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ORIGINAL ARTICLE

Trigonelline protects hippocampus against intracerebral Aβ(1–40) as a model of Alzheimer's disease in the rat: insights into underlying mechanisms

Javad Fahanik-Babaei[1] · Tourandokht Baluchnejadmojarad[1,2] · Farnaz Nikbakht[1,2] · Mehrdad Roghani[3]

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Abstract

Alzheimer's disease (AD) is a chronic neurodegenerative disorder and the most common phenotype of dementia. Trigonelline is an alkaloid found in medicinal plants such as fenugreek seeds and coffee beans with neuroprotective potential and according to existing evidences, a favorable agent for treatment of neurodegenerative disorders. In this study, the possible protective effect of trigonelline against intracerebral Aβ(1–40) as a model of AD in the rat was investigated. For induction of AD, aggregated A(1–40) (10 μg/2 ₩l for each side) was bilaterally microinjected into the hippocampal CA1 area. Trigonelline was administered *p.o.* at a dose of 100 mg/kg. The results showed that trigonelline pretreatment of Aβ-microinjected rats significantly improves spatial recognition memory in Y maze and performance in novel object recognition (NOR) task, mitigates hippocampal malondialdehyde (MDA), protein carbonyl, lactate dehydrogenase (LDH), and improves mitochondrial membrane potential (MMP), glutathione (GSH), and superoxide dismutase (SOD) with no significant change of catalase activity, nitrite level, caspase 3 activity, and DNA fragmentation. Additionally, trigonelline ameliorated hippocampal levels of glial fibrillary acidic protein (GFAP), S100b, cyclooxygenase 2 (Cox2), tumor necrosis factor α (TNFα), and interleukin 6 (IL-6) with no significant alteration of inducible nitric oxide synthase (iNOS). In addition, trigonelline pretreatment prevented loss of hippocampal CA1 neurons in Aβ-microinjected group. Therefore, our results suggest that trigonelline pretreatment in Aβ model of AD could improve cognition and is capable to alleviate neuronal loss through suppressing oxidative stress, astrocyte activity, and inflammation and also through preservation of mitochondrial integrity.

Keywords Trigonelline · Alzheimer's disease · Amyloid beta · Oxidative stress · Inflammation · Hippocampus

Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disorder and the most common phenotype of dementia. It is typified by selective neuronal loss and cognitive impairment (Campbell and Gowran 2007). AD pathogenic process accompanies a variety of cellular changes including amyloid β (Aβ) formation, enhanced oxidative stress burden, and deranged antioxidant defense system, activation of microglia and astrocytes, neurofibrillary tangles, and neuronal loss (LaFerla et al. 2007; Obulesu and Jhansilakshmi 2014; Subash et al. 2014). Aβ could induce neuronal apoptosis by targeting mitochondria as highlighted by a reduction of mitochondrial membrane potential (MMP) (Amorim et al. 2017) and its exposure is associated with glial fibrillary acid protein overexpression as a specific marker of astrogliosis (Bagheri et al. 2013). Meanwhile, Aβ induces expression of inflammation-related enzymes including inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX) (Khan et al. 2014) in addition to elevating inflammatory biomarkers such as tumor necrosis factor α (TNF α) (Min et al. 2017). Current preventive and therapeutic strategies for AD are still in contention and most of them provide only symptomatic relief in affected patients (Khan et al. 2012).

Trigonelline, chemically known as *N*-methylnicotinic acid ($C_7H_7NO_2$), is found in *Trigonella foenum-graecum*

✉ Tourandokht Baluchnejadmojarad
tmojarad@yahoo.com; baluchnejadmojarad.t@iums.ac.ir

[1] Physiology Research Center, Iran University of Medical Sciences, Tehran, Iran

[2] Department of Physiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

[3] Neurophysiology Research Center, Shahed University, Tehran, Iran

L. (fenugreek) and coffee beans (Zhou et al. 2012). Trigonelline has reported to exert hypoglycemic, neuroprotective, and memory-improving effects (Zhou et al. 2012). Additionally, it has antioxidant function and can promote the regeneration of tissues (Tharaheswari et al. 2014). Trigonelline could also accelerate dendritic and axonal regeneration (Tohda et al. 2005). Neuroprotective and anti-apoptotic effect of trigonelline in 6-hydroxydopamine rat model of Parkinson's disease has been recently informed (Mirzaie et al. 2016). Furthermore, anti-inflammatory and anti-apoptotic effect of trigonelline in diabetc mice has been established (Zhou et al. 2017). In an earlier study by our research team, beneficial effect of trigonelline on learning and memory deficits was shown in $A\beta_{1-40}$ model of AD using behavioral (passive avoidance and Morris water maze tasks) and electrophysiological (recording of long-term potentiation in medial perforant-dentate gyrus pathway as an index of synaptic plasticity) approaches. In the current study, we tried to address the question of whether trigonelline could mitigate $A\beta_{1-40}$-induced neuronal loss in the hippocampus and to further explore some modes of action.

Materials and methods

Animals

Male adult albino Wistar rats (laboratory animal breeding center of Iran University of Medical Sciences, Tehran, Iran) at an age of 12–14 weeks and a weight range of 250–295 g were housed in Plexiglas cages with woodchip bedding in groups of 2–3 per cage at a room temperature of 21–23 °C and a humidity of 30–40%) under standard 12–12 h light/dark cycle (the light period started at 07:00 a.m.). Animals were allowed to acclimate to their environment for 10 days prior to being tested and handled daily. Food and water were available ad libitum. Procedures involving animals and their care were conducted in conformity with the National Institutes of Health guidelines for the care and use of laboratory animals.

