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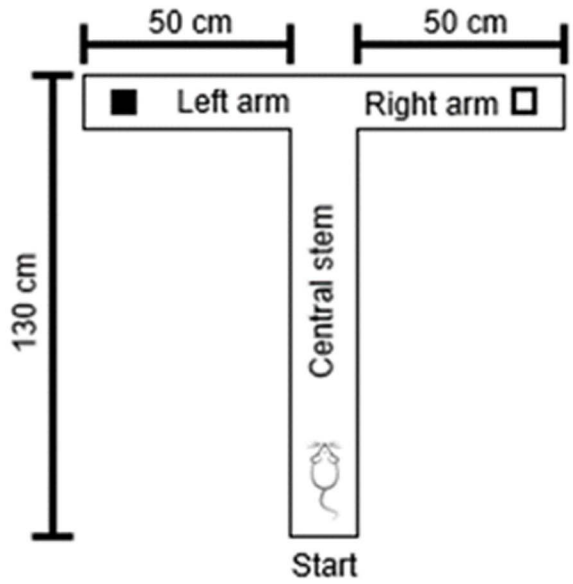

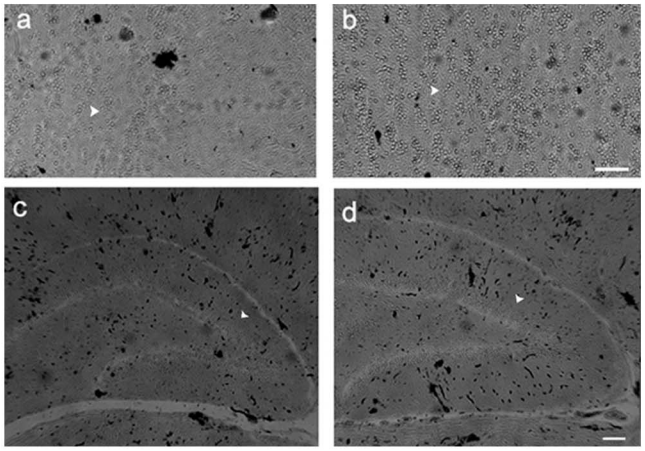

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AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate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

J Cell Neurosci Oxid Stress 2022;14(2): 1085-1094.

Potent antioxidant alpha lipoic acid reduces STZ-induced oxidative stress and apoptosis levels in the erythrocytes and brain cells of diabetic rats

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List of Abbreviations;

DIA*, Diabetes;* ***GSHPx****, Glutathione peroxidase;* ***LipPx,*** *Lipid peroxidation;* ***ROS,*** *Reactive oxygen species;* ***STZ,*** *Streptozotocin;* ***α-LA,*** *Alpha-Lipoic-Acid.*

Abstract

Diabetes, which causes oxidative stress-induced neuronal damage, is still one of the most important chronic health problems in the world. It can cause serious cellular loss and damage throughout the course of the disease. It is hypothesized that increased oxidative stress in this process increases free reactive oxygen species (ROS) and apoptotic markers and causes diabetic damage. Alpha-Lipoic acid (α-LA), which has a direct antioxidant effect in ROS reduction reactions, is also among the main components of the antioxidant system that works for free radical control and apoptosis. To understand the role of α-LA in reducing diabetes-induced oxidative damage, we examined the production of ROS in the brain cortex and erythrocytes of rats and their effects on markers of apoptosis.

Forty adult Wistar albino rats were divided into four groups as control, α-LA, diabetic (DIA), and DIA+α-LA. For the induction of diabetes, the intraperitoneal injection of a dose of streptozotocin (STZ) (45 mg/kg) was used. α-LA (50 mg/kg) was applied to the groups of α-LA and DIA+α-LA for 14 days. At the end of the experiment, the brain cortex tissue and erythrocyte samples were taken from the rats.

The levels of apoptosis, caspase 3, caspase 9, mitochondrial membrane potential, intracellular ROS, and lipid peroxidation were increased in the STZ group, although their levels were decreased in the DIA+α-LA group by the injection of α-LA. The STZ treatment-induced decreases of cell viability, reduced glutathione, and glutathione peroxidase were increased in the brain and erythrocytes by the treatment of α-LA.

In conclusion, diabetes acted a role in neuronal damage caused by increased ROS and apoptosis. We observed that α-LA induced a modulatory role on the

apoptotic, oxidant, and antioxidant parameters in the brain and erythrocyte. The neuroprotective role of α-LA treatment may be explained by its modulating activity against increased oxidative stress and apoptosis.

