The Protective Effect of N-Acetylcysteine on MK-801 Induced Neurodegeneration in Mice

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Abstract

The neurological disorders not only a decline in the quality of life for patients but also causes a global economic burden. Therefore protective medicine becomes more important for the society. MK-801 is one of the chemical agents used to understand the etiology of behavioral disorders and brain degeneration in animal models. This study aims to determine whether N-acetylcysteine (NAC) is useful to treat brain degeneration caused by MK-801, an N-methyl-D-aspartate glutamate receptor antagonist. For this, 4 groups were formed by dividing 24 male BALB/c mice into groups of six. The control group was given a saline solution (10 ml/kg-i.p.). While MK-801 (1 mg/kg-i.p.) was given both alone and with NAC (100 mg/kg-i.p.), the last group was given only NAC (100 mg/kg-i.p.). The drug administrations lasted for fourteen days. After the behavior tests (open field and elevated plus-maze) all animals were sacrificed and, brain tissues were collected for Real-time PCR, TAS-TOS analysis, and Hematoxylene-Eosine, Kluver-Barrera, and the TUNEL stains. In the MK-801 group, besides the neuron nuclear shrinkage, glial cell infiltration, vacuolization in cortical neurons, white matter damage, and apoptosis were observed. In the mice given NAC as a protector, it was observed that behavioral problems improved, antioxidant levels increased, and nuclear shrinkage, glial cell infiltration, vacuolization in the neurons and, white matter degeneration were prevented, MBP expression increased and TUNEL-positive cells significantly decreased. As a result, it has been observed that NAC may have a protective effect against brain degeneration.

Introduction

Neurodegenerative disorders are characterized by abnormal behaviors and nervous system impairments (Sharma et al. 2021). These disorders negatively affect the patient's quality of life and society. However, nervous system disorders are a general problem over the world. Protection from disorders has become more important recently because of the healing process increasing economic burden (Knapp ve Wong, 2020; Arya et al. 2021). Therefore several animal models were developed and applied to understand the etiology of these disorders for preventive medicine (Yu et al. 2011; Xiu et al. 2014; 2015; Bin-Jumah et al. 2021). One of these animal models is achieved by the MK-801 chemical agent (Yu et al. 2011; Xiu et al. 2014; Kruk-Slomka et al. 2016; Akosman et al., 2021). Previous studies have successfully shown that applying an NMDA receptor antagonist such as MK-801 revealed behavioral impairments and neurobiological degenerations in the rodent brain (Yu et al. 2011; Xiu et al. 2014; 2015; Kruk-Slomka et al. 2016; Akosman et al. 2021). Yu et al. (2011) reported that MK-801 revealed various behavioral impairments in mice such as biting, forced climbing, falling, inability to respond to light and sound stimulus and reduction in willingness to live with other animals and search for food. Besides these, in addition to behavior impairments, MK-801 applications may lead to neurodegeneration such as glial cell infiltration, vacuolization and even apoptosis (Zhang et al. 1996; Horvàth et al. 1997; Kovacic and Somanathan 2010; Xiu et al. 2014; 2015). Moreover, previous studies have reported that MK-801 caused reduction in the white matter volume of the brain, reduction in the length of myelinated nerve fibers, disintegration in the myelin layer, demyelination, shrinkage in the diameters of nerve fibers and decrease

the MBP production in mice (Xiu et al. 2014; 2015). The myelin basic protein (MBP), which is the second most frequently found protein in the central nervous system, constitutes 30% of the total protein and 10% of the dry weight of the myelin layer. Problems experienced in the white matter may lead to transmission dysfunctions in axons, and afterwards, result in several problems from psychiatric diseases and weakness in the motor system (Boggs 2006; Matute and Ransom 2012). Previous studies have reported that MK-801 applied for 14 days reduced MBP mRNA release at the end of this duration (Xiu et al. 2014; 2015). In mouse brains, MK-801 reduces the expression of the MBP gene that is associated with myelin sheath production.

In these kinds of model-based studies, demyelination level and apoptotic evaluation have essential importance. Kluver-Barrera dye is used to show the demyelination status of the brain (Xiang et al. 2005; Kipp et al. 2011; Abdel-Aziz et al. 2021). In cuprizone model demyelination studies, Kluver dye was used to evaluate the protective efficacy of various antioxidants (Sun et al. 2005; Ye et al. 2013; Abdel-Aziz et al. 2021). The previous studies have used both Kluver-Barrera and Tunel methods to evaluate brain degeneration (Lee et al. 2009; Hesse et al. 2010). However, performing brain degeneration with MK-801 is a fairly new technique, and therefore, the present study has a special importance since no study using Kluver and Tunel methods has been found in the literature.

Short-term or long-term high-dose applications of MK-801 lead to damage in the brain tissue (Sharp et al. 1991; Xiu et al. 2014; 2015). Studies have provided findings that MK-801 leads to oxidative stress by increasing reactive oxygen species in the brain (Ozyurt et al. 2007a; 2007b; Ozyurt et al. 2014). As glutamate released into the environment cannot bind to the NMDA receptor that is subjected to hypofunction by MK-801, excessive amounts of glutamate start to accumulate in the intercellular space, and the glutamatergic system balance that is disrupted based on this leads to formation of oxidative stress and injuries in nerves (Ozyurt et al. 2007a; Genius et al. 2013). Previous studies have determined that MK-801 increases oxidant substance levels in rats (Ozyurt et al. 2007a; 2007b; Ozyurt et al. 2014).

N-acetylcysteine (NAC) is a substance with strong antioxidant and anti-inflammatory properties (Scalley and Conner 1978; Dean et al. 2011). It has been used as an antioxidant in paracetamol poisoning for more than three decades. As years have passed, and the effect mechanisms of NAC have been revealed, there has been an increase in the number of trials conducted on it. NAC, which is a mucolytic, is being used in various pulmonary and renal diseases recently (Dean et al. 2011). As it easily passes through the blood-brain barrier, and successful results were obtained after its trial in Alzheimer patients as an alternative treatment method, its usage areas in psychiatry are increasingly broader today (Adair et al. 2001; Farr et al. 2003; Dean et al. 2004; Dean et al. 2011).

Reactive oxygen species that are formed during oxidative stress lead to the start of a process that may result in death by causing oxidation of the DNA, proteins and lipids in the cell. As an antioxidant, N-acetylcysteine neutralizes free radicals before they damage the cells. It does this by increasing the cysteine-glutathione ratios. It allows renewal of antioxidants like glutathione that have been degraded by free radicals in the cells (Tardiolo et al. 2018). This is because NAC is the preliminary substance of

glutathione production (Turkmen et al. 2019). This way, it increases the endogenous antioxidant defense mechanism and destroys free radicals. It was reported that such activities are also seen in the onset of neurodegenerative diseases (Tardiolo et al. 2018). In schizophrenia, against disruptions in the signal pathways, NAC strengthens NMDA receptor responses against glutamate (Himi et al. 2003; Janáky et al. 2007). NAC may be beneficial in treatment of schizophrenia by targeting to fix both oxidative stress and glutamatergic dysfunction (Carlsson 2006). NAC was tried in the clinic on schizophrenia patients, and it was reported that it had beneficial effects on the negative symptoms of schizophrenia and some other symptoms (Berk et al. 2008). While a noticeable rate of improvement was recorded in symptoms, it was reported that plasma glutathione levels increased after NAC applications (Lavoie et al. 2008). Other studies have also reported that NAC application showed beneficial outcomes in schizophrenia symptoms (Bulut et al. 2009). In toxicological studies conducted on the nervous system, it has been shown that NAC has positive, improving and protective effects against demyelination (Mirzakhani et al. 2016; Hichor et al. 2018; Zaki et al. 2018). Besides, so far, no study has been encountered to examine the effects of NAC on neurotoxicity associated with MK-801. In many experimental studies, it has been shown numerous times that NAC has neuroprotective effects against various neurotoxic agents. Furthermore, there is no study examining the effects of NAC on MK-801 induced degeneration in the brain.