The rats ($n = 60$) were randomly allocated to the following four equal-sized groups: Sham, Trigonelline-pretreated Sham, Aβ, and Trigonelline-pretreated Aβ. Trigonelline (SigmaAldrich, USA) was dissolved in distilled water and administered *p.o.* at a dose of 100 mg/kg, started 3 days before the surgery till 1 h pre-surgery. Dose of trigonelline was chosen from our earlier study on its neuroprotective and anti-apoptotic effect in 6-hydroxydopamine rat model of Parkinson's disease (Mirzaie et al. 2016). Sham animals received an equivalent volume of the vehicles.

Aβ(1-40) preparation

Aβ (1–40) (SigmaAldrich, USA) was prepared as a stock solution in sterile 0.1 M phosphate-buffered saline (pH 7.4) and aliquots were stored at −20 °C. Aβ solution was aggregated by its incubation at 37 °C for 96 h before use according to an earlier report (Piermartiri et al. 2010).

Stereotaxic surgery

On the day of surgery, the rats were anesthetized with an intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). The rats were mounted in a stereotaxic frame and after drilling two burr holes at coordinates: 3.5 mm posterior to the bregma, ±2 mm lateral to the sagittal suture, and 2.8 mm below the dura according to rat stereotaxic brain atlas (Watson 2004), A훽 (1–40) solution (10 훦g/2 훦l) was bilaterally microinjected into the CA1 area of dorsal hippocampus. Sham operated rats received vehicle solution. Finally, the skin was sutured and the animals were monitored to fully recover before returning to their home cages. For assessment of cognition, behavioral tests including single-trial Y-maze and novel object recognition tasks were used. These tests were conducted on week 3 after A훽 microinjection on 32 rats divided into 4 equal-sized experimental groups.

Y-maze task

This task is a valid test for judgement of recent working memory of a recognitive nature in rodents through assessing spontaneous alternation behavior. The black Plexiglas maze comprised three arms at angles of 120° and a central interconnecting area. Protocol for this task has been reported before (Nitta et al. 2002; Roghani et al. 2006). Shortly, each animal was placed at the end of one arm and allowed to pass freely for 8 min and subsequent entries was recorded. Alternation was taken as successive entries into the three arms based on overlapping triplets. Then, correct alternating sequences / total alternating sequences × 100 was obtained for assessment of spatial recognition capability as well as estimation of locomotor activity. All arms were wiped off with 10% ethanol between sessions to eliminate the possible effect of odorous cues.

Novel object recognition (NOR) task

This test was used to assess novel object recognition memory through judgement of differences in the exploration time of familiar and novel objects and is a valid indicator of cognition ability in rodents (Antunes and Biala 2012). Our protocol has been mentioned before (Stuart et al. 2013). Concisely, each animal received two successive 5 min object exploration sessions separated by a 4 h inter-trial interval. The objects used

here had never been seen by the animals prior to examination. Rats explored two similar objects during the first (familiarization) session, and one of the objects was replaced with a novel one in the second session. Exploration of objects, defined as chewing, licking, sniffing, or approximating vibrissae at ≤1 cm from the object was obtained and discrimination ratio was calculated from the following formula: (t [novel]-t [familiar]) / (t [novel] + t [familiar]) *100. The working space and objects were wiped off with 10% ethanol between sessions.

Biochemical measurements

Seven days following A훽, anesthetized rats ($n = 7$ from each group) were decapitated in a guillotine device and their brains were immediately harvested. The hippocampi were punched out and 5% homogenate was prepared in ice-cold Tris–HCl buffer (150 mM, pH 7.4) and in the presence of protease inhibitor cocktail from SigmaAldrich (USA) using a high-speed homogenizer (IKA, Germany). Prepared homogenate was centrifuged (5000 rpm, 4 °C, 10 min), and an aliquot of the supernatant was stored at −80 °C until being used.

Malondialdehyde (MDA) content as an index of lipid peroxidation and oxidative stress in the supernatant was determined according to an earlier report (Roghani and Baluchnejadmojarad 2010). For this purpose, supernatant was added to a solution of thiobarbituric acid and trichloroacetic acid and incubated at boiling water for 90 min. After cooling on ice, samples were centrifuged at 1000 g for 10 min and the absorbance of the supernatant was read at 532 nm. The results were calculated on tetraethoxypropane standard curve.

For determination of nitrite content as an end-product of nitric oxide and another indicator of oxidative stress, supernatant was added to and mixed with Griess reagent comprising of sulfanilamide and N-naphthyl ethylenediamine and the absorbance was read at 540 nm and concentration was calculated on sodium nitrite standard curve (Kiasalari et al. 2016).

Reduced glutathione (GSH) as a non-enzymatic intracellular defensive element was determined consistent with earlier reports (Ellman 1959; Raoufi et al. 2015). For this purpose, the homogenate was mixed with 5% trichloroacetic acid and centrifuged. Then, 0.1 ml of obtained supernatant, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5′5 dithiobis (2-nitrobenzoic acid) (DTNB) and 0.4 ml of distilled water was added and 30 min later, the absorbance was obtained at 412 nm.

For measurement of catalase activity as a defensive enzyme, Claiborne's technique was applied (Afshin-Majd et al. 2015; Claiborne 1985). In short, H2O2 was added to a mixture of 50 mM potassium phosphate buffer (pH 7.0) and supernatant and the rate of H2O2 degradation was determined by reading the absorbance changes at 240 nm.

