RESEARCH PAPER

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Minocycline protects against neuronal mitochondrial dysfunction and cognition impairment

Majid Motaghinejad[1]*, Manijeh Motevalian[2], Luis Ulloa[3], Neda Kaviani[1], Emre Hamurtekin[4]

[1] Chronic Respiratory Disease Research Center (CRDRC), National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran,
[2] Department of Pharmacology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran,
[3] Center for Perioperative Organ Protection, Department of Anesthesiology, Duke University, Durham, NC, USA,
[4] Faculty of Pharmacy, Eastern Mediterranean University, Famagusta, Turkey,
** Email: dr.motaghinejad6@gmail.com*

The potential of minocycline to protect against methylphenidate-induced neurodegeneration has been extensively reported in the literature but the mechanism of action is still unknown. This study aims to determine the role of mitochondrial chain enzymes and redox homeostasis on the neuroprotective effects of minocycline in methylphenidate-induced neurodegeneration. Wistar adult male rats were randomly assigned to the seven experimental groups: Group 1 received saline solution; Group 2 received methylphenidate (10 mg/kg, i.p.); Groups 3, 4, 5, and 6 received methylphenidate and minocycline for 21 days; Group 7 received minocycline alone. Cognition was evaluated with the Morris water maze test. Activity of the hippocampal mitochondrial quadruple complexes I, II, III and IV, mitochondrial membrane potential, adenosine triphosphate (ATP) levels, total antioxidant capacity, and reactive oxygen species were determined. Treatment with minocycline inhibited methylphenidate-induced cognitive dysfunction. Minocycline treatment increased mitochondrial quadruple complex activities, mitochondrial membrane potential, total antioxidant capacity, and ATP levels in the dentate gyrus and cornu ammonis-1 (CA1) areas of the hippocampus. Minocycline is likely to confer neuroprotection against methylphenidate-induced neurodegeneration and cognition impairment by regulating mitochondrial activity and oxidative stress.

Key words: methylphenidate, minocycline, mitochondria, cognition

INTRODUCTION

Mitochondria are dynamic organelles regulating neuronal metabolism (Lin and Sheng, 2015; Bertholet et al., 2016) and, mitochondrial morphological or biochemical changes causes neuronal dysfunction and degeneration (Llorente-Folch et al., 2015; Bertholet et al., 2016; Golpich et al., 2017; Norat et al., 2020). Mounting evidence suggest that mitochondrial dysfunction plays a critical role in neurodegeneration and neurotoxicity associated with drug abuse (Monzio Compagnoni et al., 2020; Norat et al., 2020). Oxidative stress with the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) cause mitochondrial dysfunction by inhibiting the respiratory chain (Ozcan and Ogun, 2015; Angelova and Abramov, 2018). Indeed, many psychostimulants affect neuronal mitochondria inducing neuronal dysfunction and degeneration and these effects can be more significant in the hippocampus with a direct effect in cognition (Fagundes et al., 2007; 2010; Foschiera et al., 2022).

Methylphenidate is a psychostimulant increasingly abused by patients (Clemow, 2015; Shellenberg et al., 2020). Methylphenidate induces neurobehavioral disorders including cognition impairment, neurodegeneration, and neurotoxicity associated with mitochondrial dysfunction, oxidative stress, apoptosis, and inflamma-

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tion (Banihabib et al., 2015; Motaghinejad et al., 2017; Schmitz et al., 2017a). These studies suggest a potential role of mitochondria dysfunction in the neurotoxicity induced by methylphenidate. Although we and others showed that methylphenidate induces hippocampus oxidative stress, its effects on mitochondrial function are unknown (Loureiro-Vieira et al., 2017; Schmitz et al., 2017a; Freddo et al., 2021; Foschiera et al., 2022) and thus whether it affects the mitochondrial respiratory chain, ATP production, or mitochondrial membrane potential in the areas of the hippocampus mostly affect by neurodegeneration. These studies have major clinical implications and can provide critical information to design new therapeutic strategies against neurodegeneration and drug abuse. We also reasoned that neuroprotective agents preserving mitochondrial function may provide clinical advantages for treating patients with methylphenidate abuse (Carias et al., 2018; Raoofi et al., 2020). Minocycline is a neuroprotective compound that prevents mitochondrial dysfunction, oxidative stress, apoptosis, and inflammation (Budni et al., 2016; Cankaya et al., 2019; Chauhan et al., 2021; Motaghinejad et al., 2020; Motaghinejad et al., 2021; Naderi et al., 2020). Despite the studies reporting the neuroprotective effects of minocycline against oxidative stress and mitochondrial dysfunction, its mechanism of action is unknown, and thus its effects on energy balances, mitochondrial membrane potential, mitochondrial respiratory chain, and oxidative stress (Budni et al., 2016; Cankaya et al., 2019; Motaghinejad et al., 2020; Naderi et al., 2020; Chauhan et al., 2021; Motaghinejad et al., 2021; Romero-Miguel et al., 2021). Here, we analyze the potential neuroprotective effects of minocycline against methylphenidate-induced neurotoxicity by analyzing cognition, mitochondrial quadruple complexes, ATP production, mitochondrial membrane potential, and oxidative stress in the dentate gyrus (DG) and cornu ammonis-1 (CA1) areas of the hippocampus.

METHODS

Animals

Seventy Wistar adult male rats weighing 250-300 g were purchased from Iran University of Medical Sciences. All animal were maintained at standard conditions with free access to food and water, and room temperature at 22 ± 0.5°C with 12 h light/dark cycles. All experimental procedures were approved by the Committee on Ethics in Research at the Iran University of Medical Sciences. The Research Code was 16837 and Ethical Code was: IR.IUMS.REC.1399.908. Experimental protocols are in compliance with the ARRIVE (Animal Research: Reporting of *In vivo* Experiments) guidelines (Kilkenny et al., 2010; McGrath et al., 2010).

Drugs

Methylphenidate and minocycline, with high-performance liquid chromatography (HPLC) standards purity >98.5% and purity >98.8% respectively, were purchased from Sigma-Aldrich Company (USA).

Experimental protocols and design

Animals were randomly assigned to the seven experimental groups (n=10): group 1, control animals treated with normal saline (0.7 ml/rat, i.p.) for 21 days; group 2 was treated with methylphenidate only (10 mg/kg, i.p.) for 21 days; groups 3, 4, 5, and 6 were the treated with methylphenidate (10 mg/kg, i.p.) and minocycline (10, 20, 30, and 40 mg/kg, respectively) for 21 days; group 7 treated with minocycline only (40 mg/kg, i.p.) for 21 days.

Treatment with methylphenidate was started one hour after minocycline administration. The neurodegenerative doses for methylphenidate and neuroprotective doses of minocycline were selected from previous studies (Banihabib et al., 2016; Carias et al., 2018; Comim et al., 2014; Huang et al., 2018; Martins et al., 2006; Matsukawa et al., 2009; Pinzon et al., 2008; Simon et al., 2018). The Morris water maze test to assess cognition was performed at day 22-26 post-treatment. The hippocampus were collected as previously described (Motaghinejad et al., 2021; Motaghinejad et al., 2017; Wang et al., 2015). To analyze mitochondrial complex activity, reactive oxygen species, ATP levels, total antioxidant capacity, and mitochondrial membrane potential in the granular cell of the dentate gyrus and pyramidal cells of cornu ammonis-1. Our study focuses on the hippocampus because it is the main center managing memory and learning (Anacker and Hen, 2017; Jeffery, 2018; Schlichting and Preston, 2017). The Morris water maze behavioral test was used to assess the animal's cognition and mitochondrial function in the hippocampus was analyzed at the molecular changes using the specific biomarkers.

Evaluation of behavioral changes

Morris water maze test

The Morris water maze test is a standard behavioral test to assess spatial learning and memory. It consists of a circular tank with 160 cm in diameter and 90 cm in

height. The tank is filled with water to 50 cm height. The circular tank is divided into four space quadrants (north-east, north-west, south-east, and south-west). A hidden platform was placed at one cm below the water surface in the center of the north-east quadrant. For evaluation of behavior experimenter stays in the north-west quadrant of the Morris water maze apparatus. Animal movements are analyzed with the automated infrared tracking system (CCTV B/W camera, SBC-300 (P), Samsung Electronics Co, Ltd, Korea) recording the location of the rats in the tank. The camera was located at 2.4 m above the surface of the tank and the data is automatically analyzed as reported (D'Hooge and De Deyn, 2001; Wenk, 2004; Motaghinejad et al., 2015).

Animal adaptation: During adaptation, each rat was placed at the south-west quadrant of the water tank and guided by the investigator toward the platform located below the surface at the north-east quadrant similar as previously described (D'Hooge and De Deyn, 2001; Motaghinejad et al., 2015).

Training procedure for learning assessment: Specific landmarks such as discriminate picture, door, window, etc. were inserted in the wall of the room for spatial cues for learning about the position of the platform. It should be noted that during the learning procedure if the animals could find the platform in 60 s the trial was closed by mentioned PC automatically. But if they could not find the platform in 60 s the test is completed manually by the experimenter.

Analyzed parameters included: 1. Latency time refers to the time required to find the hidden platform; 2. Traveled distance refers to the distance each animal traveled to reach the hidden platform (Bromley-Brits et al., Deng and Song, 2011; D'Hooge and De Deyn, 2001; Ghafarimoghadam et al., 2022; Motaghinejad et al., 2015; Vorhees and Williams, 2006).