The purpose of this study is to investigate the neuroprotective effects of NAC on degenerative changes in brain tissue and protective effects on behavioral impairments assessed in a mouse model occurred by MK-801 application.

Material And Method

Drugs, chemicals and kits

NAC (600 mg/20 tablet) was purchased from Basel Drug Co. (Istanbul, Turkey). MK-801 (CAS Number 77086-22-7; molecular weight 221.30 g/mol; purity ≥ 99%, a selective NMDA receptor antagonist) and all other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Total oxidant capacity (TOS) and Total antioxidant capacity (TAS) kits were purchased from Rel Assay Diagnostics (Gaziantep, Turkey). All chemicals that were used in this study were of analytical grade.

Animals

Adult (8-10-week-old) BALB/c mice (n=24) weighing 30-35 g were acquired from the Experimental Animals Research Center of Afyon Kocatepe University. The animals were housed in a light-controlled room (12 hours of light/dark, light starting at 8:00 am) at 22±1°C and 45±15% humidity with standard commercial pellet feed and free access to tap water. The permission necessary for conducting experiments on the mice was received from the Animal Experiments Local Ethics Board of Afyon Kocatepe University (AKUHADYEK-189-17), and ethical directives were adhered to throughout the experiment.

Groups and Drug Applications

After a week of adaptation, the mice were divided into four equal groups (n=6 in each group): Control, NAC, MK-801 and NAC+MK-801. To the control group, a saline solution was administered at a volume of 10 ml/kg every day in the afternoon. MK-801 (a selective NMDA receptor antagonist) was administered at a dose of 1 mg/kg every day in the afternoon, while NAC was administered at a dose of 100 mg/kg every day in the morning (Because of the half life time of the NAC). The drug applications lasted for 14 consecutive days. The dose of NAC was determined according to the description of Fukami et al. (2004), and the dose of MK-801 was determined according to the method of Xiu et al. (2014; 2015). All drugs (Sigma, USA) were dissolved in saline, the solutions were freshly prepared every day, and they were applied intraperitoneally (i.p.). On the fifteenth day, the animals were subjected to the open field test (OFT) and elevated plus-maze test (EPM).

Open Field Test

Hyperactivity was calculated in a 60 x 60 x 24 cm stainless galvanized sheet metal o.f.t. area that was separated into 36 equal squares. The squares that were nearest to the wall were labeled "peripheral," while the rest were labeled "central." The mouse was positioned in the center of the arena for o.f.t., and a video camera manually monitored the number of squares crossed by the four paws (hyperactivity) for 5 minutes (Taksande et al., 2009). After each animal was subjected to the exam, the arena was washed with 70% alcohol to remove the odor.

The Elevated Plus-Maze Test

The e.p.m. test measured exploratory hyperactivity as well as anxiety-like behaviors. A platform made of stainless galvanized sheet metal with two open arms (30 cm x 5 cm) and two enclosed arms of the same size with 15-cm-high walls was used for the EPM test. Both of the armies converged in the middle of the arena (5 cm x 5 cm). Each animal was placed in the middle of the plus maze, facing the enclosed arm, and monitored by a video camera for 5 minutes. The maze apparatus was washed with 70% alcohol after each trial. It was recorded how much time the animals spent in enclosed arms. If the animal's four legs and body moved to that zone, it was determined whether it was in the open arm, enclosed arm, or center. Mice prefer safe enclosed arms to open arms that cause anxiety (Akillioglu et al. 2012).

Sacrifice and Examination of Brain Tissue

All animals were sacrificed on the 16th day by cervical dislocation. The skulls of the animals were opened, and their brains were removed. The brains were weighed and divided into two pieces from the middle. One half of the brains were allocated for stereological-pathological examination and measurements, while the other half were allocated for TAS-TOS measurements and real-time PCR analyses.

The brain hemispheres allocated for stereological examination were consecutively sliced in compliance with the systematic uniform random sampling method (Gundersen et al., 1988) at thicknesses of 40 microns with a microtome from the polus rostralis towards the caudal. While thick cross-sections were

taken for pathological measurements, 5-micron samples were taken from the cross-sections in-between until reaching a thickness of 40 microns, and pathological assessments were made on these cross-sections. On the thick cross-sections stained by Hematoxylene-eosine, the diameters of the nuclei of cells in the hippocampus CA1 (cornu ammonis 1) region were measured with the help of the M-Shot software (x100). The measurements were made on the cells in the central zone on the thick cross-sections with a safe height interval. The safe height interval was determined as the 10-micron zone in the central zone of the cross-section after the cross-section thickness was measured with the help of a microcator. The pathological observations performed on the thin Hematoxylene-eosin stained sections for the glial cell infiltration and vacuolization were scored as follows: Observed changes were graded as normal (grade -), to severe (grade ++).in a blinded manner.

Preparation of brain tissue homogenate

The brain tissues were separately weighed after washing with a 0.9% NaCl solution and transferred into thick-walled glass tubes. Afterwards, to dilute the brain tissues, cold phosphate buffer (pH 7.4, 50 mM) was added onto them by ten times their weight, and they were homogenized in a flask full of ice for 10 s at the first speed setting (IKA Ultra Turrax-T18, Germany). The homogenization process was ended after observing that the tissues were homogenously disintegrated inside the tube at 10-s intervals. The obtained homogenates were then centrifuged at 2795 g for 10 min (Nuve NF 1000R, Ankara, Turkey). All procedures were carried out at 4°C. The brain samples were kept at -80°C before the oxidative stress parameters were assessed.

TAS-TOS measurement in brain tissue homogenate

The TOS of the tissues was measured based on the method described by Erel (2005) by using a total oxidant level kit (Rel Assay Diagnostics, Gaziantep, Turkey). The oxidants in the sample convert ferrous ion chelator complexes into ferric ions. Ferric ions form a colored complex with the chromogenic solution. The absorbance of this complex was measured at 530 nm with an ELISA reader set at 25°C to determine the TOS levels, and it was directly proportional to the oxidant amount in the sample. The results are presented as μmol H2O2 equiv/L.

The TAS of the tissues was measured based on the method described by Erel (2004) by using a TAS Rel Assay brand kit (Rel Assay Diagnostics, Gaziantep, Turkey). The measurement is made based on the discoloration of antioxidant molecules. According to the instructions of the kit, 500 μL of reagent 1 (measurement buffer) and 30 μL supernatant were combined, and the absorbance was measured at 660 nm by an ELISA kit set at 25°C to determine the TAS levels. After this, 75 μL of reagent 2 (colored ABTS solution) was added to the mixture, and the product was incubated for 10 min. The TAS levels were determined by reading the absorbance at 660 nm after incubation. Trolox was used as a calibrator, and the results are presented as mmol Trolox equiv/L.

