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Effects of curcumin and melatonin treatment in the cerebral cortex of adult rats

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Abstract. The study investigated the effect of exogenous melatonin and (or) curcumin treatment on the cerebral cortex of adult rats. In this context, malondialdehyde (MDA), nitric oxide (NO), glutathione (GSH), superoxide dismutase (SOD), nuclear factor E2-related factor 2 (Nrf2) and SIRT2 protein expression were examined. A total of 30 Wistar albino rats involved in the study were randomly divided into five groups. Over 30 days, the control groups received phosphate-buffered saline or dimethyl sulfoxide injections, and the treatment groups received melatonin, curcumin, or a combination of melatonin and curcumin injections. In the cerebral cortex homogenates, the MDA, GSH, and sum of NO were respectively determined by the thiobarbituric acid, modified Ellman and Griess test methods. The SOD and Nrf2 levels were examined using the ELISA method and SIRT2 protein expression using the Western blot technique. The study found that both melatonin and curcumin treatments significantly reduced lipid peroxidation and SIRT2 protein expression levels ($p < 0.05$) and increased the Nrf2 level in the cytoplasm ($p < 0.05$). The study revealed that curcumin and melatonin treatments reduced MDA and SIRT2 protein expression level and increased intracellular Nrf2, GSH, and SOD in the cortex tissue. We also found that the combined melatonin and curcumin treatment produced no synergistic effect.

Key words: Curcumin — Melatonin — Nuclear factor E2-related factor 2 — Oxidative stress — Sirtuin 2

Abbreviations: DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; GSH, glutathione; MDA, malondialdehyde; NOS, nitric oxide synthase; Nrf2, nuclear factor E2-related factor 2; PBS, phosphate-buffered saline; ROS, reactive oxygen species; SIRT2, sirtuin 2; SOD, superoxide dismutase.

Introduction

Oxidative stress is an imbalance between free radicals and antioxidants inside the cell. Reactive oxygen species (ROS) are formed in cells in response to environmental stress and aggravation of ROS. Antioxidant mechanisms contain enzymes like superoxide dismutase (SOD) and non-enzymatic antioxidants like glutathione (GSH). A reduction in antioxidants causes lipid peroxidation that produces malondialdehyde (MDA), an oxidative stress biomarker. The brain is a target for oxidative damage due to its high oxygen consumption and low antioxidant defense system (Jernigan et al. 2001).

Sirtuins (SIRTs1–7), class III histone deacetylase enzymes, have critical roles in the antioxidant and oxidative stress-

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related processes, including longevity, DNA repair, and metabolic disease (Nakagawa and Guarente 2011). However, the SIRT2, one of the sirtuin proteins, may behave differently in case of oxidative stress. Previous studies have reported that high SIRT2 levels in severe stress situations induce cell deaths (Cao et al. 2016); however, at low levels it protects against biological stress, neurodegenerative disease, postischemic liver injury, and hepatic fibrosis (Outeiro et al. 2007; Wang et al. 2007; Singh et al. 2018). The oxidative stress-activated SIRT2 not only deacetylates histone but also acts to deacetylate some transcription factors related to antioxidative molecules such as nuclear factor E2-related factor 2 (Nrf2). As a sensor of oxidative stress, Nrf2 located in the cytoplasm migrates to the nucleus to regulate the expression of many antioxidants and deoxification genes under oxidative stress (Arteaga et al. 2016; Wang et al. 2017).

Melatonin, a hormone released from the pineal gland, has radical scavenging antiapoptotic and anti-inflammatory properties against oxidative stress products (Tan et al. 2002). The studies on aging brain tissue and neurodegenerative diseases have shown that melatonin indirectly increases the expression and (or) function of genes through antioxidant activities (Mayo et al. 2002). Some studies have stated that in oxidative stress, melatonin up-regulates Nrf2 by reducing the levels of ROS and MDA, and modifies the transcription of GSH, and inhibits peroxynitrite formation by inhibiting nitric oxide synthase (NOS) in brain tissue (Leon et al. 2004; Yu et al. 2019). Furthermore, studies have observed that the inhibitory effect of melatonin on the SIRT2 level reduces oxidative stress and that exogenous melatonin treatment highly decreases the SIRT2 expression of aged rats and slightly middle-aged rats (Akbulut et al. 2014; Keskin-Aktan et al. 2018).