Keywords; Alpha Lipoic Acid, Apoptosis, Diabetes, Brain, Glutathione.

Introduction

Diabetes, one of the most important chronic health problems in the world, can cause serious cellular losses and damage throughout the course of the disease (Rochette et al. 2013; Düzova et al. 2021). It has been reported that diabetes causes neuronal damage and oxidative neuronal toxicity, causing damage to the central nervous system and neuronal apoptosis (Shyma et al. 2022). The molecular mechanisms and complications of diabetes are still not fully understood. Some of the current studies; provides evidence that the production of free reactive oxygen species (ROS), which causes oxidative stress in many tissues, is increased due to hyperglycemia (Rochette et al. 2013; Zhang et al. 2013; Koneri et al. 2014). It is known that ROS is eliminated by the antioxidant systems in the organism (Yang et al. 2022). Oxidative stress, which occurs when the oxidant-antioxidant balance is disrupted in the tissues, causes tissue damage and cell death (Khansari et al. 2009; Rochette et al. 2013).

Alpha-lipoic-acid (α-LA) is a substance that is synthesized in the body, can be taken in the diet, and is also soluble in both water and lipids. At the same time, α-LA is rapidly absorbed in the diet, readily crosses cell membranes, and is widely distributed in the cytosol and extracellular spaces (Shaygannia et al. 2018). α-LA molecule, which has been attributed to antioxidant properties in many studies (Ghibu et al. 2009; Park et al. 2014; Tibullo et al. 2017; Nur et al. 2017), is thought to be protective against increased ROS production with mitochondrial dysfunction (Park et al. 2014). α-LA, one of the main components of the antioxidant system working for free radical control, takes part in ROS reduction reactions. It has a direct antioxidant effect by taking part in reduction reactions thanks to the cysteine groups it contains (Tibullo et al. 2017). α-LA is an important antioxidant that can be used to prevent complications of diabetes, such as retinopathy, neuropathy and other vascular diseases due to its direct and indirect antioxidant properties (Ghibu et al. 2009). Numerous clinical studies have shown that α-LA causes a significant reduction in diabetes complications (Ametov et al. 2003; Mijnhout et al. 2012; Garcia-Alcala et al. 2015). In a previous study with human glioblastoma cell lines, the regulatory role of α-LA on ROS and apoptosis was reported (Deveci et al. 2019). It has also been reported that neuronal damage in the brain tissues of diabetic rats is reduced by α-LA (Wang and Zhao 2016; Tanbek et al. 2022). The antioxidant properties of α-LA have made it a popular dietary supplement in various pathological conditions such as diabetic polyneuropathies and neuronal damage (Ghibu et al. 2009; Liu et al. 2017). However, the detailed molecular mechanisms underlying the antioxidant effects of α-LA are still poorly known.

Mitochondrial apoptotic pathways are predicted to be important in diabetes-induced cellular damage and apoptosis. Activation of caspase 3 and 9 and an increase in ROS products can be observed as a result of disruption of signal transmission in mitochondrial membranes (Li et al. 2009; Wang and Zhao 2016). Current research shows that antioxidants protect cells from damage under oxidative stress conditions, including brain cells that are very sensitive to oxidative stress (Li et al. 2009; Wang and Zhao 2016; Valdecantos et al. 2019; Tanbek et al. 2022). In addition, increased oxidative stress may cause a decrease in cell viability (Najafi et al. 2015).

In this context, we hypothesized that suppression of oxidative stress might reduce diabetic cellular damage and apoptosis. Thus, we analyzed mitochondrial depolarization, apoptosis, ROS, caspases, and cell viability in animal's brain and erythrocyte to determine the effects of α-LA on the reduction of diabetes-induced oxidative damage. We also examined the antioxidant [glutathione (GSH), and glutathione peroxidase (GSHPx)] and lipid peroxidation (LipPx) levels in the erythrocyte and brain.

Materials and methods

Animals

40 females (190 ± 20 g) Wistar albino rats, 10-12 weeks old, were used in the study. All study procedures and animal care were approved by the Local Experimental Animal Ethical Committee in accordance with the guidelines of the International Study Plan. The animals were maintained and used according to use of Laboratory and the Guide for the Care and the Animal Welfare Act. Experimental animals were kept in cages with a temperature of 22 °C and a humidity of 60% and two

experimental animals in each cage. Throughout the study, Experimental animals were under control in a 12-hour dark light cycle. Experimental animals were provided with access to commercial feed and tap water.