Real-Time PCR

Total RNA was extracted from the brain tissue according to the instructions of the manufacturing firm using a kit with an RNA isolation capacity (GeneAll Hybrid R, RiboEx-Seoul/Korea). For this aim, a 100 mg of tissue was homogenized by dividing into small pieces with a lancet, and it was vortexed by adding 1 mL RiboEx onto it. The kit that was used for the cDNA synthesis was a HyperScript™ First strand Synthesis, GeneAll Hybrid R (Seoul/Korea). For preparing a master mix, the RNA sample, primer, dNTP mix and RNase-free distilled water were used. After the cDNA master mix was prepared, it was incubated for 5 min at 65°C. After incubation, the mixture was put onto ice. After this, onto the cDNA master mix, the 10X RTase Reaction Buffer, 0.1 M DTT, Reverse Transcriptase and RNase Inhibitor components were added, and a homogenous mixture was ensured. After preparing the master mix for cDNA synthesis, the next step was the reverse transcription reaction. The reverse transcription reaction was carried out in two steps. By leaving for 60 min at 55°C in the first step and for 5 min at 85°C in the second step, the cDNA synthesis was completed. The cDNA samples were frozen at -80°C. In the real-time PCR, for MBP amplification, the forward primer TGGAGAGATTCACCGAGGAGAGGC and reverse primer TGAAGCTCGTCGGACTCTGAGGGC primer sequences and a kit (RealAmpTM SYBR qPCR Master mix) were used. As the master mix components used for the real-time qPCR, 2X MasterMix (with SYBR-Green), ROX Dye, Forward Primer (10 µM), Reverse Primer (10 µM), cDNA Template and RNase-free distilled water were used. The real-time qPCR reaction was carried out in an Applied Biosystems™ 7500 Fast Real-Time PCR device. The PCR program was started with 1 cycle for 300 s at 95°C as the initial denaturation. This was followed by 40 cycles at 95°C for 15 s and 55-68°C for 60 s, and the program was ended with a melting curve analysis.

Kluver-barrera staining

The Kluver-barrera stain is fundamentally used to show the nervous system's myelinated parts. Thus, the myelin sheath is stained blue, and the observer easily examines demyelination. The thin sections crossed to the corpus callosum were collected and passed through the xylene and graded ethanol series (100 and 95%), then soaked in Luxol fast blue solution at 60℃ for 1 hour, and the dye was removed (except corpus callosum) by lithium carbonate solution, distilled water, and 70% ethanol. The sections was soaked in Eosin Y solution for 40 seconds, distilled water, 0.1% Cresyl violet (Sigma) solution for 1 minute, and then in 95% ethanol followed by 100% ethanol and xylene for dehydration and transparency. After mounting, corpus callosum was observed using an optical microscope. The staining density of the corpus callosum was scored as normal (grade 0), to disappearance (grade 3).

Immunuhistochemistry

The terminal deoxynucleotidyl transferase biotindUTP nick end labelling staining (TUNEL) method

TUNEL assay was performed according to manufacturer’s instructions (Abcam ab206386). The 5-µm-thick brain sections were collected and evaluated using TUNEL-positive cells. The tissues were deparaffinised at 60°C overnight and soaked in xylene, graded ethanol (100, 96 ve 80) and PBS solution. The sections were treated with deoxyribonuclease-free proteinase K at room temperature for 20 min and then washed with PBS three times (5 min for each). After being fixed at room temperature for 15 min, the

samples were washed with PBS for another three times (5 min each time). Next, each sample was added with %70 Reaction Buffer + %30 TdT Enzyme and incubated for 1.5 h at 37°C, followed by processing with Anti-Digoxigenin-Peroxidase solution for 30 min. and PBS washing three times. The samples treated DAB dilution buffer+DAB substrate solution for 10 min. After three times PBS washing the methylene green stain was performed and samples were passed through graded ethanol and xylene. Then, the samples were sealed and the apoptotic cells were observed using a light microscope. The dark brown stained cells were evaluated as Tunel-positive cells.

Statistical Analysis

The data obtained in the study were analyzed by using the SPSS 21.0 for Windows package software. In the statistical analysis, firstly a normality test was applied to see whether or not there was a normal distribution among the groups. The parametric test of analysis of variance (ANOVA) was used in the comparison of the data confirmed to be normally distributed based on the groups. Duncan's test was applied in the pairwise comparisons of the groups. The level of statistical significance for the difference between groups was taken as 0.05.

Results

Effects of NAC on schizophrenia-like behaviors induced by MK-801 applications

Half an hour after the injection of MK-801 to the mice, all the mice displayed hyperactivity, falling, trying to climb forcedly and incoordination impairments. In the group given NAC against MK-801, the impairments such as falling, trying to climb forcedly, and incoordination decreased except hyperactivity. In the o.f. test, the distance traveled (squares counted) (Fig. 1A) and the time spent in the central zone (seconds) (Fig. 1B) were examined to detect hyperactivity. Besides, time spent in the enclosed arms (sec) (Fig. 2) was examined to detect behavioral problems in the e.p.m. test. In these tests conducted the next day after the last drug application, there was no statistically significant difference between the groups ($p > 0.05$).

Figure 1

Figure 2

Effects of MK-801 and NAC on brain weight

After sacrification on the 16th day, the brains of the mice were taken out and weighed. It was observed that the mean brain weight of the group that was given only MK-801 decreased in comparison to the other groups. However, this decrease was not statistically significant (Table 1; $p > 0.05$).

Table 1
Brain weight of mice

Measurements	Control	MK-801	NAC + MK-801	NAC	P value
Brain weight (gr)	0.45 ± 0.02	0.43 ± 0.01	0.44 ± 0.03	0.44 ± 0.02	0.07

Effects of NAC on oxidative stress parameters

When the groups were compared in terms of their total oxidant statuses, it was observed that the TOS levels increased only in the MK-801 group in comparison to the other groups, but this increase was not statistically significant (Fig. 3A; $p > 0.05$).

When the groups were compared in terms of their total antioxidant statuses, it was observed that the TAS levels decreased in the MK-801 group significantly in comparison to the NAC group ($p < 0.05$), while this decrease was not statistically significant in comparison to the control and NAC + MK-801 groups (Fig. 3B; $p > 0.05$).

Figure 3

Histopathological Findings

The histopathological changes in the brains of the animals in the experiment groups are shown in Fig. 4. When the cross-sections stained with hematoxylin-eosin were examined under a light microscope, it was observed that the brains of the control group mice showed a familiar general histological structure (Fig. 4A). In the brain cortices of the mice that were given only MK-801, vacuole formations were noticeable (Fig. 4C). The vacuole formation in the brains of the mice given NAC for a protective purpose was lower in comparison to the MK-801 group (Fig. 4D; Table 2–3). In addition to this structural disorder noticed in the brain cortex, there was also intense glial cell infiltration in the brains of the mice given MK-801 (Fig. 4C; Table 2–3), while the infiltration in the group given NAC for protective purposes was close to that in the control group (Fig. 4A, D; Table 2–3).

