Metabolic Brain Disease (2019) 34:1747–1759
https://doi.org/10.1007/s11011-019-00481-6

ORIGINAL ARTICLE

Safranal, an active ingredient of saffron, attenuates cognitive deficits in amyloid β-induced rat model of Alzheimer's disease: underlying mechanisms

Tourandokht Baluchnejadmojarad[1] · Seyed-Mahdi Mohamadi-Zarch[1] · Mehrdad Roghani[2]

Received: 5 April 2019 / Accepted: 8 August 2019 / Published online: 17 August 2019

Abstract

Alzheimer's disease (AD) is the most prevalent neurodegenerative amyloid disorder with progressive deterioration of cognitive and memory skills. Despite many efforts, no decisive therapy yet exists for AD. Safranal is the active constituent of saffron essential oil with antioxidant, anti-inflammatory, and anti-apoptotic properties. In this study, the possible beneficial effect of safranal on cognitive deficits was evaluated in a rat model of AD induced by intrahippocampal amyloid beta ($A\beta_{1-40}$). Safranal was daily given *p.o.* (0.025, 0.1, and 0.2 ml/kg) post-surgery for 1 week and finally learning and memory were evaluated in addition to assessment of the involvement of oxidative stress, inflammation, and apoptosis. Findings showed that safranal treatment of amyloid β-microinjected rats dose-dependently improved cognition in Y-maze, novel-object discrimination, passive avoidance, and 8-arm radial arm maze tasks. Besides, safranal attenuated hippocampal level of malondialdehyde (MDA), reactive oxygen species (ROS), protein carbonyl, interleukin 1β (IL-1β), interleukin 6 (IL-6), tumor necrosis factor α (TNFα), nuclear factor-kappa B (NF-*k*B), apoptotic biomarkers including caspase 3 and DNA fragmentation, glial fibrillary acidic protein (GFAP), myeloperoxidase (MPO), and acetylcholinesterase (AChE) activity and improved superoxide dismutase (SOD) activity and mitochondrial membrane potential (MMP) with no significant effect on nitrite, catalase activity, and glutathione (GSH). Furthermore, safranal prevented CA1 neuronal loss due to amyloid β_{1-40}. In summary, safranal treatment of intrahippocampal amyloid $beta_{1-40}$-microinjected rats could prevent learning and memory decline via neuronal protection and at a molecular level through amelioration of apoptosis, oxidative stress, inflammation, cholinesterase activity, neutrophil infiltration, and also by preservation of mitochondrial integrity.

Keywords Alzheimer's disease · Amyloid β · Safranal · Learning and memory · Oxidative stress · Inflammation

Introduction

Alzheimer's disease (AD) is a common neurodegenerative disorder and the major cause of dementia in the elderly, associated with progressive deterioration of cognition- and memory-related processes (Dos Santos Picanco et al. 2018). AD is represented by accumulation of senile plaques due to amyloid beta (Aβ) peptides and emergence of tau protein-containing neurofibrillary tangles and progressive degeneration and loss of neurons (Jack et al. 2018). Increased oxidative stress (Butterfield et al. 2010; Cheignon et al. 2018; Collin et al. 2018) and inflammation (Bisht et al. 2018; Zhu et al. 2018) are observed in the early stages of AD, leading to varying degrees of cognitive impairment. An enhancement of apoptotic processes (Bertoni-Freddari et al. 2009; Zhang et al. 2011), disturbance of cholinergic system (Ferreira-Vieira et al. 2016), morphological and functional alterations including astrogliosis and synaptic changes (Ziegler-Waldkirch and Meyer-Luehmann 2018) and mitochondrial dysfunction (Eckert et al. 2012) are also involved in the pathogenesis of AD. In spite of great achievements for management of people with AD, however, few approved medical treatments exist for this disease. Such treatments usually act to reduce symptoms instead of targeting the pathogenic course of AD. Success rate for pharmacological therapy of AD may be higher if applied in the pre-clinical stage and before the establishment of

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

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

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

neurodegeneration (Briggs et al. 2016). Thus, novel interventions with an ability to appropriately modify biologically defined targets of AD pathogenic process are strongly required (Briggs et al. 2016; Jack et al. 2018).

Use of natural products for therapy of prevalent human disorders with a metabolic origin has expanded in recent years (Baluchnejadmojarad and Roghani 2003; Islam et al. 2017). Safranal is the main component of saffron (*Crocus sativus*) essential oil that causes its distinctive aroma (Rezaee and Hosseinzadeh 2013). Safranal being well-known as a flavoring and odorant chemical (40), is also recognized as an antioxidant (Tamaddonfard et al. 2019) and anti-inflammatory and anti-apoptotic (Delkhosh-Kasmaie et al. 2018) agent. In addition, safranal could exert gastro-protective effects in indomethacin-induced model of gastric ulcer (Tamaddonfard et al. 2019), is capable to ameliorate glutamic acid-induced oxidative injury of OLN-93 cells (Alavi et al. 2018), and to improve learning and memory deficits in streptozotocin-induced model of diabetes (Delkhosh-Kasmaie et al. 2018). Furthermore, neuroprotective potential of safranal has been shown in a model of transient cerebral ischemia (Sadeghnia et al. 2017) and it has recently been demonstrated that safranal could protect against amyloid beta-induced cell toxicity in PC12 cells (Rafieipour et al. 2019). However, the effect of safranal on cognitive deficits in amyloid β-induced model of AD and its modes of action has not been determined. Therefore, we undertake this study to demonstrate neuroprotective effect of safranal in intrahippocampal amyloid β_{1-40}-induced model of AD in the rat and to explore some underlying mechanisms.

Material and methods

Animals

Male Wistar rats (Pasteur's Institute, Tehran) (210–260 g) were kept in Plexiglas cages (3–4/cage) in an animal house (temperature: 22–24 °C; 12:12 lighting cycle; humidity: ≃ 60%). Animals had an adaptation period of 1 week before being tested. All behavioral tests were conducted between 10:00 *a.m.* and 04:00 *p.m*. The study protocol was approved by Ethics Committee of Iran University of Medical Sciences (Tehran, Iran) in 2018 (ethics no. IR.IUMS.REC.1396.32487) and it was in compliance with the policies specified in the Guide for the Care and Use of Laboratory Animals of NIH.

Experimental procedure

Rats ($n = 66$) were randomly assigned to six groups as follows: sham, safranal-treated sham at a dose of 0.2 ml/kg, amyloid β, and safranal-treated amyloid β groups receiving safranal at doses of 0.025, 0.1, or 0.2 ml/kg. For stereotaxic surgery, rats were anesthetized with ketamine (Neogen Corp., Germany; #85521V69F) and xylazine (Alfasan, Germany; 012023) (100 and 10 mg/kg, respectively; *i.p.*) and then placed in a Stoelting stereotaxic instrument (USA). After disinfecting with an iodine solution, the scalp was cut on the midline, and a burr hole was made in the skull at coordinates of 3.5 mm posterior to bregma, 2 mm lateral to sagittal suture, and 2.7–2.8 mm below the dura, using stereotaxic atlas designed for rat (Paxinos and Watson 1986). For induction of a model of AD, two μl of aggregated amyloid β_{1-40} (5 μg/μl) (Fahanik-Babaei et al. 2019) was injected into the CA1 of both sides. Amyloid β_{1-40} was prepared as a stock solution in sterile 0.1 M PBS (Bio-Idea, Tehran, Iran; #52411; pH 7.4) and aliquots were stored at −20 °C. Amyloid β (Sigma-Aldrich, USA; #072 K1289) solution was aggregated by its incubation at 37 °C for 96 h (Piermartiri et al. 2010). Safranal (Sigma-Aldrich, USA; #STBS37802V; a purity of ≥90%) was diluted in 10% Kolliphor EL (Sigma-Aldrich, USA; #C5135) and administered *p.o.* using a gavage needle at doses of 0.025, 0.1, or 0.2 ml/kg/day, one h after surgery for one week. Sham groups received the same volumes of the vehicles. Behavioral tests were conducted blind to treatments at third week after the surgery. Study design is shown in Fig. 1.

Behavioral tests

Y maze task

Short-term spatial recognition memory was assessed by analysis of spontaneous alternation in Y-maze, as reported before (Roghani et al. 2006). In brief, each rat was placed at the end of one arm and allowed to move through the arms for 8 min and sequence of arm entries was recorded. Alternation was as successive entries into the three arms on overlapping triplet sets. The maximum number of possible spontaneous alternations was obtained as the total number of arms entered minus 2, and the alternation was calculated as the ratio of actual to possible alternations × 100.

Novel object discrimination (NOD) task

This test was done similar to the protocol of earlier reports (Baluchnejadmojarad et al. 2017; Zarezadeh et al. 2017). Each animal received two successive 5 min object exploration trials with a 4 h inter-trial interval. During the familiarization, the animals were individually exposed to two identical objects, and one of the objects was replaced with a novel object in the second trial. Exploration of each object, defined as sniffing, licking, chewing, or moving vibrissae while directing the nose toward and ≤ 1 cm from the object, was recorded. The discrimination (D) ratio was calculated using the formula: (t [novel]-t [familiar]) / (t [novel] + t [familiar]) *100.

