and Ca2+ influx in rat dorsal root ganglion. Physiol Behav 106(2):122–128. https://doi.org/10.1016/j.physbeh.2012.01.014

Ozkaya D, Nazıroglu M (2020) Curcumin diminishes cisplatin-induced apoptosis and mitochondrial oxidative stress through inhibition of TRPM2 channel signaling pathway in mouse optic nerve. J Recept Signal Transduct Res 40(2):97–108. https://doi.org/10.1080/10799893.2020.1720240

Pandey M, Choudhury H, Verma RK, et al. (2020). Nanoparticles Based Intranasal Delivery of Drug to Treat Alzheimer's Disease: A Recent Update. CNS Neurol Disord Drug Targets. 19(9):648-662. https://doi.org/10.2174/1871527319999200819095620.

Roychowdhury S, Luthe A, Keilhoff G, Wolf G, Horn TF. (2002). Oxidative stress in glial cultures: detection by DAF-2 fluorescence used as a tool to measure peroxynitrite rather than nitric oxide. Glia. 38(2):103-114. https://doi.org/10.1002/glia.10024

Santo-Domingo J, Demaurex N. (2010). Calcium uptake mechanisms of mitochondria. Biochim Biophys Acta. 1797(6-7):907-912. https://doi.org/10.1016/j.bbabio.2010.01.005.

Scheltens, P, De Strooper B, Kivipelto M, et al. (2021). Alzheimer's disease. Lancet 397(10284):1577–1590. https://doi.org/10.1016/S0140-6736(20)32205-4

Sciacca MF, Kotler SA, Brender JR, Chen J, Lee DK, Ramamoorthy A. (2012). Two-step mechanism of membrane disruption by Aβ through membrane fragmentation and pore formation. Biophys J. 103(4):702-710. https://doi.org/10.1016/j.bpj.2012.06.045.

Simpson DSA, Oliver PL. (2020). ROS Generation in Microglia: Understanding Oxidative Stress and Inflammation in Neurodegenerative Disease. Antioxidants (Basel). 9(8):743. https://doi.org/10.3390/antiox9080743.

Singh BK, Tripathi M, Pandey PK, Kakkar P. (2011). Alteration in mitochondrial thiol enhances calcium ion dependent membrane permeability transition and dysfunction in vitro: a cross-talk between mtThiol, Ca(2+), and ROS. Mol Cell Biochem. 357(1-2):373-385. https://doi.org/10.1007/s11010-011-0908-0.

Staerck C, Gastebois A, Vandeputte P, et al. (2017) Microbial antioxidant defense enzymes. Microb Pathog. 110:56-65. https://doi.org/10.1016/j.micpath.2017.06.015.

Sun, Q, Jia N, Li X, Yang J, Chen G. (2019). Grape seed proanthocyanidins ameliorate neuronal oxidative damage by inhibiting GSK-3β-dependent mitochondrial permeability transition pore opening in an experimental model of sporadic Alzheimer's disease. Aging 11(12), 4107–4124. https://doi.org/10.18632/aging.102041

Uğuz AC, Öz A, Nazıroğlu M. (2016). Curcumin inhibits apoptosis by regulating intracellular calcium release, reactive oxygen species and mitochondrial depolarization levels in SH-SY5Y neuronal cells. J Recept Signal Transduct Res. 36(4):395-401. https://doi.org/10.3109/10799893.2015.1108337.

Uğuz AC, Öz A, Yılmaz B, Altunbaş S, Çelik Ö. (2015). Melatonin attenuates apoptosis and mitochondrial depolarization levels in hypoxic conditions of SH-SY5Y neuronal cells induced by cobalt chloride (CoCl 2). Turkish J Biol. 39(6):896-903.

Wang YF, Fan LM, Zhang WZ, Zhang W, Wu WH. (2004). Ca2+-

permeable channels in the plasma membrane of Arabidopsis pollen are regulated by actin microfilaments. Plant Physiol. 136(4):3892-904. https://doi.org/10.1104/pp.104.042754.

Wang, Q, Ge X, Zhang J, Chen L. (2020). Effect of lncRNA WT1-AS regulating WT1 on oxidative stress injury and apoptosis of neurons in Alzheimer's disease via inhibition of the miR-375/SIX4 axis. Aging. 12(23): 23974–23995. https://doi.org/10.18632/aging.104079

Yazgan Y, Naziroglu M (2017). Ovariectomy-induced mitochondrial oxidative stress, apoptosis, and calcium ion influx through TRPA1, TRPM2, and TRPV1 are prevented by 17beta-estradiol, tamoxifen, and raloxifene in the hippocampus and dorsal root ganglion of rats. Mol Neurobiol. 54(10):7620–7638. https://doi.org/10.1007/s12035-016-0232-5

Yildizhan K, Çinar R, Naziroğlu M. (2022). The involvement of TRPM2 on the MPP+-induced oxidative neurotoxicity and apoptosis in hippocampal neurons from neonatal mice: protective role of resveratrol. Neurol Res. 1-9. https://doi.org/10.1080/01616412.2022.2027644.

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. https://doi.org/10.1007/s12035-020-01974-7.

Zhang Q, Li J, Liu C, et al F. (2015). Protective effects of low molecular weight chondroitin sulfate on amyloid beta (Aβ)-induced damage in vitro and in vivo. Neuroscience. 305:169-82. https://doi.org/10.1016/j.neuroscience.2015.08.002.

Zhao SC, Ma LS, Chu ZH, Xu H, Wu WQ, Liu F. (2017). Regulation of microglial activation in stroke. Acta Pharmacol Sin. 38(4):445-458. https://doi.org/10.1038/aps.2016.162.