Groups

- **Control Group** (n = 10); Animals was received intraperitoneal (int) 0.9% w/v saline.

- **DIA Group** (n = 10); The animals in the group were received 45 mg / kg int STZ (a single dose) (Sözbir and Nazıroğlu 2015).

- **α-LA Group** (n = 10); α-LA (50 mg / kg / day) applied to the rats in the group of α-LA for 14 days (Hussein et al. 2012).

- **DIA+α-LA Group** (n = 10); After the induction of DIA via STZ injection, the int α-LA was given to the animals (50 mg / kg / day) in the group for 14 days.

All experimental animals dissected by either asphyxiation or cervical dislocation in accordance with experimental animal legislation. The brain cortex and erythrocyte samples were isolated. Then, the isolated brain cells samples were used for cell viability (MTT), ROS, apoptosis, mitochondrial determination, caspase 3, and 9 analyses. Remaining the brain cortex and erythrocyte samples were used for lipid peroxidation (LipPx), reduced glutathione (GSH) level, and glutathione peroxidase (GSHPx) activity analyses.

Determination of Cell Viability (MTT)

Viability assays were performed by measuring mitochondrial reductase activity with 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, Istanbul, Türkiye) as described in previous studies (Uğuz and Nazıroğlu 2012). Absorbance values of MTT were recorded by the microplate reader (Infinite pro200; Tecan Inc, Groedig, Austria) at 490 nm. The data are presented as percentage (%)-increase over the pretreatment level (Yıldızhan and Nazıroğlu 2020; Yazğan and Nazıroğlu 2021).

Determination of Intracellular ROS Production

After entering rhodamine 123 (DHR 123) cytosol, it turns into Rh 123 which is fluorescent form by oxidation. This transformation is proportional to the formation of ROS. The method of analysis has been described in detail in our previous publications (Yazğan and Nazıroğlu 2021). As measured by Yazğan and Nazıroğlu (2021), the measurement of ROS production was done with a microplate reader. Briefly, Brain tissue cells were washed with RPMI-1640 medium, incubated with DHR 123 for 25 minutes at 37 ° C (Espino et al. 2011). The fluorescence changes were determined at 488 nm excitation and 543 nm emission wavelengths in the microplate reader (Infinite PRO 200).

Determination of Apoptosis Activities

Apoptosis assay was performed using a commercial kit, Cell-APOPercentage dye. When the cell membrane integrity is impaired, APOPercentage dye is actively transported into the cell and paints the apoptotic cell in red. (Uğuz and Nazıroğlu 2012). After staining and incubation, reading was done on spectrophotometer. The method of analysis has been described in detail in our previous publications (Yıldızhan and Nazıroğlu 2020; Yazğan and Nazıroğlu 2021).

Determination of Mitochondrial Membrane Potential

JC-1 was used to determine the mitochondrial membrane potential (Bejarano et al. 2011). The method of analysis has been described in detail in our previous publications (Yazğan and Nazıroğlu 2021). As measured by Yazğan and Nazıroğlu (2021), the measurement of JC-1 activity was done with a microplate reader. Briefly, Brain tissue cells were washed with RPMI-1640 medium, incubated with 4 μM JC-1 for 45 minutes at 37 ° C (Espino et al. 2011). JC-1 signals were measured as described in a previous study (Yıldızhan and Nazıroğlu 2020; Yazğan and Nazıroğlu 2021). The fluorescence changes were determined at 488 nm excitation and 520/596 nm emission wavelengths in the microplate reader (Infinite PRO 200).

Determination of Caspase 3 and 9 Activities

For the determinations of caspase 3 and 9 activities, the brain cells were incubated at 37 °C for 1 hour with 2 ml of substrate solution as described in previous studies (Yazğan and Nazıroğlu 2021). For this, the splitting of fluorogenic substrates was used. It was then measured with a microplate reader with 360 nm excitation and emission at 460 nm. The method of analysis has been described in detail in previous publications (Yıldızhan and Nazıroğlu 2020; Yazğan and Nazıroğlu 2021).