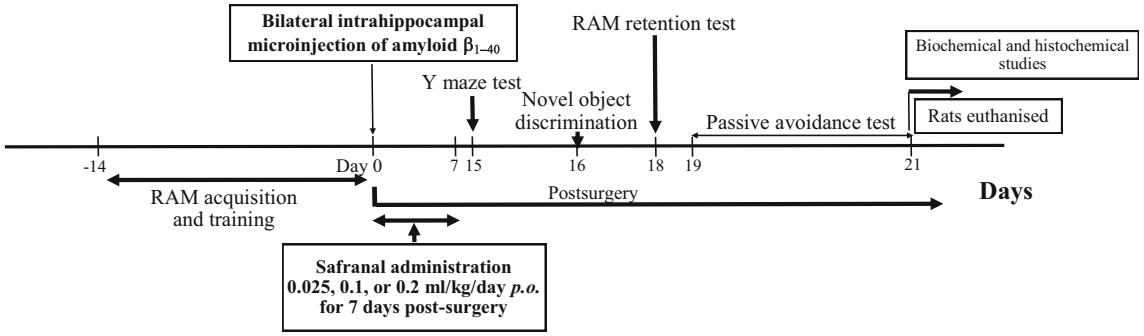

Fig. 1 Experimental protocol of the study including behavioral tests and treatments

Radial arm maze task

Protocol for this test has been described before (Cioanca et al. 2013; Hritcu et al. 2014). The maze was comprised of 8 arms, extending radially from a central area. The arms were 50 cm above the floor in a dimly lighted room with visual cues. At the end of each arm, there was a food cup. The animals were kept on a food regimen and their body weight was maintained at 85% of their initial body weight. Before the training, 3–4 rats were placed to explore the maze for 5 min and consume food freely. The animals were trained for 4 days to run to the end of the arms and consume the baited food. The training trial continued until all the 5 baits had been used up or until 5 min has passed. After adaptation, all the rats were trained with 1 trial per day for seven consecutive days. Each animal was checked for working and reference memory, in which the same 5 arms (no. 1, 2, 4, 5, and 7) were baited. When the animals made 7 or 8 correct choices and less than one error in three sessions, they were selected. Finally, number of working memory errors (entrance into an arm containing food, but previously entered) and reference memory errors (entrance into an arm that never baited) was counted. Reference memory is a long-term process for information that remains constant over repeated trials and working memory is a short-time memory process in which the information to be remembered changes in every trial (Hritcu et al. 2012; Olton and Samuelson 1976).

Passive avoidance task

The protocol of this test has been described before (Ghofrani et al. 2015). The maze was composed of an illuminated chamber connected to a dark chamber by a guillotine door. An electric shock was delivered to the grid floor. For acquisition trial, rats were placed in the lighted chamber. After a habituation period of 5 min, the guillotine door was lifted and after rat entrance into the dark chamber, the door was lowered and an electric shock (1 mA, 1 s) was applied. In this trial, the initial latency (IL) of entrance into the dark chamber was recorded. One day later, each animal was placed in the lighted chamber to assess retention and recall capability. The interval between placement in the lighted chamber and its entrance into the dark chamber was regarded as step-through latency (STL; cut-off = 5 min).

Determination of hippocampal oxidative stress

At the end of week 3 post-surgery, rats ($n = 6$ from each experimental group) were deeply anesthetized with a high dose of ketamine (150 mg/kg) and hippocampal tissue was punched out and 5% homogenate was prepared in ice-cold Tris–HCl buffer (Secoma, South Africa; #062515E0198; 150 mM, pH 7.4). The obtained homogenates were centrifuged at 5000 rpm at 4 °C.

Malondialdehyde (MDA) content of the supernatant as a biomarker of lipid peroxidation was determined by MDA assay reagent comprising of 2-thiobarbituric acid (TBA) (Sigma-Aldrich, USA; #DCBQ7125V) and trichloroacetic acid (TCA) (Sigma-Aldrich, USA; #68H48761), according to earlier studies (Baluchnejadmojarad et al. 2017; Raoufi et al. 2015).

Estimated level of ROS was assessed with the non-fluorescent substance dichlorofluorescein diacetate (Sigma-Aldrich, USA; #BCBK6827V) which is cleaved by intracellular esterase enzymes in the presence of ROS into 2,7-dichlorofluorescein that fluoresces (Arya et al. 2013; Tobon-Velasco et al. 2012). Fluorescence was measured at an excitation of 488 nm and an emission of 525 nm.

Nitrite level was determined by Griess method (Afshin-Majd et al. 2015; Majithiya and Balaraman 2006). In this assay, supernatant was added to Griess reagent comprising sulfanilamide (Sigma-Aldrich, USA; #STBB4751) and N-naphthyl ethylenediamine (Sigma-Aldrich, USA; #010 M1473) in an acidic medium.

Total protein carbonyl content as an index of protein oxidation was measured as described before (Levine et al. 1990; Shagirtha and Pari 2011). In short, tissue homogenate was centrifuged at 10000 g for 20 min to separate cytosolic fraction and this fraction was added to TCA at equal ratios. Thereafter, dinitrophenyl hydrazine (DNPH) (Merck,

Germany; #K37322281) was added and it was kept for 60 min at room temperature. Pellet was washed three times with a mixture of ethanol-ethyl acetate (Merck, Germany; #K45219764) and the pellet was solubilized by addition of guanidine hydrochloride (Merck, Germany; #B0752419) and its absorbance was read at 366 nm.

Activity of catalase was determined in accordance with Claiborne's method (Claiborne 1985; Raoufi et al. 2015). Shortly, H_2O_2 (Carlo-Erba, Algeria; #V5A560165A) was added to a mixture of 50 mM potassium phosphate buffer (pH 7.0) and supernatant and rate of H_2O_2 decomposition was monitored at 240 nm.

Superoxide dismutase (SOD) activity was determined by SOD assay kit (Cayman Chemical, USA; #0490433).

Level of reduced glutathione (GSH) was determined according to previous reports (Ellman 1959; Raoufi et al. 2015). For this test, the supernatant was mixed with 5% TCA and centrifuged. Then, obtained supernatant, phosphate buffer (pH 8.4), 5′5 dithiobis (2-nitrobenzoic acid) (DTNB) (Merck, Germany; K43541091) were added and 30 min later, the absorbance was obtained at 412 nm.

Measurement of protein content was according to Bradford method with bovine serum albumin as its standard (Bradford 1976).

Biochemical determination of hippocampal inflammation and astrogliosis

The level of the biomarkers IL-1β (MyBioSource, Inc., USA; #11513781012), IL-6 (Santa Cruz Biotechnology, Inc., USA; #H1915), TNFα (Sigma-Aldrich, USA; #1116D0715), NF-κB (Santa Cruz Biotechnology, Inc., USA; #K1915), and GFAP (Santa Cruz Biotechnology, Inc., USA; #H0414) in the hippocampal tissue was measured using enzyme-linked immunosorbent assay. The absorbance of samples was read by Synergy HT microplate reader (BioTek, Winooski, Vermont, USA) and final values were obtained.

Biochemical determination of MPO activity

Analysis of MPO activity as a marker of neutrophil infiltration was according to an earlier study (Pulli et al. 2013). For this purpose, hippocampal tissue (right side) was homogenized in CTAB buffer (50 mM cetyltrimethylammonium bromide (Merck, Germany; S5771719) in 50 mM potassium phosphate buffer at pH = 6) and centrifuged at 15,000 g for 20 min. For estimation of peroxidase activity, sample was mixed with 0.75 mM H_2O_2 and 3,3′,5,5′-tetramethylbenzidine-HCl (Sigma-Aldrich, USA; #STBB6012V) (2.9 mM in 14.5% DMSO (Merck, Germany; #802912) and 150 mM sodium phosphate buffer at pH 5.4) and the plate was incubated at 37 °C for 5 min. The reaction was stopped by adding 2 M H_2SO_4 and absorption was measured at 450 nm.

Biochemical determination of apoptosis biomarkers

Caspase 3 activity was determined by an assay kit from Abcam (Cambridge, USA; #ab39401). DNA fragmentation was assessed using Cell Death Detection ELISA Plus kit (Sigma-Aldrich, USA; #11287100).

Measurement of MMP

Mitochondrial membrane potential (MMP) as a reliable index of mitochondrial integrity and cell health was determined according to earlier reports (Ding et al. 2013; Ma et al. 2010). The hippocampal supernatant was re-centrifuged (10,000 rpm for 15 min) and the resultant precipitate encompasses mitochondrial fraction. Mitochondrial fraction was incubated with 0.2 μmol/l of rhodamine 123 (Sigma-Aldrich, USA; #MKBC2653V) at 37 °C for 5 min and then the MMP was determined. Fluorescence signals excited at 488 nm and emission was followed at 525 nm using a fluorescent microplate reader and fluorescence intensity was finally reported as arbitrary fluorescence unit (AFU).

Measurement of hippocampal acetylcholinesterase (AChE) activity

AChE activity in the hippocampal lysate was determined by a modified method of Ellman's according to Isomae et al. (Isomae et al. 2003). The activity of AChE was determined by measuring the formation of the yellow product obtained from the reaction between Ellman's reagent and the thiocholine formed by enzymatic degradation of acetylthiocholine at a wavelength of 412 nm, according to kit protocol (Abcam, USA; #GR3179205-5).