Memory assessment: Memory assessment was performed on day 5. The hidden platform was removed from the tank and each animal was randomly placed in one quadrant of the water tank (other than the north- east quadrant), and the system determine the time of the animals in each quadrant and the specific percentage of time that the animal spent at the north-east quadrant (Bromley-Brits et al., 2011; D'Hooge and De Deyn, 2001; Ghafarimoghadam et al., 2022; Motaghinejad et al., 2015a; 2015b; Vorhees and Williams, 2006).

Molecular studies

Hippocampus were collected as reported (Yilmaz et al., 2021). For isolation of hippocampus each animals was anesthetized by administration of sodium thiopental (50 mg /kg, i.p.), and then the whole brains of rats was dissected and their hippocampus removed by standard surgical procedure which its guide was published in previous studies (Bose et al., 2021; Fernández-Vizarra et al., 2006). Cold homogenization buffer (25 mM 4-morpholinepropanesulfonic acid, 400 mM sucrose, 4 mM magnesium chloride ($MgCl_2$), 0.05 mM ethylene glycol tetra-acetic acid (EGTA), pH 7.3) was prepared and isolated hippocampal tissue was kept in this buffer. Hippocampus tissue and homogenization buffer was mixed and centrifuged for 15 min at 450 g, and then its supernatant fluid was re-centrifuged with 12000 g for 12 min. After his process its sediment was mixed in mentioned homogenization buffer and re-suspend. This mixture kept at 0°C until use. For evaluation of total protein in tissues assay Bradford Tanique (BT) was used by using a special kit (Dc-Bio- Rad) (California, USA). Basis of this technique is that a series of serial dilution (0.1-1.0 mg/ml) of well-known protein (such as bovine serum albumin) was prepared and Bradford reagent (1 portion Bradford: 4 portions dH_2O) was added to each of tubes/wells. Mentioned serial dilutions of bovine serum albumin with various concentrations were used to drawing the standard curve. In other sides serial dilution (10, 15, 20, 25, and 30 µl) of the mentioned homogenized hippocampal tissue was mixed with Bradford reagent and color density of each dilution/concentration was recorded in wave length of 630 nm. Finally by using of standard curve protein content of each dilution/concentration was calculates. This procedure and protein quantification was used measurements of mitochondrial enzymes activates (Bose et al., 2021; Fernández-Vizarra et al., 2006).

Mitochondrial complex enzymes chain evaluation: Mitochondria I, II, III and IV complexes activity was evaluated by immunosorbent enzyme assay kits for (Abcam, Cambridge, MA, USA UU: # AB109721, # AB109908, # AB109911, respectively). Briefly, mitochondrial complex I activity was determined measuring the oxidation of NADH to NAD^+ and analyzing absorbance at 450 nm. Mitochondrial complex II activity was determined measuring the catalysis of the electronic transfer of succinct to the ubiquinone and analyzing absorbance at 550 nm. Mitochondrial complex III activity was determined measuring the reaction speed of the conversion of cytochrome C oxidized into its reduced form and analyzing absorbance at 600 nm. Mitochondrial complex IV activity determined by measuring the oxidation of the reduced form of cytochrome C and its absorbance read at 550 nm. All procedures were performed according to the manufacturer's instructions, and values were reported as activity/mg of protein/min (Bénit et al., 2006; Kirby et al., 2007).

Measurement of reactive oxygen species (ROS) levels: Intracellular reactive oxygen species (ROS) levels were determined using fluorescein-labeled dye, 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA), which is converted to the fluorescent 2,7-dichlorofluorescein (DCF) *via* de-esterification and oxidation reaction (Lin et al., 2012; Gao et al., 2019). Briefly, 2,7-dichlorodihydrofluorescein diacetate was mixed with hippocampus tissue and stored in lab room temperature for 45 min. Then, the samples are washed 3 times, and fluorescence intensity of fluorescent 2,7-dichlorofluorescein was read at 535 nm and ROS level in hippocampus was reported as fold of control (Gao et al., 2019; Lin et al., 2012; Wang et al., 2003).

Measurement total antioxidant: Total antioxidant capacity levels were measured with commercial kits (cat# MAK187, Sigma-Aldrich, MO) and all procedures were performed following the manufacture guidelines (Modesti et al., 2019; Salvi et al., 2020). Total antioxidants levels in homogenized hippocampus tissue were evaluated according to its capacity to convert Cu^{2+} to its reduced form, Cu^{+}. Cu^{+} chelates with a colorimetric probe and absorbance of this complex of Cu^{+} and colorimetric probe can be read at 570 nm wave lengths. Total antioxidant capacity levels in hippocampus were reported as nM/µg on hippocampus homogenates (Modesti et al., 2019; Salvi et al., 2020).

Measurement of adenosine triphosphate (ATP): ATP synthesis enzyme the level of ATP determinate by commercial kit (cat# A22066, Molecular probes, OR). ATP synthase activity was determined with D-luciferin and measured at wavelength of 560 nm. The ATP level in hippocampus was reported as ATP/µg on hippocampus homogenates (Salvi et al., 2020; Kepchia et al., 2022).

Measurement of mitochondrial membrane potential: Mitochondrial membrane potential was evaluated by using commercial kit (cat # 600880, Cayman, MI) using JC1 dye according to manufacturer guidelines. This dye enters the mitochondria to form J-aggregates (Ex/Em=560/590 nm) or J-monomers (Ex/Em=485/535 nm). The ratio of J-aggregate to J-monomers intensity indicates the mitochondrial membrane potential. Low ratios indicate low membrane potentials. Mitochondrial membrane potentials were reported as JC1 ratio (J-aggregate to J-monomers ratio) on hippocampus homogenates (Wang et al., 2003; Sakamuru et al., 2016; Salvi et al., 2020).

Statistical analyses

Statistical analyses were performed with GraphPad Prism v.7 Software (GraphPad Software, La Jolla, CA). The sample size (n=10/group) was determined with power analyses of our previous studies. The figures are representative of experiments that were repeated twice in different days and the data are expressed as mean ± standard error of the mean (SEM).

Analyses of three or more groups were performed with one-way ANOVA test with multiple pair-wise comparisons for repeated measures of independent samples with normal distribution. Normality and homogeneity of variance were confirmed with Kolmogorov- Smirnov analyses. The least significant difference (LSD) test was used when the ANOVA F omnibus was significant, if not, the Dunnett's T3 method was used. For non-normal distributions, nonparametric Kruskal-Wallis test was performed with SPSS 23.0. The degrees of freedom and the confidence level of the Type I error are reported. $p<0.05$ and $p<0.001$was considered statistically significant.

RESULTS

Treatment with minocycline improved methylphenidate-induced cognitive dysfunction

The Morris water maze tests after four days of training showed that methylphenidate treatment (10 mg/kg, i.p.) increased both the latency time (one-way ANOVA, $F_{(6,49)}=18.63$; $p<0.001$) and traveled distance (one-way ANOVA, $F_{(6,49)}=15.73$; $p<0.001$) as compared to control animals (Fig. 1A, B). Minocycline treatment (10, 20, 30, and 40 mg/kg) dramatically decreased both the latency time (one-way ANOVA, $F_{(6,49)}=18.63$; $p<0.001$) and traveled distance (one-way ANOVA, $F_{(6,49)}=15.73$; $p<0.001$) in a concentration dependent manner as compared to methylphenidate treated animals (Fig. 1A, B). Minocycline alone (40 mg/kg) affected neither the latency time nor the traveled distance in the Morris water maze tests (Fig. 1A, B). The swimming speed was not altered during training trials in any of the animal groups (one-way ANOVA, $F_{(6,49)}=1.082$; $p=0.09$; Fig. 1C). Methylphenidate treatment decreased the percentage of time of the animals in the target quadrant as compared to sham group (one-way ANOVA, $F_{(6,49)}=6.562$; $p<0.001$; Fig. 1D). Minocycline treatment progressively increased the percentage of time that the animals spent in target quadrant as compared to methylphenidate treated animals (one-way ANOVA, $F_{(6,49)}=6.562$; $p<0.001$; Fig. 1D). Minocycline treatment (40 mg/kg) did not affect the percentage of time of the animals in target quadrant by itself (Fig. 1D).