The Determination of Lipid Peroxidation, GSH, and GSHPx Levels in Erythrocyte and Brain

The lipid peroxidation (LipPx) levels as malondialdehyde (MDA) in the hemolyzed brain homogenate and erythrocyte hemolysate were spectrophotometrically (Shimadzu-UV1800, Kyoto, Japan) measured with the Thiobarbituric-acid reaction. The total protein contents in the hemolyzed cells homogenate were measured by method of Lowry et al. (1951) with bovine serum albumin as the standard. For assaying the GSHPx activity, a spectrophotometric (37 °C and 412 nm) method of Lawrence and Burk (1976) was used in the brain and erythrocyte as described in previous studies. The extraction and wavelength details of analyses were indicated in our studies (Nazıroğlu et al. 2012; Yıldızhan and Nazıroğlu 2020; Yazğan and Nazıroğlu 2021). The unit of μmol/g protein was used in the brain and erythrocyte cells for the expression of the LipPx and GSH levels. The activity of GSHPx in the brain and erythrocyte cells was expressed as IU/ g protein.

Statistical Analyses

All results were expressed as means ± standard deviation (SD) and p values less than $p \leq 0.05$ were regarded as significant. To determine the effect of treatment, data were analyzed using one way ANOVA. Post-hoc test was used in all data with a statistically significant difference. Presence of significance was assessed with Tukey HSD test. The SPSS statistical program was used to analyze the data (version 17.0 software, SPSS Inc. Chi, USA).

Results

α-LA Treatment Modulated STZ-Induced Cell Viability and Apoptosis in The Brain of Diabetic Rats

In addition to an increase in cytosolic ROS production, an increase in caspase activations due to mitochondrial activity results in apoptosis (Keil et al. 2011; Nazıroğlu 2012; Ureshino et al. 2019). Therefore, JC-1 is an important parameter of mitochondrial activity and is used as an important indicator of caspase activity and apoptosis. α-LA treatment modulated STZ-induced cell viability and apoptosis in the brain are shown in Fig 1. The MTT values were markedly ($p \leq 0.001$) lower in the DIA groups than in the controls (Fig 1A). The MTT values were higher in the α-LA and DIA+α-LA groups than in the DIA groups ($p \leq 0.001$). Apoptosis values were seriously ($p \leq 0.001$) higher in the DIA groups than in the controls (Fig 1B). In the brain cells, the apoptosis values were seriously ($p \leq 0.001$) lower in the α-LA and DIA+α-LA groups than in the DIA groups.

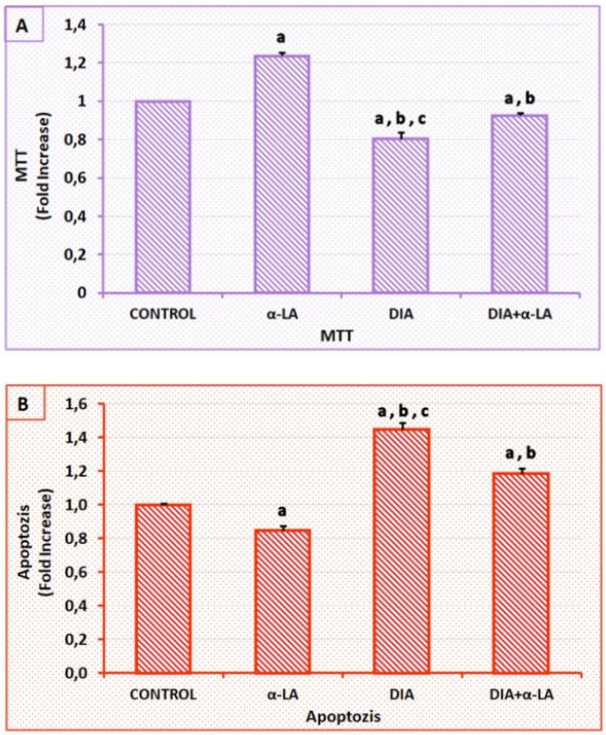

***Figure 1**. Effects of α-LA (50 mg/kg for 14 days) administrations on MTT (**A**) and apoptosis (**B**) levels in brain of diabetes (STZ)-induced rats. Values are presented as mean ± SD of eight separate experiments and expressed as fold-increase over the pre-treatment level (experimental/control). ($^{a}p \leq 0.001$ versus control. $^{b}p \leq 0.001$ versus α-LA group, $^{c}p \leq 0.001$ versus DIA+α-LA).*

α-LA Treatment Modulated STZ-Induced Mitochondrial Membrane Depolarization and ROS in The Brain of Diabetic Rats

The electron transport system of mitochondria causes JC-1 loss in mitochondria, resulting in excess ROS production (Joshi and Bakowska 2011). Therefore, JC-1 is an important parameter of mitochondrial activity and is used as an important indicator of ROS generation in cells. α-LA treatment modulated STZ-induced JC-1 and ROS in the brain are shown in **Fig 2**. The JC-1 and ROS levels were seriously ($p \leq 0.001$) higher in the DIA groups than in the control (**Fig 2A, B**). Also, the JC-1 and ROS levels in the brain were lower in the α-LA and DIA + α-LA groups

compared to the DIA group ($p \leq 0.001$).