Histological evaluation

For this purpose, the rats ($n = 4$–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 followed by 100–150 ml of fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer. Following perfusion, brains were removed from the skull, post-fixed for one week, hippocampal block was prepared and embedded in paraffin, hippocampal block was cut into 5 μm coronal sections and prepared for Nissl (Cresyl violet acetate, #C1791, 124 K3737) staining. Neuronal counting was done in CA1 area of the hippocampus in at least 4 sections at a plane 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, Italy). Cells with

a clear membrane and a visible nucleolus were included in counting process. The analysis was repeated two times for each section and done blind to the treatments.

Statistical analysis

All results are expressed as mean ± S.E.M. Kolmogorov-Smirnov test was used to confirm parametric distribution of data. Then, the parametric one-way ANOVA test was used for data analysis and if a difference was noted to be significant, pair-wise comparison was done using the Tukey *post-hoc* test. In all analyses, significance level was set at <0.05.

Results

Recognition short-term memory in Y maze task

Figure 2a shows the performance of animals in the Y-maze task to assess recognition aspect of short-term spatial memory. Obtained data as a percentage of alternation score showed that alternation score is significantly lower in amyloid β and amyloid β + safranal0.025 groups as compared to the sham group ($p < 0.001$) and no such significant reduction was obtained for amyloid β + safranal0.1 and amyloid β + safranal0.2 groups. In contrast, safranal treatment of amyloid β group at a dose of 0.2 ml/kg significantly prevented

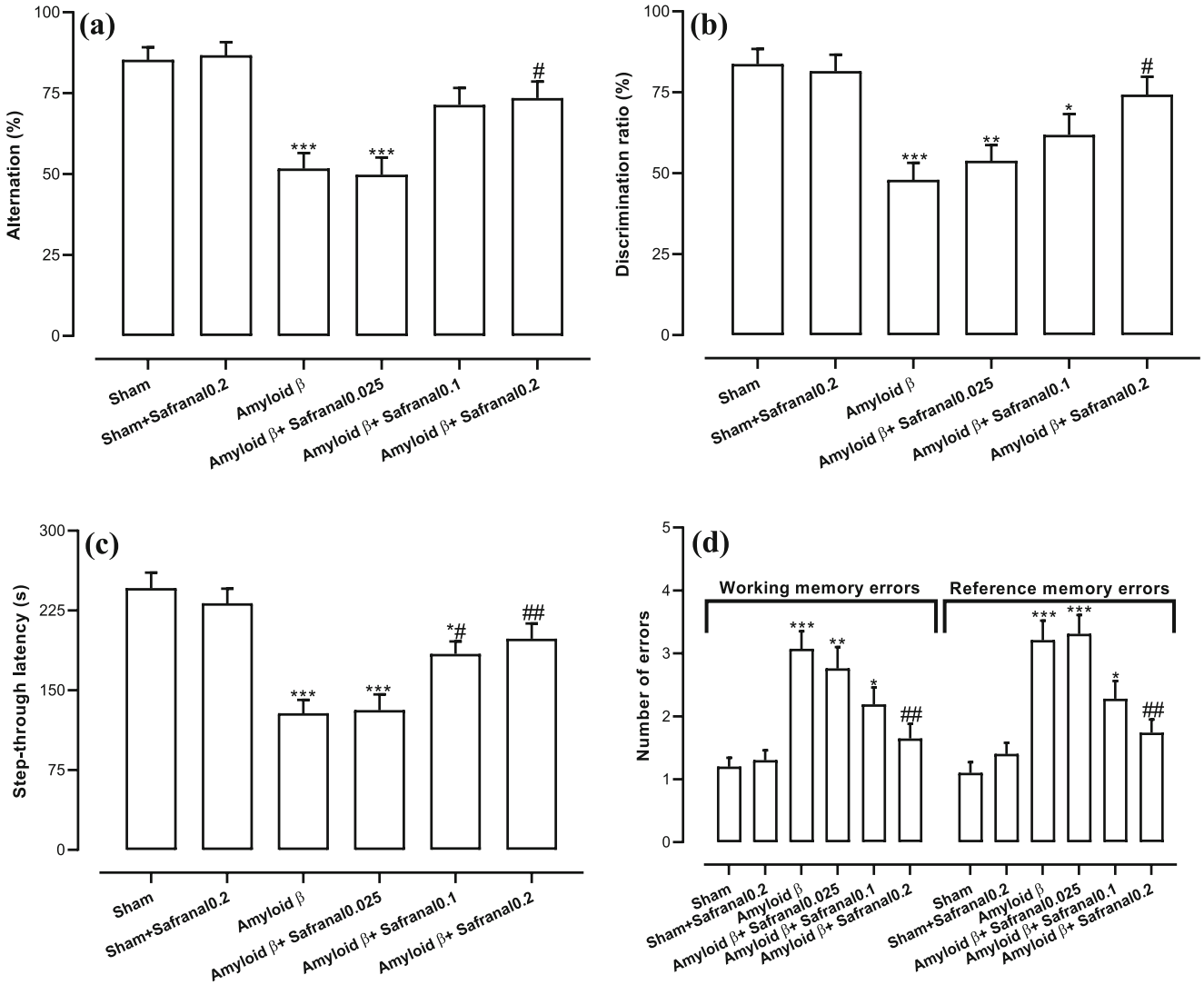

Fig. 2 Alternation percentage in Y-maze task (**a**), discrimination ratio in novel object discrimination (**b**), step-through (STL) latency in passive avoidance test (**c**), and working and reference memory errors in radial arm maze (**d**). Amyloid β_{1-40} was bilaterally microinjected into hippocampal CA1 to induce AD model and safranal was *p.o.* administered at doses of 0.025, 0.1, or 0.2 ml/kg/day post-surgery for 1 week. Behavioral tests were conducted on week 3 after the surgery. Values are means ± S.E.M. $n = 10–11$ for each group. * $p < 0.05$, ** $p < 0.01$, ** $p < 0.001$ (versus Sham); # $p < 0.05$, ## $p < 0.01$ (versus amyloid β)

reduction of alternation score relative to amyloid β group ($p = 0.031$).

Novel object discrimination

A significant reduction of discrimination ratio percentage was obtained for amyloid β ($p < 0.001$), amyloid β + safranal0.025 ($p = 0.007$), and amyloid β + safranal0.1 ($p = 0.029$) when compared to sham group and no such significant reduction was noted for amyloid β + safranal0.2 group. In contrast, discrimination ratio was significantly higher in amyloid β + safranal0.2 group in comparison with amyloid β group ($p = 0.030$) (Fig. 2b).

Passive avoidance test

Figure 2c displays the performance of rats in the passive avoidance task as reported by initial latency (IL) and step-through latency (STL). Regarding IL, there was no significant difference amongst the groups (data not shown). With regard to STL, it significantly decreased in amyloid β ($p < 0.001$), amyloid β + safranal0.025 ($p < 0.001$), and amyloid β + safranal0.1 ($p = 0.027$) versus sham group and no such significant decrease was found out for amyloid β + safranal0.2 group. In addition, STL was significantly greater in amyloid β + safranal0.1 ($p = 0.023$) and amyloid β + safranal0.2 ($p = 0.007$) relative to amyloid β.

Radial arm maze test

Figure 2d shows the performance of rats in radial arm maze task as a valid indicator of long-term spatial memory. In this regard, working and reference memory errors were significantly greater in amyloid β, amyloid β + safranal0.025, and amyloid β + safranal0.1 ($p < 0.05$–0.001) when compared to sham. In contrast, these errors were significantly lower in amyloid β + safranal0.2 group relative to amyloid β group ($p = 0.008$).

Hippocampal biomarkers of oxidative stress

Amyloid β group had a significantly higher level of MDA (Fig. 3a) as a valid biomarker of lipid peroxidation ($p = 0.008$), ROS (Fig. 3b) ($p = 0.06$) and protein carbonyl (Fig. 3d) ($p = 0.08$) and a significant decrease of catalase

