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Article

Neuroprotective effects of sinapic acid involve the iron regulatory role on the rotenone-induced Parkinson's disease model

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In the last decades, ferroptosis and its relationship with Parkinson's disease have gained significant attention. Compounds that affect ferroptosis and iron-dependent pathways in particular, have possible candidates for study in this context.Sinapic acid is an iron-chelator and high antioxidant bioactive phenolic acid. Its neuroprotective action, due to the antioxidant capacity, has been shown in several experimental models.However, the relationship between iron and antioxidant actions is still misunderstood and therefore, in the current study, we tried to investigate the effects of sinapic acid in rotenone-induced Parkinson's disease with the aspect of ferroptosis and iron-dependent alterations.The Parkinson's disease model was induced by a single dose intrastriatal and intrategmental rotenone (5µg/µl) injection.Sinapic acid (30mg/kg) was orally administered during a 28-day period after the Parkinson's disease model was validated.Our results demonstrated that sinapic acid treatment attenuated rotenone-induced increase of serum transferrin and iron levels.Furthermore, sinapic acid inhibited rotenone-induced heme oxygenase-1(HO-1) increase and decrease of glutathione peroxidase-4 (GPx-4) levels in brain tissue. Also, sinapic acid treatment decreased motor impairment, likely as a result of the ameliorative effects on the tyrosine hydroxylase immunoreactivity loss after the rotenone insult.Our study suggests that the iron regulatory role of sinapic acid possibly plays a role in the protective effect on rotenone-induced neuronal damage.

Keywords: Sinapic acid. Parkinson's disease. Rotenone. Iron.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder which is commonly seen in the aged people (Greenamyre, Hastings, 2004). Its primary pathologic mechanism is progressive and irreversible neuronal loss in dopamine-containing neurons in the substantia nigra pars compacta (SNpc) (Mastroberardino *et al.*, 2009). Although pharmacologic treatments are available, none of them are able to halt or reverse neuronal damage, and they are only being exploited for symptomatic therapy. Therefore, identification of new targets or possible treatment options is urgently needed.

Oxidative stress and results of peroxidation of lipid and protein structures of cells are commonly accepted mechanisms behind PD pathology. Free oxygen radicals and peroxynitrites are in a constant production cycle due to the impaired mitochondrial balance. It has been already established that the dysregulated redox state and altered iron homeostasis are closely related, because of iron being a cellular element also responsible for oxidative stress. Additionally, recent understanding on

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new cellular cell death pathways (pyroptosis, paraptosis, anoikis) and identification of iron-dependent death, which was termed as ferroptosis in PD pathology, made this relationship more tempting for investigation as possible new targets (Mou *et al.*, 2019). Dopamine metabolism, neurotransmitter regulation and several physiological processes, are directly dependent on cellular iron status and that makes iron levels more critical in PD. Additionally, it has been established that rotenone, which is a well-known mitochondrial complex I inhibitor, induces oxidative stress and causes nigral iron accumulation, which is detrimental for neuronal survival (Ayton, Lei, 2014). Afterwards, plenty of research about iron accumulation in the striatal area provided information to consider iron chelation as being an alternative option to protect neurons of the substantia nigra pars compacta (Guerreiro *et al.*, 2006; Kaur *et al.*, 2003). Demonstration of the neuroprotective actions of iron chelators in Parkinson's disease and their ability of cause a decrease in the ferroptosis, led us propose that iron chelation might be act as protection for the survival of nigral neurons. Before the discovery of the direct ferroptosis inhibitor ferrostatin-1, phenolic compounds with the ability of affecting ferroptosis, showed neuroprotection in PD (Kose *et al.*, 2019). Ferrostatin-1 also protected dopaminergic neuroblastoma cells against rotenone-induced oxidative stress, which suggests effect of rotenone on ferroptosis. Considered together, iron chelator therapy in Parkinson's disease seems to be a rational approach (Kabiraj *et al.*, 2015).

Sinapic acid is a hydroxycinnamic acid derivative with reported numerous beneficial activities in the central nervous system (Zych *et al.*, 2018). It has already been known that polyphenols such as sinapic acids have iron-binding capabilities and protect plants by withholding iron from pathogens. Additionally, these molecules can protect against iron-induced free radical damage to cells, hence prevent complexation of the metals by proteins. Sinapic acid is the one of the phenolic acids which also has the iron-chelating capacity (Hynes, O'Coinceanainn, 2004). Although antioxidant and neuroprotective action of sinapic acid has been shown in several animal studies, the role of sinapic acid on the iron regulation and ferroptosis behind neuroprotective actions, are still missing. Therefore, we sought to investigate possible iron regulatory role of sinapic acid in a rotenone-induced PD model.

MATERIAL AND METHODS

Animals

Thirty (30) Sprague-Dawley male rats (240-260 g) were used in this study. Before the experiments, animals which showed abnormal motor behaviors were excluded from the study.

Chemicals and treatment groups

Sinapic acid, apomorphine hydrochloride, rotenone, and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Apomorphine was dissolved in saline and sinapic acid was dissolved in 10 % Cremophore (Sigma-Aldrich, USA) solution, then administered with oral gavage at 30 mg/kg doses. The sinapic acid dose was chosen according to previous reports which have demonstrated neuroprotective actions (Kanchana *et al.*, 2011; Lee, 2018; Lee *et al.*, 2012). Rotenone was freshly dissolved in DMSO on the day of the administration. Animals were randomly divided before experiments in the following two groups: control (n=10) and rotenone (n=20).

