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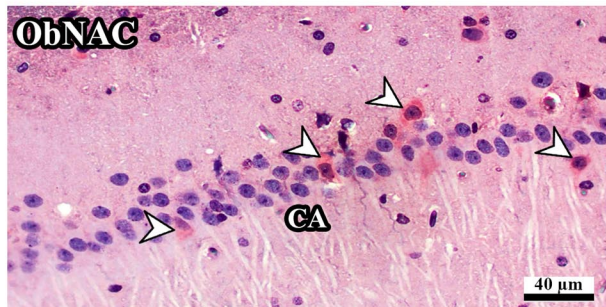
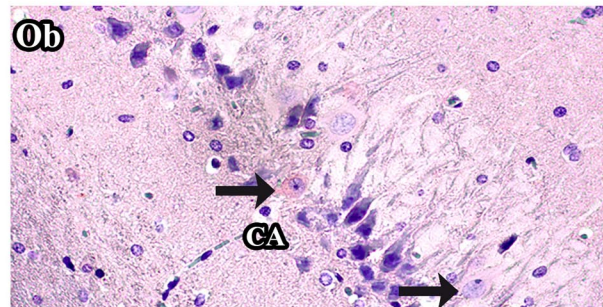
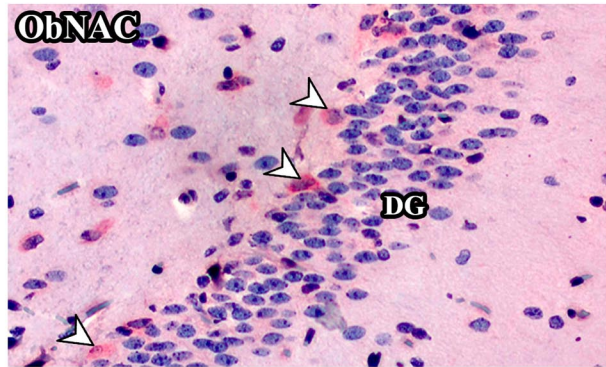
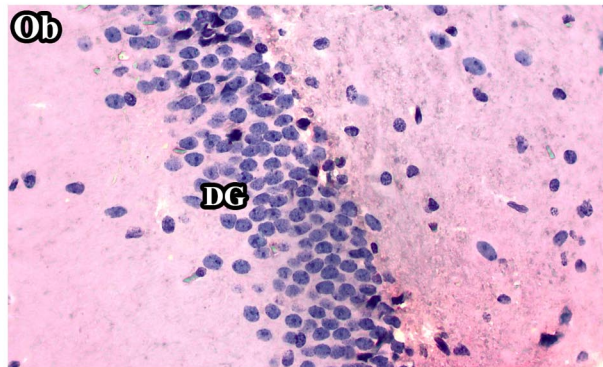
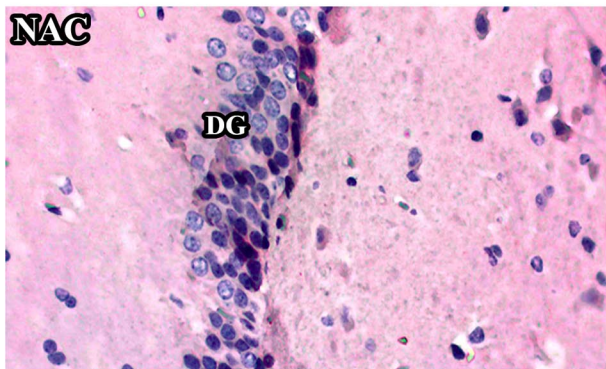
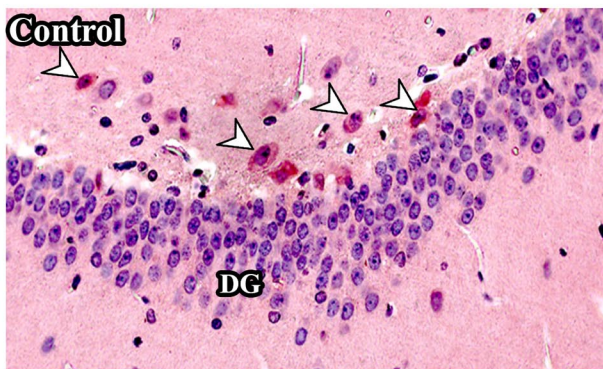
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#### AIM AND SCOPE

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** ( $\text{Na}^+$ -  $\text{K}^+$  Channels,  $\text{Cl}^-$  channels,  $\text{Ca}^{2+}$  channels, ADP-Ribose and metabolism of  $\text{NAD}^+$ , 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  $\text{NAD}^+$  on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

##### D- Gene and Oxidative Stress

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

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#### 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.