Superoxide dismutase (SOD) activity was determined as indicated in earlier reports (Kiasalari et al. 2013; Wang et al. 2005). In brief, supernatant was incubated with xanthine and xanthine oxidase in potassium phosphate buffer for 40 min and nitroblue tetrazolium was added. Blue formazan production was monitored at 550 nm.

Bradford method was applied to measure protein concentration with bovine serum albumin as its standard (Bradford 1976).

Measurement of caspase 3 activity as a biomarker of apoptotic pathway was based on the hydrolysis of the p-nitroaniline (pNA) moiety by the enzyme and according to an earlier report (Movsesyan et al. 2002). Briefly, 20 μl of tissue homogenate was incubated with assay buffer (50 nmol/L HEPES, pH 7.4, 0.2% CHAPS, 20% sucrose, 2 mmol/L EDTA, 10 mmol/L dithiothreitol, and 50 μ mol/L of chromogenic pNA specific apopain substrate (Z-Asp-IH-Val-Asp-pNA)) at 37 °C. The amount of chromogenic pNA released was measured with Synergy HT microplate reader (BioTek, USA) at 450 nm and the values were expressed as OD. Additionally, DNA fragmentation as another consistent biomarker of apoptosis was measured by means of Cell Death Detection ELISA kit (SigmaAldrich, USA) as reported before (Kiasalari et al. 2016; Morroni et al. 2013).

The activity of lactate dehydrogenase (LDH) as an enzyme of intracellular metabolic pathway and a marker indicating ischemic and/or stressful conditions was determined by measuring the rate of oxidation of NADH at 340 nm according to an earlier report (Al-Bayaty and Abdulla 2012). Briefly, the reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.5), 0.5 mM sodium pyruvate, 0.1 mM NADH and the required amount of cytosolic fraction to reach to a volume of 1 ml. The reaction was started at 25 °C by addition of NADH and the rate of oxidation of NADH was measured at 340 nm in spectrophotometer. The enzyme activity was calculated using extinction coefficient 6.22 mM^{1} cm^{-1}. One unit of the enzyme was defined as the amount producing the oxidation of 1 μmol of NADH/min. Activity of the enzyme was reported as unit/mg protein.

Measurement of MMP

For measurement of MMP as an indicator of mitochondrial integrity and cell health, the supernatant was centrifuged at 10,000 rpm for 15 min. The precipitate contains mitochondrial fraction from hippocampal tissue. Assessment of changes in MMP was conducted as previously reported (Ding et al. 2013; Ma et al. 2010). Brain mitochondria were incubated with 0.2 μmol/l of rhodamine 123 (SigmaAldrich, USA) at 37 °C for 5 min, and then the MMP was measured. Fluorescence signals of mitochondria were excited at 488 nm and emission was monitored at a wavelength of 525 nm using a fluorescent

microplate reader and fluorescence intensity was finally reported.

Determination of hippocampal GFAP, iNOS, S100, Cox2, TNFα, and IL-6

The hippocampal level of GFAP as a specific marker of astrocytes and astrogliosis, Cox2 as an enzyme in inflammatory pathway, IL-6 and TNFa as reliable inflammatory biomarkers, and iNOS and S100 were determined using sandwich enzyme-linked immunosorbent assay technique and commercial kits (for TNFa from SigmaAldrich (USA), for IL-6 kit from MyBioSource, Inc. (USA), and for others from Cloud-Clone Corp. (USA)).

Histological evaluation

After conductance of behavioral tests at week 3, some rats ($n = 5$ from each experimental group) were deeply anesthetized with a high dose of ketamine (150 mg/kg) and perfused through the ascending aorta with 50 ml of heparinized normal saline and then by 50–75 ml of fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). Following perfusion, brains were removed from the skull, post-fixed for 1 week, left hippocampal block was prepared and embedded in paraffin, hippocampal block was cut into 7 μm coronal sections and prepared for Nissl (Cresyl violet acetate) staining. Neuronal counting was done in CA1 area of the hippocampus in at least four sections at a level range between −3.6 and − 4.3 mm from the bregma (according to the coordinates of the stereotaxic atlas of Paxinos and Watson in an area of 0.1 mm^2 using an image capturing and analysis system (Bel Engineering Co., Italy). Cells with visible cytoplasmic boundaries and clear nucleolus were included in counting. The counting process repeated two times for each section and it was done blind to the treatments received.

Data analysis

Parametric distribution of data was checked out using Kolmogorov–Smirnov test. For all experimental tests, data were analyzed using one-way ANOVA followed by Tukey's post-hoc test. All results have been shown as means ± SEM. In all statistical comparisons, $P < 0.05$ was considered significant.

Results

Figure 1a displays performance of rats in single-trial Y-maze paradigm that assesses short-term spatial and recognition memory. Obtained data indicated that alternation score in Aβ-microinjected rats was significantly lower as compared to sham group ($p < 0.01$). Additionally, trigonelline treatment of Aβ group at a dose of 100 mg/kg significantly increased alternation relative to Aβ group ($p < 0.05$). Moreover, sham+ trigonelline group showed no significant alteration as compared to control one.

We also used NOR test to check recognition memory. Our data indicated that there is a significant decrease in discrimination ratio in Aβ group relative to sham group ($p < 0.01$) and such significant difference was also obtained to a lower degree for trigonelline-pretreated Aβ group ($p < 0.05$). In contrast, discrimination ratio was significantly higher in Aβ + trigonelline group when compared to Aβ group ($p < 0.05$) (Fig. 1b). Once more, no significant change was noted for sham+trigonelline group versus sham.