Experimental design

The animals were anesthetized with the intraperitoneally administration of ketamine hydrochloride (80 mg/kg) and xylazine (10 mg/kg), then placed in the stereotaxic apparatus (World Precision Instruments, US). Hairs of the animals were shaved, their heads were incised in the fronto-occipital direction, then burr holes were drilled after bregma was identified. All procedures were performed under sterile conditions. The following coordinates were selected for the sites of rotenone administration (5 µg) with 28G Hamilton syringe: substantia nigra pars compacta (SNc, AP:5.0 mm, L:2.00 mm, DV:8.0 mm) and ventral tegmental area (VTA, AP:5.0 mm, L:1.0 mm, DV:7.8 mm) according to the Paxinos rat brain atlas (Paxinos, Watson,

2009). After each injection, two additional minutes were granted for complete drug diffusion. The control group (n=10) only received DMSO (1 µL) as a rotenone vehicle. DMSO was selected according to the previous studies and laboratory practices (Blevins, Stanley, Reidelberger, 2002). Following the surgery, animals were monitored for abnormal turning and motor behaviors, and those that showed these behaviors were excluded from the experiment. Following the tenth day of the rotenone injection, the PD model was validated with an apomorphine-induced rotation test. Eight animals from each group, which showed successful development of Parkinson's disease model, were used for further experiments. Next, equal numbers of animals (n=8 per group) were administered with sinapic acid (30 mg/kg, p.o.) and 10% Cremophore as a sinapic acid vehicle, for 28 days. Following the last drug administration, behavioral tests were performed, and the animals were subsequently decapitated. Experiment schedule is represented in Figure 1.

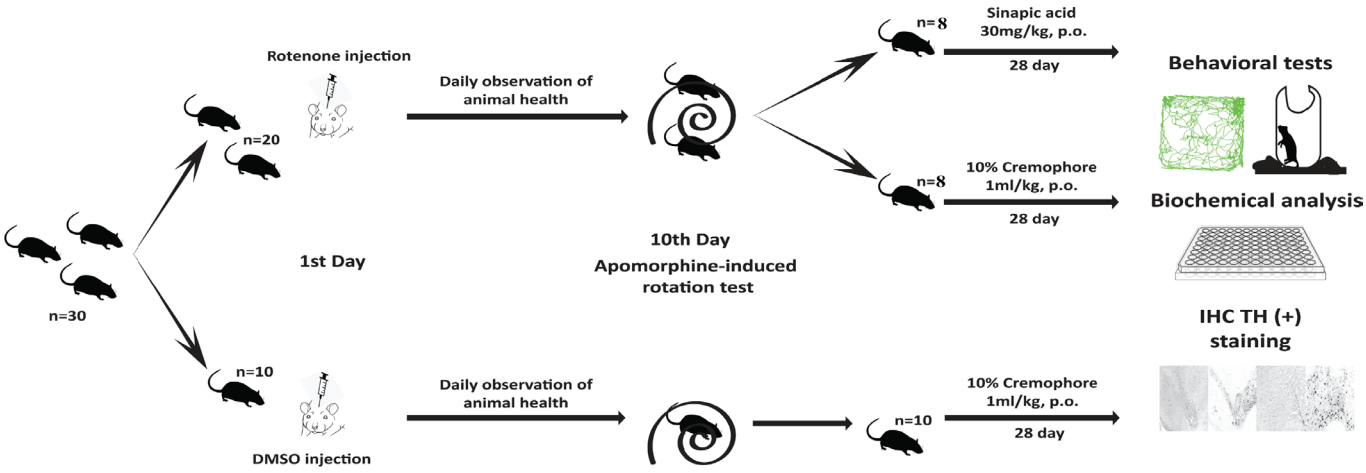

FIGURE 1 - Schematic illustration of the experimental timeline. The number of animals used in each group was represented by the "n" letter.

Apomorphine induced rotation test

The animals were tested with apomorphine for turning behavior due to the changed sensitivity to the dopamine agonists, after unilateral rotenone injection (Zare *et al.*, 2015). Apomorphine 0.1 mg/kg (dissolved in a 0.2 mg/mL ascorbic acid in 0.9% saline solution) was administered subcutaneously, and animals that turned 360° more than 7 times in one minute were accepted as a successful model development (Jerussi, Glick, 1975).

Open-field and cylinder test

Evaluation of motor performance and deficits are performed with an open-field test. In our study, the test was performed using a square (100x100 cm) plane with a 50 cm high wall plexiglass apparatus. The rats were placed in the center of the platform and observed for 5 minutes by two blinded observers. Immobility times and the total traveled distance of rats were quantified using the ToxTrac free software (Rao *et al.*, 2019). Voluntary movement during exploratory activity was assessed using the cylinder test. Rats were placed in a transparent glass cylinder and evaluated for motor deficits. Because of the rotenone injection, rats would preferentially use the ipsilateral forelimb: the two blinded observers counted the number of contacts made by each forelimb during a period of 5 minutes. The test score was calculated according to the following formula: (contralateral side+1/2 both)/ (ipsilateral side+contralateral side+both); scores less than 0.5 suggested motor impairment of the contralateral limb (Lundblad *et al.*, 2002).

Measurement of serum levels of iron, transferrin, HO-1 and GPx-4

After the behavioral tests and before cardiac perfusion, blood samples from the rats were obtained. The samples were centrifuged after clotting, and serum was separated before quantification of serum iron and transferrin levels. Additionally, serum levels of HO-1, GPx-4 were determined using commercially available ELISA (Enzyme-like Immunosorbent Assay) kits (Ayaydin *et al*., 2020).