Table 2
Vacuolization in neurons, focal glial cell infiltrations, nucleus diameter measurements (μm) and myeline degeneration ratio according to results of histopathological assessments

Measurements	Control	NAC	MK-801	NAC + MK-801	*P* value
Vacuolization in neurons	1.16 ± 0.40[b]	1.16 ± 0.40[b]	2.16 ± 0.98[a]	1.50 ± 0.54[ab]	0.040
Focal glial cell infiltrations	1.00 ± 0.00[b]	1.00 ± 0.00[b]	2.50 ± 0.83[a]	1.33 ± 0.51[b]	0.000
Hippocampus neuron nucleus shrinkage	18.7 ± 0.80[a]	18.2 ± 1.20[a]	16.7 ± 0.50[b]	19.2 ± 1.3[a]	0.000
Myelin degeneration ratio	0.0 ± 0.00[c]	0.0 ± 0.00[c]	2.2 ± 0.75[a]	1.3 ± 0.63[ab]	0.000
Tunel positive cells	0.1 ± 041[c]	0.1 ± 0.35[c]	2.6 ± 0.51[a]	1.8 ± 0.75[ab]	0.000

[a,b] In the same column, values with different letters show statistically significant differences in vacuolization and focal glial cell infiltration in neurons among the different groups ($p < 0.05$). n = 6 per group (data expressed as mean ± SD). Abbreviations: NAC, *N*-acetylcysteine

Table 3
Effects of repeated MK-801 (1mg/kg), *N*-acetylcysteine (NAC-100 mg/kg), and NAC (100 mg/kg) + MK-801 (1 mg/kg) on histopathological changes in the brain of mice.

Tissue	Histopathological changes	Control	NAC	MK-801	NAC + MK-801
Brain	Vacuolization in neurons	− (5/6) +(1/6)	− (5/6) +(1/6)	− (2/6) +(1/6) ++(3/6)	− (3/6) +(3/6)
	Focal glial cell infiltration in neurons	− (6/6)	− (6/6)	− (1/6) +(1/6) ++(4/6)	− (4/6) +(2/6)

The findings were evaluated in a blinded manner and scored as follows: normal (grade -), to severe (grade ++).

Figure 4

Hematoxyline-eosin staining of neurons in the hippocampal CA1 region of mice indicated extensive damage with a large number of dark-stained shrunken neurons and shrunken cell nuclei in the MK-801 group (Fig. 5C; Table 2). In contrast, the NAC + MK-801 group represented significantly reduced

degenerative neurons. The neurons in this group were in the normal posture with well-outlined nuclei (Fig. 5D; Table 2).

Figure 5

Examination of the demyelination

1. Kluver-Barrera stain

In Kluver-Barrera stained sections, corpus callosum stained entirely blue in the control group (Fig. 6A). However in MK-801 group, no to pale staining was observed (Fig. 6C). In NAC + MK-801 group staining level placed between the MK-801 and control groups ($p < 0.05$; Fig. 6D; Table 2).

Figure 6

2. Mbp Expression Findings

Figure 7 presents the data on the mRNA expression of MBP in the structure of the myelin sheath of the groups. It was observed that the MBP mRNA expression levels in the NAC + MK-801 group increased in comparison to the MK-801 group, and the NAC application increased the MBP mRNA expression (Fig. 7.; $p < 0.01$).

Figure 7

Observation of TUNEL-positive cells

The TUNEL-positive cells were detected in the MK-801 samples (Fig. 8C). However, TUNEL-positive cells were statistically decreased in the samples of NAC + MK-801 (Fig. 8D; Table 2; $p < 0.05$).

Figure 8

Discussion

This study aimed to investigate the protective effect of NAC against behavioral impairments and degeneration that arise in the brain after MK-801 application in mice. While it was found that MK-801 application in the mice led to falling, trying to climb forcedly, hyperactivity, incoordination and unwillingness to food, it was observed that it also led to vacuole formation, glial cell infiltration in the brain, shrinkage in the hippocampal neuron nuclei and reduction in the MBP release, demyelination and apoptosis. After administering NAC for protective purposes against MK-801, although the hyperactivity continued in the mice, they started to walk more regularly, had reduced behaviors like falling and trying to climb forcedly, had lower glial cell infiltration rates, and shrinkage in their hippocampus cell nuclei were prevented and demyelination with apoptosis were significantly decreased. However, on the next day the symptoms were disappeared.

The cause of difficulty in motor activities started to be researched in animal models, and it was seen that this situation especially arose as a result of disruptions in cell function in oligodendrocytes in the white matter and myelination problems (Walther and Strik 2012). Degeneration of the myelin layer may suggest not only schizophrenia but also multiple sclerosis (MS), which is another disease related to problems in myelin sheath production (Karussis 2014). The myelin sheath is subjected to degeneration also in this disease, and as a result, problems in motor activities such as difficulty in walking are encountered. The layer in the brain that is rich in myelin sheaths is the white matter region. Degeneration of the myelin layer found in the white matter results weakness in the motor system in patients after a certain time. It was observed in previous studies that MK-801 leads to volumetric shrinkage in the white matter of the brain, and it was found that this shrinkage was caused by degeneration in the neurons and myelin sheaths found in this region (Xiu et al. 2014; 2015). Similarly, in the present study decreasing MBP levels may cause the demyelination noticed by the Kluver-Barrera stain. However, protective aimed NAC administered group appeared better results.

In the studies of Ye et al. (2013), Abdel-Aziz et al. (2021), and Sun et al. (2021), neuroprotective effects of Progesterone, Telmisartan (antihypertensive drug), and Catalpol (main active ingredient of the Rehmannia glutinosa) were revealed on the cuprizone model of demyelination. In these studies, Kluver-Barrera dye was also used while determining the degree of demyelination. In addition to determining the degree of demyelination, these studies also examined the apoptosis. In the present study, similar to the studies mentioned above, the degree of demyelination and apoptosis were investigated with the same methods and the protective role of the NAC was detected.

In the study by Jatana et al. (2006), where the protective activity of NAC on the brain was assessed, the authors observed the protective effect of hypothermia and subsequently applied NAC in the hypoxic brain. While a highly substantial loss was observed in the brain volumes of the hypoxic animals when they were examined 2 and 4 weeks later, the brain volume values of the rats that were applied hypothermia + NAC were found very close to those in the sham group that received no intervention. Likewise, when the brains of the rats in the group that was applied hypothermia + NAC were examined, an increase was determined in myelin sheath production and MBP release. Previous studies have reported that MK-801 applied for 14 days reduced MBP mRNA release at the end of this duration (Xiu et al. 2014; 2015). In mouse brains, MK-801 reduces the expression of the MBP gene that is associated with myelin sheath production. In this study, it was similarly observed that the reduced MBP mRNA release was increased by administering NAC.

In previous studies MK-801-induced neurotoxicity decreased, and locomotor problems disappeared by using various antioxidants such as CAPE (caffeic acid phenethyl ester), omega 3 fatty acid and melatonin (Ozyurt et al. 2007a; 2007b; Ozyurt et al. 2014). In this study, although it was shown that MK-801 applications increased the TOS levels and reduced the TAS levels, it was demonstrated that these differences were not significant, and the combined applications of NAC with MK-801 did not create a significant change in the TOS and TAS levels. These results may have been caused by the animals being sacrificed two days after the last injections. However, Ozyurt et al. (2007a) remarked that CAPE decreased

the apoptotic cells appeared with MK-801 administration. Except Ozyurt et al. (2007a), Zhang et al. (1996) also pointed out MK-801 dependent apoptosis in the brain and protective effect of the Cycloheximide. Takadera et al. (1999) also mentioned the Insulin-Like Growth Factor I protected the neuronal cell death occurred with the MK-801 administration. Similarly to the studies above, in the present study the protective effect of the NAC also observed on the neuronal death depended to the MK-801 administration.

In this study, when the diameters of the cell nuclei in the hippocampus CA1 region of the brain were estimated, a statistically significant reduction was determined in the diameters of the nuclei of the mice given MK-801. Previous study similarly measured the shrinkage level of the nuclei's in the same area to estimate the protective feature of resveratrol (Akosman et al. 2021). According to Bonde et al. (2002) the shrinkage in the neuron nuclei may be an indicator of the presence of neurodegeneration. It is also known that MK-801 applications significantly reduce NMDA receptor release in the hippocampus (Kim et al. 2014). In both previous studies and this study, when the brains of the mice in the groups given MK-801 were examined under light microscopes, vacuole formations and glial cell infiltrations as neurodegeneration indicators were also noticed (Olney et al. 1989; Sharp et al. 1991). However, protective aimed NAC administration improved the nuclear shrinkage, vacuole formation and glial cell infiltration, significantly.