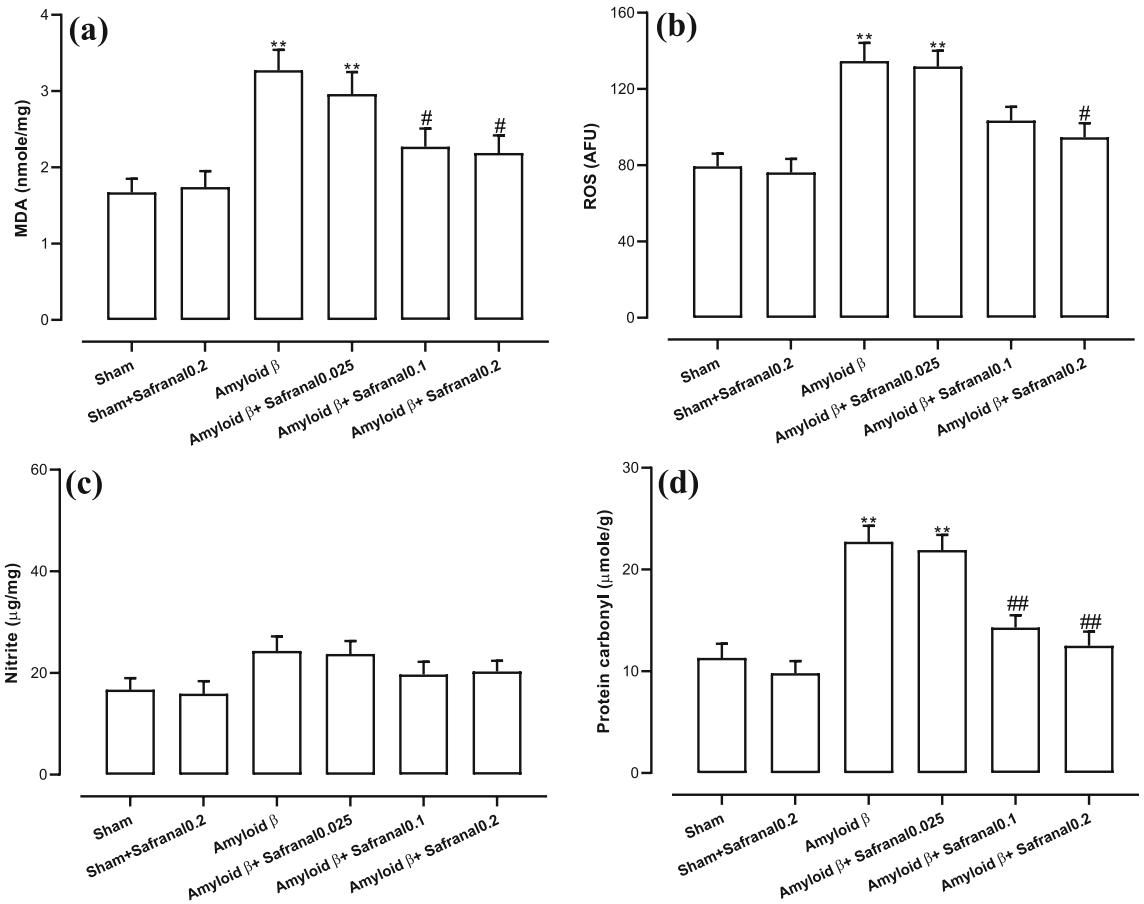

Fig. 3 Hippocampal level of malondialdehyde (MDA) (**a**), estimated level of reactive oxygen species (ROS) (**b**), nitrite (**c**), and protein carbonyl (**d**). $n = 6$ for each group. Values are means ± S.E.M. ** $p < 0.01$ (versus Sham); # $p < 0.05$, ## $p < 0.01$ (versus amyloid β)

(Fig. 4a) (p = 0.032), SOD (Fig. 4b) (p = 0.009), and glutathione (GSH) (Fig. 4c) (p = 0.035) with no significant alteration

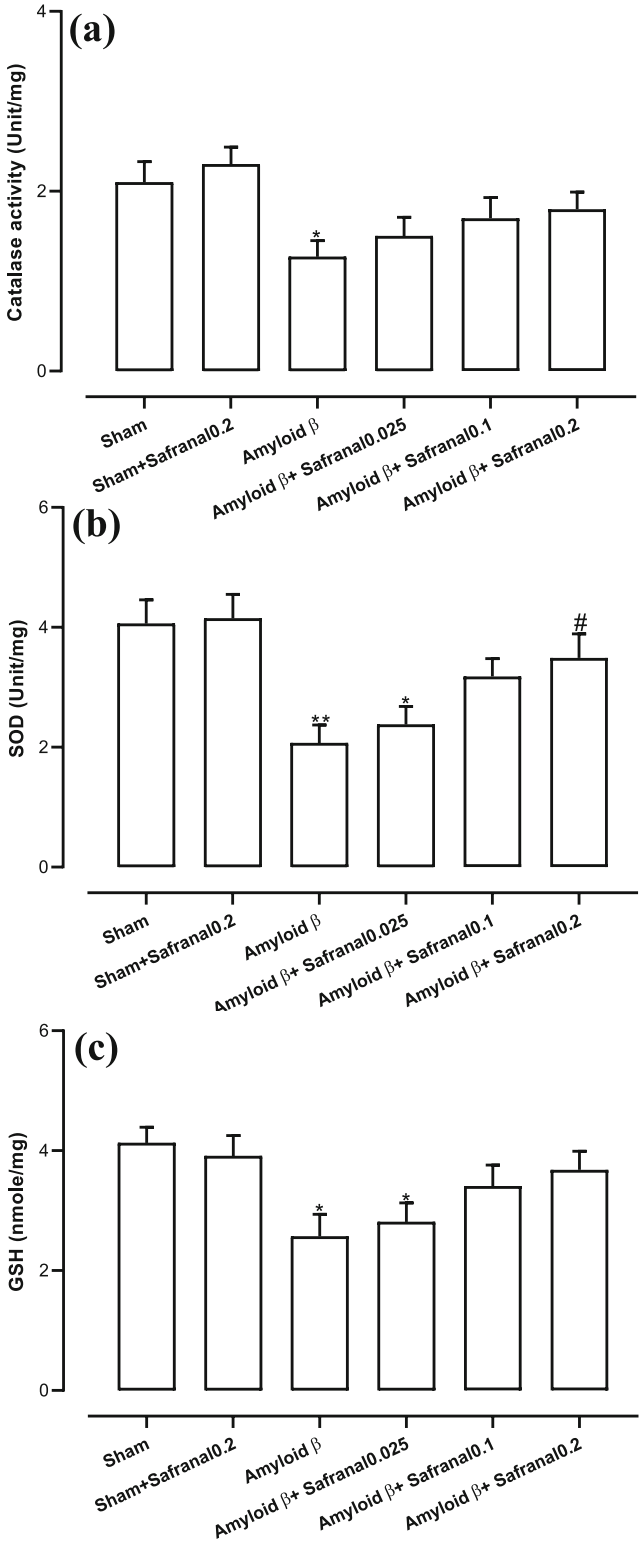

Fig. 4 Hippocampal level of catalase activity (**a**), superoxide dismutase activity (SOD) (**b**), and reduced glutathione (GSH) (**c**). n = 6 for each group. Values are means ± S.E.M. * p < 0.05, ** p < 0.01 (versus Sham); # p < 0.05 (versus amyloid β)

of nitrite level (Fig. 3c) when compared to sham group. Additionally, treatment of amyloid β group with safranal at a dose of 0.2 ml/kg significantly and partially reversed MDA (p = 0.037), ROS (p = 0.031), protein carbonyl (p = 0.008), and SOD (p = 0.041) with no significant and beneficial effect regarding nitrite, catalase, and GSH.

Hippocampal biomarkers of astrogliosis, inflammation, apoptosis, and neutrophil infiltration

Obtained data showed that hippocampal levels of inflammation-related indices including IL-1β (Fig. 5a) (p = 0.008), IL-6 (Fig. 5b) (p = 0.009), TNFα (Fig. 5c) (p = 0.007), and NF-kB (Fig. 6b) (p < 0.001) are significantly higher in amyloid β group as compared to sham group. In contrast, treatment of amyloid β group with safranal at a dose of 0.2 ml/kg significantly and partly reversed IL-1β (p = 0.034), IL-6 (p = 0.029), TNFα (p = 0.021), and NF-kB (P = 0.004) when compared to amyloid β group.

Regarding apoptosis-related biomarkers, amyloid β group had a significantly higher level of caspase 3 activity (Fig. 5e) (p = 0.007) and DNA fragmentation (Fig. 5f) (p = 0.008) relative to amyloid β group. In addition, amyloid β + safranal0.2 group had a significantly lower level of these apoptotic biomarkers (p = 0.041 and p = 0.37, respectively).

Hippocampal MPO activity as a consistent index of neutrophil infiltration increased in amyloid β group versus sham group (Fig. 5d) (p = 0.006) and safranal treatment of amyloid β group at doses of 0.1 or 0.2 ml/kg was able to significantly reduce it (p = 0.027 and p = 0.018, respectively).

Hippocampal level of GFAP as a reliable biomarker of astrogliosis was significantly greater in amyloid β group when compared to sham group (Fig. 6c) (p < 0.001) and safranal treatment of amyloid β group at doses of 0.1 (p = 0.028) or 0.2 ml/kg (p = 0.005) was able to significantly reduce it.

Hippocampal level of AChE and MMP

AChE activity (Fig. 6a) elevated (p = 0.008) and MMP (Fig. 6d) significantly decreased (p = 0.004) in amyloid β group versus sham group. In contrast, safranal treatment of amyloid β group at a dose of 0.2 ml/kg (p = 0.043 and p = 0.035, respectively) was capable to significantly and partially reverse inappropriate changes of these parameters.