# Regulatory role of phospholipase A2 inhibitor in oxidative stress and inflammation induced by an experimental mouse migraine model

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## List of Abbreviations;

**ACA**, N-(p-Amylcinnamoyl) anthranilic acid; **Cas 3**, Caspase 3; **Cas 9**, Caspase 9; **GSHPx**, Glutathione peroxidase; **GSH**, Glutathione; **GTN**, Glyceryl trinitrate; **IL 1 $\beta$** , Interleukin 1 beta; **IL 6**, Interleukin 6; **PLA2**, phospholipase A2; **OS**, Oxidative stress; **ROS**, Reactive oxygen species; **TNF  $\alpha$** , Tumor Necrosis Factor Alpha; **TRP**, Transient receptor potential; **TRPM2**, TRP melastatin 2

## Abstract

Migraine is a complex neurological problem whose primary symptom is headache and is common in the human population. It is well known that neuroinflammation plays a vital role in the pathogenesis of migraine, with adverse effects on the nervous system, including headache disorders such as migraine. The infusion of the nitric oxide donor glyceryl trinitrate (GTN) is often used in experimental models of migraine because it is the best-

known model of migraine provocation. N-(p-amylicinnamoyl) anthranilic acid (ACA) has been shown to inhibit both TRPM2 and phospholipase A2 (PLA2). Recent research has explored potential interventions to mitigate GTN-induced neurotoxicity. One such candidate is ACA, a compound with anti-inflammatory and antioxidant properties.

Thirty-six C57BL/6j black mice were divided into the control groups of ACA, GTN, and ACA+GTN. Mice in the ACA groups were treated intraperitoneally with ACA (25 mg/kg) for three days. Mice in the GTN groups were treated intraperitoneally with a single dose of GTN (10 mg/kg) for migraine induction. Brain tissue and erythrocyte samples were taken from the mice at the end of the experiment.

The levels of inflammatory cytokines (TNF  $\alpha$ , IL 1 $\beta$ , and IL 6), apoptosis, intracellular ROS, lipid peroxidation, caspase 3, caspase 9, and mitochondrial membrane potential increased in the GTN group. However, their levels were decreased in the ACA+GTN group by the injection of ACA. The treatment of ACA regulated the GTN treatment-induced decreases of glutathione levels,

glutathione peroxidase activation, and cell viability in the brain and erythrocytes.

In conclusion, GTN plays a role in neurotoxicity caused by increased apoptosis and ROS. We observed that ACA modulated the brain and erythrocyte oxidant, antioxidant parameters, and apoptotic processes. The neuro-protective role of PLA2 antagonist (ACA) treatment may be explained by its modulating activity against increased apoptosis and oxidative stress.

**Keywords:** Apoptosis, Brain, Glutathione, Experimental migraine, Inflammatory cytokines

## Introduction

Migraine is a complex neurological problem whose primary symptom is headache and is common in the human population (Yazğan & Nazıroğlu, 2021). The most recent findings of the Global Burden of Disease study show that migraine is the second leading cause of disability worldwide and even the leading cause among young women (Aguilar-Shea et al. 2021). Non-pharmacological and pharmacological treatments are available for migraine (Amin et al., 2018). However, recurrent attacks of migraine are currently not completely treatable. The inadequate treatment of migraine attacks is a major socio-economic problem worldwide. The idea that migraine is a neurovascular disease is widely accepted.

The infusion of the nitric oxide donor glyceryl trinitrate (GTN) is often used in experimental models of migraine because it is the best-known model of migraine provocation (Ashina et al., 2013; Marone et al., 2018). Although the pathophysiological mechanisms of migraine remain unclear, in experimental studies with GTN or its derivative, possible causes of migraine have been tried to be explained through different means. It is widely accepted that neuroinflammation plays a vital role in the pathogenesis of migraine. (Kursun et al. 2021). In experimental models of migraine induced by GTN, it has been reported that GTN causes neuroinflammation by increasing the level of cytokine expression (Hou et al., 2017; Yazğan & Nazıroğlu, 2021). Oxidative stress results from the overproduction of reactive oxygen species (ROS) (Su et al., 2010), which are produced throughout the body during aerobic metabolism (Owen et al., 2022). Recent studies show that oxidative stress is one of the most critical factors in the etiology of migraine (Ishii et al., 2011; Yazğan & Nazıroğlu, 2021). It has been reported that

oxidative stress (OS) and apoptosis induced by the elevation of cytosolic free  $\text{Ca}^{2+}$  as a result of mitochondrial membrane depolarisation in the brain are significant in the pathophysiology of migraine (Abushik et al., 2014; Yazğan & Nazıroğlu, 2021). In migraine, neuronal death is induced by programmed cell death (apoptosis) (Park et al., 2020). Among several pathophysiological pathways of apoptosis in the brain, as in many other tissues, mitochondrial dysfunction is an essential molecular pathway (Nazıroğlu et al., 2015). Mitochondria protect neurons against elevated intracellular  $\text{Ca}^{2+}$  (Özgül & Nazıroğlu, 2012). Imbalances in cytosolic free  $\text{Ca}^{2+}$  concentrations can disrupt mitochondrial function and cause OS and caspase activation, leading to neuronal death (Argun et al., 2014).