Curcumin has powerful anti-inflammatory, antiproliferative, antioxidant, and anti-tumor effects (Bala et al. 2006; Mansouri et al. 2012); its antioxidant effects are visible in various brain regions, such as the cerebral cortex, hippocampus, and cerebellum of aged rats (Bala et al. 2006; Akbulut et al. 2008). Studies have reported that the protective effect of curcumin emerges with the activity modulation of redox-sensitive and survival pathways such as Nrf2 and SIRT1 (Motaghinejad et al. 2015). As a natural compound, curcumin induces Nrf2 (Na et al. 2013).

Without correctly targeting the cause of oxidative stress or the intrinsic oxidant system, sufficient therapeutic options to treat many diseases may not be achieved. The specific target to cope with oxidative stress may be Nrf2. Although studies in the literature have separately revealed the oxidative stress relationship between antioxidant Nrf2 and SIRT2, to our best knowledge, there is no holistic research on the healthy cerebral cortex of rats. In light of this information, the current study aimed to investigate the relationship of exogenous melatonin and (or) curcumin treatments with the levels of lipid peroxidation (MDA), NOx, antioxidants (GSH and SOD), the level of transcription factor Nrf2, and the SIRT2 protein expression in the adult rat cerebral cortex.

Materials and Methods

Animals and treatment

Adult (12–13-month-old; weighing 358.06 ± 29.05 g Wistar albino) male rats were obtained from and housed in the Gazi University Laboratory Animal Breeding and Experimental Research Center (GUDAM). The conditions of care for the rats was determined as a controlled environment of 150 lx light intensity, 22 ± 2°C temperature, 55 ± 10% humidity on a 12:12 h light/dark cycle and fed with standard ad libitum rat chow and regular tap water. The research was conducted according to the ethics committee criteria (permission: GU ET-11.056).

The rats were randomly divided into five groups ($n = 6$ *per* group): two control groups (PBS, DMSO) and three treatment groups (MLT, CUR, MLT+CUR). PBS control group: phosphate buffered saline (1% ethanol) was injected subcutaneously (sc). DMSO control group: dimethyl sulfoxide (1%) was injected intraperitoneally (ip). MLT group: injected with melatonin (sc, 10 mg/kg/day; M5250-16, Sigma-Aldrich, Germany), and PBS solution (1% ethanol). CUR group: injected with curcumin (ip, 30 mg/kg/day; 1385-56 Sigma-Aldrich, Germany), and DMSO solution (1%). MLT+CUR group: injected with melatonin (sc, 10 mg/kg/day with 1% ethanol: PBS solution) and curcumin (ip, 30 mg/kg/day with 1% DMSO). The dosage and duration of the treatments were based on previous research (Akbulut et al. 2008, 2014). All rats in the study groups were injected daily at 5:00 pm for 30 days. On the 31st day, all rats were sacrificed at 9:00 am; the brains were removed, and the hemispheres separated. All cerebral cortex tissues of the cortical lobes were harvested and collected into a tube and then stored in a freezer at −80°C for further study.

The measurement of MDA and GSH specification in the cerebral cortex tissue

Lipid peroxidation levels in cerebral cortex tissue were measured using the reagent thiobarbituric acid method (Casini et al. 1986). Cerebral cortex tissue (40 mg) was homogenized in ice-cold trichloroacetic acid (TCA: 0.72 ml, 10% w/v, Sigma-Aldrich, Germany) by a sonicator (Sonics vibrocell, Sonics&Materials, Inc.,Germany). Then, the homogenized tissue was centrifuged (MPW-350, Med. Instrument, Poland) at 4000 rpm for 15 min at +4°C. The supernatant was collected and centrifuged for 8 min again.