Histological findings

In this research study, number of Nissl-stained neurons in the hippocampal CA1 area was compared between the groups (Fig. 7). Our findings showed that amyloid β (p < 0.001) and amyloid β + safranal0.025 (p = 0.008) groups had a significantly lower number of CA1 neurons relative to sham group and

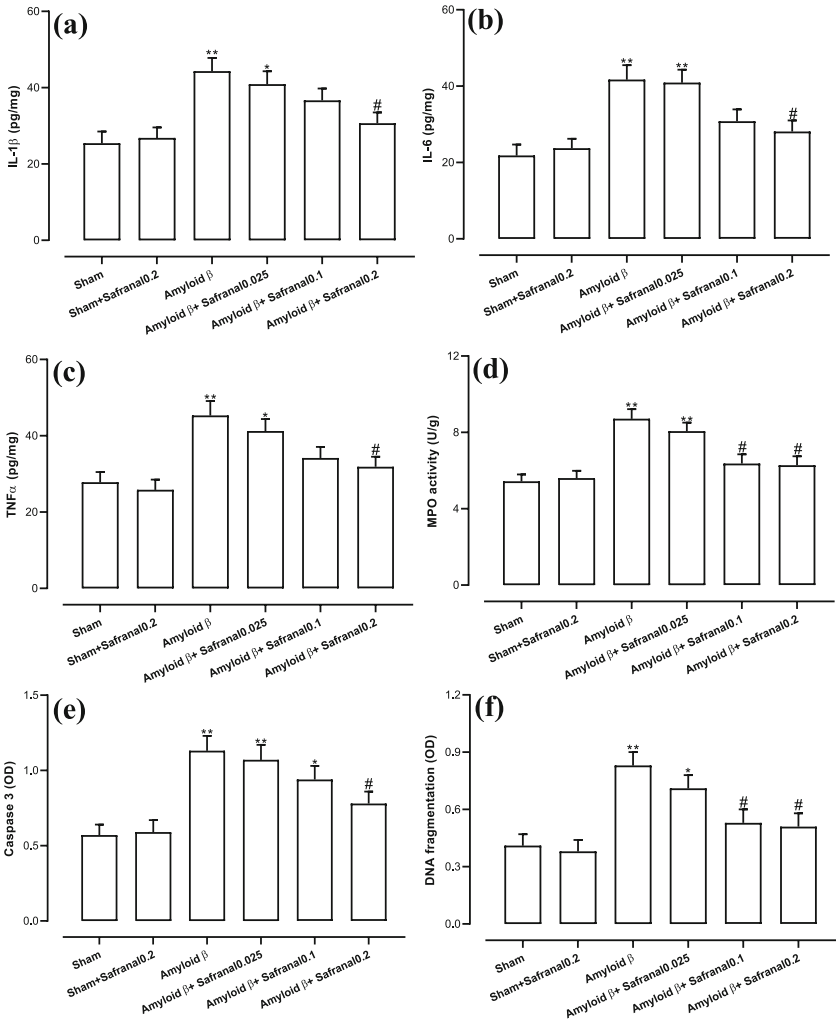

Fig. 5 Hippocampal level of interleukin 1β (IL-1 β) (**a**), interleukin 6 (IL-6) (**b**), tumor necrosis factor α (TNF α) (**c**), myeloperoxidase (MPO) activity (**d**), caspase 3 activity (**e**), and DNA fragmentation (f). n = 6 for each group. Values are means ± S.E.M. * $p < 0.05$, ** $p < 0.01$ (versus Sham); # $p < 0.05$ (versus amyloid β)

safranal treatment of amyloid β group at doses of 0.1 or 0.2 ml/kg significantly prevented this reduction as compared to amyloid β group ($p = 0.037$ and $p = 0.024$, respectively).

Discussion

Earlier reports have shown that intrahippocampal amyloid β microinjection is associated with deficits in learning and memory processes (Ghofrani et al. 2015; Liu et al. 2015; Rasoolijazi et al. 2007). In this respect, amyloid β_{1-40}-induced learning and memory disturbances occur for behavioral tasks including Y maze (Fahanik-Babaei et al. 2019), passive avoidance (Tsai et al. 2015), novel object discrimination (Fahanik-Babaei et al. 2019; Santos et al. 2017), and radial arm maze (Hashimoto et al. 2011) that have been consistent with findings of this study. Additionally, oral administration of safranal mitigated cognitive deficits induced by intrahippocampal injection of amyloid β_{1-40}. Previous studies have shown the beneficial effect of saffron or its active ingredient safranal in different models of cognitive deficits (Delkhosh-Kasmaie et al. 2018; Linardaki et al. 2017; Papandreou et al. 2011).

In this research study, at the end of week 3 following intrahippocampal microinjection of amyloid β_{1-40}, hippocampal level of oxidative stress biomarkers including MDA as a consistent indicator of lipid peroxidation, ROS, and protein carbonyl elevated and level and/or activity of antioxidants consisting of catalase, SOD, and GSH depressed that is in agreement with earlier research studies (Fahanik-Babaei et al. 2019; Guerra de Souza et al. 2018; Lin et al. 2019). Oxidative stress burden as a causative factor strongly participates in pathogenesis of AD (Bisht et al. 2018). There are research evidence indicating incremental and inappropriate effect of oxidative damage during progression of AD with subsequent emergence of clinical and pathological symptoms of AD such as cognitive decline (Bonda et al. 2010; Swomley and Butterfield 2015). Oral safranal in this study was capable to ameliorate oxidative stress and to improve SOD activity following intrahippocampal $A\beta_{1-40}$. Such beneficial effects for safranal to lower oxidative stress in toxic conditions have already been reported (Ahmad et al. 2017; Pan et al. 2016).

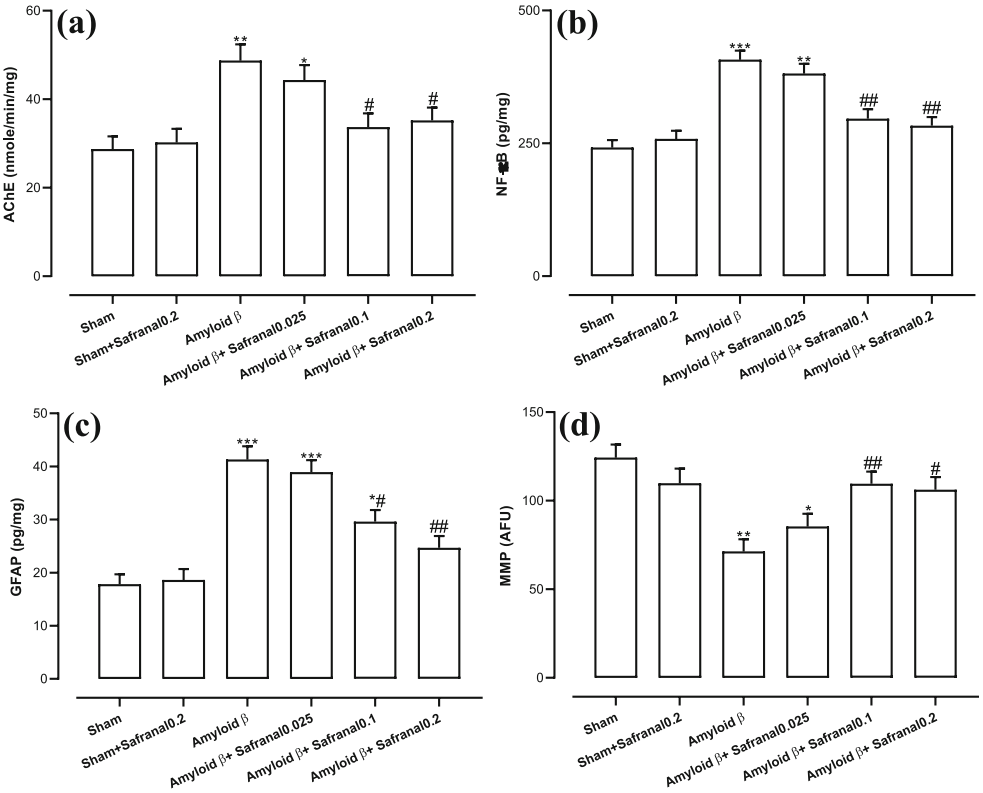

Fig. 6 Hippocampal level of acetylcholinesterase (AChE) activity (**a**), nuclear factor-kappaB (NF-*k*B) (**b**), glial fibrillary acidic protein (GFAP) (**c**), and mitochondrial membrane potential (MMP) (d). n = 6 for each group. Values are means ± S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ (versus Sham); # $p < 0.05$, ## $p < 0.01$ (versus amyloid β)

In this study, we also determined inflammatory biomarkers comprising IL-1β, IL-6, TNFα, and NF-κB in hippocampal tissue. These markers had a significant and notable elevation to varying degrees following intrahippocampal $A\beta_{1-40}$. Development of inflammation in the brain is also a distinctive pathological feature of neurodegenerative diseases such as AD (Kim et al. 2014; Pimplikar 2014). Brain inflammation is largely attributed to activation and mobilization of astrocytes and microglia. Amyloid β challenge stimulate the latter cells to produce and release a variety of pro-inflammatory cytokines with subsequent damage of the neurons (Li et al. 2011). NF-κB itself plays a pivotal