For assessment of oxidative stress, we measured hippocampal level of MDA, nitrite, protein carbonyl in addition to GSH and activity of SOD and catalase. With respect to hippocampal oxidative stress indices, Aβ-injected group showed significantly elevated level of MDA (Fig. 2a) ($P < 0.01$) and a significantly lower level of GSH (Fig. 2c) ($P < 0.05$), catalase activity (Fig. 2d) ($P < 0.01$), and SOD activity (Fig. 2e) ($P < 0.01$) relative to sham group. Additionally, no significant change was observed for hippocampal nitrite (Fig. 2b) in Aβ-injected rats versus sham group. Furthermore, pretreatment of Aβ-injected group with trigonelline significantly lowered MDA level ($P < 0.05$) and significantly elevated GSH level and SOD activity ($P < 0.05$) as compared to Aβ-injected group. Meanwhile trigonelline had no significant effect on nitrite level and catalase activity in Aβ-injected group.

We also measured hippocampal level of protein carbonyl as another biomarker of oxidative stress ($P < 0.05$) in addition to assessment of DNA fragmentation and activity of LDH and caspase 3. In this respect, Aβ-injected group showed a significantly greater level of protein carbonyl (Fig. 3a) ($P < 0.005$) and higher activity of LDH (Fig. 3b) ($P < 0.01$) with no significant elevation of caspase 3 activity (Fig. 3c) and DNA fragmentation (Fig. 3d) as compared to sham group. Additionally, trigonelline pretreatment of Aβ group significantly lowered hippocampal level of protein carbonyl ($P < 0.05$) and LDH activity ($P < 0.05$) with no significant alteration of caspase 3 activity and DNA fragmentation. Furthermore, we measured MMP to assess mitochondrial energy metabolism condition. In this regard, MMP significantly decreased in Aβ group (Fig. 3e) ($P < 0.005$) relative to sham and trigonelline was capable to significantly elevate MMP in Aβ group ($P < 0.05$).

Our ELISA assessment showed that Aβ group had an elevated level of GFAP (Fig. 4a) ($P < 0.01$), iNOS (Fig. 4b) ($P < 0.05$), S100B (Fig. 4c) ($P < 0.005$), and COX-2 (Fig. 4d) ($P < 0.01$), TNFα (Fig. 5a) ($P < 0.05$), and IL-6 (Fig. 5b) ($P < 0.01$). Additionally, trigonelline pretreatment of Aβ-injected group significantly lowered GFAP ($P < 0.05$), S100 ($P < 0.01$), Cox2 ($P < 0.05$), TNFα ($P < 0.05$), and IL-6 ($P < 0.05$) with no significant alteration of iNOS.

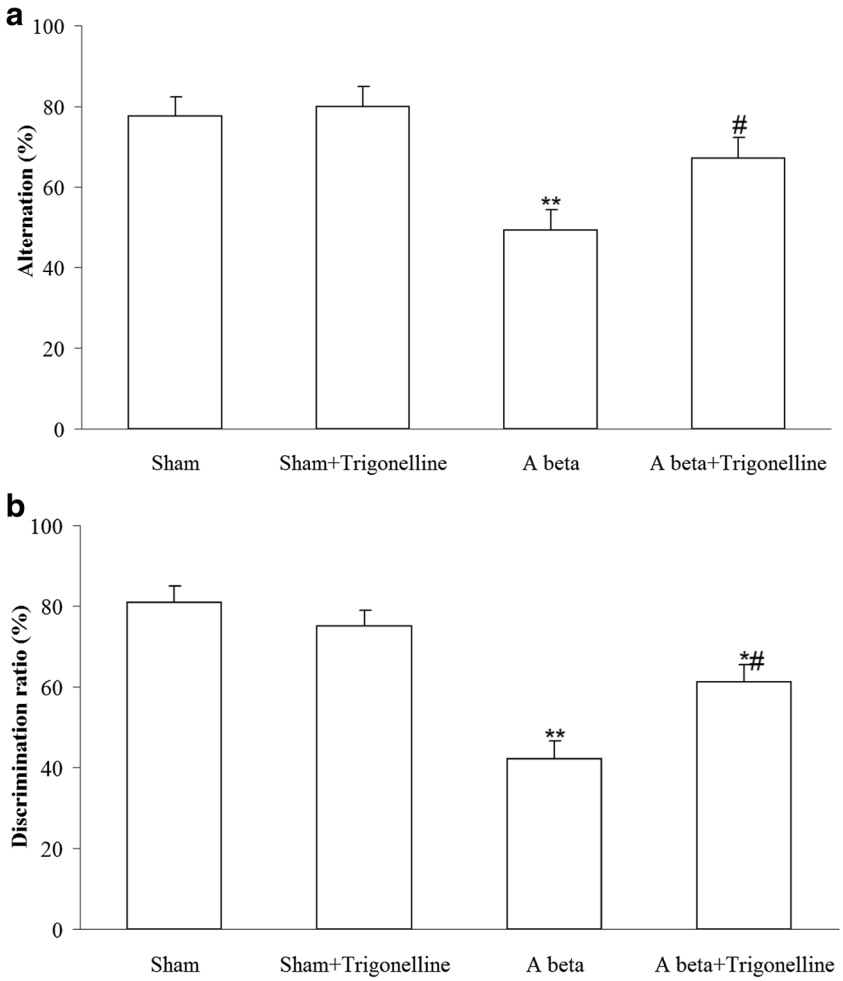

Fig. 1 Performance of animals in Y- maze (**a**) and novel object recognition (**b**) tests, as shown by alternation behavior and discrimination ratio, respectively. These tests were conducted on week 3 following Aβ injection. Trigonelline was administered at a dose of 100 mg/kg/day for 3 days till 1 h pre-surgery. ($n = 8$ for each group). * $p < 0.05$, ** $p < 0.01$ (as compared to sham); # $p < 0.05$ (as compared to Aβ)

In this study, we also counted the number of hippocampal CA1 pyramidal neurons to evaluate the protective potential of trigonelline in Aβ-induced model of AD (Fig. 6). Our results showed that Aβ group had a significantly lower number of neurons as compared to sham group ($P < 0.01$) and trigonelline-pretreated Aβ group had a significantly higher number of neurons ($P < 0.05$).