After centrifugation, 10 µl butylated hydroxytoluene (BHT: 1% w/v, Sigma-Aldrich, Germany) was added to 250 µl of the supernatant. Then 250 µl thiobarbituric acid (TBA: 0.67% w/v, Sigma-Aldrich, Germany) was incubated in boiling water (100°C) for 15 min. After cooling, the last centrifugation was applied at 4000 rpm. The absorbance of the collected supernatant was measured immediately at 532 nm. The cortex tissue lipid reoxidation level was expressed as equivalent MDA (nmol/g tissue) using the extinction coefficient of 1.56×10^5 $mol^{-1}\cdot cm^{-1}$.

The glutathione (GSH) level was determined using the modified Ellman method. After the 0.3 M Na_2HPO_4 and dithiobisnitrobenzoate solution (0.4 mg/ml 1% sodium citrate; Sigma-Aldrich, Germany) were added to the homogenate tissue, the absorbance was measured spectrophotometrically at 412 nm (BioTek ELx800, USA). The cerebral cortex tissue GSH levels (µmol/g) were calculated using the extinction coefficient of 13,000 $mol^{-1}\cdot cm^{-1}$.

The measurement of NOx specification in the cortex tissue

The sum of nitric oxides (NOx) in the cerebral cortex tissue was determined using the Griess methods (Aykaç et al. 1985). The cerebral cortex tissue (0.05 g) was homogenized in the PBS (0.5 ml) and centrifuged at 4000 rpm for 5 min at +4°C. Firstly, the supernatant (400 µl) was added to 200 µl sodium hydroxide (NaOH, Sigma-Aldrich, Germany) and incubated at room temperature for 5 min. Then 200 µl zinc sulfate (10% ZnSO4, Sigma-Aldrich, Germany) was added. The mixture was vortexed and centrifuged at 5000 rpm for 5 min at +4°C. Finally, 500 µl of the obtained sample was centrifuged at 14000 rpm for 5 min at +4°C, and the sample was ready for NOx measurement. The 100 µl sample, 100 µl vanadium chloride (VCl_2), 50 µl sulfanilamide, 50 µl NEDD complex (Sigma-Aldrich, Germany) were incubated at 37°C for 30 min. The absorbance measurement of the standard solution was performed spectrophotometrically at 540 nm.

Western blot analysis of SIRT2 expression in the cerebral cortex tissue

The total protein concentration of the cerebral cortex tissue was measured using the Bradford method (Bradford 1976). In the first step, each sample was loaded with an equal protein extract (20 µg) up to a volume of 40 µl, separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE 12%) and transferred to a nitrocellulose membrane (Bio-Rad, USA). The membrane blocking was done with non-fat dry milk (3.5% w/v, Santa Cruz Biotechnology, Inc., USA) and bovine serum albumin (1.5% w/v, Bioshop, Canada) dissolved in tris buffered saline (TBS, Sigma-Aldrich, Germany) containing 0.1% Tween 20 (Merck Millipore, Germany) (TBST).

In the second step, the membranes were incubated in TBST (5% w/v) non-fat dry milk at the proposed dilution with primary antibodies (SIRT2 (A-5) Sc: 28298, Dilution: 1:500; Beta Actin (R22) Sc: 130657, Dilution: 1:1000, Santa Cruz Biotecnology, Inc. USA) at +4°C overnight and then with seconder antibodies (goat anti-mouse IgG-HRP: Sc-2030, Dilution: 1:5000, Santa Cruz Biotecnology, Inc. USA) for 1.5 h at room temperature. After washing each membrane three times for 10 min with TBST, in the third step, the membranes were exposed to the chemiluminescence (ECL, WP 20002, Invitrogen, USA) detection reagent for 5 min in a dark environment to make them visual using the Gen-box image system. In the fourth step, ImageJ software (Windows version of NIH Image, http://rsb.info.nih.gov/nih-image/) was used to quantify the protein bands, and the obtained data were normalized to β-actin expression levels.