Several antioxidant defense systems are present in the brain. Glutathione peroxidase (GSHPx) is responsible for the reduction of hydro- and organic peroxides in the presence of reduced glutathione (GSH) (Lee et al., 2010). GSH is the major thiol antioxidant and maintains thiol redox in cells. Oxidative stress induces lipid peroxidation. Lipid peroxidation has been implicated in many neurological disorders, including migraine (Li & Jiang, 2019). According to recent clinical reports, GSHPx activity is low in adults and children with migraines (Vurucu et al., 2013).

N-(p-*amyl cinnamoyl*) anthranilic acid (ACA) has been shown to inhibit both Transient receptor potential (TRP) melastatin 2 (TRPM2) and phospholipase A2 (PLA2) (Harteneck et al., 2007). Activation of PLA2 is a pathway underlying neuroinflammation, while activation of TRPM2 is associated with increased intracellular  $\text{Ca}^{2+}$  (Çakır et al., 2017). In many cells, PLA2 activity and arachidonic acid release are intertwined with TRP channel function, leading to cellular responses. These cellular pathways are involved in cellular events such as inflammation, OS, and mitochondrial depolarisation, which play an essential role in migraine pathophysiology (Çakır et al., 2019). Therefore, ACA may act as a chemical that modulates these two cellular pathways.

In this study, we investigated the effect of ACA, a TRPM2 channel blocker and PLA2 enzyme inhibitor, on the mouse brain in an experimental migraine model. We aimed to investigate brain injury by analyzing cytokine production, cell viability, and caspase activation in GTN-induced mouse brains. We also examined oxidative stress and enzymatic antioxidants in brain and erythrocyte tissue. We aimed to evaluate whether ACA would have a

protective effect in the experimental GTN migraine mouse model.

## Materials and Methods

### Animals and experimental groups

In this study, 36 C57BL/6j black mice were purchased from Burdur Mehmet Akif University (BMAU), and ethics committee approval was obtained. Mice were housed in cages at a standard ambient temperature of  $23 \pm 1$  °C under a 12 h dark/light cycle, with free access to food and water.

Black mice were divided into four groups, and mice in the control (Cont) groups were not treated with GTN and ACA. Mice in the ACA groups received 25 mg/kg ACA intraperitoneally daily for three days (Yazgan & Naziroglu, 2021). Mice in the GTN groups received 10 mg/kg GTN intraperitoneally with a single dose for migraine induction (Naziroglu et al., 2015; Yazgan & Naziroglu, 2021). Mice in the ACA+GTN group received 25 mg/kg ACA intraperitoneally daily for three days. Then, they received 10 mg/kg GTN intraperitoneally with a single dose for migraine induction. After the experimental steps were completed, mice in all groups were sacrificed.

### Preparation of brain tissue homogenate and erythrocyte preparations

After the experiment, all mice were sacrificed under ketamine and xylazine anesthesia. The brain cells and erythrocyte samples were isolated. Tissues were taken for analysis. Then, the isolated brain cell samples were used for intracellular ROS, caspase -3 and -9 (Cas 3 and Cas 9), mitochondrial membrane potential, apoptosis, cell viability (MTT) analyses, and cytokine [Interleukin 1 beta (IL 1 $\beta$ ), Interleukin 6 (IL 6), and Tumor Necrosis Factor Alpha (TNF  $\alpha$ )] analyses. Brain and erythrocyte samples were used for lipid peroxidation, GSH, and GSHPx activity analyses.

### Cell Viability Assay

To test cell viability in brain tissue cells, we used the standard manual method MTT (4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) as detailed in our previous studies (Yazgan & Naziroglu, 2021; Yazgan et al., 2023). Brain tissue cells in 96-well black plates were incubated for four hours at 37 °C to convert the MTT tetrazolium compound to formazan. The absorbance values

of MTT were read using a microplate reader (490 nm) (PRO200/Infinite, Tecan/Austria).

### The Analyses of Caspase 3 and 9 and Apoptosis

The cleavage of specific fluorogenic substrates was used to analyze the activities of Cas 3 and 9 in brain tissue homogenate [AcDEVD/AMC (Cas 3) and AcLEHD/AMC (Cas 9)] (Bachem/Switzerland). They were incubated with 2 ml of substrate solution for one hour at 37 °C as described in previous studies (Yazgan & Naziroglu, 2021; Yazgan et al., 2023). The 360 nm was used as the excitation wavelength in the microplate reader (PRO200/Infinite, Tecan/Austria), although 460 nm was used as the emission wavelength (Yildizhan & Naziroglu, 2020).

The apoptosis assay analysis method is described in detail in our previous publications. Apoptosis assay (Cell-APOPercentage dye) was performed using a commercial kit. When the cell membrane integrity is impaired, APOPercentage dye is actively transported into the cell and paints the apoptotic cell red. According to the manufacturer's instructions, the detection of apoptosis was measured as a loss of asymmetry in the membranes of apoptotic brain tissue homogenate using a microplate reader (PRO200/Infinite, Tecan/Austria) (Biocolor Ltd. Northern-Ireland) (Yazgan & Naziroglu, 2021; Yazgan et al., 2023). Apoptosis, Cas 3, and Cas 9 data were presented as a fold increase (experiment/control).