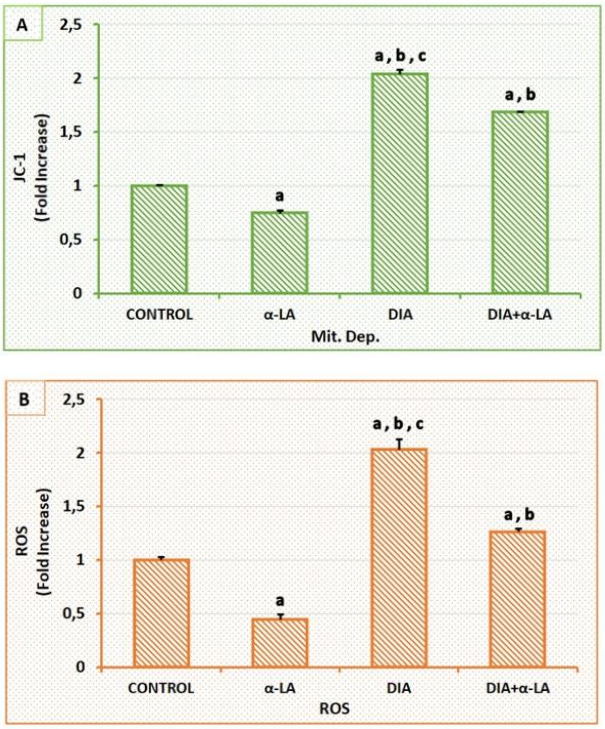

***Figure 2**. Effects of α-LA (50 mg/kg for 14 days) administration on JC-1 (**A**) and ROS (**B**) levels in the brain of diabetes (STZ)-induced rats. Values are presented as mean ± SD of eight separate experiments and expressed as fold-increase over the pre-treatment level (experimental/control). ($^{a}p \leq 0.001$ versus control, $^{b}p \leq 0.001$ versus α-LA group, $^{c}p \leq 0.001$ versus DIA+α-LA).*

α-LA Treatment Modulated STZ-Induced Caspase 3 and 9 in The Brain of Diabetic Rats

α-LA treatment modulated STZ-induced caspase 3 and 9 in the brain are shown in **Fig 3**. The caspase 3 and 9 levels in brain were seriously ($p \leq 0.001$) higher in the DIA groups than in the control (**Fig 3A, B**). Also, the caspase 3 and 9 activities were lower in the α-LA and DIA + α-LA groups compared to the DIA group ($p \leq 0.001$).

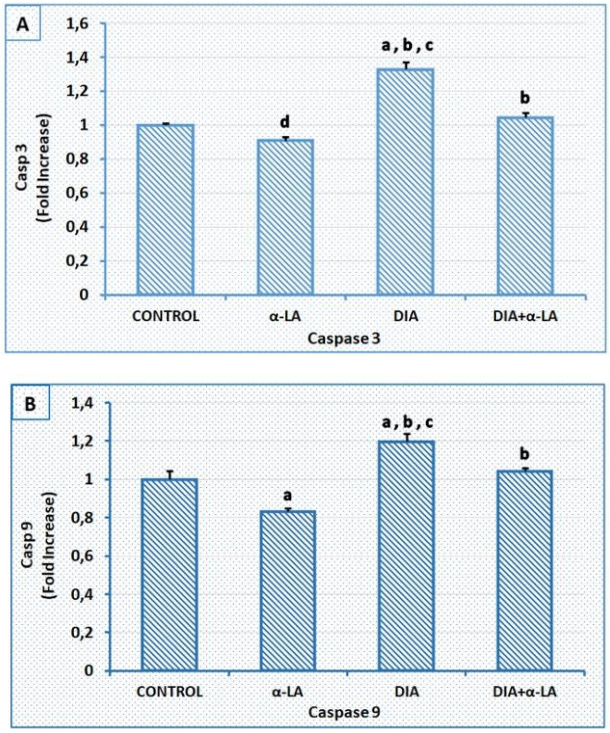

***Figure 3**. Effects of α-LA (50 mg/kg for 14 days) administration on caspase 3 (**A**) and caspase 9 (**B**) activities levels in the brain of diabetes (STZ)-induced rats. Values are presented as mean ± SD of eight separate experiments and expressed as fold-increase over the pre-treatment level (experimental/control). ($^{a}p \leq 0.001$ and $dp \leq 0.05$ versus control, $^{b}p \leq 0.001$ versus α-LA group, $^{c}p \leq 0.001$ versus DIA+α-LA).*