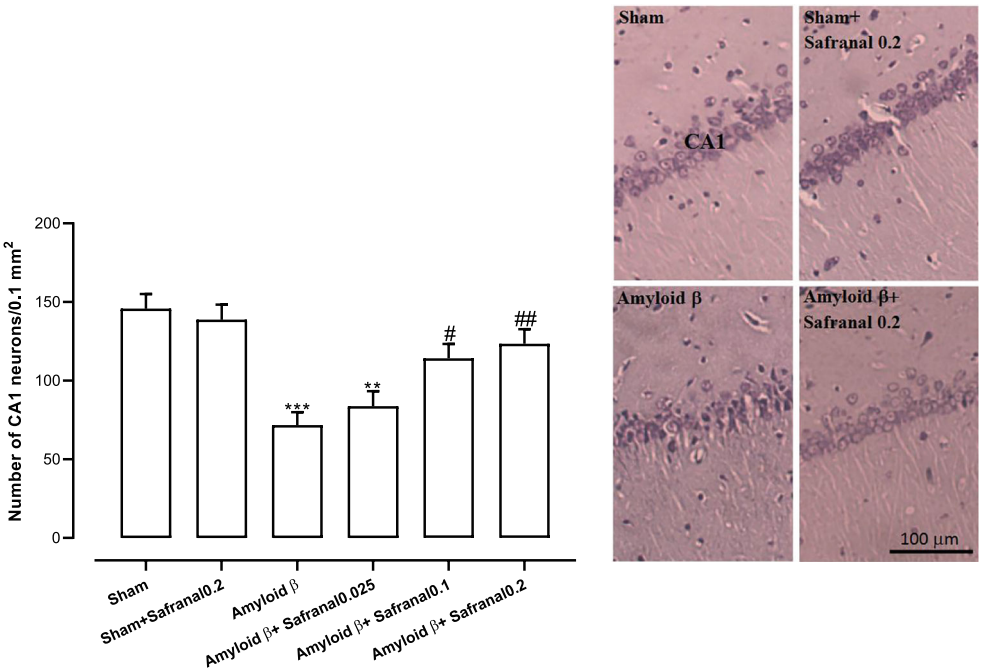

Fig. 7 Number of hippocampal CA1 pyramidal neurons in Nissl staining and related coronal sections through the hippocampus. $n = 4–5$ for each group. Values are means ± S.E.M.** $p < 0.01$, *** $p < 0.001$ (versus Sham); # $p < 0.05$, ## $p < 0.01$ (versus amyloid β)

role in amyloid β-induced production and release of proinflammatory cytokines (Bales et al. 1998). In agreement with our findings, earlier reports have also shown anti-inflammatory potential of safranal (Gholamnezhad et al. 2013; Tamaddonfard et al. 2013).

In the present study, we had a higher activity of AChE in the hippocampus of amyloid β-challenged group and safranal attenuated this change. Cholinesterases overexpression and/or activity in an indicator of central cholinergic dysfunction (Mufson et al. 1991) and this dysfunction could play a pivotal role in AD pathogenesis (Wang et al. 2014). However, there is no evidence whether safranal has direct and/or indirect effects on cholinesterase in AD that itself requires further studies.

To assess mitochondrial health status following amyloid β and safranal, MMP was determined. It has been shown that amyloid β exposure accentuates production of deleterious free radicals with subsequent mitochondrial dysfunction and cell damage (Reddy 2006). Besides, it has been claimed that sporadic AD may be linked to mitochondrial dysfunction (Mancuso et al. 2010; Swerdlow et al. 2010). In our study, safranal prevented MMP decline due to amyloid β, indicating its ability to preserve mitochondrial health. Consistent with these findings, it has shown that crocin as another active ingredient of saffron is able to reduce cell lysis, lipid peroxidation, ROS production, MMP decline, and cellular proteolysis (Yousefsani et al. 2018) and is able to protect PC12 cells against 1-methyl-4-phenylpyridinium toxicity through attenuation of mitochondrial dysfunction and endoplasmic reticulum stress (Zhang et al. 2015).

In this study, we also observe a prominent and significant elevation of apoptotic biomarkers including caspase 3 and DNA fragmentation in the hippocampal tissue in amyloid β_{1-40}-microinjected group that was consistent with earlier reports (Boland and Campbell 2004; Davis et al. 1999; Liu and Zhao 2004). In contrast to these findings, some reports exist indicating that amyloid β_{1-40} could not significantly raise apoptotic indices like DNA condensation and fragmentation and caspase 3 activation in some cell types (Chen and Dong 2009). Safranal treatment of amyloid β group was successful to lower apoptosis. Consistent with this finding, anti-apoptotic potential of safranal has been reported before (Pan et al. 2016; Rafieipour et al. 2019).

Intrahippocampal amyloid β_{1-40} is also associated with increased expression of GFAP that is clearly suggestive of an inflammatory event and occurrence of astrogliosis (Cirillo et al. 2015; Deng et al. 2017). Besides, safranal treatment reduced hippocampal GFAP that is most probably related to its anti-inflammatory effect and is in agreement with an earlier report regarding its anti-inflammatory activity. In support of this fact, it has been reported that safranal could attenuate expression of GFAP and inflammatory cytokines including TNF-α and IL-1β in a rat model of spinal nerve transection (Zhu and Yang 2014).

One of the limitations of this study was the lack of a correlation analysis. Although such analysis could enrich our study, however, due to many biochemical and behavioral parameters of different natures that were measured, it was not practically possible to have such an analysis. Another limitation of this study was that we did not evaluate the time course of the beneficial effect of safranal treatment post-Aβ for a longer duration, in other words, how long its effects persist. Such analyses and studies are strongly warranted to be included and/or conducted in future studies.

Collectively, it is concluded that safranal treatment of intrahippocampal amyloid beta-microinjected rats could prevent learning and memory decline via neuronal protection and at a molecular level through amelioration of oxidative stress, inflammation, acetylcholinesterase (AChE) activity, neutrophil infiltration, and also by way of preservation of mitochondrial integrity. This may be considered as a subordinate therapeutic supplement for reducing the development of neurodegenerative diseases like AD.

Acknowledgements This work was approved and financially supported by a grant from Research Council of Iran University of Medical Sciences (grant no. 96-04-30-32487).

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest for this research project.

References

Afshin-Majd S, Khalili M, Roghani M, Mehranmehr N, Baluchnejadmojarad T (2015) Carnosine exerts neuroprotective effect against 6-hydroxydopamine toxicity in hemiparkinsonian rat. Mol Neurobiol 51:1064–1070. https://doi.org/10.1007/s12035-014-8771-0

Ahmad N, Ahmad R, Abbas Naqvi A, Ashafaq M, Alam MA, Ahmad FJ, Al-Ghamdi MS (2017) The effect of safranal loaded mucoadhesive nanoemulsion on oxidative stress markers in cerebral ischemia. Artif Cells Nanomed Biotechnol 45:775–787. https://doi.org/10.1080/21691401.2016.1228659

Alavi MS, Fanoudi S, Fard AV, Soukhtanloo M, Hosseini M, Barzegar H, Sadeghnia HR (2018) Safranal attenuates excitotoxin-induced oxidative OLN-93 cells injury. Drug Res (Stuttg). https://doi.org/10.1055/a-0790-8200

Arya A, Sethy NK, Singh SK, Das M, Bhargava K (2013) Cerium oxide nanoparticles protect rodent lungs from hypobaric hypoxia-induced oxidative stress and inflammation. Int J Nanomedicine 8:4507–4520. https://doi.org/10.2147/ijn.s53032

Bales KR, Du Y, Dodel RC, Yan GM, Hamilton-Byrd E, Paul SM (1998) The NF-kappaB/Rel family of proteins mediates Abeta-induced neurotoxicity and glial activation. Brain Res Mol Brain Res 57:63–72

Baluchnejadmojarad T, Roghani M (2003) Garlic extract attenuates time-dependent changes in the reactivity of isolated aorta in streptozotocin-diabetic rats. Life Sci 73:2281–2289

Baluchnejadmojarad T, Kiasalari Z, Afshin-Majd S, Ghasemi Z, Roghani M (2017) S-allyl cysteine ameliorates cognitive deficits in

streptozotocin-diabetic rats via suppression of oxidative stress, inflammation, and acetylcholinesterase. Eur J Pharmacol 794:69–76. https://doi.org/10.1016/j.ejphar.2016.11.033

Bertoni-Freddari C, Fattoretti P, Casoli T, Di Stefano G, Balietti M, Giorgetti B, Perretta G (2009) Neuronal apoptosis in Alzheimer's disease: the role of age-related mitochondrial metabolic competence. Ann N Y Acad Sci 1171:18–24. https://doi.org/10.1111/j.1749-6632.2009.04886.x

Bisht K, Sharma K, Tremblay ME (2018) Chronic stress as a risk factor for Alzheimer's disease: roles of microglia-mediated synaptic remodeling, inflammation, and oxidative stress. Neurobiol Stress 9: 9–21. https://doi.org/10.1016/j.ynstr.2018.05.003

Boland B, Campbell V (2004) Abeta-mediated activation of the apoptotic cascade in cultured cortical neurones: a role for cathepsin-L. Neurobiol Aging 25:83–91

Bonda DJ, Wang X, Perry G, Nunomura A, Tabaton M, Zhu X, Smith MA (2010) Oxidative stress in Alzheimer disease: a possibility for prevention. Neuropharmacology 59:290–294. https://doi.org/10.1016/j.neuropharm.2010.04.005

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Briggs R, Kennelly SP, O'Neill D (2016) Drug treatments in Alzheimer's disease. Clin Med (Lond) 16:247–253. https://doi.org/10.7861/clinmedicine.16-3-247