Discussion

This study was undertaken to assess whether trigonelline pretreatment could protect hippocampal CA1 neurons against intracerebral microinjection of Aβ(1–40) as a model of AD in the rat. Our results indicated that trigonelline could improve memory function and alleviate neuronal loss through suppression of oxidative stress, apoptotic pathway, astrocyte activity, inflammation, and through preservation of mitochondrial integrity.

Deposition of Aβ in the brain is responsible for learning and memory deficits and Aβ could induce a valid model of AD (Yamaguchi and Kawashima 2001). In this regard, it has been shown intrahippocampal injection of Aβ leads to lower performance of animals in Y-maze (Ghofrani et al. 2015) and novel object recognition (He et al. 2013) tasks that is consistent with our obtained data. In this study, trigonelline administration at a dose of 100 mg/kg was capable to improve cognitive deficits in Aβ group. In line with our findings, it has shown that trigonelline due to its anti-oxidative and anti-inflammatory potential is capable to alleviate lipopolysaccharide-induced learning and memory impairment in the rat (Khalili et al. 2018) and also through up-regulation of cerebral level of brain-derived neurotrophic factor (Chowdhury et al. 2018).

Our histological findings regarding CA1 neuronal loss following $A\beta_{(1–40)}$ were consistent with previous reports (Colom et al. 2011; Zhang et al. 2014). This clearly indicate intracerebral $A\beta_{(1–40)}$ causes neurodegeneration in the hippocampus. In addition, trigonelline was capable to ameliorate neuronal loss due to Aβ(1–40) that could be attributed to its neuroprotective potential through possibly a variety of intracellular pathways.

Oxidative stress as a major pathogenic mechanism for many disorders including AD is due to an imbalance in pro-