Tissue homogenization and determination of brain levels of HO-1 and GPx-4, SOD and CAT

Rats were trans-cardially perfused with a heparinized phosphate buffered saline (PBS, pH 7.4) solution and brain tissues were quickly isolated. Samples were homogenized in PBS with glass homogenizer and centrifuged at 20 000 g for 5 minutes (Amsen, de Visser, Town, 2009). The protein amounts of samples were quantified using Lowry's method and levels in the brain tissue of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), HO-1 and GPx-4 were determined with commercially available ELISA kits (Figure S1)(Li *et al*., 2019; Lowry *et al*., 1951).

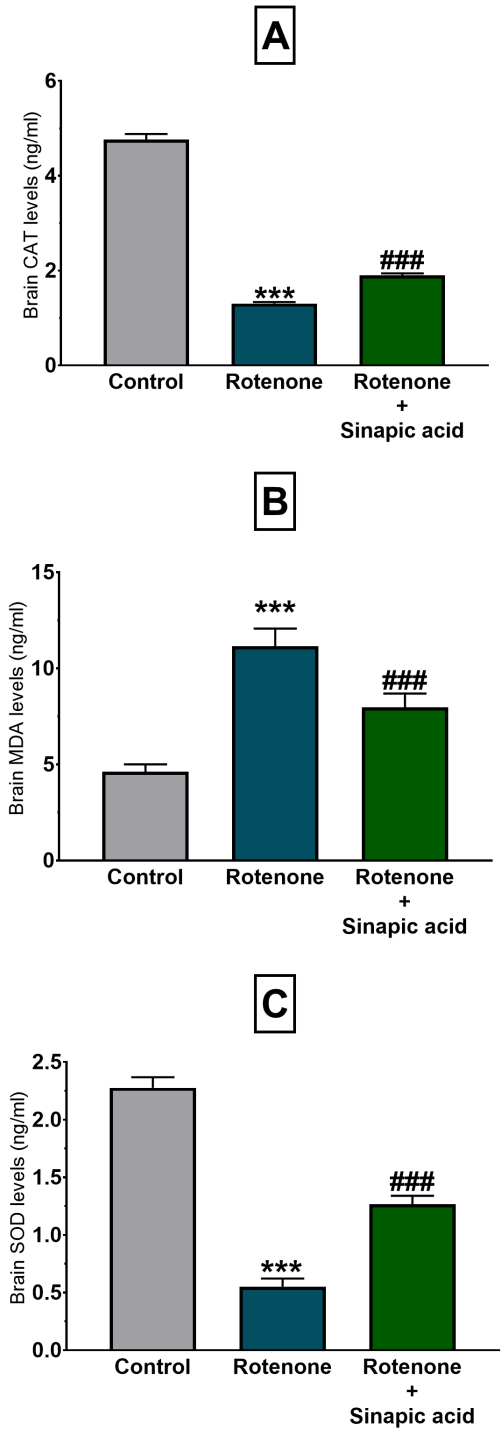

FIGURE S1 - Brain iron (A) and transferrin (B) levels after sinapic acid treatment and rotenone insult. In parallel with serum results sinapic acid significantly decreased rotenone-induced increase in iron and transferrin levels in the brain tissue. All data expressed as mean ± SD. ***$p<0.001$ versus control, $^{###}p<0.001$, $^{#}p<0.05$versus rotenone group.

Histopathological and immunohistochemical analysis

Necropsy was performed on all rats following the cardiac perfusion with a heparinized-4% paraformaldehyde solution. After cardiac perfusion, brain tissues of the rats were trimmed according to the rat brain atlas and were fixed in a paraformaldehyde solution for 24 h at +4°C, then washed under running water and dehydrated with gradient ethanol series before two (2) xylene treatments (Paxinos, Watson, 2009). Samples were embedded in paraffin wax, 5 µm thick sections were cut using the rotary microtome (Leica, RM2125), then stained using hematoxylin and eosin for examination under a light microscope (Nikon Eclipse E400, Japan). Immunohistochemical (IHC) staining was performed on 5-µm sections using the streptavidin-biotin-immunperoxidase complex method (Lab Vision™ UltraVision™ LP Detection System, Thermo Fisher Scientific) for detection of antibodies. Sections were deparaffinized in xylene and sections were then dehydrated through graded alcohols (100%, 96%, 80% and 70% ethanol). Endogenous peroxidase activity was blocked with a 3% hydrogen peroxide solution for 10 minutes. The sections were rinsed with a phosphate-buffered saline (PBS, pH 7.2) followed by heat (600 watts in a microwave) in citrate buffer (pH 6.0) for 15 minutes for antigen retrieval. Sections were preincubated in a blocking solution (Thermo Scientific Large Volume Ultra V Block) at room temperature for 10 minutes to stop nonspecific binding. All sections were incubated with the primary antibody (anti-TH antibody: Elabscience, Cat No E-AB-70077, dilution: 1/300) overnight at 4 °C and then rinsed with PBS at room temperature. Subsequently, the sections were incubated with a biotin-labeled secondary antibody (Biotinylated goat anti-polyvalent) for 10 minutes at room temperature. Then sections were washed with PBS three times for 5 minutes and incubated with the streptavidin-peroxidase for 30 minutes at room temperatures. At the end of this procedure, the slides were washed 3 times with PBS for 5 minutes, then in a streptavidin-bitoin-peroxidase complex (SABC)(Zymed Laboratories) for 30 minutes at room temperature. Labeling was performed by 3-amino-9-ethylcarbazole (AEC) as the chromogen. Sections were counterstained with Mayer's hematoxylin and examined under a light microscope (Nikon Eclipse E400, Japan). The number of immunohistochemically positive and negative cells was established by counting a total of 500 cells in randomly selected ×40 high-power magnification fields, per brain sample.