### The Analyses of Intracellular ROS Production and Mitochondrial Membrane Potential

Dihydro-rhodamine 123 (DHR123) was used to test intracellular ROS formation in brain cells. The DHR123 non-fluorescent stains are oxidized to fluorescent intercalators [rhodamine 123 (Rh123)] by superoxide radicals (Joshi & Bakowska, 2011; Keil et al., 2011). The ROS Production analysis method is described in detail in our previous publications. (Yazgan and Yazgan 2022, Yazgan et al. 2023). Brain tissue cells were washed with DMEM medium and incubated with two  $\mu$ M DHR 123 for 25 minutes (37 °C) in the dark. The fluorescence changes (488 nm excitation value and 543 nm emission value) were measured in a microplate reader (PRO200/Infinite). Rh123 microplate reader results were expressed as a fold increase.

The levels of mitochondrial membrane potential were assayed in the microplate reader (PRO200/Infinite) using a fluorescent stain (JC1 Cayman, Istanbul/Turkey). The mitochondrial membrane potential (JC 1) analysis

method is described in detail in our previous publications (Yazgan & Naziroglu, 2021; Yazgan et al., 2023). Brain tissue cells were washed with DMEM medium and incubated with four  $\mu\text{M}$  JC 1 for 45 minutes at (37 °C) in the dark. The fluorescence changes (Excitation: 488 nm. Emission: 520/ 596 nm) were measured in a microplate reader (PRO200/Infinite, Tecan/Austria). The results of mitochondrial membrane potential in the microplate reader were expressed fold increase (experiment/control).

### The Analyses of Inflammatory Cytokines in the Brain Homogenate

Supernatants obtained from brain tissue homogenate of C57BL/6j black mice were collected by centrifugation at 1500 g for 5 min. The levels of IL 1 $\beta$  (Cat:E0045Mo), IL 6 (Cat:E0049Mo), and TNF  $\alpha$  (Cat:E0117Mo) were assayed in a microplate reader (PRO200/Infinite, Tecan/Austria) at 450 nm wavelength using a commercial assay kit (BT Lab/China) (Yazgan & Naziroglu, 2021; Yildizhan & Naziroglu, 2020). The expression of IL 1 $\beta$  and IL 6 activity concentration levels in brain tissues is expressed in pg/ml, and the expression of TNF  $\alpha$  activity is expressed in ng/ml.

### The Analyses of Glutathione, Glutathione Peroxidase, and Lipid Peroxidation in the Erythrocyte and Brain Homogenate

Optical densities (absorbance) of total protein, GSH, GSHPx, and malondialdehyde (MDA) were determined using a spectrophotometer (UV1800/Shimadzu, Kyoto/Japan). The details of the analyses are described in previous studies (Yazgan & Yazgan, 2022; Yazgan & Naziroglu, 2021; Yildizhan & Naziroglu, 2020). Lipid peroxidation levels as MDA were measured by thiobarbituric-acid reaction, GSHPx activity was measured using the method of Lawrence and Burk (1976) as described in previous studies, and GSH activity was measured using the method of Sedlak and Lindsay (1968) as described in previous studies (Yazgan & Naziroglu, 2021; Yildizhan & Naziroglu, 2020). The expression of MDA and GSH activity concentration levels in erythrocyte and brain homogenate is expressed in  $\mu\text{mol/g}$  protein, and the expression of GSHPx activity concentration levels is defined in IU/ g protein.

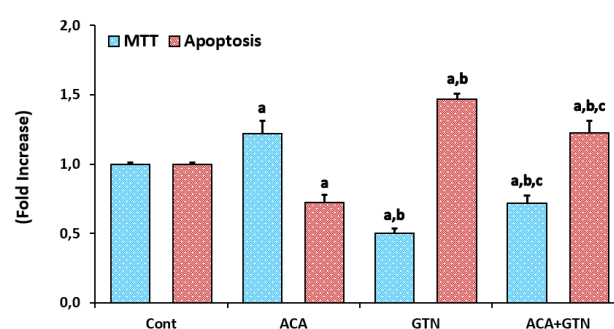
### Statistical Analyses

All data are presented as mean  $\pm$  SD. SPSS software (version 17.0) was used for data analysis. One-way ANOVA was used to assess differences between groups. For all data with a statistically significant difference, the post-hoc Tukey test was used.  $p \leq 0.05$  was considered to be statistically significant.