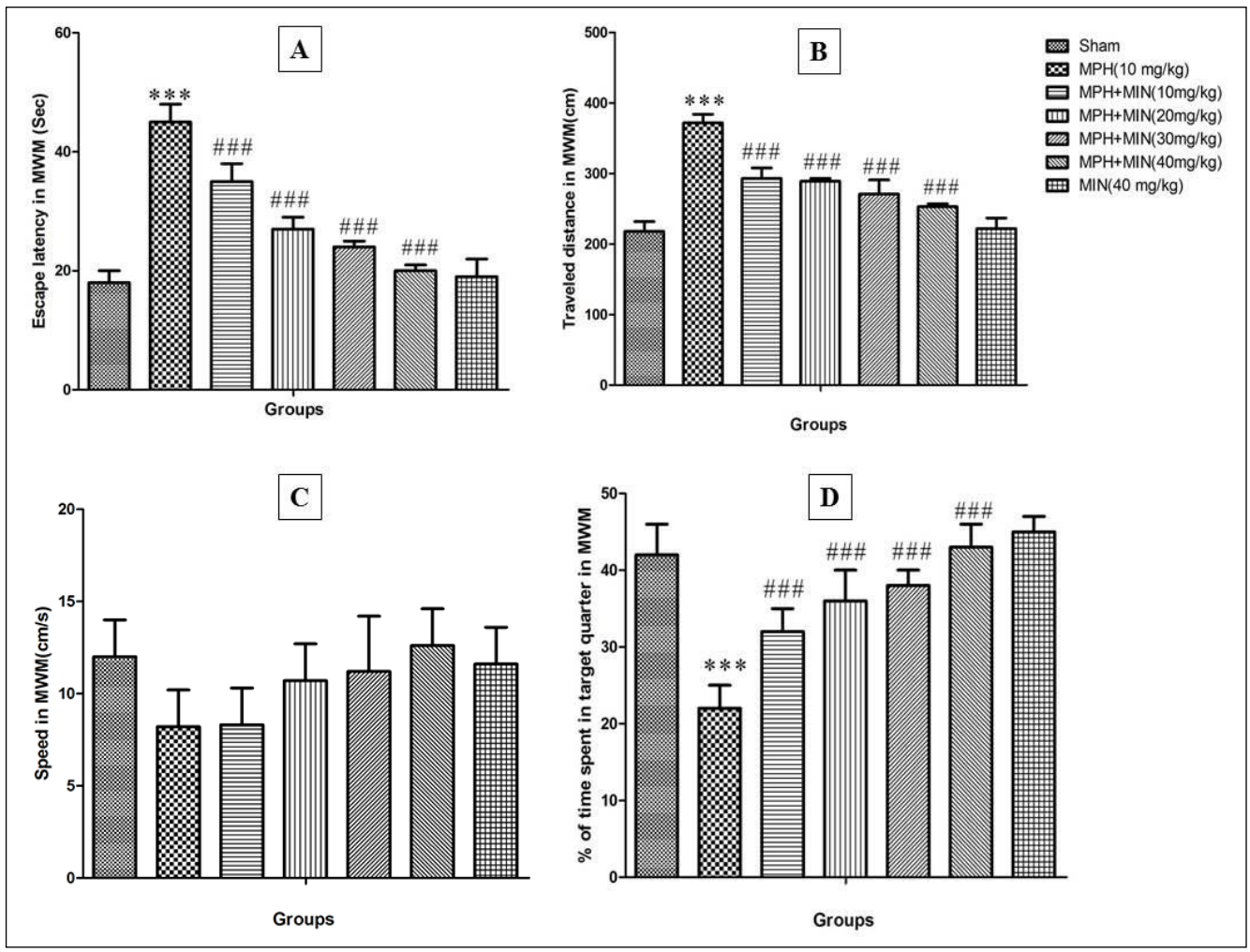

Fig. 1. Behavior of rats in the Morris water maze test. Escaped latency (A), traveled distance (B), swimming speed (C) and percent of presences in target quarter (D) in all groups. All data are expressed as Mean ± SEM (n=10). *** p<0.001 *vs.* sham group. ### p<0.001 *vs.* 10 mg/kg of methylphenidate. MPH: Methylphenidate. MIN: Minocycline.

Minocycline treatment restored mitochondrial enzymatic activity in the hippocampus

Methylphenidate treatment significantly reduced the activity of mitochondrial complexes I, II, III, and IV in the dentate gyrus (one-way ANOVA, $F_{(6,49)}$=5.827 for complex I, $F_{(6,49)}$=19.04 for complex II, $F_{(6,49)}$=12.50 for complex III and $F_{(6,49)}$=3.527 for complex IV; p<0.001; Fig. 2A-D) and cornu ammonis-1 (one-way ANOVA, $F_{(6,49)}$=11.84 for complex I, $F_{(6,49)}$=9.246 for complex II, $F_{(6,49)}$=12.50 for complex III and $F_{(6,49)}$=6.914 for complex IV; p<0.001; Fig. 3A-D) areas of hippocampus of rats as compared to those from the sham group. The main effect of methylphenidate was inhibiting complex II and III activity by 81% and 82.5%, respectively whereas the lowest effect was inhibiting complex I activity by 70% in the dentate gyrus and cornu ammonis-1. Minocycline treatment restored the enzymatic activity of the mitochondrial complexes I, II, III, and IV in both dentate gyrus (one-way ANOVA, $F_{(6,49)}$=5.827 for complex I, $F_{(6,49)}$=19.04 for complex II, $F_{(6,49)}$=12.50 for complex III and $F_{(6,49)}$=3.527 for complex IV; p<0.001; Fig. 2A-D) and cornu ammonis-1 (one-way ANOVA, $F_{(6,49)}$=11.84 for complex I, $F_{(6,49)}$=9.246 for complex II, $F_{(6,49)}$=12.50 for complex III and $F_{(6,49)}$=6.914 for complex IV; p<0.001; Fig. 3A-D) in methylphenidate treated rats in a concentration dependent manner. Minocycline treatment was the most effective by restoring 98% on complex III activity at 40 mg/kg in both the dentate gyrus and cornu ammonis-1. Actually, minocycline restored normal activity of complexes I, II, III, and IV at dose 10, 20, 30, and 40 mg/kg, respectively. Minocycline treatment (40 mg/kg) did not significantly affect the enzymatic activity of any of these mitochondrial complexes by itself (Fig. 2 and Fig. 3A-D).

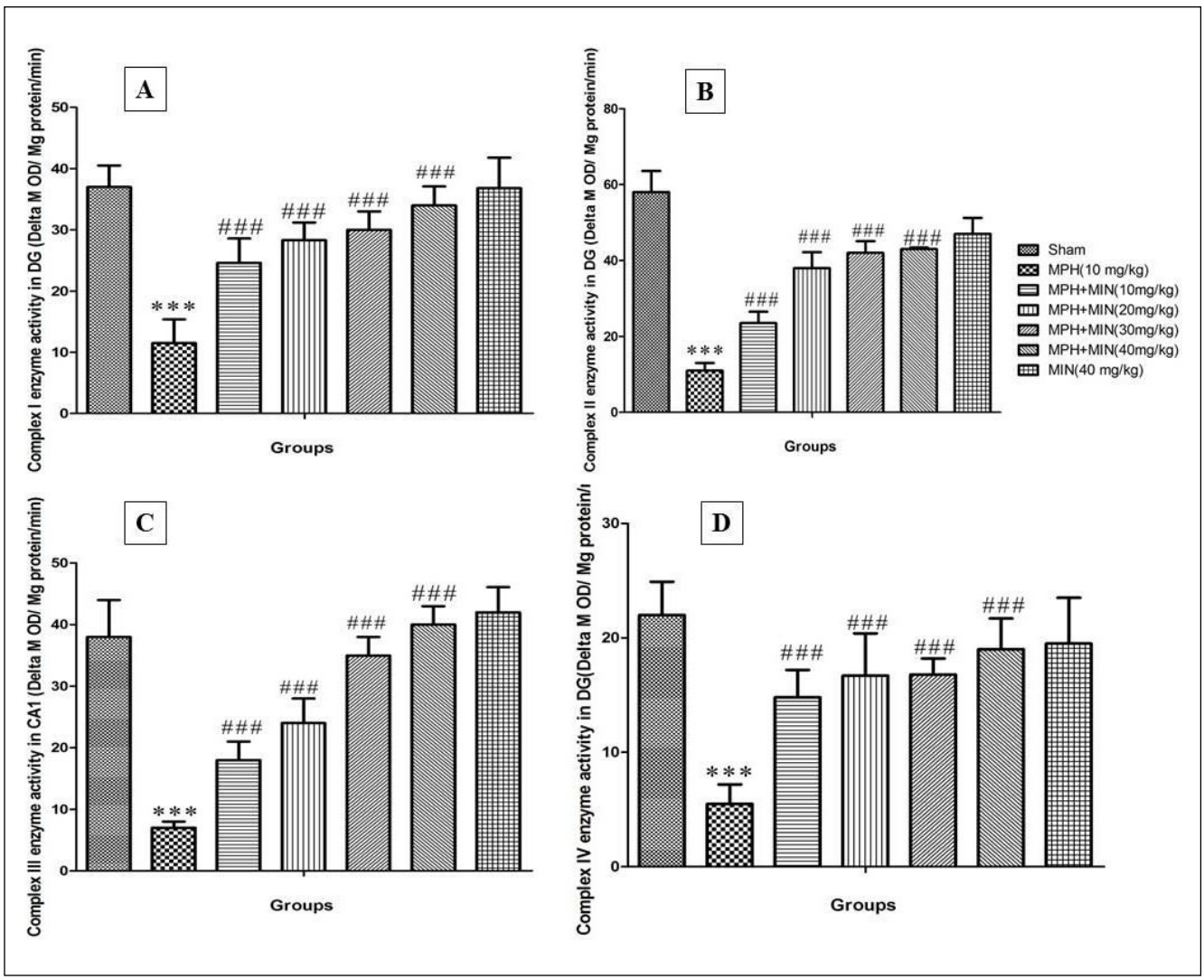

Fig. 2. Alterations of complex I (A), complex II (B), complex III (C) and complex III (D) in dentate gyrus dentate gyrus (DG) area of rat isolated hippocampus in all groups. All data are expressed as Mean ± SEM (n=10). *** p<0.001 *vs.* sham group. ### p<0.001 *vs.* 10 mg/kg of methylphenidate. MPH: Methylphenidate. MIN: Minocycline.