In recent years, various studies have revealed that NAC has a protective effect against neurotoxicity and myelin degeneration (Mirzakhani et al. 2016; Zaki et al. 2018). In the study by Zaki et al. (2018), where NAC was applied for protective purposes against cisplatin toxicity, it was reported that the myelin sheath on the sciatic nerve was affected by cisplatin toxicity, this led to folding, dissolution and degeneration in the myelin sheath, whereas NAC had a protective effect in the NAC-given rats, and in these rats, the structure of the myelin sheath was close to that in the control group. In another study, where Mirzakhani et al. (2016) investigated the protective activity of NAC, it was stated that NAC applied on rats whose sciatic nerves were damaged by compression increased the recovery in the sciatic nerves and reduced the negative changes in the myelin sheath. Abd-Ellah et al. (2018) investigated the protective activity of the NAC and folic acid combination against aspartame neurotoxicity and reported that NAC had a protective effect against degeneration in the myelin layer. The researchers stated that NAC could be used for protective purposes against neurotoxicity. Haber et al. (2018) applied NAC combined with minocycline plus for protection against trauma-related damage induced on the brain and determined that NAC protected the myelin layer and stimulated remyelination. In a study by Hichor et al. (2018) examining the protective role of NAC on mice against sciatic nerve damage, the researchers detected damage in the myelin layer on the sciatic nerve, and it was observed that NAC application noticeably reduced this damage. NAC was also successful in bringing the myelin protein values within normal limits. Furthermore, the movement problems of the animals given NAC disappeared, and their values were normalized.

Conclusion

In this study, it was observed that NAC that was used for protective purposes against schizophrenia symptoms induced with MK-801 protected the brain and neurons against degeneration, repaired locomotor activity disorders to a certain extent, increased the antioxidant levels in the brain and could have protective effects on myelin sheath production. Consequently, although NAC has neuroprotective effects, it is needed to conduct more studies on it to discover its exact benefits.

Declarations

Compliance with ethical standards

Ethical Approval The Animal Experiments Local Ethics Board, Afyon Kocatepe University, Afyon, (Registration number: AKUHADYEK-189-17) has approved this study.

Consent to Participate Not applicable

Consent to Publish Not applicable

Conflict of interest The authors declare that they have no conflict of interest.

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Availability of data and materials The datasets used/analyzed in this study are available from the corresponding author on reasonable request.

Authors' contributions: The idea of the research was recommended by M.S.A, who also took part in the design of the study. M.S.A. and R.T. performed the experimental work and wrote/drafted/edited the manuscript and interpreted the results. M.S.A., R.T. and H.H.D. performed the laboratory analyses. All authors were involved in revising the manuscript critically for important intellectual content, and all authors approved the final version to be published.

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Figures

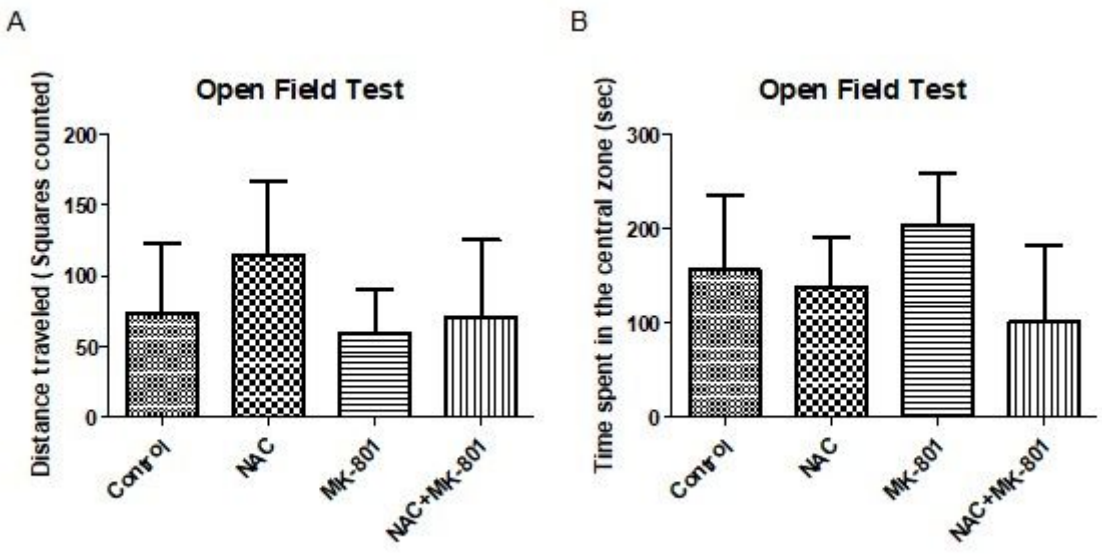

Figure 1

Effects of NAC on schizophrenia-like behaviors induced by MK-801 applications. Mice were administered with NAC (100 mg/kg, intraperitoneally [ip]) in the morning and with MK-801 (1 mg/kg, intraperitoneally [ip]) in the afternoon for 14 consecutive days, and placed on an open field device 24 hours later. The distance traveled (A) in 5 min on the open field device did not significant differences between the groups. (B) Time spent in the central zone in 5 min on the open field device did not significant differences between the groups; n=6 per group (data expressed as mean ± SD). Abbreviations: sec, seconds; NAC, *N*-acetylcysteine.

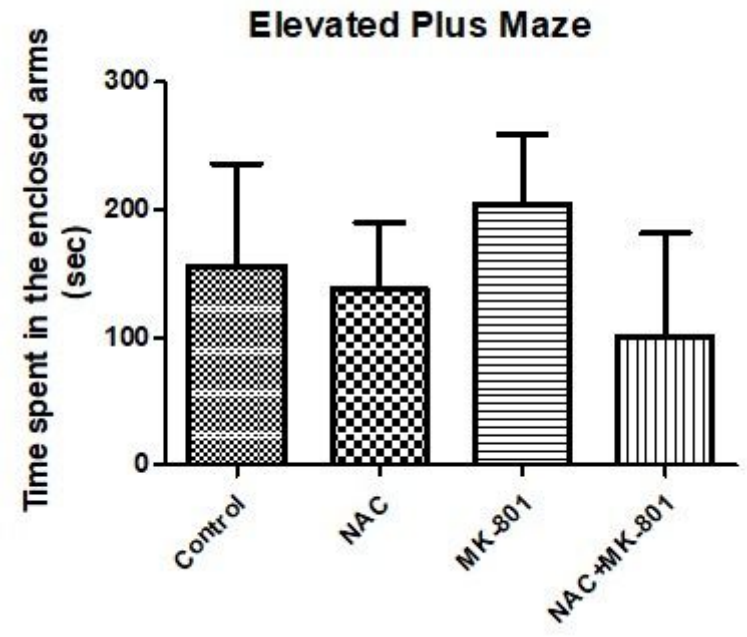

Figure 2

Effects of NAC on schizophrenia-like behaviors induced by MK-801 applications. Mice were administered with NAC (100 mg/kg, intraperitoneally [ip]) in the morning and with MK-801 (1 mg/kg, intraperitoneally [ip]) in the afternoon for 14 consecutive days, and placed on an elevated plus maze device 24 hours later. Time spent in the enclosed arms in 5 min on the elevated plus maze device did not significant differences between the groups; n=6 per group (data expressed as mean ± SD). Abbreviations: sec, seconds; NAC, *N*-acetylcysteine.