Measurement of SOD and Nrf2 levels by ELISA

SOD and Nrf2 protein levels were detected using a commercial ELISA kit (for SOD: Cat No: E1082Ra, for Nrf2: Cat No: E0168Ra, Bioassay Technology Laboratory, Shangai, China). The measurements were performed at 450 nm using Chromate 4300 ELISA reader (Awareness Technology, Inc. Martin Hwy. Palm City, USA) according to the protocol of the ELISA assay kit. The concentration of SOD and Nrf2 (ng/ml) in the sample was calculated using the instructions provided within the kit, normalized to the total protein, and expressed as ng/mg protein.

Statistical analysis

The current study presented all quantitative results as mean ± standard deviation (SD). The one-way ANOVA was used to determine statistically significant differences between test results, the p*ost-hoc* test Least significant difference (LSD) to separately compare the groups, and the Pearson's correlation coefficient to calculate correlations between groups. The level of statistical significance was taken as $p < 0.05$.

Results

MDA levels of cerebral cortex tissue

MDA levels were compared between groups. There was no significant difference between the two control groups: PBS and DMSO (6.63 ± 1.79 and 6.52 ± 0.77 nmol/g tissue, respectively, $p > 0.05$). There was no significant difference between the three treatment groups: MLT, CUR, and MLT+CUR (4.77 ± 0.35; 4.63 ± 1.02; 4.96 ± 0.49 nmol/g tissue, respectively, $p < 0.05$). However, a significant difference existed between treatment groups and control groups.

In all three treatment groups (MLT, CUR and MLT+CUR), the MDA levels were significantly reduced compared to both control groups (Fig. 1A).

GSH levels of cerebral cortex tissue

As for GSH levels, no significant difference existed between the two control groups (PBS, DMSO) and MLT group (2.50 ± 0.11; 2.43 ± 0.09; 2.54 ± 0.07 μmol/g tissue, respectively, $p > 0.05$). There was no significant difference between CUR and combined MLT+CUR groups and the PBS control group. However, the GSH levels were significantly higher in the CUR and combined MLT+CUR groups (2.61 ± 0.14; 2.82 ± 0.08 μmol/g tissue, $p < 0.05$, respectively) compared to the DMSO control group (Fig. 1B).

SOD levels of cerebral cortex tissue

The level of SOD protein was examined by the ELISA method, and there was no significant difference between the control groups (PBS; 0.19 ± 0.08, DMSO; 0.27 ± 0.05 ng/mg protein, $p > 0.05$). However, CUR (2.11 ± 0.62 ng/mg protein) group showed a significant increase in the level of SOD compared to the control groups and other treatment groups (MLT and combined MLT+CUR; 0.61 ± 0.26; 0.46 ± 0.10 ng/mg protein, respectively, $p < 0.05$). Also, there was a statisti-