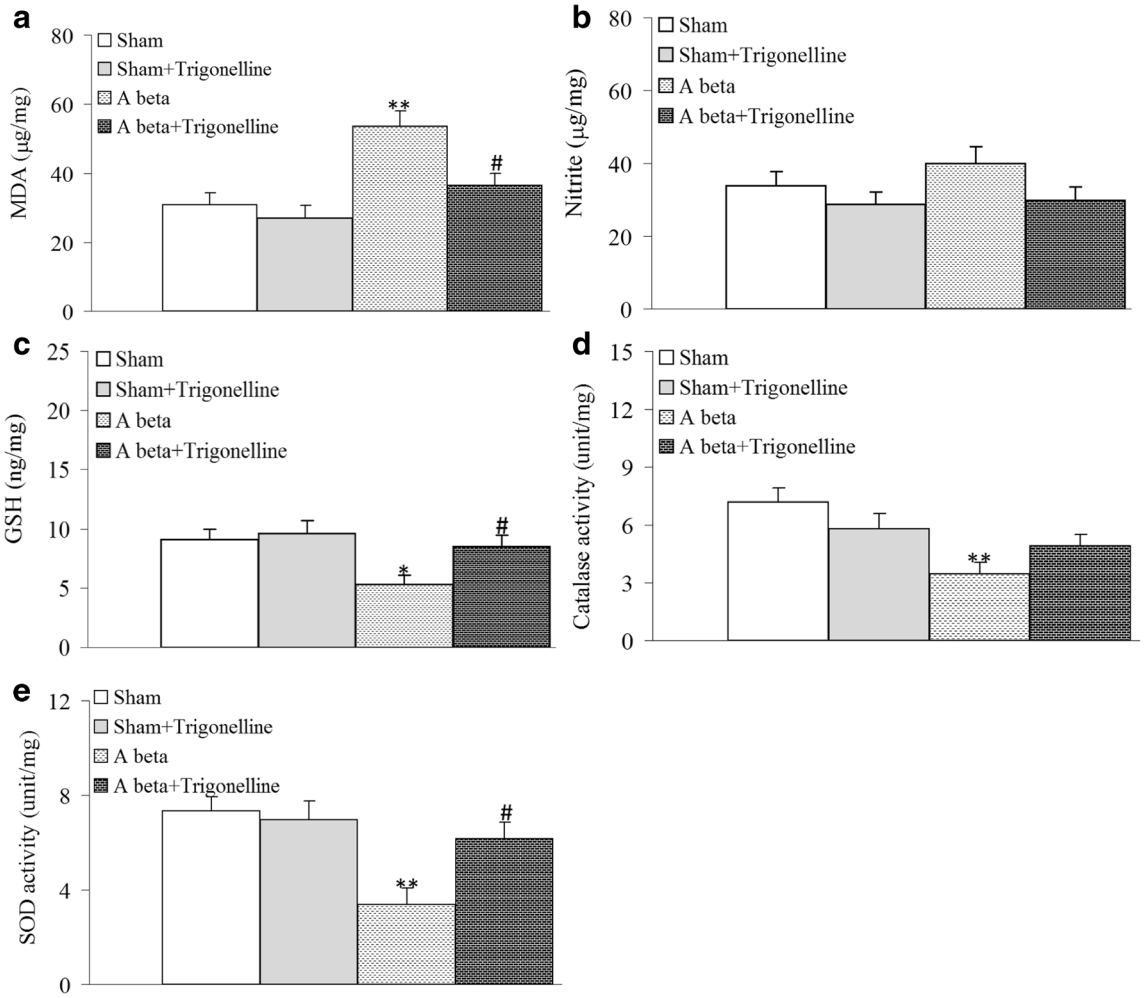

Fig. 2 Hippocampal level of MDA (**a**), nitrite (**b**), GSH (**c**), catalase activity (**d**), and SOD activity (E) in different groups. The parameters were measured in duplicate and 7 days after bilateral intrahippocampal Aβ(1–40) microinjection. Trigonelline was administered at a dose of 100 mg/kg/day for 3 days till 1 h pre-surgery. ($n = 7$ for each group). * $p < 0.05$, ** $p < 0.01$ (in comparison with sham); # $p < 0.05$ (in comparison with A beta)

oxidant/antioxidant homeostasis (Butterfield 2004; Zhu et al. 2005). This imbalance is as a result of increased free radicals generation or a decrease in activity of antioxidant defensive system. In our study, following intrahippocampal microinjection of Aβ(1–40), tissue level of MDA as a specific marker of lipid peroxidation elevated, clearly indicating enhanced oxidative stress in the hippocampus. Increased level of MDA has also been reported in Aβ-induced model of AD (Zhang et al. 2015). A significant increase in protein carbonyl following exposure of cultured neurons to Aβ(1–40) has been indicative of oxidative damage to cellular proteins (Aksenov et al. 1998), that was also observed in our study. In addition, antioxidant elements including GSH, SOD, and catalase were depressed following intrahippocampal microinjection of Aβ(1–40) in our study, that were also consistent with earlier reports (Rege et al. 2015). Regarding nitrite, we did not observe a significant elevation in Aβ group that was contrary to a previous study (Bagheri et al. 2011). In this study, trigonelline was capable to ameliorate oxidative stress and to augment antioxidant defensive system in Aβ group. Consistent with our findings, it has been shown that trigonelline could decrease oxidative stress biomarkers in high-fat high-fructose diet-induced insulin resistance in rats (Afifi et al. 2017) and in type 2 diabetic Goto-Kakizaki rats (Yoshinari et al. 2013). However, there is still no report on the anti-oxidative effect of trigonelline in AD. However, Mirzaie et al. have reported that trigonelline could alleviate oxidative stress in 6-hydroxydopamine rat model of Parkinson's disease (Mirzaie et al. 2016). Inflammation increased following cerebral microinjection of Aβ, as shown by higher hippocampal levels of TNFα and IL-6. A higher rate of inflammation has also been reported in Aβ-induced model of AD (Li et al. 2015). In contrast, trigonelline pretreatment was able to significantly reduce level of inflammatory indices that is also in agreement with earlier reports on its anti-inflammatory potential (Khalili et al. 2018; Zhou et al. 2017)