Statistical analysis

All data obtained from experiments were analyzed using the SPSS (v. 21.0, SPSS Inc., Chicago, IL). Shapiro-Wilk's test was used for normality and data was analyzed with one-way analysis of variance test (ANOVA). Tukey's post-hoc test was performed for multiple comparison and p value less than 0.05 was considered significant.

RESULTS

Sinapic acid mitigated motor impairment and forelimb akinesia

Rotenone caused a significant decrease in the total travelled distance, while increasing immobility time compared to the control group ($p<0.001$ and $F(2, 13)=31.7$, $p<0.001$ and $F(2, 13)=86.1$, respectively). Sinapic acid significantly ameliorated the effects of rotenone on the open-field tests ($p<0.01$) (Figure 2A, B). Additionally, rotenone caused significant increase in the asymmetrical forelimb use ($p<0.001$ and $F(2, 11) = 58$). Although, sinapic acid did not return asymmetrical forelimb usage to the control levels, it significantly mitigated rotenone-induced asymmetrical forelimb usage ($p<0.001$) (Figure 2C).

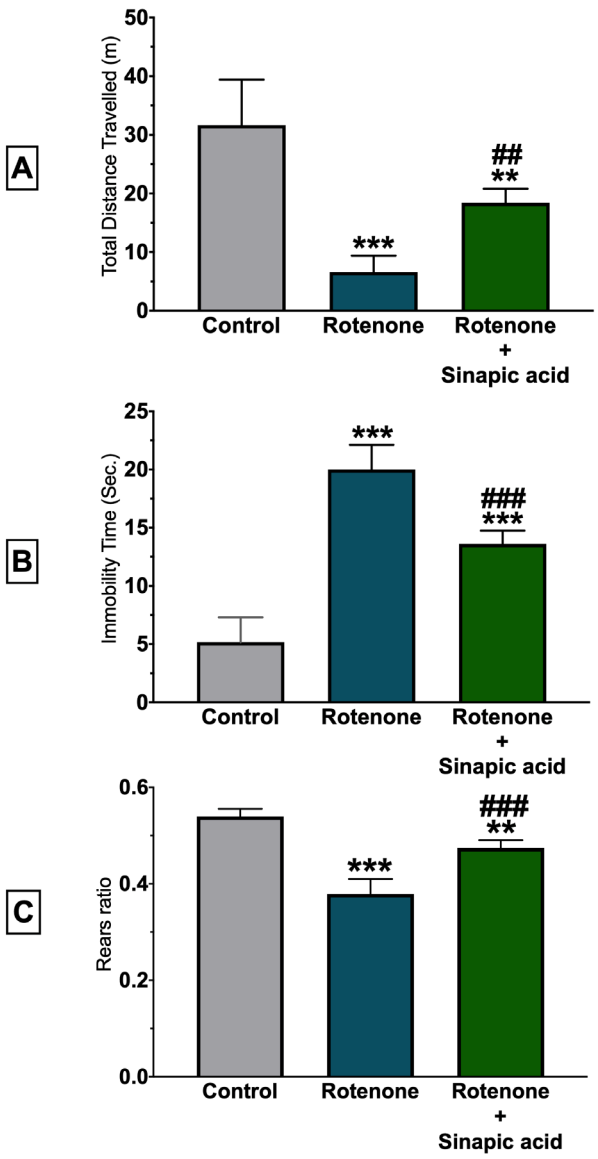

FIGURE 2 - Decreased traveled distance (A), increased immobility time (B), and impaired rears ratio (C) due to the rotenone insult were significantly alleviated by sinapic acid (n=8 for each group). All data expressed as mean ± SD. ***p<0.001, **p<0.01 versus control group, ###p<0.001, ##p<0.01 versus rotenone group.

Sinapic acid decreased serum iron and transferrin levels

Serum iron levels were significantly increased by rotenone (p<0.001 and F (2, 9) = 40.9). However, sinapic acid treatment significantly decreased the serum iron levels (p<0.05) (Figure 3A). Moreover, serum transferrin levels significantly increased (p<0.001 and F (2, 14) = 22.6) with the rotenone injection and that increase was inhibited by sinapic acid treatment (p<0.001) (Figure 3B).

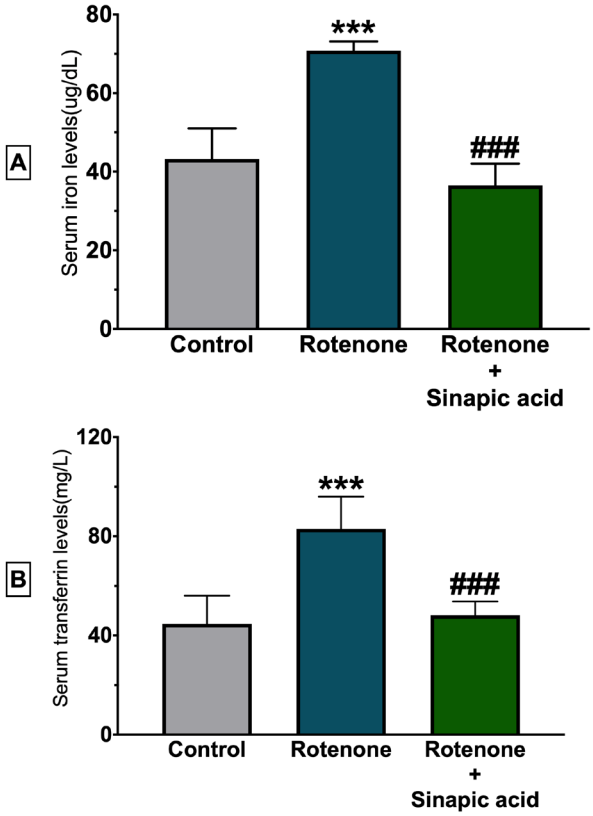

FIGURE 3 - Rotenone significantly increased serum iron and transferrin levels (B). But sinapic acid significantly prevented rotenone-induced increase in the iron and transferrin levels (n=8 for each group). All data expressed as mean ± SD. ***p<0.001 versus control group, ###p<0.001 versus rotenone group.