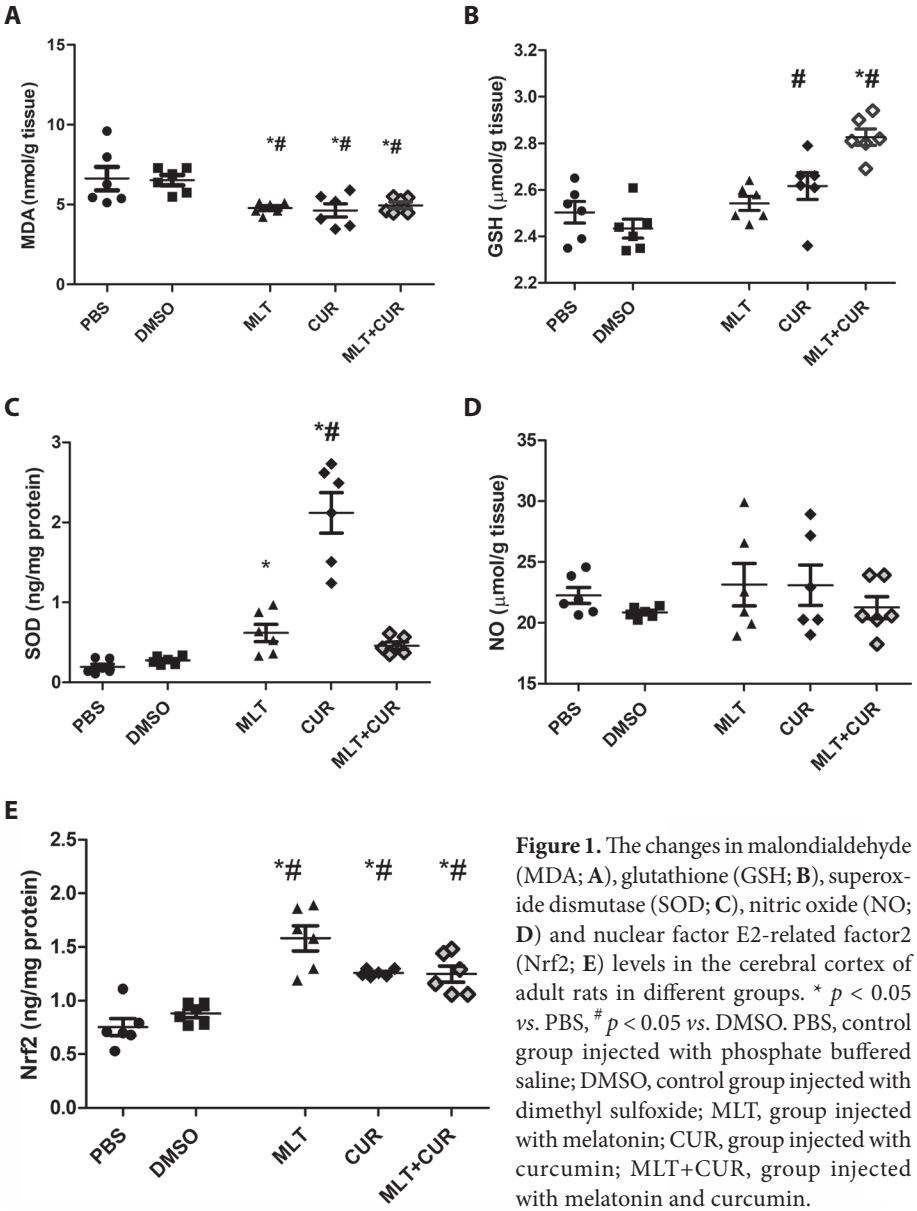

Figure 1. The changes in malondialdehyde (MDA; **A**), glutathione (GSH; **B**), superoxide dismutase (SOD; **C**), nitric oxide (NO; **D**) and nuclear factor E2-related factor2 (Nrf2; **E**) levels in the cerebral cortex of adult rats in different groups. * $p < 0.05$ *vs*. PBS, # $p < 0.05$ *vs*. DMSO. PBS, control group injected with phosphate buffered saline; DMSO, control group injected with dimethyl sulfoxide; MLT, group injected with melatonin; CUR, group injected with curcumin; MLT+CUR, group injected with melatonin and curcumin.

cally significant difference between the MLT group and the PBS control group in the level of SOD ($p < 0.05$) (Fig. 1C).

NO levels of cerebral cortex tissue

There was no significant difference in NO levels between all groups PBS, DMSO, MLT, CUR, and MLT+CUR (22.25 ± 1.61; 20.85 ± 0.43; 23.13 ± 4.27; 23.08 ± 4.08; 21.25 ± 2.24 μmol/g tissue, respectively, $p > 0.05$ (Fig. 1D).

Nrf2 levels of cerebral cortex tissue

As for the level of Nrf2 protein examined by the ELISA method, the control groups (PBS; 0.75 ± 0.19, DMSO; 0.87 ± 0.09) ng/mg protein, $p > 0.05$) showed no significant difference between each other. In the treatment groups, there was no significant difference between the CUR and MLT+CUR treatment groups ($p > 0.05$), however, the Nrf2 level was significantly higher in the MLT group compared to the CUR and MLT+CUR groups ($p < 0.05$). Additionally, all the treatment groups, MLT, CUR, and MLT+CUR (1.58 ± 0.29; 1.25 ± 0.03; 1.24 ± 0.18 ng/mg protein, respectively, $p < 0.05$), demonstrated a significant increase in Nrf2 levels compared to the control groups (Fig. 1E).