α-LA Treatment Modulated STZ-Induced Lipid Peroxidation (MDA), Reduced Glutathione (GSH), and Glutathione Peroxidase (GSHPx) in The Brain and Erythrocyte of Diabetic Rats

Reduced glutathione (GSH) levels, glutathione peroxidase (GSHPx) activities, and lipid peroxidation (MDA) levels results are shown in **Fig 4** (brain) and **Fig 5** (erythrocyte) respectively. Brain GSH concentration (**Fig 4A**) and GSHPx activity (**Fig 4B**) were lower in the DIA group than in the control and α-LA groups, although the MDA levels (**Fig 4C**) was higher in the DIA group than in the control and α-LA groups ($p \leq 0.05$). However, the GSHPx activity and GSH levels were increased in the DIA+α-LA group by the α-LA treatment, although MDA levels was decreased in the DIA+α-LA group by the α-LA treatment ($p \leq 0.05$). Erythrocyte GSH concentration (**Fig 5A**) and GSHPx activity (**Fig 5B**) were lower in the DIA group than in the control and α-LA groups, although the

MDA levels (**Fig 5C**) was higher in the DIA group than in the control and α-LA groups ($p \leq 0.05$). However, the GSHPx activity and GSH levels were increased in the DIA+α-LA group by the α-LA treatment, although MDA levels was decreased in the DIA+α-LA group by the α-LA treatment ($p \leq 0.05$). These results clearly indicated that DIA induced increase of MDA is decreased in the brain and erythrocyte by α-LA treatment through upregulation of GSH level and GSHPx activity.

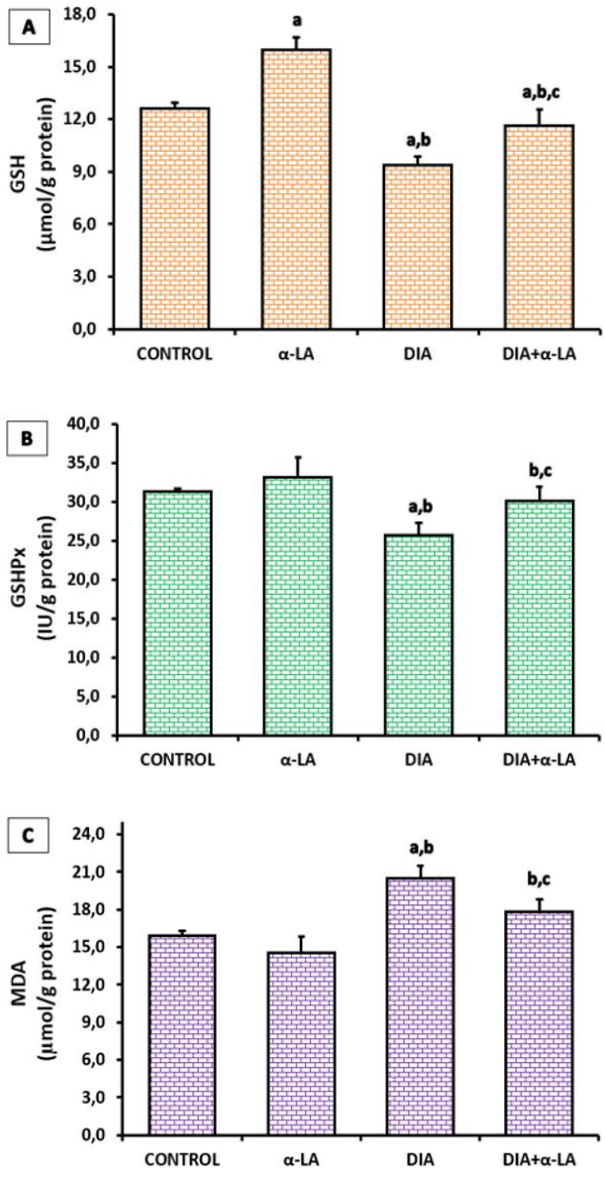

Figure 4. *Effects of α-LA (50 mg/kg for 14 days) administration on reduced glutathione (GSH) (**A**), glutathione peroxidase (GSHPx) (**B**), and lipid peroxidation (MDA) (**C**) levels in the brain of diabetes (STZ)-induced rats. Values are presented as mean ± SD of eight separate experiments and expressed as fold-increase over the pre-treatment level (experimental/control). ($^{a}p \leq 0.05$ versus control, $^{b}p \leq 0.05$ versus α-LA group, $^{c}p \leq 0.05$ versus DIA).*