Minocycline treatment reduced oxidative stress in the dentate gyrus and cornu ammonis-1

Methylphenidate administration (10 mg/kg) increased ROS levels in both the dentate gyrus (one-way ANOVA, $F_{(6,49)}$=32.18; p<0.001) and cornu ammonis-1 (one-way ANOVA, $F_{(6,49)}$=47.49; p<0.001) in the hippocampus of the experimental rats as compared to those from sham rats (Fig. 4A, B). Minocycline treatment dramatically reduced oxidative stress and ROS levels in methylphenidate-treated rats in the dentate gyrus (one-way ANOVA, $F_{(6,49)}$=32.18; p<0.001) and cornu ammonis-1 (one-way ANOVA, $F_{(6,49)}$=47.49; p<0.001) areas of hippocampus (Fig. 4A, B). Minocycline treatment did not significantly affect the ROS levels by itself (Fig. 4A, B).

Methylphenidate (10 mg/kg) significantly reduced the total antioxidant capacity in both the dentate gyrus (one-way ANOVA, $F_{(6,49)}$=28.90; p<0.001) and cornu ammonis-1 (one-way ANOVA, $F_{(6,49)}$=16.56; p<0.001) in the hippocampus in the experimental rats as compared to those from the sham group (Fig. 5A, B). Minocycline treatment dramatically reduced oxidative stress and total antioxidant capacity in methylphenidate-treated rats in both the dentate gyrus (one-way ANOVA, $F_{(6,49)}$=28.90; p<0.001) and cornu ammonis-1 (one-way ANOVA, $F_{(6,49)}$=16.56; p<0.001) in the hippocampus in the experimental rats as compared to those from the sham group (Fig. 5A, B).

Minocycline treatment did not significantly affect total antioxidant capacity by itself (Fig. 5A, B).

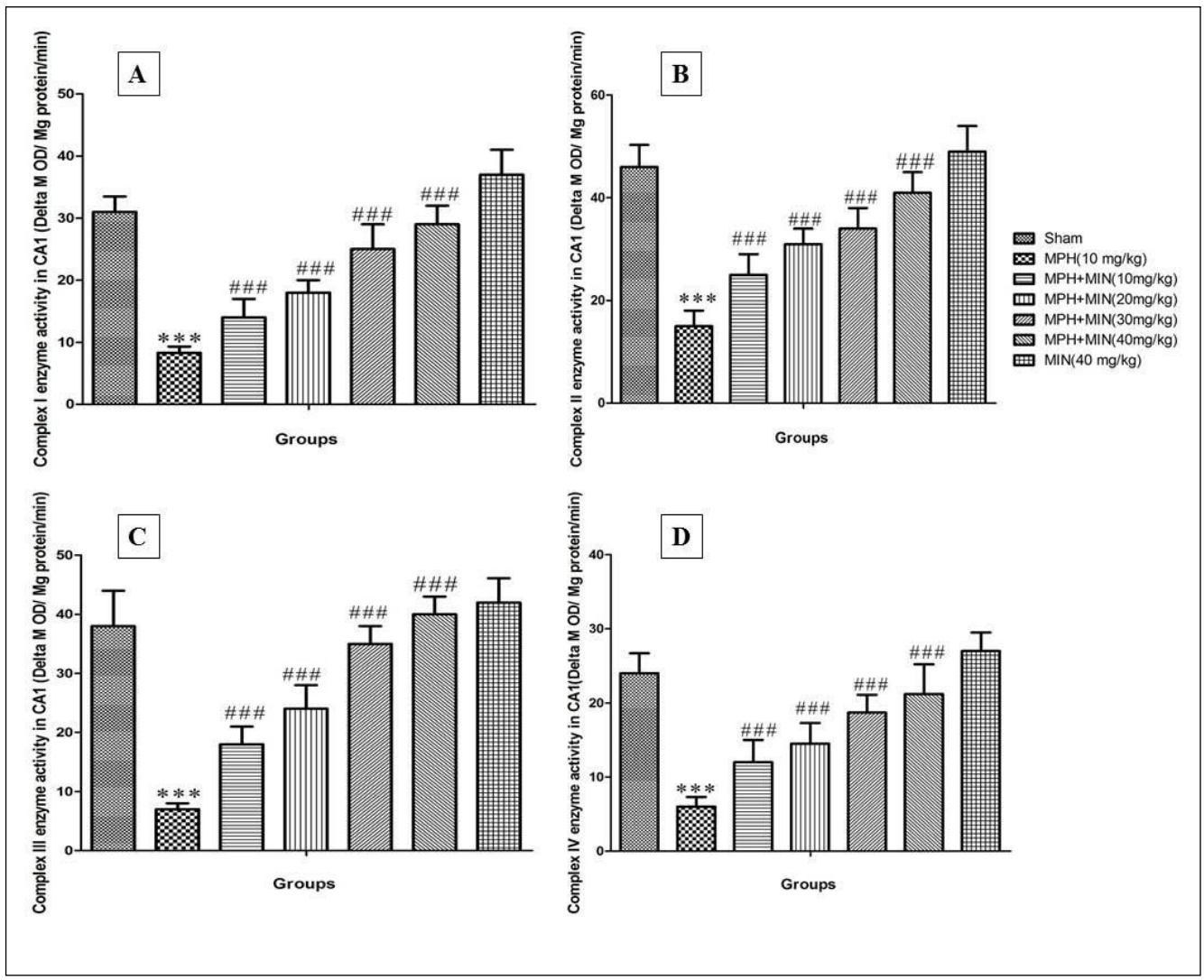

Fig. 3. Alterations of complex I (A), complex II (B), complex III (C) and complex III (D) in cornu ammonis-1 (CA1) area of rat isolated hippocampus. All data are expressed as Mean ± SEM (n=10). *** p<0.001 *vs.* sham group. ### p<0.001 *vs.* 10 mg/kg of methylphenidate. MPH: Methylphenidate. MIN: Minocycline.

Minocycline treatment restored ATP synthesis in the dentate gyrus and cornu ammonis-1

Methylphenidate administration (10 mg/kg) decreased ATP levels in both the dentate gyrus (one-way ANOVA, $F_{(6,49)}$=13.38; p<0.001) and cornu ammonis-1 (one-way ANOVA, $F_{(6,49)}$=163.7; p<0.001) in the hippocampus in experimental rats as compared to those from the sham rats (Fig. 6A, B). Minocycline treatment dramatically increased ATP levels in methylphenidate-treated rats in both the dentate gyrus (one-way ANOVA, $F_{(6,49)}$=13.38; p<0.001) and cornu ammonis-1 (one-way ANOVA, $F_{(6,49)}$=163.7; p<0.001) in the hippocampus in the experimental rats as compared to those from the sham group (Fig. 6A, B). Minocycline treatment did not significantly affect the ATP levels by itself (Fig. 6A, B).

Minocycline treatment restored mitochondrial membrane potential in the dentate gyrus and cornu ammonis-1

JC1 assay indicated that methylphenidate treatment significantly reduced the mitochondrial membrane potential activity in the dentate gyrus (one-way ANOVA, $F_{(6,49)}$=15.07; p<0.001) and cornu ammonis-1 (one-way ANOVA, $F_{(6,49)}$=23.14; p<0.001) in the hippocampus of methylphenidate-treated rats as compared to those from sham rats (Fig. 7A, B). Minocycline dramatically

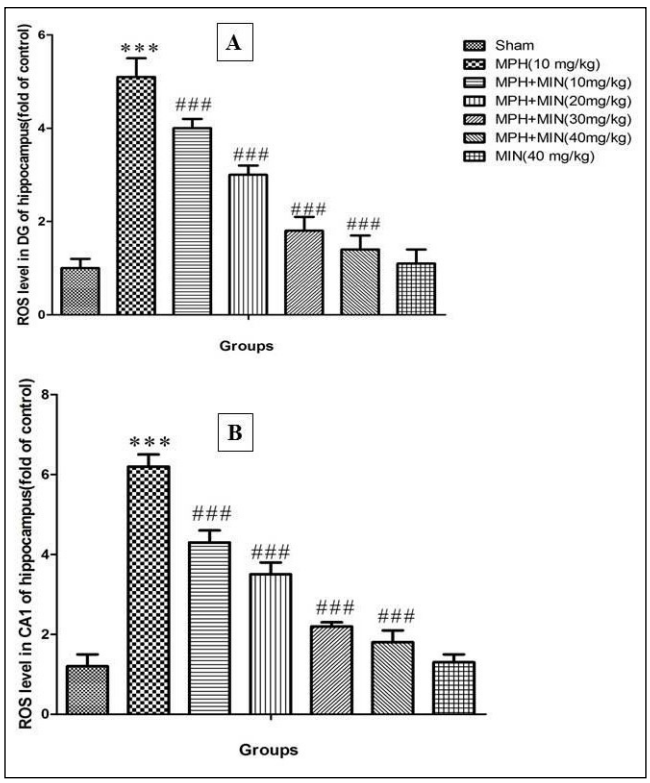

Fig. 4. Alterations of reactive oxygen species (ROS) level in dentate gyrus (DG) (A) and cornu ammonis-1 (CA1) (B) area of rat isolated hippocampus. All data are expressed as Mean ± SEM (n=10). *** p<0.001 *vs.* sham group. ### p<0.001 *vs.* 10 mg/kg of methylphenidate. MPH: Methylphenidate. MIN: Minocycline.