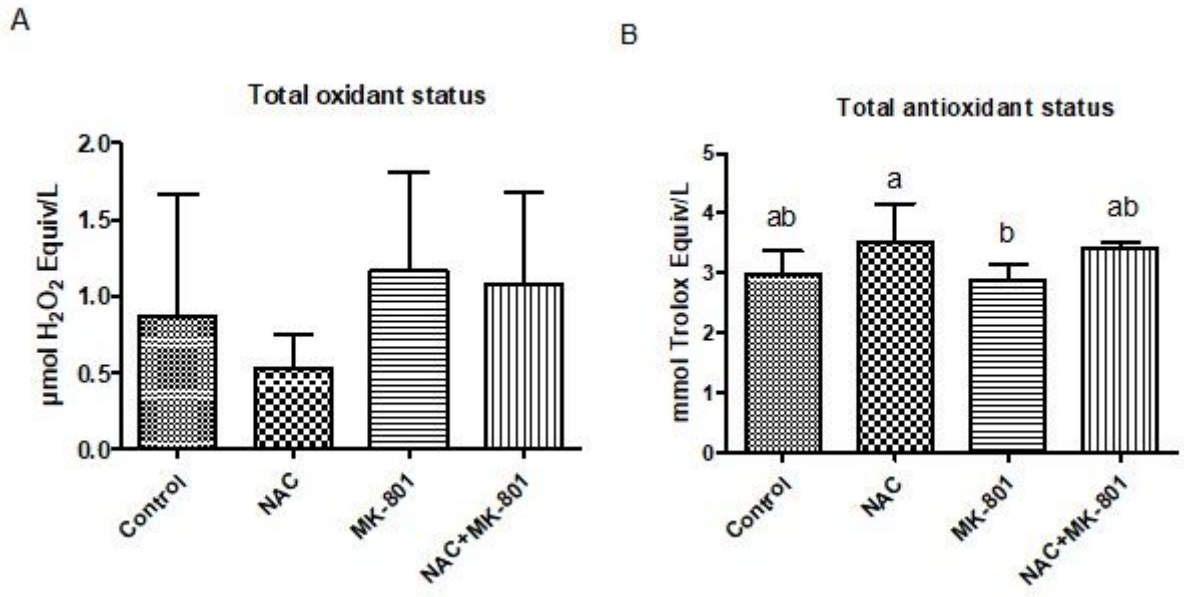

Figure 3

Effects of NAC and MK-801 on levels of TOS and, TAS among the different groups. Mice were administered with NAC (100 mg/kg, intraperitoneally [ip]) in the morning and with MK-801 (1 mg/kg, intraperitoneally [ip]) in the afternoon for 14 consecutive days. 48 hours later, mice brains were obtained. TOS and, TAS were measured by a colorimetric method as described in the methodological section. Values bearing different letters on the bars show statistically significant differences on levels of TOS (A) and, TAS (B) among the different groups ($p < 0.05$); n=6 per group (data expressed as mean ± SD). Abbreviations: TOS, total oxidant status; TAS, total antioxidant status; NAC, *N*-acetylcysteine.

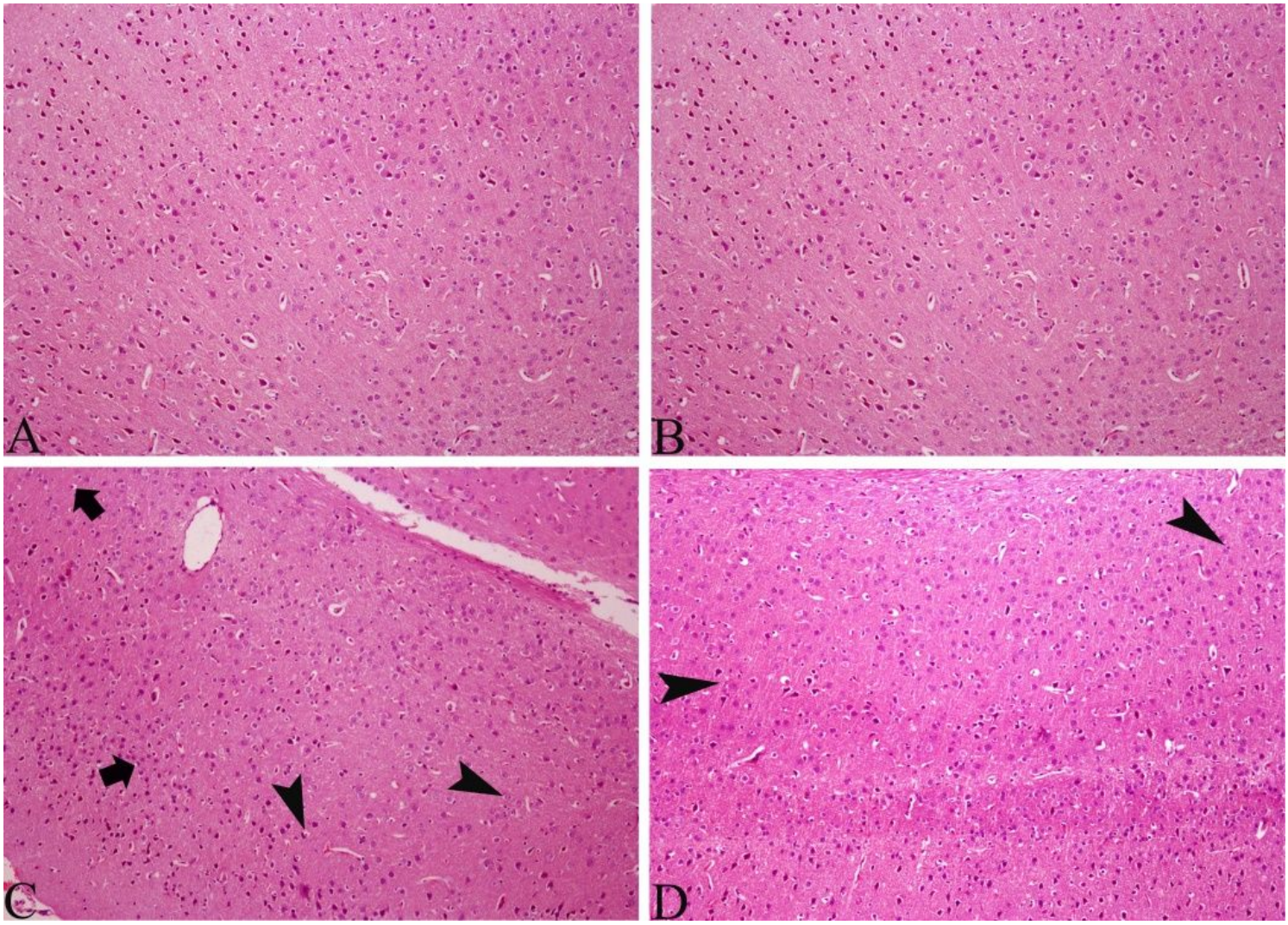

Figure 4

Effects of *N*-acetylcysteine on histopathological changes induced by MK-801 applications. Representative figures were stained with Hematoxylene and eosine . The original magnification was ×20 and the scale bars represent 100 μm. Arrows indicate vacuolization (C) and arrow head indicate focal glial cell infiltration in neurons (C, D) in the brain. A. Control group; B. NAS group; C. Animals treated with 1 mg/kg/day MK-801 group; D. Animals treated with 100 mg/kg/day NAC and 1 mg/kg/day MK-801 (NAC+MK-801 group)