SIRT2 protein expression in the cerebral cortex tissue

SIRT2 protein expression in the cerebral cortex tissue did not differ significantly between the PBS and DMSO control groups (2.36 ± 0.17; 1.89 ± 0.21, respectively, $p > 0.05$). And no significant difference existed between the MLT, CUR, and MLT+CUR treatment groups ($p < 0.05$). However, Western blotting results revealed that the MLT and CUR treatment groups had low SIRT2 protein expressions in the cerebral cortex tissue. MLT, CUR, and MLT+CUR groups (0.88 ± 0.10; 0.93 ± 0.07; 0.78 ± 0.09, respectively, $p < 0.05$) showed a significant decrease in SIRT2 protein expression compared to the control groups. However, no significant difference existed between the MLT, CUR, and MLT+CUR treatment groups ($p > 0.05$) (Fig. 2A, B).

According to the correlation test results, the SIRT2 expression was positively correlated with MDA ($r = 0.722$; $p < 0.001$) and negatively correlated with GSH, SOD and NRF2 ($r = -0.526$, $p = 0.01$; $r = -0.485$, $p = 0.035$; $r = -0.787$, $p < 0.001$, respectively). The Nrf2 level was reversely correlated with MDA ($r = -0.690$; $p < 0.01$) and insignificantly correlated with GSH, SOD, and NO ($p > 0.05$).

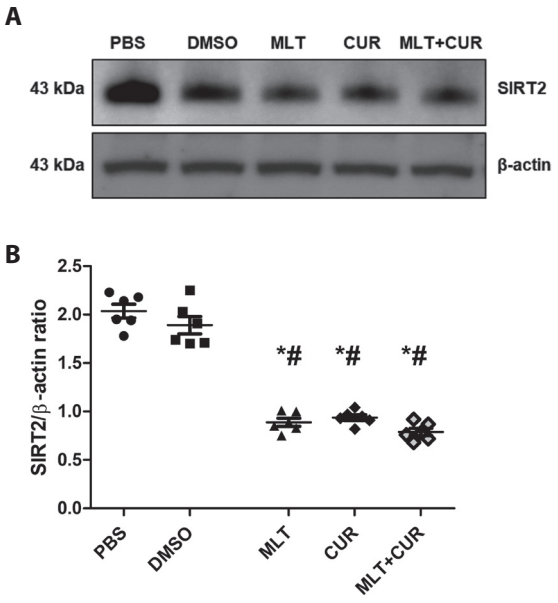

Figure 2. The result of Western blot of SIRT2 protein expression in the cerebral cortex tissue of adult rats in different groups. **A.** Representative image of SIRT2 and β-actin protein band. **B.** Sirt2/β-actin ratio. * $p < 0.05$ *vs.* PBS, # $p < 0.05$ *vs.* DMSO. For abbreviations see Figure 1.

Discussion

Many studies have already examined oxidative stress and found synchronous increases in MDA levels in the brain and other tissues with age (Farooqui et al. 1987; Sahoo and Chainy 1997; Driver et al. 2000). The first step of this study was to demonstrate the effect of melatonin and curcumin on oxidative stress markers and antioxidants in the cerebral cortex of adult rats. The study found that exogenous melatonin, curcumin, and combined melatonin and curcumin treatments reduce MDA levels, an indicator of lipid peroxidation, but make no significant difference in NOx levels compared to control groups. It has also been shown that melatonin and curcumin reduce MDA and increase GSH and SOD levels. Similar to our study, many studies have revealed that melatonin and curcumin increase antioxidant enzymes such as SOD and GSH, and decrease MDA (Farzaei et al. 2018; He et al. 2018; Moniruzzaman et al. 2018). The current study has revealed that the effect of combined melatonin and curcumin treatment on MDA, GSH, NOx, and SOD was similar to the effects of melatonin and curcumin individually. In other words, there is no synergistic effect of combined melatonin and curcumin therapy. It may be because the melatonin and curcumin treatment have regular effects rather than a linear increase in decreasing oxidative stress.