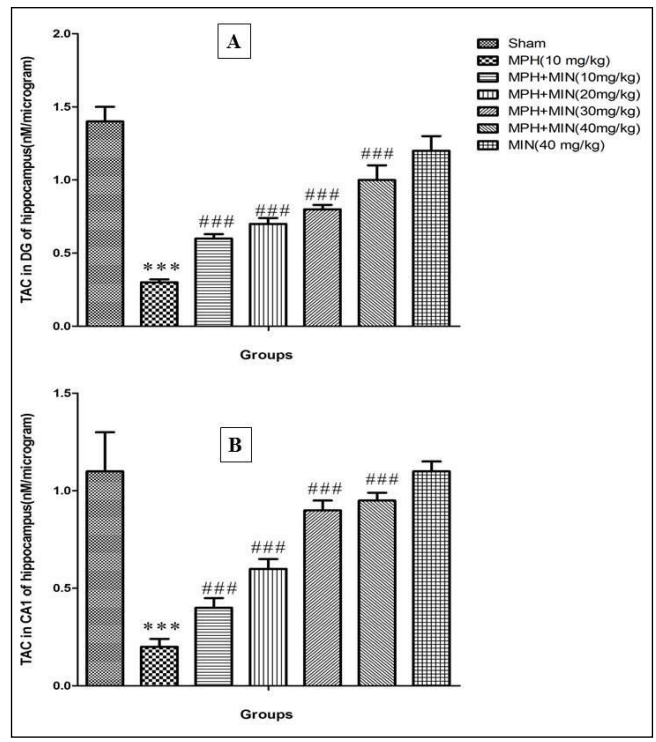

Fig. 5. Alterations of total antioxidant capacity (TAC) amounts in dentate gyrus (DG) (A) and cornu ammonis-1 (CA1) (B) area of rat isolated hippocampus. All data are expressed as Mean ± SEM (n=10). *** p<0.001 *vs.* sham group. ### p<0.001 *vs.* 10 mg/kg of methylphenidate. MPH: Methylphenidate. MIN: Minocycline.

increased and restored mitochondrial membrane potential activity in methylphenidates treated rats in both dentate gyrus (one-way ANOVA, $F_{(6,49)}$=15.07; p<0.001) and cornu ammonis-1 (one-way ANOVA, $F_{(6,49)}$=23.14; p<0.001) in the hippocampus in the experimental rats as compared to those from the sham group (Fig. 7A, B). Minocycline treatment did not significantly affect the mitochondrial membrane potential activity by itself (Fig. 7A, B).

DISCUSSION

This study evaluates the neuroprotective effects of minocycline in methylphenidate- induced cognition impairment and mitochondrial dysfunction in the hippocampus of rats. Minocycline treatment inhibited methylphenidate-induced oxidative stress and restored mitochondrial membrane potential, ATP synthesis, and total antioxidant capacity in the dentate gyrus and cornu ammonis-1 of the hippocampus.

Our results indicate that treating animals with of 10 mg/kg of methylphenidate increased the latency time and traveled distances in the Morris water maze tests and reduced the percentage of time of the animals in the target quadrant. Methylphenidate administration did not affect the traveled speed of the animals by itself. Our results concur with previous studies about the potential of methylphenidate to improve cognition impairment (Banihabib et al., 2016; Carias et al., 2018; Motaghinejad et al., 2015). Although the methylphenidate-induced neurobehavioral, cognition, and mood disturbances were previously reported, the molecular mechanism of action was unknown (Banihabib et al., 2016; Carias et al., 2018; Kapur, 2020; Motaghinejad et al., 2015). Our results show that administration of 10, 20, 30, and 40 mg/kg of minocycline in methylphenidate addicted rats decreased the latency time and traveled distances and increased of presence time of the animals in the target quadrant. Minocycline treatments did not affect the speed of animals. Minocycline treatment alone did not impact the Morris water maze behaviors by itself. Our results also concur with previous experimental and clinical results about the protective effects of minocycline in some neurodegenerative and cognitive disorders (Hiskens et al., 2021; Jiang et

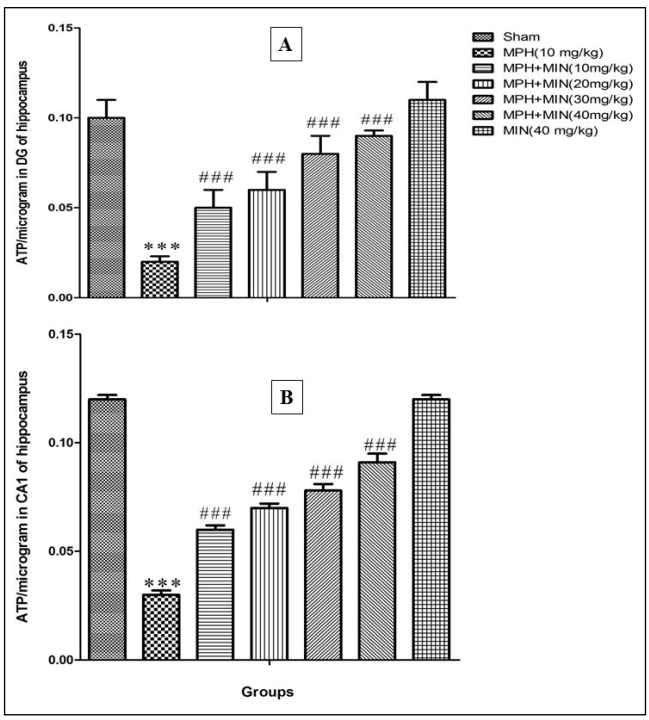

Fig. 6. Alterations of adenosine triphosphate (ATP) level in dentate gyrus (DG) (A) and cornu ammonis-1 (CA1) (B) area of rat isolated hippocampus. All data are expressed as Mean ± SEM (n=10). *** p<0.001 *vs.* sham group. ### p<0.001 *vs.* 10 mg/kg of methylphenidate. MPH: Methylphenidate. MIN: Minocycline.

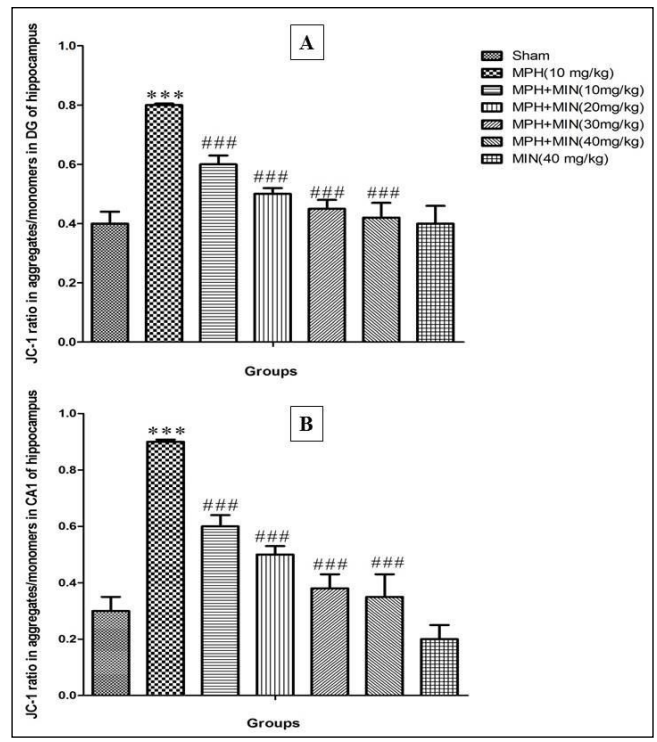

Fig. 7. Alterations of mitochondrial membrane potential (MMP) level in dentate gyrus (DG) (A) and cornu ammonis-1 (CA1) (B) area of rat isolated hippocampus. All data are expressed as Mean ± SEM (n=10). *** p<0.001 *vs.* sham group. ### p<0.001 *vs.* 10 mg/kg of methylphenidate. MPH: Methylphenidate. MIN: Minocycline.

al., 2015; Naderi et al., 2017; Zhao et al., 2015). Previous similar studies showed that minocycline can affect molecular mechanisms evoked in cognition and can affect learning and memory during neurodegenerative disorders and drug abuse (Deakin et al., 2019; El-Shimy et al., 2015; Zhao et al., 2015). According to our data, minocycline can inhibit methylphenidate-induced cognition impairment, but its molecular mechanism was previously unknown. One major cause of cognition impairment is mitochondrial dysfunction and the role of mitochondria on learning and memory has been well established previously (Fernandez et al., 2019; Khacho, Harris and Slack, 2019; Lanzillotta et al., 2019; Lejri et al. 2019). In particular, mitochondrial dysfunction leads to severe deficiencies in hippocampal-dependent loss of memory and learning (Fernandez et al., 2019; Khacho et al., 2019; Lejri et al., 2019). Our data suggest that there is a strong link between mitochondrial dysfunction and cognition dysfunction, thus mitochondrial modulation appears critical to the neuroprotective effects of minocycline (Fernandez et al., 2019; Khacho et al., 2019; Lejri et al., 2019).