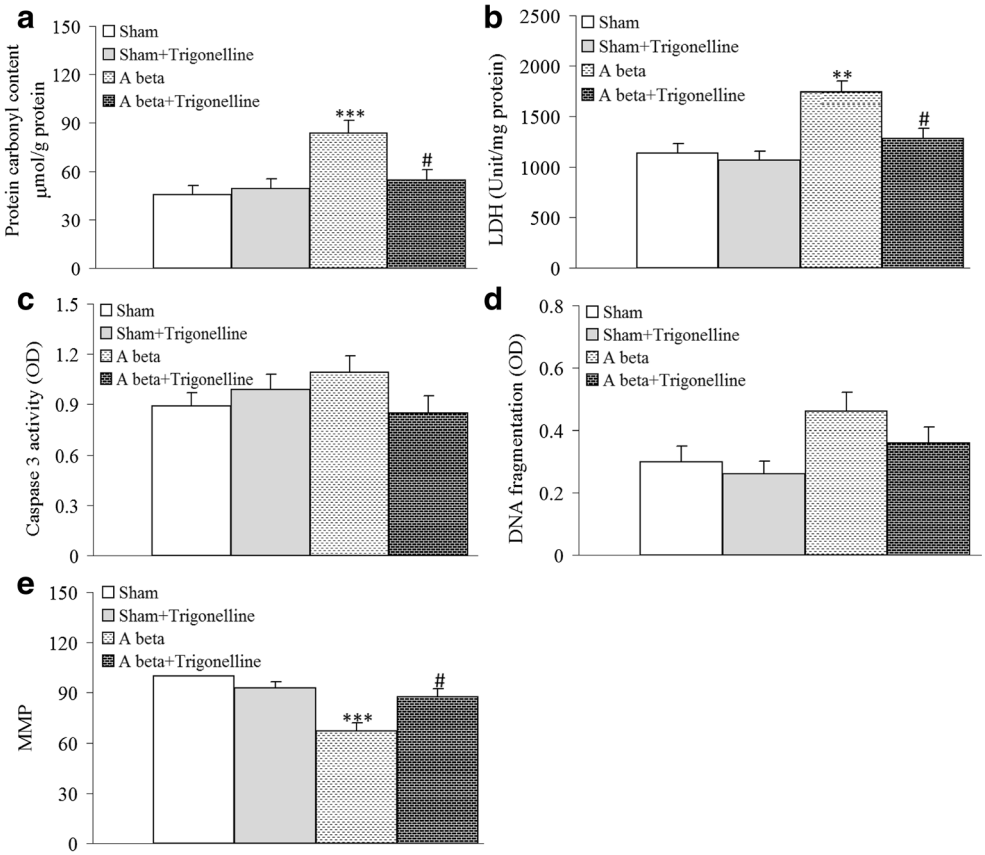

Fig. 3 Hippocampal level of protein carbonyl (**a**), LDH activity (**b**), caspase 3 activity (**c**), DNA fragmentation (**d**), and mitochondrial membrane potential (MMP) (**e**) in different groups. The parameters were measured in duplicate and 7 days after bilateral intrahippocampal Aβ(1–40) microinjection. Trigonelline was administered at a dose of 100 mg/kg/day for 3 days till 1 h pre-surgery. ($n = 7$ for each group). ** $p < 0.01$, *** $p < 0.005$ (in comparison with sham); # $p < 0.05$ (in comparison with A beta)

Increased activity of LDH was also observed in Aβ group. Dead or plasma membrane-damaged cells release LDH enzyme (Korzeniewski and Callewaert 1983) and amount of released LDH correlates with the number of damaged neurons (Liu et al. 2017). Trigonelline pretreatment of Aβ group significantly lowered LDH activity, indicating a lower cytotoxicity and less neuronal damage in the hippocampal tissue. Consistent with this finding, it has shown that natural trigonelline could protect against isoproterenol-induced myocardial injury as indicated by a lower serum activity of LDH (Panda et al. 2013).

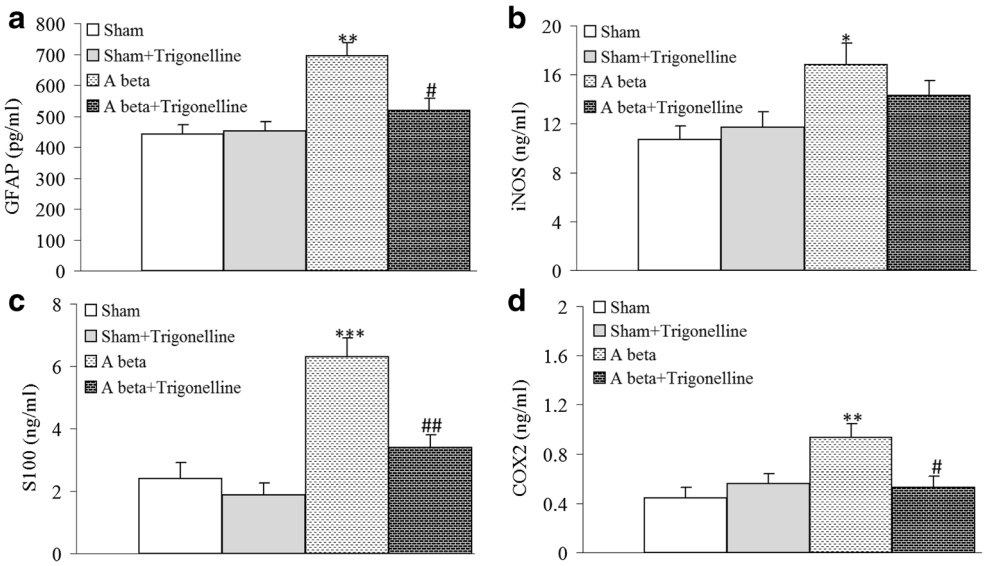

Fig. 4 Hippocampal level of GFAP (**a**), iNOS (**b**), S100b (**c**), and Cox2 (**d**) in different groups, as measured by sandwich Elisa. The parameters were measured in duplicate and 7 days after bilateral intrahippocampal Aβ(1–40) microinjection. Trigonelline was administered at a dose of 100 mg/kg/day for 3 days till 1 h pre-surgery. (n = 7 for each group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ (in comparison with sham), # $p < 0.05$, ## $p < 0.01$ (in comparison with A beta)