The second step of the study was to demonstrate the effect of the treatments on the expression level of SIRT2 and cytoplasmic Nrf2. Previous studies have reported that Nrf2, a sensor of oxidative stress, regulates the transcription of enzymes responsible for GSH synthesis and GSH utilization (Krajka-Kuźniak et al. 2017). The present study determined that curcumin significantly increases the GSH

and SOD levels and the cytoplasmic Nrf2 level in the adult rat cerebral cortex tissue compared to the control group. Melatonin treatment showed a significant increase in Nrf2 levels and SOD, however, the increase in GSH levels was insignificant. Melatonin can possibly cause an elevation in SOD *via* Nrf2. Curcumin treatment can also affect the level of GSH and SOD through Nrf2.

Many studies in the literature in pathological condition have revealed the oxidative stress components used in this study. Some studies in the literature have shown that melatonin increases gene expression and (or) activity of antioxidant enzymes such as GSH and SOD when oxidative damage is induced (Barlow-Walden et al. 1995; Mayo et al. 2002). Another study reported that melatonin in diabetes mellitus rats reduces oxidative stress in the hippocampus, increases SOD and Nrf2, but has no effect on nitrite and GSH (Albazal et al. 2021). Furthermore, Tresguerras et al. (2008) found that melatonin reduces oxidative stress and increases SIRT2 levels in aged rats. Another study has shown that melatonin increases antioxidant enzymes (GSH and SOD) and reduces oxidative stress through activating the Nrf2-ARE pathway in experimental traumatic brain injury (Ding et al. 2014).

Melatonin's indirect antioxidative action is mediated by Nrf2 activation (Hardeland 2018). It was found that the level of Nrf2 decreases in the postmortem cerebral cortex tissue and in different pathological conditions (Yao et al. 2016; Martin-Hernandez et al. 2018; Zhang et al. 2018). In addition, it has been shown in the literature that melatonin treatment improves different pathological findings by increasing Nrf2 activation (Vriend and Reither 2015; Garstkiewicz et al. 2017). Moreover, it has been shown that melatonin activates Nrf2 through up-regulation of SIRT 1 in order to reduce oxidative stress (Arioz et al. 2019; Fang et al. 2019; Shi et al. 2019). While the effect of melatonin therapy has been mostly investigated with SIRT1 in aging and inflammation models, its effects on SIRT2 are also remarkable. In a study of aging models, it was shown that melatonin treatment did not change SIRT2 levels in the neural apoptosis in the dentate gyrus (Kireev et al. 2013), but SIRT2 activity decreased in response to aging-related oxidative stress in the aged rat colon (Akbulut et al. 2014). In the literature, we could not find any study showing SIRT2/Nrf2 levels after melatonin treatment. In the current study, finding that MDA and SIRT2 decreased and Nrf2 increased after melatonin treatment in the cerebral cortex tissue is a significant result.

The study has found that compared to melatonin, curcumin is more potent in decreasing MDA levels, increasing GSH and SOD antioxidant activity, and affecting GSH and SOD antioxidants through an increase in Nrf2. The study findings support the literature studies: Curcumin is a potent inhibitor of lipid peroxidation and lowers age-related MDA levels in different regions of the brain (Rajakumar and Rao 1994). In addition, curcumin increases the activity of antioxidant enzymes such as SOD and GSH (Bala et al. 2006).