Our study shows that methylphenidate (10 mg/kg; i.p.) significantly decreased the enzymatic activity of mitochondrial respiratory chain in mitochondrial complexes I, II, III, and IV in both the dentate gyrus and cornu ammonis-1 areas of the hippocampus. Previous studies suggested that mitochondria dysfunction and oxidative stress can contribute to methylphenidate-induced neurotoxicity and neurodegeneration (Comim et al., 2014; Fagundes et al., 2010; Réus et al., 2013). Our results show that minocycline can activate mitochondrial complexes I, II, III, and IV in both areas of the hippocampus of methylphenidate-treated rats. Even minocycline restored all the four mentioned mitochondrial complexes; it appears more effective in improving complex III ubiquinol-cytochrome c oxidoreductase activity. Our data are consistent with previous studies showing the potential of minocycline to improve mitochondrial function (Dai et al., 2017; Fagundes et al., 2010; Mozafari et al., 2020).

Our data show that methylphenidate significantly decreased mitochondrial membrane potential in both areas of the hippocampus; this detrimental effect of methylphenidate can cause oxidative stress, inflammation, and apoptosis (Foschiera et al., 2022; Freddo et al., 2021; Schmitz et al., 2017b; 2018). The effects of methylphenidate on mitochondrial membrane po-

tential can further cause mitochondrial dysfunction and neurodegeneration (Foschiera et al., 2022; Freddo et al., 2021; Schmitz et al., 2017b; 2018). Our findings also indicate that minocycline can increase mitochondrial membrane potential in both areas of the hippocampus in methylphenidate-treated rats. Our data are consistent with previous results showing that mitochondrial biogenesis and regulation by minocycline play a critical role in oxidative stress, inflammation, and apoptosis during neurodegeneration (Budni et al., 2016; Dai et al., 2017; Dean et al., 2012; Gieseler et al., 2009; Parvardeh et al., 2022). Our study shows that methylphenidate can affect ATP synthase and reduced ATP levels in both regions of the hippocampus in line with the methylphenidate-induced dysfunction in mitochondrial quadruple complexes and membrane potential. As discussed above methylphenidate-induced mitochondrial dysfunction in the hippocampus was not previously reported, but indirect evidence indicated that mitochondria are mainly responsible for the pathologic events caused by methylphenidate (Gomes et al., 2009; Réus et al., 2013; Comim et al., 2014). In other words, methylphenidate effects on mitochondria dysfunction can cause neurodegeneration and neurotoxicity (Gomes et al., 2009; Réus et al., 2013). Our data show that minocycline increased ATP synthesis and ATP levels in both areas of the hippocampus of methylphenidate-treated rats. Our study also confirms previous results about the effects of minocycline in mitochondrial function (Gieseler et al., 2009; Haj-Mirzaian et al., 2019; Mozafari et al., 2020). Previous studies reported the potential of minocycline to increase ATP levels in neurodegenerative disorders (Ghavimi et al., 2021; Haj-Mirzaian et al., 2019), but its effects on ATP synthase and ATP levels in methylphenidate-induced neurotoxicity was unknown.

Minocycline neuroprotective effects include inhibiting oxidative stress, inflammation, apoptosis and restore mitochondrial respiratory chain, ATP synthase, and mitochondrial membrane potential (Budni et al., 2016; Dai et al., 2017; Dean et al., 2012; Gieseler et al., 2009; Parvardeh et al., 2022). However, the role of minocycline in other mitochondrial activities is still unclear and needs further study, but mitochondria functions appeal pivotal for the neuroprotective effects of minocycline (Gieseler et al., 2009; Haj-Mirzaian et al., 2019; Mozafari et al., 2020). Our study also shows that methylphenidate reduced total antioxidant capacity in the hippocampus. This result is consistent with previous data showing that methylphenidate can induce oxidative stress and reduce antioxidant activities in neurons (Schmitz et al., 2012; Coelho-Santos et al., 2019). By contrast, minocycline can increase total antioxidant capacity in both regions of the hippocampus of methylphenidate-treated rats. Previous studies suggested that minocycline neuroprotective effects are mediated by its antioxidant potential (Budni et al., 2016; Dai et al., 2017; Dean et al., 2012; Gieseler et al., 2009; Parvardeh et al., 2022). Our previous studies showed that methylphenidate can induces oxidative stress in both regions of the hippocampus, whereas of our new results show the efficacy of minocycline to reverse these pathological effects.

Methylphenidate neurotoxicity appears to be mediated by mitochondrial dysfunction, oxidative stress and the production of free radicals (Gomes et al., 2009; Fagundes et al., 2010; Coelho-Santos et al., 2016). Minocycline neuroprotective properties are mediated by inhibiting oxidative stress, reducing the production of free radicals, including both reactive oxygen/nitrogen species (ROS/RNS) (Garcia-Martinez et al., 2010; Lin et al., 2003). Minocycline acts as a free radical scavenger during neurodegeneration as shown in our study (Kraus et al., 2005; Shultz and Zhong, 2017). Minocycline activation of mitochondrial complexes I, II, III, IV, ATP synthase and membrane potential can increase anti-oxidative defenses, reduce the production of free radicals and neurodegeneration in the hippocampus in methylphenidate-treated rats. These effects can modulate methylphenidate-induced neurobehavioral disturbances such as cognition impairment. Our results show the strong potential of minocycline to protect methylphenidate-induced mitochondrial dysfunction and oxidative stress. Our results improve the current knowledge about the minocycline neuroprotective effects against some neurotoxic agents such as methylphenidate and suggest the potential clinical advantages of minocycline to restore mitochondria function in neurodegenerative disorders (Salehi et al., 2019; Verghese and Abdijadid, 2022).

CONCLUSION

Our study reveals that methylphenidate induces cognition impairment likely by mitochondrial dysfunction and oxidative stress in the dentate gyrus and cornu ammonis-1 areas of hippocampus contributing to neurodegeneration. Our study reveals the promising clinical implications of minocycline to reverse these pathogenic oxidative effects and mitochondrial dysfunction in methylphenidate-induced neurotoxicity and neurodegeneration. Our results provide new insights into the molecular mechanism of methylphenidate and minocycline in the hippocampus, but they warrant further analyses to determine the specific molecular and cellular mechanisms and their clinical implications.

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REFERENCES

Anacker C, Hen R (2017) Adult hippocampal neurogenesis and cognitive flexibility – linking memory and mood. Nat Rev Neurosci 18: 335–346.

Angelova PR, Abramov AY (2018) Role of mitochondrial ROS in the brain: from physiology to neurodegeneration. FEBS Lett 592: 692–702.

Banihabib N, Haghi ME, Zare S, Farrokhi F (2016) The effect of oral administration of methylphenidate on hippocampal tissue in adult male rats. Neurosurg Q 26: 315–318.

Banihabib N, Ilkhani Pour M, Zare S, Farrokhi F (2015) Oral administration of methylphenidate induces hippocampal necrosis in juvenile rats. Neurosurg Q 25: 411–414.

Bénit P, Goncalves S, Dassa EP, Brière JJ, Martin G, Rustin P (2006) Three spectrophotometric assays for the measurement of the five respiratory chain complexes in minuscule biological samples. Clin Chim Acta 374: 81–86.

Bertholet A, Delerue T, Millet A, Moulis M, David C, Daloyau M, et al. (2016) Mitochondrial fusion/fission dynamics in neurodegeneration and neuronal plasticity. Neurobiol Dis 90: 3–19.

Bose U, Broadbent JA, Juhász A, Karnaneedi S, Johnston EB, Stockwell S, et al. (2021) Protein extraction protocols for optimal proteome measurement and arginine kinase quantitation from cricket Acheta domesticus for food safety assessment. Food Chem 348: 129110.

Bromley-Brits K, Deng Y, Song W (2011) Morris water maze test for learning and memory deficits in Alzheimer's disease model mice. J Vis Exp 20: 2920.

Budni J, Garcez M, Medeiros J, Cassaro E, Bellettini-Santos T, Mina F, Quevedo J (2016) The anti-inflammatory role of minocycline in Alzheimer's disease. Curr Alzheimer Res 13: 1319–1329.

Cankaya S, Cankaya B, Kilic U, Kilic E, Yulug B (2019) The therapeutic role of minocycline in Parkinson's disease. Drugs Context 8: 212553.

Carias E, Hamilton J, Robison LS, Delis F, Eiden R, Quattrin T, et al. (2018) Chronic oral methylphenidate treatment increases microglial activation in rats. J Neural Transm 125: 1867–1875.

Chauhan P, Kakkar AK, Singh H, Gautam C (2021) Minocycline for the management of multiple sclerosis: repositioning potential, opportunities, and challenges. Expert Rev Neurother 21: 35–43.

Clemow DB (2015) Misuse of methylphenidate. Curr Top Behav Neurosci 34: 99–124.

Coelho-Santos V, Cardoso FL, Magalhães A, Ferreira-Teixeira M, Leitão RA, Gomes C, et al. (2019) Effect of chronic methylphenidate treatment on hippocampal neurovascular unit and memory performance in late adolescent rats. Eur Neuropsychopharmacol 29: 195–210.

Coelho-Santos V, Socodato R, Portugal C, Leitao RA, Rito M, Barbosa M, et al. (2016) Methylphenidate-triggered ROS generation promotes caveolae-mediated transcytosis via Rac1 signaling and c-Src-dependent caveolin-1 phosphorylation in human brain endothelial cells. Cell Mol Life Sci 73: 4701–4716.