Butterfield DA, Bader Lange ML, Sultana R (2010) Involvements of the lipid peroxidation product, HNE, in the pathogenesis and progression of Alzheimer's disease. Biochim Biophys Acta 1801:924–929. https://doi.org/10.1016/j.bbalip.2010.02.005

Cheignon C, Tomas M, Bonnefont-Rousselot D, Faller P, Hureau C, Collin F (2018) Oxidative stress and the amyloid beta peptide in Alzheimer's disease. Redox Biol 14:450–464. https://doi.org/10.1016/j.redox.2017.10.014

Chen Y, Dong C (2009) Abeta40 promotes neuronal cell fate in neural progenitor cells. Cell Death Differ 16:386–394. https://doi.org/10.1038/cdd.2008.94

Cioanca O, Hritcu L, Mihasan M, Hancianu M (2013) Cognitive-enhancing and antioxidant activities of inhaled coriander volatile oil in amyloid beta(1-42) rat model of Alzheimer's disease. Physiol Behav 120:193–202. https://doi.org/10.1016/j.physbeh.2013.08.006

Cirillo C, Capoccia E, Iuvone T, Cuomo R, Sarnelli G, Steardo L, Esposito G (2015) S100B inhibitor Pentamidine attenuates reactive gliosis and reduces neuronal loss in a mouse model of Alzheimer's disease. Biomed Res Int 2015:508342–508311. https://doi.org/10.1155/2015/508342

Claiborne A (1985) Catalase activity. In: CRC handbook of methods for oxygen radical research, edited by Greenwald RA. Boca Raton, FL: CRC.: 283–284

Collin F, Cheignon C, Hureau C (2018) Oxidative stress as a biomarker for Alzheimer's disease. Biomark Med 12:201–203. https://doi.org/10.2217/bmm-2017-0456

Davis J, Cribbs DH, Cotman CW, Van Nostrand WE (1999) Pathogenic amyloid beta-protein induces apoptosis in cultured human cerebrovascular smooth muscle cells. Amyloid 6:157–164

Delkhosh-Kasmaie F, Farshid AA, Tamaddonfard E, Imani M (2018) The effects of safranal, a constitute of saffron, and metformin on spatial learning and memory impairments in type-1 diabetic rats: behavioral and hippocampal histopathological and biochemical evaluations. Biomed Pharmacother 107:203–211. https://doi.org/10.1016/j.biopha.2018.07.165

Deng Y, Long L, Wang K, Zhou J, Zeng L, He L, Gong Q (2017) Icariside II, a broad-Spectrum anti-cancer agent, reverses Beta-amyloid-induced cognitive impairment through reducing inflammation and apoptosis in rats. Front Pharmacol 8:39. https://doi.org/10.3389/fphar.2017.00039

Ding J, Yu HL, Ma WW, Xi YD, Zhao X, Yuan LH, Feng JF, Xiao R (2013) Soy isoflavone attenuates brain mitochondrial oxidative stress induced by beta-amyloid peptides 1-42 injection in lateral cerebral ventricle. J Neurosci Res 91:562–567. https://doi.org/10.1002/jnr.23163

Dos Santos Picanco LC, Ozela PF, de Fatima de Brito M, Pinheiro AA, Padilha EC, Braga FS, de Paula da Silva CHT, Dos Santos CBR, Rosa JMC, da Silva Hage-Melim LI (2018) Alzheimer's disease: a review from the pathophysiology to diagnosis, new perspectives for pharmacological treatment. Curr Med Chem 25:3141–3159. https://doi.org/10.2174/0929867323666161213101126

Eckert GP, Renner K, Eckert SH, Eckmann J, Hagl S, Abdel-Kader RM, Kurz C, Leuner K, Muller WE (2012) Mitochondrial dysfunction–a pharmacological target in Alzheimer's disease. Mol Neurobiol 46: 136–150. https://doi.org/10.1007/s12035-012-8271-z

Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82: 70–77

Fahanik-Babaei J, Baluchnejadmojarad T, Nikbakht F, Roghani M (2019) Trigonelline protects hippocampus against intracerebral Abeta(1-40) as a model of Alzheimer's disease in the rat: insights into underlying mechanisms. Metab Brain Dis 34:191–201. https://doi.org/10.1007/s11011-018-0338-8

Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM (2016) Alzheimer's disease: targeting the cholinergic system. Curr Neuropharmacol 14:101–115

Ghofrani S, Joghataei MT, Mohseni S, Baluchnejadmojarad T, Bagheri M, Khamse S, Roghani M (2015) Naringenin improves learning and memory in an Alzheimer's disease rat model: insights into the underlying mechanisms. Eur J Pharmacol 764:195–201. https://doi.org/10.1016/j.ejphar.2015.07.001

Gholamnezhad Z, Koushyar H, Byrami G, Boskabady MH (2013) The extract of Crocus sativus and its constituent Safranal, affect serum levels of endothelin and Total protein in sensitized Guinea pigs. Iran J Basic Med Sci 16:1022–1026

Guerra de Souza AC, Goncalves CL, de Souza V, Hartwig JM, Farina M, Prediger RD (2018) Agmatine attenuates depressive-like behavior and hippocampal oxidative stress following amyloid beta (Abeta1-40) administration in mice. Behav Brain Res 353:51–56. https://doi.org/10.1016/j.bbr.2018.06.032

Hashimoto M, Tozawa R, Katakura M, Shahdat H, Haque AM, Tanabe Y, Gamoh S, Shido O (2011) Protective effects of prescription n-3 fatty acids against impairment of spatial cognitive learning ability in amyloid beta-infused rats. Food Funct 2:386–394. https://doi.org/10.1039/c1fo00002k

Hritcu L, Cioanca O, Hancianu M (2012) Effects of lavender oil inhalation on improving scopolamine-induced spatial memory impairment in laboratory rats. Phytomedicine 19:529–534. https://doi.org/10.1016/j.phymed.2012.02.002

Hritcu L, Noumedem JA, Cioanca O, Hancianu M, Kuete V, Mihasan M (2014) Methanolic extract of Piper nigrum fruits improves memory impairment by decreasing brain oxidative stress in amyloid beta(1-42) rat model of Alzheimer's disease. Cell Mol Neurobiol 34:437–449. https://doi.org/10.1007/s10571-014-0028-y

Islam MA, Khandker SS, Alam F, Khalil MI, Kamal MA, Gan SH (2017) Alzheimer's disease and natural products: future regimens emerging from nature. Curr Top Med Chem 17:1408–1428. https://doi.org/10.2174/1568026617666170103163054

Isomae K, Morimoto S, Hasegawa H, Morita K, Kamei J (2003) Effects of T-82, a novel acetylcholinesterase inhibitor, on impaired learning and memory in passive avoidance task in rats. Eur J Pharmacol 465: 97–103

Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R (2018) NIA-AA

research framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement 14:535–562. https://doi.org/10.1016/j.jalz.2018.02.018

Kim M, Kim SO, Lee M, Lee JH, Jung WS, Moon SK, Kim YS, Cho KH, Ko CN, Lee EH (2014) Tetramethylpyrazine, a natural alkaloid, attenuates pro-inflammatory mediators induced by amyloid beta and interferon-gamma in rat brain microglia. Eur J Pharmacol 740: 504–511. https://doi.org/10.1016/j.ejphar.2014.06.037

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER (1990) Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 186: 464–478

Li C, Zhao R, Gao K, Wei Z, Yin MY, Lau LT, Chui D, Yu AC (2011) Astrocytes: implications for neuroinflammatory pathogenesis of Alzheimer's disease. Curr Alzheimer Res 8:67–80

Lin LW, Tsai FH, Lan WC, Cheng YD, Lee SC, Wu CR (2019) Steroid-enriched fraction of achyranthes bidentata protects amyloid beta peptide 1-40-induced cognitive dysfunction and neuroinflammation in rats. Mol Neurobiol. https://doi.org/10.1007/s12035-018-1436-7

Linardaki ZI, Lamari FN, Margarity M (2017) Saffron (Crocus sativus L.) tea intake prevents learning/memory defects and Neurobiochemical alterations induced by aflatoxin B1 exposure in adult mice. Neurochem Res 42:2743–2754. https://doi.org/10.1007/s11064-017-2283-z

Liu Q, Zhao B (2004) Nicotine attenuates beta-amyloid peptide-induced neurotoxicity, free radical and calcium accumulation in hippocampal neuronal cultures. Br J Pharmacol 141:746–754. https://doi.org/10.1038/sj.bjp.0705653

Liu H, Deng Y, Gao J, Liu Y, Li W, Shi J, Gong Q (2015) Sodium hydrosulfide attenuates Beta-amyloid-induced cognitive deficits and Neuroinflammation via modulation of MAPK/NF-kappaB pathway in rats. Curr Alzheimer Res 12:673–683

Ma W, Yuan L, Yu H, Ding B, Xi Y, Feng J, Xiao R (2010) Genistein as a neuroprotective antioxidant attenuates redox imbalance induced by beta-amyloid peptides 25-35 in PC12 cells. Int J Dev Neurosci 28: 289–295. https://doi.org/10.1016/j.ijdevneu.2010.03.003