### Results

#### ACA Treatment Modulated GTN-Induced Cell Death and Apoptosis in the Brain of Mice with Experimental Migraine

The increased cytosolic ROS production increases mitochondrial activity-dependent caspase activation, resulting in apoptosis. Therefore, JC 1 is an important parameter of mitochondrial activity and an essential indicator of caspase activity and apoptosis (Keil et al., 2011; Naziroglu, 2012; Ureshino et al., 2019). In Figure 1, GTN-induced increased apoptosis and decreased cell viability in the brain of mice, showing that ACA treatment modulates this state. The MTT values were markedly ( $p \leq 0.05$ ) lower in the GTN groups than in the control (Figure 1). The MTT values were higher in the ACA and ACA+GTN groups than in the GTN groups ( $p \leq 0.05$ ). Apoptosis values were seriously ( $p \leq 0.05$ ) higher in the GTN groups than in the control (Figure 1). In the brain cells, the apoptosis values were seriously ( $p \leq 0.05$ ) lower in the ACA and ACA+GTN groups than in the GTN groups.

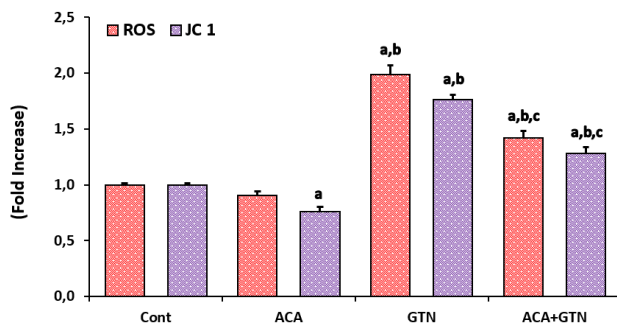


**Figure 1.** Effects of administration of ACA (25 mg/kg for three days) on MTT and apoptosis levels in the brain of the migraine model (GTN, single dose intraperitoneally, 10 mg/kg)-induced mice. Values are presented as mean  $\pm$  SD of nine separate experiments and expressed as fold-increase over the pre-treatment level (experimental/control). (<sup>a</sup> $p \leq 0.05$  versus Cont, <sup>b</sup> $p \leq 0.05$  versus ACA group, <sup>c</sup> $p \leq 0.05$  versus ACA+GTN)

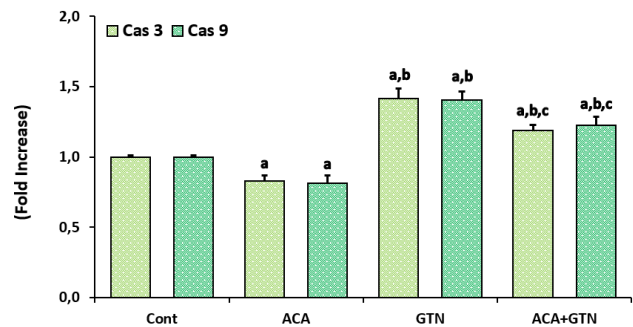


### ACA Treatment Modulated GTN-Induced Mitochondrial Membrane Depolarization, ROS, Cas 3 and 9 in the Brain of Mice with Experimental Migraine

The electron transport system of mitochondria causes loss of JC 1 in mitochondria, leading to excessive ROS production. Therefore, JC 1 is an important parameter of mitochondrial activity and an essential indicator of cell ROS production (Joshi & Bakowska, 2011). Figure 2 and Figure 3 GTN-induced increased JC 1, ROS, Cas 3, and Cas 9 in the brain of mice, showing that ACA treatment modulates this state. The JC 1 and ROS levels were seriously ( $p \leq 0.05$ ) higher in the GTN groups than in the control (Figure 2). Also, the JC 1 and ROS levels in the brain were lower in the ACA and ACA+GTN groups compared to the GTN group ( $p \leq 0.05$ ). The Cas 3 and Cas 9 levels in the brain were seriously ( $p \leq 0.05$ ) higher in the GTN groups than in the control (Figure 3). Also, the Cas 3 and Cas 9 activities were lower in the ACA and ACA+GTN groups compared to the GTN group ( $p \leq 0.05$ ).