Sinapic acid mitigated rotenone-induced HO-1 and GPx-4 alterations

Rotenone significantly decreased serum (p<0.05 and F (2, 15) = 32.3) levels of HO-1 and sinapic acid increased HO-1 levels more than 70% (p<0.001) (Figure 4A). Furthermore, rotenone increased brain HO-1 levels significantly (p<0.001 and F (2, 26) = 50.4) and that increase was significantly alleviated by sinapic acid treatment (p<0.001) (Figure 4B). Moreover, GPx-4 levels were found to be decreased by rotenone in the brain tissue (p<0.001 and F (2, 26) = 50.4), which was in line with HO-1 results that rotenone-induced decrease significantly attenuated by sinapic acid treatment, (p<0.001) (Figure 4C).

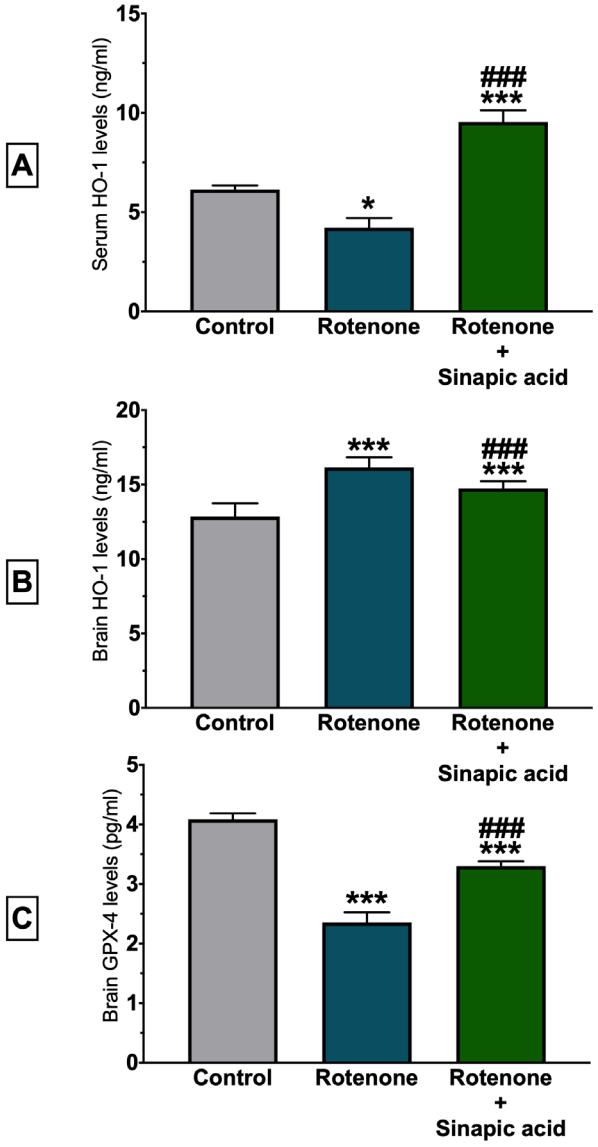

FIGURE 4 - While serum HO-1 levels (A) decreased after rotenone, brain levels of HO-1 were increased (B). Rotenone significantly decreased GPx-4 levels in the brain tissue (C). However, sinapic acid increased serum HO-1 levels (A), which attenuated rotenone-induced increase in the brain tissue (B). Additionally, sinapic acid prevented rotenone-induced decrease of GPx-4 in the brain (C).All data expressed as mean ± SD. ***p<0.001, *p<0.05 versus control group, ###p<0.001 versus rotenone group.

Sinapic acid improves rotenone-induced decrease of SOD and CAT levels and prevented lipid peroxidation

Rotenone caused a significant decrease in the SOD (p<0.001, F(2,27)=108, Figure 5A) and CAT (p<0.001, F(2,15)=581, Figure 5B) levels. Additionally, rotenone also caused a significant increase in the lipid peroxidation in the brain, as expected (p<0.001, F(2,26)=216, Figure 5C). In contrast sinapic acid prevented a rotenone-induced decrease in the CAT (p<0.001, Figure 5B) and SOD (p<0.001, Figure 5A) levels. Furthermore, sinapic acid also prevented a rotenone-induced increase in the lipid peroxidation (p<0.001, Figure 5C).