Comim CM, Gomes KM, Reus GZ, Petronilho F, Ferreira GK, Streck EL, et al. (2014) Methylphenidate treatment causes oxidative stress and alters energetic metabolism in an animal model of attention-deficit hyperactivity disorder. Acta Neuropsychiatr 26: 96–103.

D'Hooge R, De Deyn PP (2001) Applications of the Morris water maze in the study of learning and memory. Brain Res Rev 36: 60–90.

Dai C, Ciccotosto GD, Cappai R, Wang Y, Tang S, Xiao X, Velkov T (2017) Minocycline attenuates colistin-induced neurotoxicity via suppression of apoptosis, mitochondrial dysfunction and oxidative stress. J Antimicrobial Chemother 72: 1635–1645.

Deakin B, Suckling J, Dazzan P, Joyce E, Lawrie SM, Upthegrove R, et al. (2019) Minocycline for negative symptoms of schizophrenia and possible mechanistic actions: the BeneMin RCT. Efficacy Mechanism Evaluation 6: 1–66.

Dean OM, Data-Franco J, Giorlando F, Berk M (2012) Minocycline: therapeutic potential in psychiatry. CNS Drugs 26: 391–401.

El-Shimy IA, Heikal OA, Hamdi N (2015) Minocycline attenuates Aβ oligomers-induced pro-inflammatory phenotype in primary microglia while enhancing Aβ fibrils phagocytosis. Neurosci Lett 609: 36–41.

Fagundes AO, Aguiar MR, Aguiar CS, Scaini G, Sachet MU, Bernhardt NM, et al. (2010) Effect of acute and chronic administration of methylphenidate on mitochondrial respiratory chain in the brain of young rats. Neurochem Res 35: 1675–1680.

Fagundes AO, Rezin GT, Zanette F, Grandi E, Assis LC, Dal-Pizzol F, et al. (2007) Chronic administration of methylphenidate activates mitochondrial respiratory chain in brain of young rats. Int J Dev Neurosci 25: 47–51.

Fagundes AO, Scaini G, Santos PM, Sachet MU, Bernhardt NM, Rezin GT, et al. (2010) Inhibition of mitochondrial respiratory chain in the brain of adult rats after acute and chronic administration of methylphenidate. Neurochem Res 35: 405–411.

Fernández-Vizarra E, Fernández-Silva P, Enríquez JA (2006) Isolation of mitochondria from mammalian tissues and cultured cells. In: Cell Biology. Elsevier, pp. 69–77.

Fernandez A, Meechan DW, Karpinski BA, Paronett EM, Bryan CA, Rutz HL, et al. (2019) Mitochondrial dysfunction leads to cortical under-connectivity and cognitive impairment. Neuron 102: 1127–1142.

Foschiera LN, Schmitz F, Wyse AT (2022) Evidence of methylphenidate effect on mitochondria, redox homeostasis, and inflammatory aspects: Insights from animal studies. Prog Neuropsychopharmacol Biol Psychiatry 116: 110518.

Freddo N, Soares SM, Fortuna M, Pompermaier A, Varela ACC, Maffi VC, et al. (2021) Stimulants cocktail: Methylphenidate plus caffeine impairs memory and cognition and alters mitochondrial and oxidative status. Prog Neuropsychopharmacol Biol Psychiatry 106: 110069.

Gao X, Yang J, Li Y, Yu M, Liu S, Han Y, et al. (2019) Lanthanum chloride induces autophagy in rat hippocampus through ROS-mediated JNK and AKT/mTOR signaling pathways. Metallomics 11: 439–453.

Garcia-Martinez E. M, Sanz-Blasco S, Karachitos A, Bandez MJ, Fernandez-Gomez FJ, Perez- Alvarez S, et al. (2010) Mitochondria and calcium flux as targets of neuroprotection caused by minocycline in cerebellar granule cells. Biochem Pharmacol 79: 239–250.

Ghafarimoghadam M, Mashayekh R, Gholami M, Fereydani P, Shelley-Tremblay J, Kandezi N, et al. (2022) A review of behavioral methods for the evaluation of cognitive performance in animal models: Current techniques and links to human cognition. Physiol Behav 244: 113652.

Ghavimi H, Bayani Ershadi AS, Dastvar S, Hosseini MJ (2021) The effects of minocycline in improving of methamphetamine withdrawal syndrome in male mice. Drug Chem Toxicol 45: 2319–2327.

Gieseler A, Schultze AT, Kupsch K, Haroon MF, Wolf G, Siemen D, Kreutzmann P (2009) Inhibitory modulation of the mitochondrial permeability transition by minocycline. Biochem Pharmacol 77: 888–896.

Golpich M, Amini E, Mohamed Z, Azman Ali R, Mohamed Ibrahim N, Ahmadiani A (2017) Mitochondrial dysfunction and biogenesis in neurodegenerative diseases: pathogenesis and treatment. CNS Neurosc Therap 23: 5–22.

Gomes KM, Inácio CG, Valvassori SS, Réus GZ, Boeck CR, Dal-Pizzol F, Quevedo J (2009) Superoxide production after acute and chronic treatment with methylphenidate in young and adult rats. Neurosci Lett 465: 95–98.

Haj-Mirzaian A, Ramezanzadeh K, Tafazolimoghadam A, Kazemi K, Nikbakhsh R, et al. (2019) Protective effect of minocycline on LPS-in-