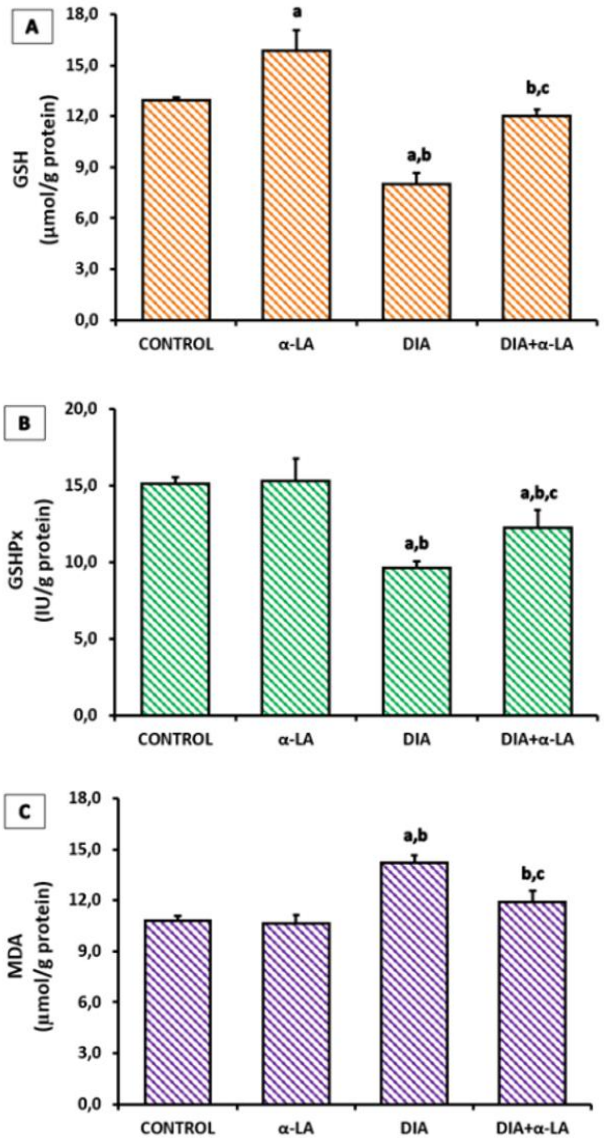

Figure 5. *Effects of α-LA (50 mg/kg for 14 days) on reduced glutathione (GSH) (**A**), glutathione peroxidase (GSHPx) (**B**), and lipid peroxidation (MDA) (**C**) levels in the erythrocyte of diabetes (STZ)-induced rats. Values are presented as mean ± SD of eight separate experiments and expressed as fold-increase over the pre-treatment level (experimental/control). ($^{a}p \leq 0.05$ versus control, $^{b}p \leq 0.05$ versus α-LA group, $^{c}p \leq 0.05$ versus DIA).*

Discussion

Diabetes is a chronic disease that causes severe cellular damage and tissue loss. This metabolic disease affects all organs, including the brain, over a long period of time (Tanbek et al. 2022). Considering the results of this study, we think that hyperglycemia-induced cytosolic ROS increase, mitochondrial dysfunction, and oxidative stress play a role in the pathophysiological processes of diabetic neuronal damage. Many studies support these data (Umeda et al. 2006; Pabbidi et al. 2008; Uchida et al. 2011). Consistent with previous studies, increased cytosolic ROS in diabetes groups triggered an increase in caspase activity via mitochondrial membrane depolarization. (Kahya et al. 2017; Zhang et al. 2020; Düzova et al. 2021). The increase in ROS production associated with hyperglycemia is considered to be related to the degenerative processes in diabetes **(**Rochette et al. 2013). In the results of this study, we showed that increased cellular oxidative stress in brain tissues caused a decrease in cell viability. The results of the diabetes groups confirm the knowledge that oxidative stress and increased mitochondrial depolarization may be responsible for the increase in ROS (Uslusoy et al. 2017; Kahya et al. 2017).