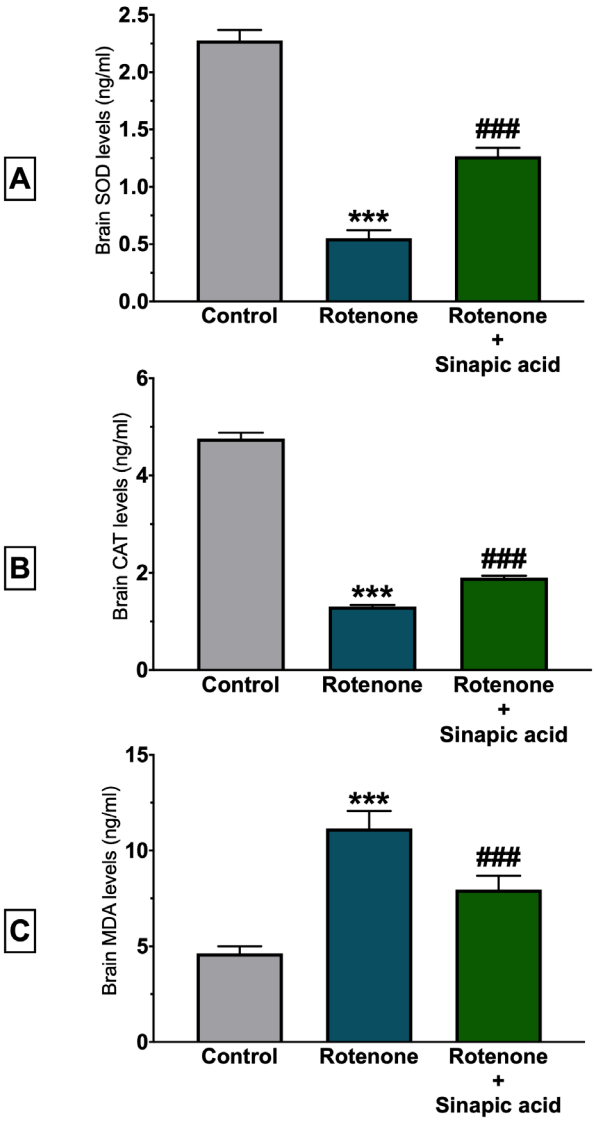

FIGURE 5 - Biochemical analysis of brain SOD (A), CAT (B) and MDA (C) levels in all experimental groups. Rotenone significantly decreased SOD and CAT levels. That decrease was significantly attenuated by sinapic acid. Additionally, rotenone caused lipid peroxidation as expected. Sinapic acid inhibited an increase in lipid peroxidation. All data expressed as mean ± SD. ***p<0.001, ###p<0.001, versus rotenone group.

Effect of sinapic acid on TH immunoreactivity and nigral neurons

Dopaminergic neuron damage was interpreted with tyrosine hydroxylase immunoreactivity. Sections from the control group showed normal substantia nigra neurons with normal nucleus and basophilic cytoplasm. Also, positive reaction was noticed in tyrosine hydroxylase (TH) in dopaminergic substantia nigra neurons (Figure 6A). Remarkably, decreased tyrosine hydroxylase (TH) activity in the dopaminergic substantia nigra neurons was observed in the rotenone (Figure 6B). Rotenone significantly decreased TH immunoreactivity ($p<0.001$, $F(2, 15) = 48.5$) (Figure 6B,7). In contrast, sinapic acid notably improved the decrease of tyrosine hydroxylase immunoreactivity ($p<0.001$)(Figures 6C,7).