In addition, the current study revealed that curcumin decreases SIRT2 protein expression and increases cytoplasmic Nrf2 level in the cerebral cortices of adult rats. Similarly, Keskin-Aktan et al. (2018) documented that curcumin decreases the expression level of SIRT2 in the hippocampus. Unlike the current study, Liu et al. (2016) stated that curcumin decreases cytoplasmic Nrf2 expression level and increases nuclear Nrf2 expression level compared to oxidant-treated cells. Many studies in the literature include both curcumin (Yang et al. 2009; Lima et al. 2011; Tapia et al. 2012; Shahcherahi et al. 2021) and acute oxidative stress (Sandberg et al. 2014; Choi 2019) induced Nrf2. The general opinion in the literature is that Sirt2 activity is stimulated by acute oxidative stress and in response, Nrf2 decreases at both total and nuclear levels (Yang et al. 2017). In addition, the literature shows that curcumin treatment causes a time-dependent Nrf2 activation. In various models, it has been found that maximal activation of Nrf2 is between 8 and 48 h after curcumin treatment (Yang et al. 2009; Jiang et al. 2011; Lima et al. 2011). In our study, an acute oxidative stress situation was not created. However, we showed the effect of curcumin and melatonin treatment on physiological oxidative stress in the cortex tissue of middle-aged rats.

In the study, the level of SIRT2 protein expression was significantly reduced in all treatment groups compared with the control groups. The effects of SIRT2 on oxidative stress are contradictory in the literature. Sighn et al. stated that higher SIRT2 activity is required for protection against oxidative stress (Singh et al. 2017, 2018; Qu et al. 2020). In other studies, inhibition or reduction of SIRT2 levels for protective effects was demonstrated in many models (Outeiro et al. 2007; Wang et al. 2007; Lynn et al. 2008; Luthi-Carter et al. 2010; Ponnusamy et al. 2014; Sarikhani et al. 2018; Li et al. 2020). In the literature, the confusing roles of SIRT2, which is abundant in brain tissue, are mentioned; in other words, it is stated that it can function differently under different conditions (Chen et al. 2021). Additionally, there is disagreement on the causal connection between oxidative stress and SIRT2 expression (Li et al. 2020).

We can say that the limitation of the study is the lack of an elderly or oxidative stress group. If we had such an induced oxidative stress group, after the melatonin and curcumin treatment we could show the effect of melatonin and curcumin treatment on the SIRT2 and Nrf2 levels in the cortex tissue. And we could also compare the SIRT2 and Nrf2 levels in response to the physiological oxidative stress and induced oxidative stress. Another limitation is that SIRT2 and Nrf2 activations were not analysed – perhaps we could have defended our conclusions more strongly.

The present study is important in terms of showing the effects of a one-month melatonin or curcumin treatment on

the expression of SIRT2 and Nrf2 levels in the cell cytoplasm, in reducing physiologically oxidative stress. The study has holistically sought to show the correlation between physiological oxidative stress, antioxidant enzymes, Nrf2 protein, and SIRT2 in the adult rat cerebral cortex with melatonin, curcumin, or combined melatonin and curcumin treatment. As a result, the current study revealed that melatonin and curcumin treatments reduce lipid peroxidation and SIRT2 protein expression and increase the level of Nrf2 in the cytoplasm. The antioxidant molecules (GSH and SOD) are induced by both curcumin and melatonin treatment. In the study, the combined melatonin and curcumin treatment introduced no synergistic effect. Melatonin and curcumin treatments separately may act on antioxidant molecules in the cerebral cortices through different pathways. Inhibition of SIRT2 may have a protective role by reducing physiological oxidative stress in the cerebral cortex. SIRT2 protein expression has a strong positive correlation with MDA, a negative correlation with Nrf2, and a strong negative correlation with GSH and SOD.

This study indicates a direction for our future studies by trying to reveal the effect of the melatonin and curcumin treatment on Nrf2. We consider that explaining the causative relationship between the curcumin treatment and Nrf2. In future studies, we believe that explaining the causative relationship between the curcumin treatment and Nrf2 will be critical in correcting oxidative stress.

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Ethical approval. All the experiments were performed in accordance with the criteria by ethics committee decision G.Ü. ET-11.056.

Conflict of interest. The authors have no conflicts of interest to declare that are relevant to the content of this article.

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