It has been shown that α-LA increases antioxidant enzyme activities and reduces complications in neurological diseases with high ROS increase such as diabetes, Alzheimer's, Down Syndrome and brain ischemia (Sousa et al. 2019). Numerous studies have reported that oxidative stress causes neuron damage in the brain in diabetic rats (Najafi et al. 2015; Düzova et al. 2021; de Tanbek al. 2022; Shyma et al. 2022). There are also studies showing that α-LA reduces oxidative stress-induced damage in the brain tissue of diabetic rats (Piotrowski et al. 2001; Gomes et al. 2014). α -LA is considered to be protective against increased ROS production with mitochondrial dysfunction (Park et al. 2014). The modulatory roles of α-LA on SOD, CAT, GSHPx activities and LipPx in diabetic rat brain tissue were reported (Tanbek et al. 2022). However, we did not find any report examining the effect of α-LA on antioxidant parameters in brain cortex cells and erythrocyte of diabetic rats and cytosolic ROS, mitochondrial depolarization, caspase activities, apoptosis, and cell viability in the brain tissues. In this direction, we analyzed the effects of α-LA on apoptosis, intracellular ROS and caspase activities in brain cells, and on GSH, GSHPx and LipPx levels in brain cortex cells and erythrocytes.

In various animal and human experimental models, administration of α-LA caused an increase in GSH level and GSHPx activity. (Khansari et al. 2009; de Sousa et al. 2019; Tanbek et al. 2022; Shyma et al. 2022). In DIA groups of rats with diabetes model created with STZ: we found that GSH and GSHPx activities decreased and LipPx increased in brain tissue and erythrocyte. On the other hand, we determined that after α-LA treatment, it increased GSH and GSHPx activities and decreased LipPx in diabetic rat brain tissue and erythrocyte. Our results showing the oxidant-antioxidant status in diabetic rat brain tissues were consistent with similar reports in the literature. (Najafi et al. 2015; de Sousa et al. 2019; Tanbek et al. 2022). This may be related to the antioxidant activity of α-LA.

We detected an increase in mitochondrial membrane depolarization, caspase 3 and caspase 9 activation, as well as cytosolic ROS analysis results in diabetes groups. Therefore, according to the results of this study, oxidative stress caused by diabetes has led to the idea that it may cause neuronal damage by disrupting cellular signaling pathways. The presence of neuronal damage and cell death in the experimental diabetes model has been reported in the literature (Piotrowski et al. 2001; Amer et al. 2021; Shyma et al. 2022). In this study, we determined that caspase 3 and caspase 9 activities increased and cell viability decreased in brain tissue. As a result, diabetes caused disruption of cellular signals, increased production of ROS, mitochondrial dysfunction, and in parallel, caused apoptosis.

These indicated the presence of brain tissue damage and were consistent with previous studies. It has been reported that α-LA exerts neuroprotective effects in neurological and psychological diseases, obesity, and diabetes in human and animal experimental models. (Rocamonde et al. 2012; Miao et al. 2013; de Sousa et al. 2019). We determined that ROS and caspase activation decreased in α-LA treated groups, whereas cell viability increased. α-LA treatment applied to DIA groups modulated STZ-induced cell damage and apoptosis and increased cell viability in the brain. α-LA may be effective in repairing cell damage in brain tissue with its antioxidant and neuroprotective effects.

In conclusion, similar reports in the literature and results of this study showed that the increase in oxidative stress caused by hyperglycemia in diabetes weakens the antioxidant defense in the brain tissue and causes the

oxidant-antioxidant balance to deteriorate. In addition, our results showing that apoptotic signals were induced and caspases activities increased due to increased ROS determined that diabetes may be associated with neuronal damage in brain tissue. Thanks to its antioxidant and neuroprotective effects, α-LA treatment may be effective in preventing complications related to diabetes.

Acknowledgments

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

Authorship Contributions

Dr. Betül Yazğan and Dr. Yener Yazğan formulated the hypothesis and was responsible for writing the report. Dr. Betül Yazğan and Dr. Yener Yazğan was also responsible for the MDA, GSH level, GSHPx activity and animal experiments such as the induction of STZ and injection of α-LA. Dr. Yener Yazğan performed the microplate reader analyses**.**

Ethics declarations

In the current study, there is no study with human and human participants. All study procedures and animal care were approved by the Local Experimental Animal Ethical Committee of SDU (Protocol number: 2017-05-04. Date: 24.08.2017).

Conflict of Interest

The authors declare that they have no conflicts of interest.

Financial Disclosure

This study was carried out with financial support from BSN Health, Analyses, Innov., Consult., Org., Agricul., Trade Ltd, Göller Bölgesi Teknokenti, Isparta, Türkiye (Project No: 2017-02). The owner of the project is Dr. Betül Yazğan.

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