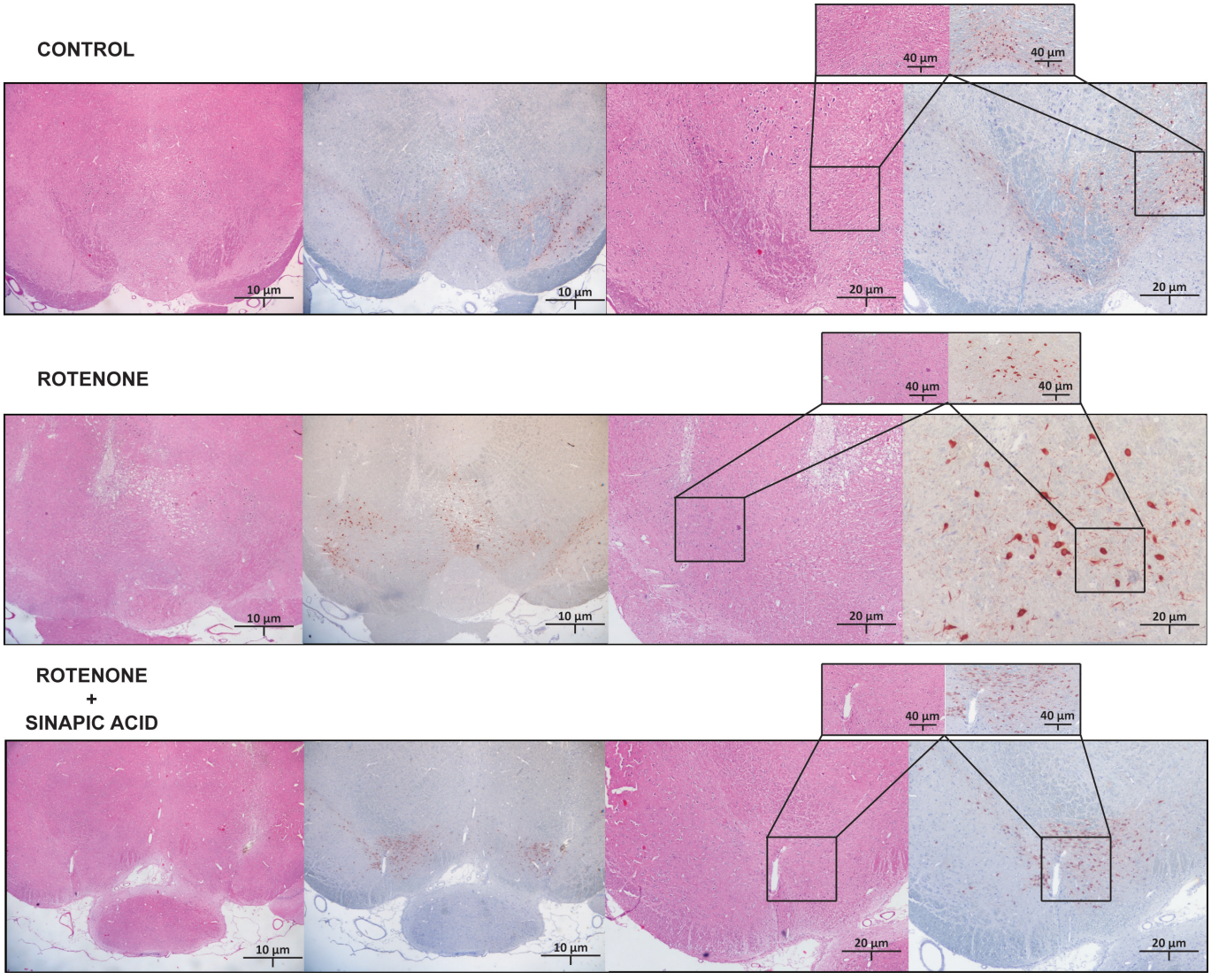

FIGURE 6 - Healthy neurons with TH immunopositivity in the control group. Rotenone injection resulted in neuronal degeneration and intense vacuolations with shrinkage and necrosis. In sinapic acid group, neurons showed slight vacuolization, but these neurons were immunohistochemically positive for tyrosine hydroxylase.

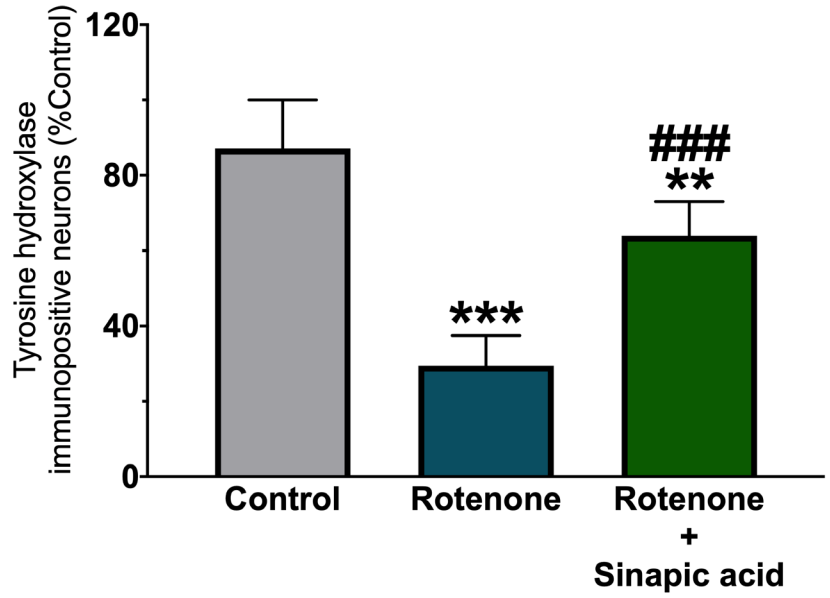

FIGURE 7 - Number of TH immunopositive neurons. Intact side was accepted as control. Rotenone significantly decreased TH (+) neuron percentage. Sinapic acid alleviated rotenone-induced loss of TH (+) neurons. All data expressed as mean ± SD. ***p<0.001, **p<0.01 versus control group, ###p<0.001, versus rotenone group.

DISCUSSION

In the current study, sinapic acid treatment caused a significant improvement in motor symptoms with decreasing TH positive neuronal loss in the striatum. Additionally, sinapic acid treatment decreased serum iron and transferrin levels, along with the decreased lipid peroxidation and oxidative stress parameters. To the best of our knowledge, this is the first study regarding the investigation of neuroprotective effects of sinapic acid in the rotenone-induced PD model, with the aspect of the ferroptosis and iron relationship.

It has been established that natural compounds such as flavonoids have the high antioxidant capacity and demonstrated neuroprotective actions in PD models (Tamilselvam *et al.*, 2013). Rotenone is a complex I inhibitor and commonly used for experimental models owing to its ability of mimicking motor deficiencies seen in humans (Sherer *et al.*, 2003). It has been demonstrated that low levels of endogenous antioxidants cause an increase in reactive species and exacerbate dopamine oxidation, which results with highly reactive toxic iron complexes in brains from PD patients (Sutachan *et al.*, 2012). Therefore, antioxidant compounds gained significant attention to alleviate detrimental cellular stress seen in the PD. Superoxide dismutase and catalase are well-defined antioxidant enzymes which play a role in the cellular regulation of oxidative stress. Decreased levels of SOD and CAT is demonstrated as an ordinary result of oxidative stress mediated Parkinson's disease (Percario *et al.*, 2020). Several antioxidant compounds showed ameliorative effects on rotenone-induced motor impairment. In particular, well known compounds such as vitamin E, coenzyme Q10, mitoquinone and melatonin, demonstrated neuroprotective effects on PD models with divergent mechanisms of actions (Filograna *et al.*, 2016; Murphy, 2014; Janda *et al.*, 2012). Our results are in line with previous studies which reported that sinapic acid improved SOD and CAT levels, which might play a role in the neuroprotective action. Therefore, alleviated motor impairment by sinapic acid due to the rotenone, in line with other reports which antioxidant compounds, was shown to protect against rotenone insult (Zare *et al.*, 2015).