Majithiya JB, Balaraman R (2006) Metformin reduces blood pressure and restores endothelial function in aorta of streptozotocin-induced diabetic rats. Life Sci 78:2615–2624. https://doi.org/10.1016/j.lfs.2005.10.020

Mancuso M, Orsucci D, LoGerfo A, Calsolaro V, Siciliano G (2010) Clinical features and pathogenesis of Alzheimer's disease: involvement of mitochondria and mitochondrial DNA. Adv Exp Med Biol 685:34–44

Mufson EJ, Presley LN, Kordower JH (1991) Nerve growth factor receptor immunoreactivity within the nucleus basalis (Ch4) in Parkinson's disease: reduced cell numbers and co-localization with cholinergic neurons. Brain Res 539:19–30

Olton DS, Samuelson RJ (1976) Remembrance of places passed: spatial memory in rats. J Exp Psychol Anim Behav Process 2:97–116

Pan PK, Qiao LY, Wen XN (2016) Safranal prevents rotenone-induced oxidative stress and apoptosis in an in vitro model of Parkinson's disease through regulating Keap1/Nrf2 signaling pathway. Cell Mol Biol (Noisy-Le-Grand, France) 62: 11-17. https://doi.org/10.14715/cmb/2016.62.14.2

Papandreou MA, Tsachaki M, Efthimiopoulos S, Cordopatis P, Lamari FN, Margarity M (2011) Memory enhancing effects of saffron in aged mice are correlated with antioxidant protection. Behav Brain Res 219:197–204. https://doi.org/10.1016/j.bbr.2011.01.007

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd edn. Academic Press, New York

Piermartiri TC, Figueiredo CP, Rial D, Duarte FS, Bezerra SC, Mancini G, de Bem AF, Prediger RD, Tasca CI (2010) Atorvastatin prevents hippocampal cell death, neuroinflammation and oxidative stress following amyloid-beta(1-40) administration in mice: evidence for dissociation between cognitive deficits and neuronal damage. Exp Neurol 226:274–284. https://doi.org/10.1016/j.expneurol.2010.08.030

Pimplikar SW (2014) Neuroinflammation in Alzheimer's disease: from pathogenesis to a therapeutic target. J Clin Immunol 34(Suppl 1): S64–S69. https://doi.org/10.1007/s10875-014-0032-5

Pulli B, Ali M, Forghani R, Schob S, Hsieh KL, Wojtkiewicz G, Linnoila JJ, Chen JW (2013) Measuring myeloperoxidase activity in biological samples. PLoS One 8:e67976. https://doi.org/10.1371/journal.pone.0067976

Rafieipour F, Hadipour E, Emami SA, Asili J, Tayarani-Najaran Z (2019) Safranal protects against beta-amyloid peptide-induced cell toxicity in PC12 cells via MAPK and PI3 K pathways. Metab Brain Dis 34: 165–172. https://doi.org/10.1007/s11011-018-0329-9

Raoufi S, Baluchnejadmojarad T, Roghani M, Ghazanfari T, Khojasteh F, Mansouri M (2015) Antidiabetic potential of salvianolic acid B in multiple low-dose streptozotocin-induced diabetes. Pharm Biol 53: 1803–1809. https://doi.org/10.3109/13880209.2015.1008148

Rasoolijazi H, Joghataie MT, Roghani M, Nobakht M (2007) The beneficial effect of (−)-epigallocatechin-3-gallate in an experimental model of Alzheimer's disease in rat: a behavioral analysis. Iran Biomed J 11:237–243

Reddy PH (2006) Amyloid precursor protein-mediated free radicals and oxidative damage: implications for the development and progression of Alzheimer's disease. J Neurochem 96:1–13. https://doi.org/10.1111/j.1471-4159.2005.03530.x

Rezaee R, Hosseinzadeh H (2013) Safranal: from an aromatic natural product to a rewarding pharmacological agent. Iran J Basic Med Sci 16:12–26

Roghani M, Joghataie MT, Jalili MR, Baluchnejadmojarad T (2006) Time course of changes in passive avoidance and Y-maze performance in male diabetic rats. Iran Biomed J 10:99–104

Sadeghnia HR, Shaterzadeh H, Forouzanfar F, Hosseinzadeh H (2017) Neuroprotective effect of safranal, an active ingredient of *Crocus sativus* , in a rat model of transient cerebral ischemia. Folia Neuropathol 55:206–213. https://doi.org/10.5114/fn.2017.70485

Santos VV, Stark R, Rial D, Silva HB, Bayliss JA, Lemus MB, Davies JS, Cunha RA, Prediger RD, Andrews ZB (2017) Acyl ghrelin improves cognition, synaptic plasticity deficits and neuroinflammation following amyloid beta (Abeta1-40) administration in mice. Journal of Neuroendocrinology 29. https://doi.org/10.1111/jne.12476

Shagirtha K, Pari L (2011) Hesperetin, a citrus flavonone, protects potentially cadmium induced oxidative testicular dysfunction in rats. Ecotoxicol Environ Saf 74:2105–2111. https://doi.org/10.1016/j.ecoenv.2011.06.002

Swerdlow RH, Burns JM, Khan SM (2010) The Alzheimer's disease mitochondrial cascade hypothesis. J Alzheimers Dis 20(Suppl 2): S265–S279. https://doi.org/10.3233/JAD-2010-100339

Swomley AM, Butterfield DA (2015) Oxidative stress in Alzheimer disease and mild cognitive impairment: evidence from human data provided by redox proteomics. Arch Toxicol 89:1669–1680. https://doi.org/10.1007/s00204-015-1556-z

Tamaddonfard E, Farshid AA, Eghdami K, Samadi F, Erfanparast A (2013) Comparison of the effects of crocin, safranal and diclofenac on local inflammation and inflammatory pain responses induced by carrageenan in rats. Pharmacol Rep 65:1272–1280

Tamaddonfard E, Erfanparast A, Farshid AA, Imani M, Mirzakhani N, Salighedar R, Tamaddonfard S (2019) Safranal, a constituent of saffron, exerts gastro-protective effects against indomethacin-induced gastric ulcer. Life Sci. https://doi.org/10.1016/j.lfs.2019.03.054

Tobon-Velasco JC, Vazquez-Victorio G, Macias-Silva M, Cuevas E, Ali SF, Maldonado PD, Gonzalez-Trujano ME, Cuadrado A, Pedraza-Chaverri J, Santamaria A (2012) S-allyl cysteine protects against 6-hydroxydopamine-induced neurotoxicity in the rat striatum: involvement of Nrf2 transcription factor activation and modulation

of signaling kinase cascades. Free Radic Biol Med 53:1024–1040. https://doi.org/10.1016/j.freeradbiomed.2012.06.040

Tsai FS, Wu LY, Yang SE, Cheng HY, Tsai CC, Wu CR, Lin LW (2015) Ferulic acid reverses the cognitive dysfunction caused by amyloid beta peptide 1-40 through anti-oxidant activity and cholinergic activation in rats. Am J Chin Med 43:319–335. https://doi.org/10.1142/s0192415x15500214

Wang H, Yu Y, Chen W, Cui Y, Luo T, Ma J, Jiang XC, Qin S (2014) PLTP deficiency impairs learning and memory capabilities partially due to alteration of amyloid-beta metabolism in old mice. J Alzheimers Dis 39:79–88. https://doi.org/10.3233/jad-130812

Yousefsani BS, Mehri S, Pourahmad J, Hosseinzadeh H (2018) Crocin prevents sub-cellular organelle damage, proteolysis and apoptosis in rat hepatocytes: a justification for its Hepatoprotection. Iran J Pharm Res 17:553–562

Zarezadeh M, Baluchnejadmojarad T, Kiasalari Z, Afshin-Majd S, Roghani M (2017) Garlic active constituent s-allyl cysteine protects against lipopolysaccharide-induced cognitive deficits in the rat: possible involved mechanisms. Eur J Pharmacol 795:13–21. https://doi.org/10.1016/j.ejphar.2016.11.051

Zhang H, Liu Y, Lao M, Ma Z, Yi X (2011) Puerarin protects Alzheimer's disease neuronal cybrids from oxidant-stress induced apoptosis by inhibiting pro-death signaling pathways. Exp Gerontol 46:30–37. https://doi.org/10.1016/j.exger.2010.09.013

Zhang GF, Zhang Y, Zhao G (2015) Crocin protects PC12 cells against MPP(+)-induced injury through inhibition of mitochondrial dysfunction and ER stress. Neurochem Int 89:101–110. https://doi.org/10.1016/j.neuint.2015.07.011

Zhu KJ, Yang JS (2014) Anti-allodynia effect of safranal on neuropathic pain induced by spinal nerve transection in rat. Int J Clin Exp Med 7:4990–4996

Zhu M, Wang X, Sun L, Schultzberg M, Hjorth E (2018) Can inflammation be resolved in Alzheimer's disease? Ther Adv Neurol Disord 11:1756286418791107. https://doi.org/10.1177/1756286418791107

Ziegler-Waldkirch S, Meyer-Luehmann M (2018) The role of glial cells and synapse loss in mouse models of Alzheimer's disease. Front Cell Neurosci 12:473. https://doi.org/10.3389/fncel.2018.00473