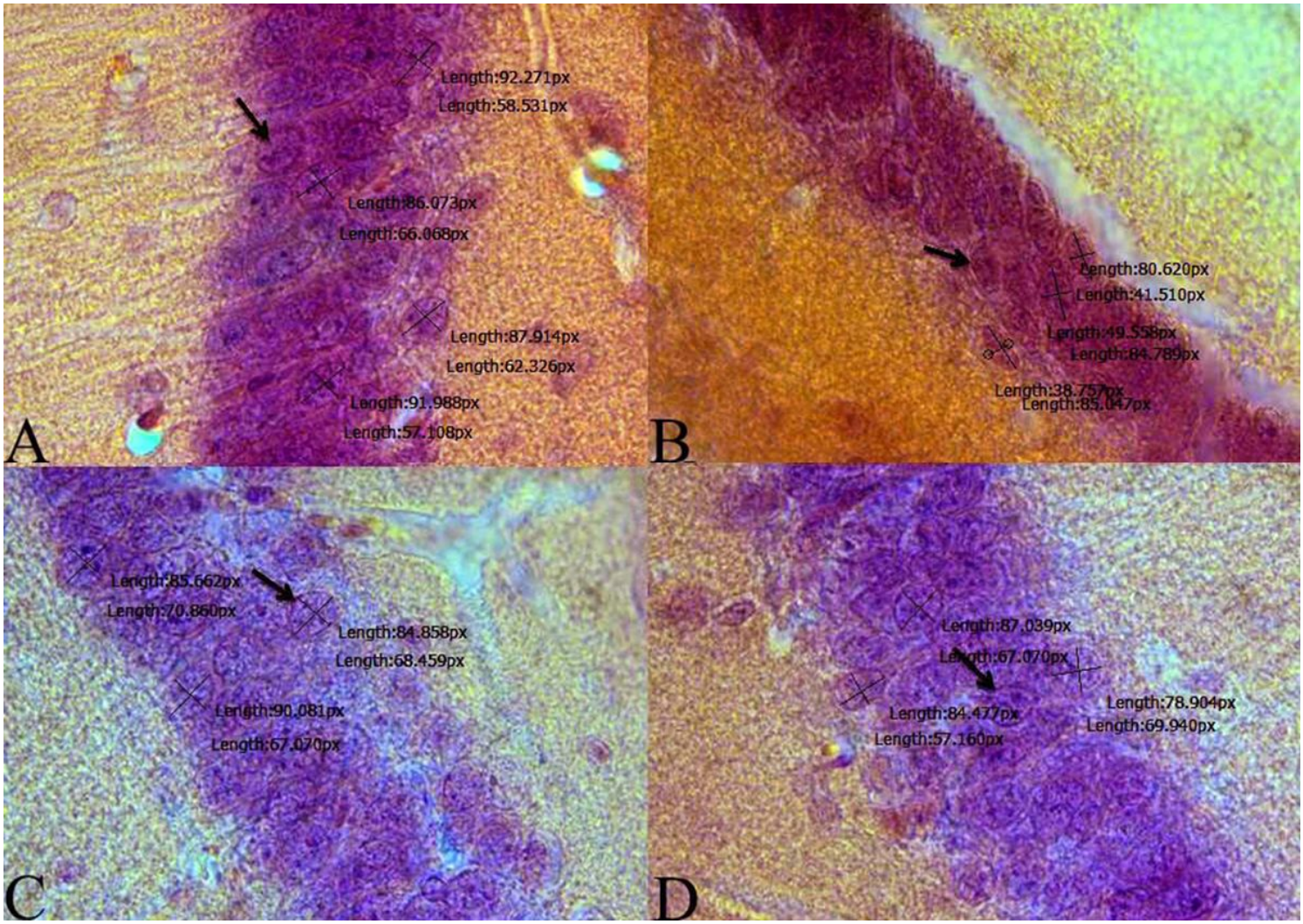

Figure 5

Hippocampal CA1 neuronal cell line (Arrow). The measurements were performed on the nuclei of the neurons. The original magnification was ×100. A. Control group; B. NAC group; C. Animals treated with 1 mg/kg/day MK-801 group; D. Animals treated with 100 mg/kg/day NAC and 1 mg/kg/day MK-801 (NAC+MK-801 group)