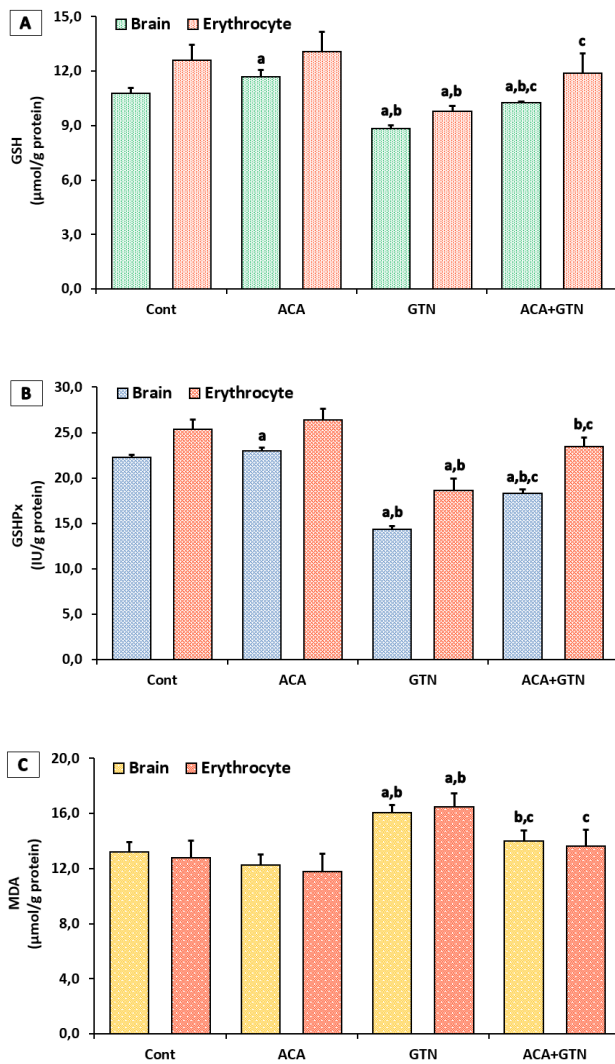
**Figure 2.** Effects of administration of ACA (25 mg/kg for three days) on ROS and JC 1 levels in the brain of the migraine model (GTN, single dose intraperitoneally, 10 mg/kg)-induced mice. Values are presented as mean  $\pm$  SD of nine separate experiments and expressed as fold-increase over the pre-treatment level (experimental/control). (<sup>a</sup> $p \leq 0.05$  versus Cont, <sup>b</sup> $p \leq 0.05$  versus ACA group, <sup>c</sup> $p \leq 0.05$  versus ACA+GTN)



**Figure 3.** Effects of administration of ACA (25 mg/kg for three days) on caspase 3 and 9 (Cas 3, and Cas 9) levels in the brain of the migraine model (GTN, single dose intraperitoneally, 10 mg/kg)-induced mice. Values are presented as mean  $\pm$  SD of nine separate experiments and expressed as fold-increase over the pre-treatment level (experimental/control). (<sup>a</sup> $p \leq 0.05$  versus Cont, <sup>b</sup> $p \leq 0.05$  versus ACA group, <sup>c</sup> $p \leq 0.05$  versus ACA+GTN)

### ACA Treatment Modulated GTN-Induced Decreased Glutathione and Glutathione Peroxidase Activity and Lipid Peroxidation in the Erythrocytes and Brains of Mice with Experimental Migraine

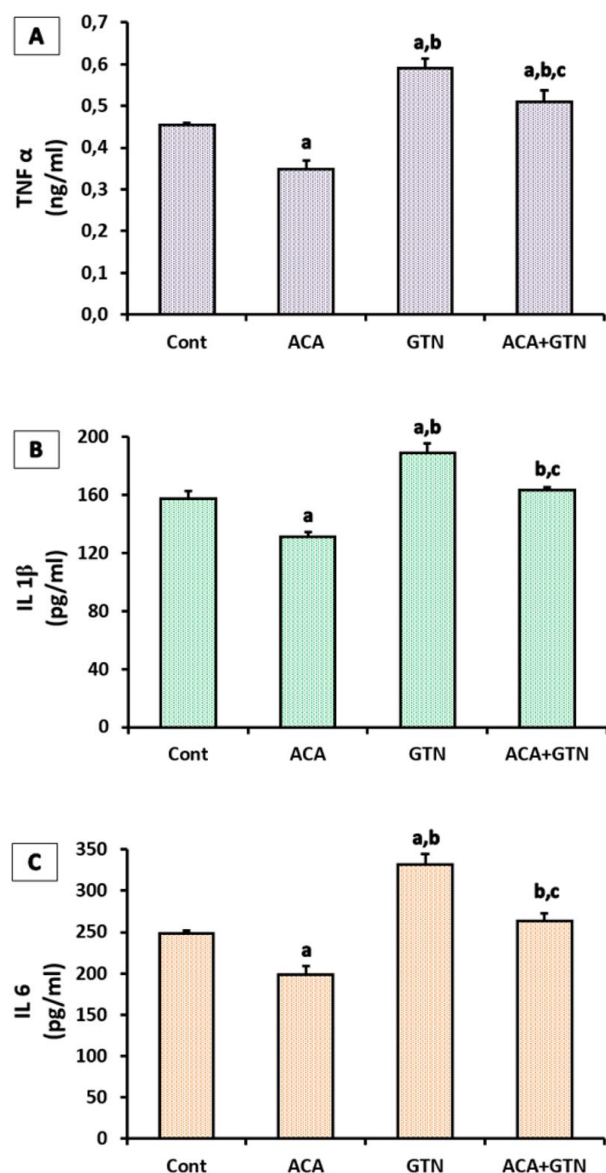
In Figure 4, GTN-induced increased lipid peroxidation (MDA) and decreased GSH levels, and GSHPx activities in the brain and erythrocyte of mice, showing that ACA treatment modulates this state. Brain and erythrocyte GSH concentration (Figure 4A) and GSHPx activity (Figure 4B) were lower in the GTN group than in the control and ACA groups. However, the MDA levels (Figure 4C) were higher in the GTN group than in the control and ACA groups ( $p \leq 0.05$ ). However, in the ACA+GTN group, GSHPx activity and GSH levels increased with ACA treatment, while MDA levels decreased with ACA treatment ( $p \leq 0.05$ ). These results indicated that the GTN-induced increase of MDA is reduced in the brain and erythrocyte by ACA treatment through upregulation of GSH level and GSHPx activity.