duced mitochondrial dysfunction and decreased seizure threshold through nitric oxide pathway. Eur J Pharmacol 858: 172446.
Hiskens MI, Vella RK, Schneiders AG, Fenning AS (2021) Minocycline improves cognition and molecular measures of inflammation and neurodegeneration following repetitive mTBI. Brain Inj 35: 831–841.
Huang R, Liang S, Fang L, Wu M, Cheng H, Mi X, Ding Y (2018) Low-dose minocycline mediated neuroprotection on retinal ischemia-reperfusion injury of mice. Mol Vis 24: 367.
Jeffery KJ (2018) The hippocampus: from memory, to map, to memory map. Trends Neurosci 41: 64–66.
Jiang Y, Liu Y, Zhu C, Ma X, Ma L, Zhou L, et al. (2015) Minocycline enhances hippocampal memory, neuroplasticity and synapse-associated proteins in aged C57 BL/6 mice. Neurobiol Learn Mem 121: 20–29.
Kapur A (2020) Is methylphenidate beneficial and safe in pharmacological cognitive enhancement? CNS Drugs 34: 1045–1062.
Kepchia D, Huang L, Currais A, Liang Z, Fischer W, Maher P (2022) The Alzheimer's disease drug candidate J147 decreases blood plasma fatty acid levels via modulation of AMPK/ACC1 signaling in the liver. Biomed Pharmacother 147: 112648.
Khacho M, Harris R, Slack RS (2019) Mitochondria as central regulators of neural stem cell fate and cognitive function. Nature Rev Neurosci 20: 34–48.
Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010) Animal research: reporting in vivo experiments: the ARRIVE guidelines. British J Pharmacol 160: 1577–1579.
Kirby DM, Thorburn DR, Turnbull DM, Taylor RW (2007) Biochemical assays of respiratory chain complex activity. Methods Cell Biol 80: 93–119.
Kraus RL, Pasieczny R, Lariosa-Willingham K, Turner MS, Jiang A, Trauger JW (2005) Antioxidant properties of minocycline: neuroprotection in an oxidative stress assay and direct radical-scavenging activity. J Neurochem 94: 819–827.
Lanzillotta C, Di Domenico F, Perluigi M, Butterfield DA (2019) Targeting mitochondria in Alzheimer disease: rationale and perspectives. CNS Drugs 33: 957–969.
Lejri I, Agapouda A, Grimm A, Eckert A (2019) Mitochondria- and oxidative stress-targeting substances in cognitive decline-related disorders: from molecular mechanisms to clinical evidence. Oxid Med Cell Longev 2019: 9695412.
Lin F, Xie B, Cai F, Wu G (2012) Protective effect of Puerarin on β-amyloid-induced neurotoxicity in rat hippocampal neurons. Arzneimittelforschung 62: 187–193.
Lin MY, Sheng ZH (2015) Regulation of mitochondrial transport in neurons. Exp Cell Res 334: 35–44.
Lin S, Wei X, Xu Y, Yan C, Dodel R, Zhang Y, et al. (2003) Minocycline blocks 6-hydroxydopamine-induced neurotoxicity and free radical production in rat cerebellar granule neurons. Life Sci 72: 1635–1641.
Llorente-Folch I, Rueda C, Pardo B, Szabadkai G, Duchen M, Satrustegui J (2015) The regulation of neuronal mitochondrial metabolism by calcium. J Physiol 593: 3447–3462.
Loureiro-Vieira S, Costa VM, de Lourdes Bastos M, Carvalho F, Capela JP (2017) Methylphenidate effects in the young brain: friend or foe? Int J Dev Neurosci 60: 34–47.
Martins MR, Reinke A, Petronilho FC, Gomes KM, Dal-Pizzol F, Quevedo J (2006) Methylphenidate treatment induces oxidative stress in young rat brain. Brain Res 1078: 189–197.
Matsukawa, N, Yasuhara, T, Hara, K, Xu, L, Maki, M, Yu, G, et al. (2009) Therapeutic targets and limits of minocycline neuroprotection in experimental ischemic stroke. BMC Neurosci 10: 126.
McGrath J, Drummond G, McLachlan E, Kilkenny C, Wainwright C (2010) Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Brit J Pharmacol 160: 1573–1576.
Modesti M, Baccelloni S, Brizzolara S, Aleandri MP, Bellincontro A, Mencarelli F, Tonutti P (2019) Effects of treatments with ozonated water in the vineyard (cv Vermentino) on microbial population and fruit quality parameters. BIO Web Conf 13: 04011.
Monzio Compagnoni G, Di Fonzo A, Corti S, Comi GP, Bresolin N, Masliah E (2020) The role of mitochondria in neurodegenerative diseases: the lesson from Alzheimer's disease and Parkinson's disease. Mol Neurobiol 57: 2959–2980.
Motaghinejad M, Farokhi N, Motevalian M, Safari S (2020) Molecular, histological and behavioral evidences for neuroprotective effects of minocycline against nicotine-induced neurodegeneration and cognition impairment: Possible role of CREB-BDNF signaling pathway. Behav Brain Res 386: 112597.
Motaghinejad M, Fatima S, Karimian M, Ganji S (2016) Protective effects of forced exercise against nicotine-induced anxiety, depression and cognition impairment in rat. J Basic Clin Physiol Pharmacol 27: 19–27.
Motaghinejad M, Mashayekh R, Motevalian M, Safari S (2021) The possible role of CREB-BDNF signaling pathway in neuroprotective effects of minocycline against alcohol-induced neurodegeneration: molecular and behavioral evidences. Fundam Clin Pharmacol 35: 113–130.
Motaghinejad M, Motevalian M, Ebrahimzadeh A, Khajehamedi Z (2015) Reduction of methylphenidate induced anxiety, depression and cognition impairment by various doses of venlafaxine in rat. Int J Prev Med 6: 52.
Motaghinejad M, Motevalian M, Fatima S (2017) Mediatory role of NMDA, AMPA/kainate, GABAA and Alpha2 receptors in topiramate neuroprotective effects against methylphenidate induced neurotoxicity in rat. Life Sciences 179: 37–53.
Motaghinejad M, Motevalian M, Larijani SF, Khajehamedi Z (2015) Protective effects of forced exercise against methylphenidate-induced anxiety, depression and cognition impairment in rat. Adv Biomed Res 4: 134.
Motaghinejad, M, Motevalian, M, Shabab B (2017) Possible involvements of glutamate and adrenergic receptors on acute toxicity of methylphenidate in isolated hippocampus and cerebral cortex of adult rats. Fundam Clin Pharmacol 31: 208–225.
Mozafari H, Amiri S, Mehr SE, Momeny M, Amini-Khoei H, Bijani S, Hosseini MJ (2020) Minocycline attenuates depressive-like behaviors in mice treated with the low dose of intracerebroventricular streptozotocin; the role of mitochondrial function and neuroinflammation. Mol Biol Rep 47: 6143–6153.
Naderi Y, Panah, Y, Barreto GE, Sahebkar A (2020) Neuroprotective effects of minocycline on focal cerebral ischemia injury: a systematic review. Neural Regen Res 15: 773–782.
Naderi Y, Sabetkasaei M, Parvardeh S, Zanjani TM (2017) Neuroprotective effect of minocycline on cognitive impairments induced by transient cerebral ischemia/reperfusion through its anti- inflammatory and anti-oxidant properties in male rat. Brain Res Bull 131: 207–213.
Norat P, Soldozy S, Sokolowski JD, Gorick CM, Kumar JS, Chae Y, et al. (2020) Mitochondrial dysfunction in neurological disorders: Exploring mitochondrial transplantation. NPJ Regen Med 5: 22.
Ozcan A, Ogun M (2015) Biochemistry of reactive oxygen and nitrogen species. In: Basic Principles and Clinical Significance of Oxidative Stress. doi: 10.5772/61193.
Parvardeh S, Sheikholeslami MA, Ghafghazi S, Pouriran R, Mortazavi SE (2022) Minocycline improves memory by enhancing hippocampal synaptic plasticity and restoring antioxidant enzyme activity in a rat model of cerebral ischemia-reperfusion. Basic Clin Neurosci 13: 225–236.
Pinzon A, Marcillo A, Quintana A, Stamler S, Bunge MB, Bramlett HM, Dietrich WD (2008) A re-assessment of minocycline as a neuroprotective agent in a rat spinal cord contusion model. Brain Res 1243: 146–151.
Raoofi A, Aliaghaei A, Abdollahifar MA, Boroujeni ME, Javadinia SS, Atabati H, Abouhamzeh B (2020) Long-term administration of high-dose methylphenidate-induced cerebellar morphology and function damage in adult rats. J Chem Neuroanat 103: 101712.
Réus GZ, Scaini G, Furlanetto CB, Morais MO, Jeremias IC, Mello-Santos LM, et al. (2013) Methylphenidate treatment leads to abnormalities on krebs cycle enzymes in the brain of young and adult rats. Neurotoxicity Res 24: 251–257.

Romero-Miguel D, Lamanna-Rama N, Casquero-Veiga M, Gómez-Rangel V, Desco M, Soto-Montenegro ML (2021) Minocycline in neurodegenerative and psychiatric diseases: an update. Eur J Neurol 28: 1056–1081.

Sakamuru S, Attene-Ramos MS, Xia M (2016) Mitochondrial membrane potential assay. In High-throughput screening assays in toxicology (pp. 17–22): Springer.

Salehi P, Shahmirzadi ZY, Mirrezaei FS, Boushehri FS, Mayahi F, Songhori M, et al. (2019) A hypothetic role of minocycline as a neuroprotective agent against methylphenidate- induced neuronal mitochondrial dysfunction and tau protein hyper-phosphorylation: possible role of PI3/Akt/GSK3β signaling pathway. Med Hypotheses 128: 6–10.

Salvi A, Liu H, Salim S (2020) Involvement of oxidative stress and mitochondrial mechanisms in air pollution-related neurobiological impairments. Neurobiol Stress 12: 100205.

Schlichting ML, Preston AR (2017) The hippocampus and memory integration: building knowledge to navigate future decisions. In: The hippocampus from cells to systems. Springer, pp. 405–437.

Schmitz F, Pierozan P, Biasibetti-Brendler H, Ferreira FS, dos Santos Petry F, Trindade VMT, et al. (2018) Methylphenidate disrupts cytoskeletal homeostasis and reduces membrane-associated lipid content in juvenile rat hippocampus. Metab Brain Dis 33: 693–704.

Schmitz F, Pierozan P, Rodrigues AF, Biasibetti H, Grings M, Zanotto B, et al. (2017a) Methylphenidate decreases ATP levels and impairs glutamate uptake and Na+, K+-ATPase activity in juvenile rat hippocampus. Mol Neurobiol 54: 7796–7807.

Schmitz F, Pierozan P, Rodrigues AF, Biasibetti H, Grunevald M, Pettenuzzo LF, et al. (2017b) Methylphenidate causes behavioral impairments and neuron and astrocyte loss in the hippocampus of juvenile rats. Mol Neurobiol 54: 4201–4216.

Schmitz F, Scherer EB. d. S, Da Cunha M J, Da Cunha AA, Lima DD, Delwing D, et al. (2012) Chronic methylphenidate administration alters antioxidant defenses and butyrylcholinesterase activity in blood of juvenile rats. Mol Cell Biochem 361: 281–288.

Shellenberg TP, Stoops WW, Lile JA, Rush CR (2020) An update on the clinical pharmacology of methylphenidate: therapeutic efficacy, abuse potential and future considerations. Expert Rev Clin Pharmacol 13: 825–833.

Shultz RB, Zhong Y (2017) Minocycline targets multiple secondary injury mechanisms in traumatic spinal cord injury. Neural Regener Res 12: 702.

Simon DW, Aneja RK, Alexander H, Bell MJ, Bayır H, Kochanek PM, Clark RS (2018) Minocycline attenuates high mobility group box 1 translocation, microglial activation, and thalamic neurodegeneration after traumatic brain injury in post-natal day 17 rats. J Neurotrauma 35: 130–138.

Verghese C, Abdijadid S (2022) Methylphenidate. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-.

Vorhees CV, Williams MT (2006) Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nature Protoc 1: 848–858.

Wang WX, Visavadiya NP, Pandya JD, Nelson PT, Sullivan PG, Springer JE (2015) Mitochondria-associated microRNAs in rat hippocampus following traumatic brain injury. Exp Neurol 265: 84–93.

Wang X, Sharma RK, Gupta A, George V, Thomas Jr AJ, Falcone T, Agarwal A (2003) Alterations in mitochondria membrane potential and oxidative stress in infertile men: a prospective observational study. Fertil Steril 80: 844–850.

Wenk GL (2004) Assessment of spatial memory using the radial arm maze and Morris water maze. Curr Protoc Neurosci 26: 8.5 A.1–8.5 A.12.

Yilmaz I, Karaarslan N, Ozbek H (2021) Practical performance of hippocampal tissue resection in rats in pharmacomolecular research. Turk Neurosurg 31: 112–118.

Zhao Y, Xiao M, He W, Cai Z (2015) Minocycline upregulates cyclic AMP response element binding protein and brain-derived neurotrophic factor in the hippocampus of cerebral ischemia rats and improves behavioral deficits. Neuropsychiatr Dis Treat 11: 507–516.