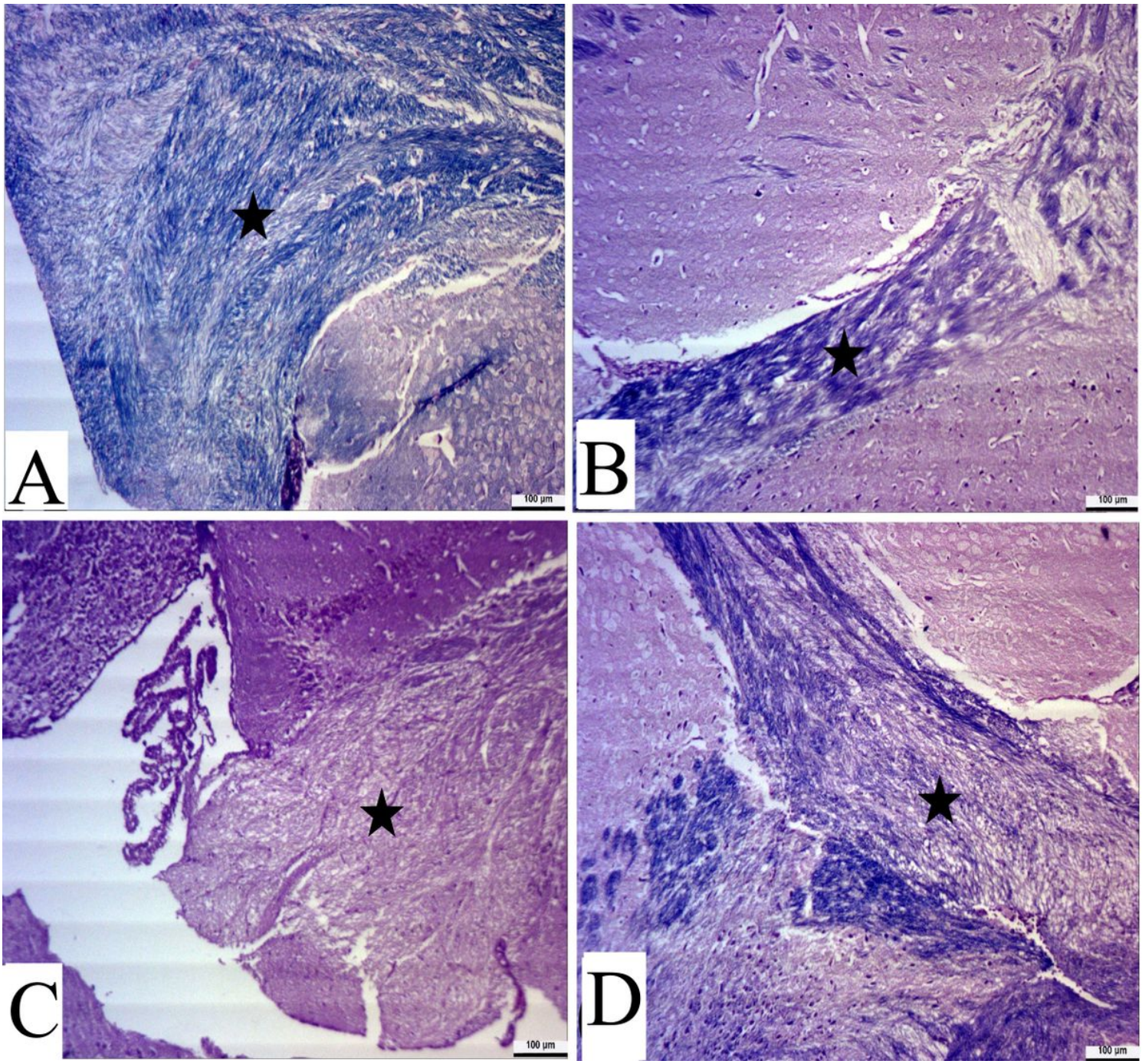

Figure 6

Kluver-Barrera stain of the brain. Stars indicate the corpus callosum. A. Control group; B. NAC group; C. Animals treated with 1 mg/kg/day MK-801 group; D. Animals treated with 100 mg/kg/day NAC and 1 mg/kg/day MK-801 (NAC+MK-801 group) X4

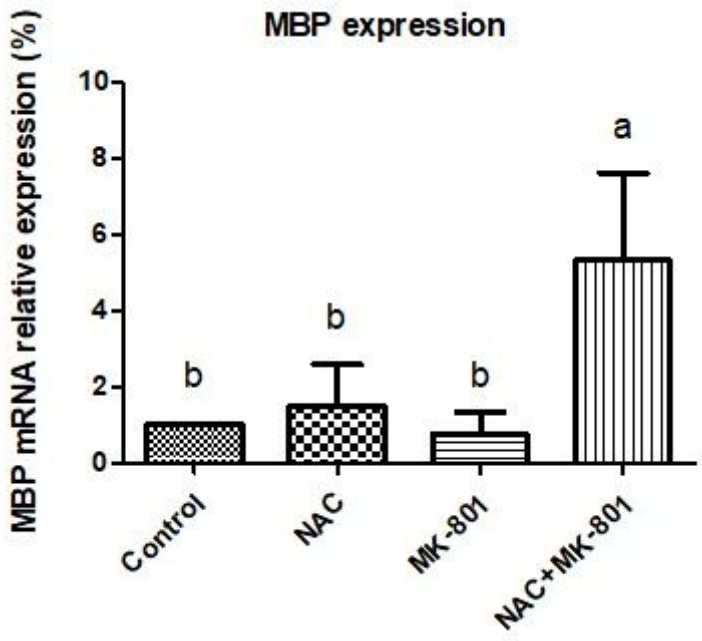

Figure 7

Effects of *N*-acetylcysteine and MK-801 on the MBP mRNA expression levels among the different groups. Mice were administered with NAC (100 mg/kg, intraperitoneally [ip]) in the morning and with MK-801 (1 mg/kg, intraperitoneally [ip]) in the afternoon for 14 consecutive days. 48 hours later, mice brains were obtained and processed for RT-PCR. Values bearing different letters on the bars show statistically significant differences on the MBP mRNA expression levels among the different groups ($p < 0.05$). n=6 per group (data expressed as mean ± SD). Abbreviations: MBP, myelin basic protein; NAC, *N*-acetylcysteine.

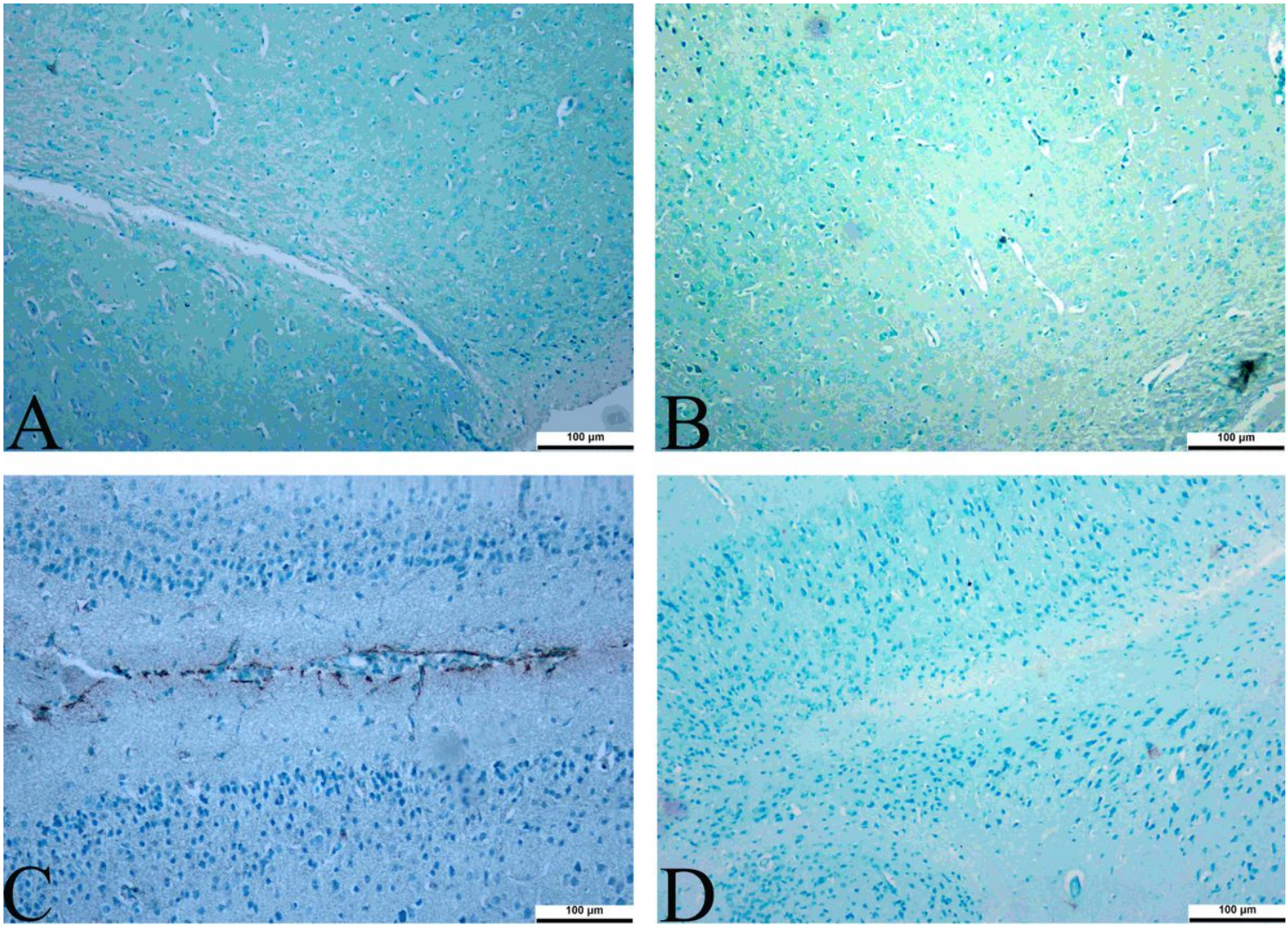

Figure 8

Tunel metod of the brain. Dark brown stained cells indicate the apoptosis (+) cells. A. Control group; B. NAC group; C. Animals treated with 1 mg/kg/day MK-801 group; D. Animals treated with 100 mg/kg/day NAC and 1 mg/kg/day MK-801 (NAC+MK-801 group).