**Figure 4.** Effects of ACA (25 mg/kg for three days) administration on glutathione (GSH) (A), glutathione peroxidase (GSHPx) (B), and lipid peroxidation (MDA) (C) levels in the Erythrocyte and Brain of the migraine model (GTN, single dose intraperitoneally, 10 mg/kg)-induced mice. Values are presented as mean  $\pm$  SD of nine separate experiments and expressed as fold-increase over the pre-treatment level (experimental/control). (<sup>a</sup> $p \leq 0.05$  versus Cont, <sup>b</sup> $p \leq 0.05$  versus ACA group, <sup>c</sup> $p \leq 0.05$  versus ACA+GTN)

#### ACA Treatment Modulated GTN-Induced Inflammatory Cytokines in the Brain of Mice with Experimental Migraine

In Figure 5, GTN-induced increased inflammatory cytokines [TNF  $\alpha$  (A), IL 1 $\beta$  (B), and IL 6 (C)] in the brain of mice, showing that ACA treatment modulates this state. The TNF  $\alpha$  (Figure 5A), IL 1 $\beta$  (Figure 5B), and IL 6 (Figure 5C) levels were seriously ( $p \leq 0.05$ ) higher in the GTN groups than in the control. Also, the TNF  $\alpha$ , IL 1 $\beta$ , and IL 6 levels in the brain were lower in the ACA and ACA+GTN groups compared to the GTN group ( $p \leq 0.05$ ).



**Figure 5.** Effects of ACA (25 mg/kg for three days) administration on cytokines [TNF  $\alpha$  (A), IL 1 $\beta$  (B), and IL 6 (C)] levels in the brain of the migraine model (GTN, single dose intraperitoneally, 10 mg/kg)-induced mice. Values are presented as mean  $\pm$  SD of nine separate experiments and expressed as fold-increase over the pre-treatment level (experimental/control). (<sup>a</sup> $p \leq 0.05$  versus Cont, <sup>b</sup> $p \leq 0.05$  versus ACA group, <sup>c</sup> $p \leq 0.05$  versus ACA+GTN)

#### Discussion

PLA2 and TRPM2 channels are known to be involved in neuroinflammatory and neurodegenerative processes, including migraine (Çakır et al., 2017; Yazgan & Nazıroğlu, 2021; Filippone et al., 2022). ACA is an inhibitor of PLA2 and TRPM2 (Harteneck et al., 2007). We investigated ACA's protective effects against migraine headaches and neurotoxicity in the GTN-induced migraine

model. Recent studies have reported that GTN causes an increase in cytosolic ROS and mitochondrial depolarisation, which is associated with an increase in intracellular  $\text{Ca}^{2+}$  and increased inflammatory cytokines (Nazıroğlu et al., 2015; Fila et al., 2023). Increased cytosolic ROS production and increased mitochondrial activity-dependent caspase activation lead to apoptosis (Keil et al., 2011). This study found increased ROS, mitochondrial membrane depolarization, and caspase activation in an experimental migraine model. Our results showed that increased Cas 3 and 9 levels due to mitochondrial activity lead to apoptosis and decreased cell viability in the nerve cells.

The presence of neuronal damage and cell death in the experimental migraine model has been reported in the literature (Ishii et al., 2011; Filippone et al., 2022; Wang et al., 2022). Yazğan and Nazıroğlu (2021) reported GTN-mediated apoptosis, cytosolic ROS, Cas 3, and Cas 9 increases and decreased cell viability in trigeminal ganglion in GTN-induced migraine in trigeminal ganglion. In this study, we found that increased mitochondrial depolarisation activity increases cytosolic ROS and Cas 3 and 9 activities in brain tissue of the GTN group. Furthermore, our analyses showed that apoptosis increased and cell viability decreased in parallel in the GTN group. These results are consistent with previous brain tissue damage studies (Maniskas et al., 2018; Gao et al., 2022). ACA, a TRPM2 channel modulator and PLA2 inhibitor may prevent pathological cellular processes by inhibiting these two pathways that can be pathologically activated in cell membranes. Based on the results of this study, we found that ACA could regulate these pathological processes induced by GTN. In the GTN+ACA group, there was a decrease in cytosolic ROS levels and caspases, as well as inhibition of mitochondrial depolarisation and caspase activation, compared to the GTN group. In addition, ACA increased cell viability by reducing GTN-induced apoptosis in the brain. These effects of ACA may be due to its modulation of PLA2 and TRPM2 channels, reducing cytokine release and inhibiting cytosolic  $\text{Ca}^{2+}$  increase. In this context, ACA may produce neuroprotective effects.