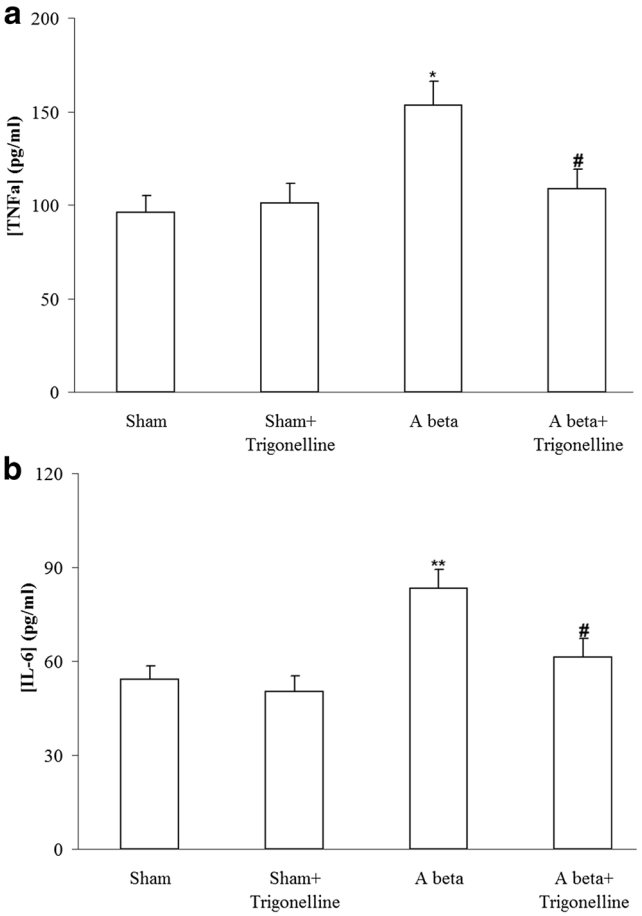

Fig. 5 Hippocampal level of TNFα (**a**) and IL-6 (**b**) as valid biomarkers of inflammation in different groups. The parameters were measured in duplicate and 7 days after bilateral intrahippocampal Aβ(1–40) microinjection. Trigonelline was administered at a dose of 100 mg/kg/day for 3 days till 1 h pre-surgery. ($n = 6$ for each group). * $p < 0.05$, ** $p < 0.01$ (in comparison with sham), # $p < 0.05$ (in comparison with A beta)

In this study, we did not observe a prominent and significant elevation of apoptosis in the hippocampal tissue in Aβ-injected group, as measured by caspase 3 activity and DNA fragmentation. This fact may be related to our 1-week time lag following intrahippocampal microinjection of Aβ. This important issue may be taken into account in similar studies in the future.

There are also evidences that direct interaction of Aβ with mitochondria induces free radicals overproduction, mitochondrial dysfunction, and finally cell death (Reddy 2006). Moreover, it is postulated that the sporadic form of AD may be triggered by mitochondrial dysfunction. MMP is an important parameter of mitochondrial function that its depression is indicative of cell death (Mancuso et al. 2010; Swerdlow et al. 2010). In our study, trigonelline was capable to prevent MMP depression following Aβ, indicating its ability to maintain mitochondrial function.

We also found increased levels of GFAP, S100b, and Cox2 in hippocampal homogenate from Aβ-lesioned rats that is indicative of an inflammatory phenomenon in the lesioned tissue and that has also been reported before (Bagheri et al. 2013; Cirillo et al. 2015; Deng et al. 2017). Additionally, trigonelline pretreatment reversed this neurotoxin effect that is most probably related to its anti-inflammatory effect and is in agreement with an earlier report regarding its anti-inflammatory activity (Zhou et al. 2017).

Aβ exposure in rats induces expression of iNOS mRNA as well as an increase of iNOS activity in the hippocampus (Tran et al. 2001). In our study, we found that injection of Aβ into the hippocampus increased iNOS and had no significant impact on the level of nitrite and this finding is similar to results obtained by other studies (Bagheri et al. 2011; Valles et al. 2010). As shown in the present study, trigonelline had no significant impact on decreasing level of nitrite or iNOS.

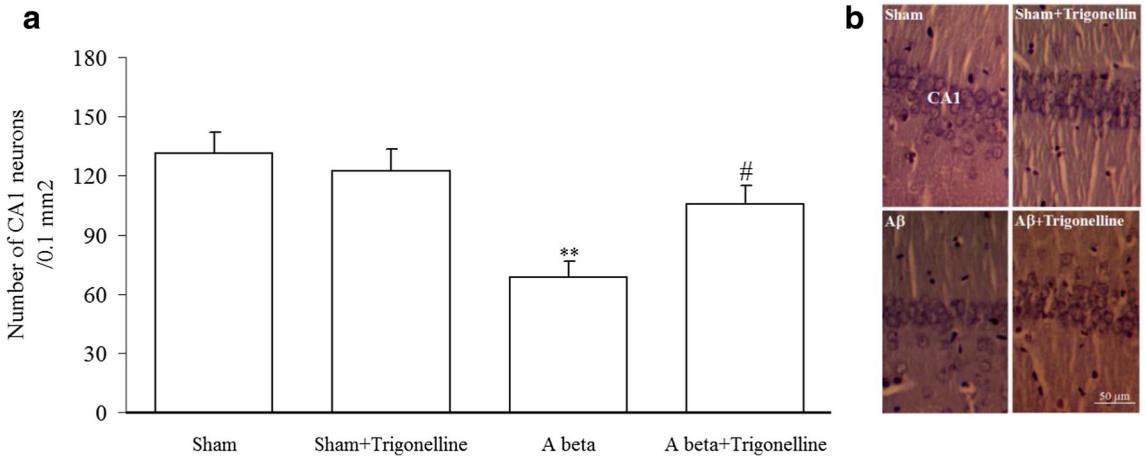

Fig. 6 Number of Nissl-stained neurons in CA1 area of hippocampus and photomicrographs of coronal sections (**a**) at the end of week 1 post-surgery. ($n = 5$ for each group). ** $p < 0.01$ (in comparison with sham); # $p < 0.05$ (in comparison with A beta)

However, participation of NO and NO-producing enzymes in neurodegenerative diseases remains controversial. Some authors believe that increased NOS expression and NO production serve as one of the causative factors of neuronal death in AD (Benzing and Mufson 1995; Tohgi et al. 1999), whereas others suppose that deficit in NO synthesis results in cell death in selectively vulnerable brain regions under neurodegenerative conditions (Gargiulo et al. 2000; Hyman et al. 1992; Tao et al. 1999). These contradictions emphasize an important and still unclear function of reactive nitrogen species in redox imbalance observed in AD. One of the limitations of the present work was the lack of a dose-response design. In this respect, our selected dose for trigonelline was chosen from an earlier study (Mirzaie et al. 2016).

Collectively, our results indicate that trigonelline pretreatment in Aβ model of AD could alleviate hippocampal neuronal loss through suppressing oxidative stress, astrocyte activity, and inflammation and also through preservation of mitochondrial integrity.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interests.

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