Iron accumulation in the substantia nigra is one of the suggested mechanisms of PD and considered as a possible target for therapeutic interventions (Sian-Hulsmann *et al.*, 2011). Ablation of iron regulatory protein expressions (IRP2) resulted in iron accumulation and caused parkinsonism in

mice. Transferrin transport complexes have been known as iron importers into the mitochondria of SNc neurons (LaVaute *et al*., 2001). Because of the direct relationship between soluble iron in cytoplasm and pigmented neurons in the striatal area, deferiprone, which is the one of iron-chelating agent, is now in the phase 2 trials (Martin-Bastida *et al*., 2017). Therefore, it led us to propose chemicals with iron-chelating properties could have neuroprotective action in experimental models seen in our study and a number of other studies. Several flavonoids and phenolic acids have been shown to decrease serum iron levels (Lesjak, Srai, 2019). Sinapic acid is one of the compounds claimed which have the ability to decrease iron levels, as seen in our study (Chen, 2016). Due to the reducing noxious effects of iron in the substantia nigra, sinapic acid could inhibit rotenone-induced TH (+) loss. Therefore, it is rationale to think that neuroprotective action of sinapic acid on in our model might be result of iron regulatory effects.

Although the presence of HO-1 is limited to healthy substantia nigra pars compacta in the aging, oxidative stress and susceptible neurons in the central nervous system have the ability to up-regulate HO-1 immunopositivity (Schipper, Liberman, Stopa, 1998). In addition to its antioxidant action, HO-1 is responsible for the degradation of free ferrous iron (Fe^{2+}) and protects the cell against Fenton reaction-caused cellular stress (Baker, Anderson, Baker, 2003). The role of HO-1 in neurodegenerative diseases has been already demonstrated and, like in aging, HO-1 up-regulation against cellular stress is possibly dependent on Nrf2 activation (Loboda *et al*., 2016). However, this up-regulation in glial cells also promotes energy failure and directly affects the iron metabolism and mitochondrial activity (Song *et al*., 2014). Additionally, overexpression of HO-1 in astrocytes of transgenic mice showed parkinsonian features, despite the inactivation of monoamine oxidase (Song *et al*., 2017). These contrary reports support our hypothesis and explain the difference between serum and brain levels of HO-1 seen in our study. The decrease seen in the rotenone-induced elevation of HO-1 levels in our study, confirms the relationship between sinapic acid's antioxidant and iron-dependent actions. We suggested that due to its ability to decrease serum iron levels, rotenone-induced decrease in HO-1 upregulation was mediated by sinapic acid.

It has already been demonstrated that rotenone causes significant lipid peroxidation, which results in neuronal death in the PD models (Verma, Nehru, 2009). Therefore, in our study we investigated MDA levels to evaluate lipid peroxidation and the effect of sinapic acid, in addition to the GPx-4 levels. Our results showed similarity with previous studies, that sinapic acid prevented lipid peroxidation in hemi-parkinsonian disease model (Zare *et al*., 2015). Furthermore, to investigate the effect of sinapic acid on rotenone-induced lipid peroxidation and its relationship with ferroptosis, we also investigated both serum and tissue levels of GPx-4. GPx-4 is a membrane-bound glycoprotein and widely expressed in the brain tissue (Wang *et al*., 2013). It has been recently proposed as one of the main elements in ferroptosis, responsible for lipid and cardiolipin peroxidation in cells (Cardoso *et al*., 2017). Moreover, GPx-4 also regulates ATP status in the oxidative stress and is found to be a relevant element in the progression of the neurodegeneration (Friedmann *et al*., 2014). Oxidative and nitrosative stress directly affects mitochondrial cytochrome C release and lipids on plasma membranes, and GPx-4 has been shown as a major protector against these cellular stressors (Nomura *et al*., 2000). We tried to analyze serum levels of GPx-4 in the experimental groups, however we failed to detect GPx-4 isoform in the serum as claimed in other studies (Wang *et al*., 2013). Rotenone, possibly due to inducing ROS and RNS levels to increase, decreased brain levels of GPx-4, as expected. On the contrary, sinapic acid administration reduced rotenone-induced GPx-4 decreases, in agreement with other reports that claimed iron-chelation and resulting decreased serum iron levels, might cause an increase in the GPx-4 levels (Cardoso *et al*., 2017). Considered together, our results on lipid peroxidation status (MDA levels) are in line with GPx-4 levels. In parallel with decreased plasma levels of iron seen in sinapic acid, our results suggest that this was the possible reason behind increased GPx-4 levels against rotenone insult in the dopaminergic neurons.

In conclusion, our study suggests that the neuroprotective effect of sinapic acid is due to a mechanism of reduction of ferroptosis and iron regulation. As far as we know, this is the first study on iron and iron-

dependent action of sinapic acid in rotenone-induced Parkinson's disease model. Consequently, our study might illuminate the role of iron-homeostasis in PD and will provide additional insights to the investigation of new treatment options.

DECLARATIONS

This study was funded by the Ondokuz Mayis University Scientific Research Council (1904.19.007).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in this study.

ETHICS APPROVAL

All experiments were performed to minimize animal suffering and according to the Guide for the Care and Use of Laboratory Animals adopted by the National Institutes of Health (USA) as well as the Declaration of Helsinki. Ethical approval was obtained from the Ondokuz Mayis University Ethics Committee for Animal Experiments (HADYEK 2019-19).

DECLARATIONS

Funding

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COMPETING INTEREST

Authors declare there is are conflicts of interest.

ETHICS APPROVAL

Animals were obtained from the Ondokuz Mayis University (Samsun, Turkey) vivarium and maintained in constant conditions (22±0.5°C and 55% humidity, 12/12-night cycles). All experiments were performed to minimize animal suffering and in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the National Institutes of Health (USA) and the Declaration of Helsinki, with the approval from the Ondokuz Mayis University Ethics Committee for Animal Experiments (HADYEK 2019-19).

AUTHOR CONTRIBUTIONS

Conception and design: BA, CG. Data acquisition, data analysis and interpretation: MK, SB, NK, TG. Drafting the article or critically revising it for important intellectual content: BA, SB, TG. Final approval of the version to be published: all authors.

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