Recent studies show that oxidative stress is one of the most critical factors in the etiology of migraine (Ishii et al., 2011; Abushik et al., 2014; Togha et al., 2019; Gross et al., 2021). Increased intracellular calcium and ROS with pathological activation of receptors and channels in

cellular membranes in brain cells cause mitochondrial membrane depolarisation and, consequently, oxidative stress (Bütün et al., 2015; Yazğan & Nazıroğlu, 2021; Fila et al., 2023). Lipid peroxidation and decreased GSH and GSHPx activity are associated with many neurological disorders, including migraine (Nazıroğlu et al., 2015; Islam, 2017). In a study, GSHPx activity was low in adults and children with migraine (Vurucu et al., 2013). According to recent clinical reports, a decrease in plasma GSH levels and GSHPx activity in clinical and experimental migraine has been reported in the literature (Nazıroğlu et al., 2015; Tripathi et al., 2018; Togha et al., 2019). Our analyses revealed that MDA levels and cytosolic ROS increased in the GTN group, whereas GSH levels and GSHPx activity decreased in brain and erythrocyte samples. We found that ACA administration prevented the GTN-induced decrease in GSHPx enzyme activity and GSH level in the brain and also prevented lipid peroxidation. A similar study reported that ACA administration prevented the OKA-induced decrease in SOD and GSHPx enzyme activity and GSH levels in the cortex and hippocampus and prevented lipid peroxidation (Çakır et al., 2017). The present study showed that GTN disrupted brain and erythrocyte tissue oxidant/antioxidant balance.

PLA2 activation and intracellular  $\text{Ca}^{2+}$  increase are the underlying causes of neuroinflammation in migraine (Kayan et al., 2012; Çakır et al., 2017). This pathway also plays a role in the pathogenesis of various neurodegenerative diseases, including migraine (Sun et al., 2010). Neuroinflammation, which means increased activation of the immune system in the brain, plays a vital role in migraine pathology (Kurşun et al., 2021). In neuroinflammation, an inflammatory process modulated by the production of cytokines and reactive oxygen species occurs in the brain and spinal cord (Norden et al., 2016). Thus, increased levels of inflammatory cytokines in the brain are accepted as an indicator of neuroinflammation (Zhang et al., 2019; Lama et al., 2022). Increased levels of various cytokines such as  $\text{TNF } \alpha$ , IL 1 $\beta$ , and IL 6 have been associated with the modulation of pain threshold and sensitization of nerve fibers, and this has been associated with migraine (Bruno et al., 2007; Yamanaka et al., 2021). Clinical studies found that IL 1 $\beta$ , IL 6, IL 8, and  $\text{TNF } \alpha$  levels increased during migraine attacks and decreased at the end of the attack (Perini et al., 2005; Wang et al., 2015; Yücel et al., 2016). In a rat model, it was observed that IL

1 $\beta$  and IL 6 cytokines increased in cerebrospinal fluid and brain after GTN administration (Reuter et al., 2001). In another GTN-induced migraine model, it was reported that the transcriptional activity of inflammatory cytokines (IL 6 and TNF  $\alpha$ ), CGRP, and nNOS increased in the trigeminal ganglia, cervical spinal cord, and medulla-pons (Greco et al., 2017). In our study, the levels of neuroinflammatory markers TNF  $\alpha$ , IL 1 $\beta$ , and IL 6 were increased in the brains of mice in the GTN group. TNF  $\alpha$ , IL 1 $\beta$ , and IL 6 levels in the brain were significantly decreased in the GTN+ACA group compared to the GTN group. Thus, in our study, by the literature, we found that ACA, which inhibits both TRPM2 and PLA2, decreased neuroinflammation in GTN-induced migraine.

In conclusion, according to the results of this study, in the GTN-induced migraine model, GTN weakened antioxidant defense in brain tissue and disrupted oxidant-antioxidant balance. Our results were consistent with similar reports in the literature. In addition, our results showed that caspase activities increased due to increased cytosolic ROS. Thus, apoptotic signals were induced, and cell viability decreased. Therefore, migraine may be associated with neuronal damage in brain tissue. Our analyses suggest that ACA, which prevents cytosolic ROS increase and cytokine release, may produce neuroprotective effects. We propose that ACA may exert these effects by preventing cytosolic Ca<sup>2+</sup> increase and cytokine release through inhibition of TRPM2 and PLA2.

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The authors declare no conflict of interest in the current study.

### Authorship Contributions

Dr. B Yazğan and Dr. Y Yazğan formulated the hypothesis and was responsible for writing the report. Dr. B Yazğan and Dr. Y Yazğan were also accountable for the MDA, GSH level, GSHPx activity, cytokines analyses, and animal experiments such as the induction of GTN and injection of ACA. Dr. Y Yazğan performed the microplate reader analyses.

### Ethics declarations

The current study has no study with human and human participants. All study procedures and animal care were approved by the Local Experimental Animal Ethical

Committee of Burdur Mehmet Akif University (BMAU) (Decision: 78/653. Date: 17.06.2020).

### Conflict of Interest

The authors declare that they have no conflicts of interest.

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