Research in Pharmaceutical Sciences, June 2023; 18(3): 270-278
Received:08-06-2022
Peer Reviewed:13-07-2022
Revised: 09-10-2022
Accepted: 13-02-2023
Published: 14-3-2023

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Betaine attenuates oxidative stress and cognitive dysfunction in an amyloid β-induced rat model of Alzheimer's disease

Fatemeh Alipourfard[1], Hooman Shajiee[1,*], Farzaneh Nazari-Serenjeh[2,*], Vida Hojati[1], And Masoud Alirezaie[3]

[1]Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran
[2]Department of Biology, Payame Noor University (PNU), Tehran, Iran
[3]Division of Biochemistry, Faculty of Veterinary Medicine, Lorestan University, Khorràmabad, Iran

Abstract

Background and purpose: Increasing evidence indicates that oxidative stress is an important factor in the pathogenesis and progression of Alzheimer's disease (AD). Betaine is trimethylglycine with antioxidant and neuroprotective properties. The present study aimed to evaluate the possible beneficial effects of betaine on oxidative stress and memory deficits induced by intrahippocampal injection of amyloid beta (Aß) in an AD model.

Experimental approach: Forty adult male Wistar rats were divided into 5 equal groups: the control and Aß groups which received oral gavage of saline (1 mL daily) for 14 days. The other 3 groups (betaine + Aß) received betaine (5, 10, and 15 mg/kg, orally) for 14 consecutive days. On the 15th day, all of the groups were injected bilaterallyintrahippocampal of Aß (5 µg/µL), except controls that were injected with normal saline as a vehicle. Seven days after the Aß injection, memory was assessed in a passive avoidance test. Changes in catalase activities and glutathione peroxidase, glutathione, and malondialdehyde concentrations were investigated to determine the antioxidant activity in the rat hippocampus.

Findings/Results: Data showed that betaine pretreatment of Aß-injected rats improved memory in avoidance tasks. In addition, betaine pretreatment attenuated oxidative stress.

Conclusion and implications: The current findings showed that oral administration of betaine could prevent Aß-induced impairment of memory possibly through suppression of oxidative stress in the hippocampus area of rats.

Keywords:Alzheimer's disease; Betaine; Amyloid beta; Learning and memory; Oxidative stress.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized clinically by cognitive dysfunction and memory loss (1). Abnormal accumulation of extracellular senile plaque of the amyloid-ß peptide (Aß) and intracellular hyper-phosphorylated-tau proteins are the main neuropathological hallmarks of AD (2). However, studies show that in brain areas involved in AD, the levels of oxidative damage biomarkers are increased indicating an important role of oxidative stress in the pathogenesis of AD (3). In addition, several lines of evidence have shown that one of the main mechanisms by which Aß accumulation contributed to AD pathogenesis is the induction of oxidative stress (4,5). Therefore, the prevention of oxidative stress is considered a potential target to inhibit AD progression and pathogenesis (6).

Betaine (trimethylglycine) is endogenously synthesized from choline. Betaine is recognized as a methyl donor agent in the conversion of homocysteine to methionine *via*betaine-homocysteinemethyltransferase(7).

*Corresponding authors:
H. Shajiee, Tel & Fax: +98-2325261034
Email: shajiee@damghan.ac.ir
F. Nazari-Serenjeh, Tel: +98-۲۱۲۲۴۴۲۰۳۶, Fax: +98-21۲۲٤٥٥٤۲۹
Email: nazari.f@pnu.ac.ir

Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/1735-5362.371583

Elevated plasma homocysteine induces memory impairment and AD-like pathological changes in the brain (8,9), subsequently, betaine prevents the toxic accumulation of homocysteine. On the other hand, it is well-documented that betaine has antioxidant properties and supplementation with betaine was able to protect the brain against oxidative stress inducers such as ethanol and levodopa (10,11).

In recent years, the modulatory effects of betaine on memory and hippocampus function have been reported (12-14). Research on mechanisms by which betaine improves memory performance has been carried out. A previous report demonstrated that the administration of betaine ameliorates memory impairments induced by water-immersion restraint stress *via* the changes in the hippocampal neuronal system (15). Furthermore, betaine increases long-term potentiation, expression of the synapse-associated protein, and the number of dendritic spines in the hippocampus of homocysteine-injected rats (8). Recent studies by Ibi and co-workers indicated that betaine prevents the progression of AD by improving the expression of genes involved in antioxidant activity and synapse formation in the hippocampus (16,17). It has been reported that betaine induces autophagy that leads to the inhibition of Aß accumulation (18). Although, the antioxidant properties of betaine have been widely studied and betaine has been introduced as an antioxidant agent. The effect of betaine on the antioxidant system in Aßinduced model of AD has not yet been well studied.

Therefore, in the present study, we investigated the effects of betaine on Aß-induced memory impairment in the passive avoidance learning task. We also investigated the effect of betaine treatment on Aß-induced changes in oxidative stress markers in the hippocampus area of rats. For this goal, glutathione peroxidase (GPx), catalase (CAT) activities, glutathione (GSH) levels, and malondialdehyde (MDA) concentrations as lipid peroxidation markers were measured.

MATERIALS AND METHODS

Animals

In the present study, 40 adults male Wistar rats weighing 200-220g were used. The animals were housed under standard laboratory conditions with a natural 12/12-h light/dark photocycle with free access to food and water. All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals under the supervision of the ethics committee of Lorestan University of Medical Sciences with Approval No. LU. ECRA 2019/15.

Treatments and kits

Aß peptide (Aß1-42) (Enzo, Life Sciences, USA) and betaine(Betafin® 96%) (Biochem Company, Germany) were dissolved in sterile saline (0.9%). GPx, CAT, GSH, and MDA kits were obtained from Kiazist, Life Sciences, Iran.

Experimental design

Forty rats were divided into 5 equal groups (n = 8) including the control (normal saline as a vehicle was injected) and Aß groups which received oral gavage of saline (1 mL,daily) for 14 days. The other 3 groups (betaine + Aß) received betaine (5, 10, and 15 mg/kg, orally) for 14 consecutive days. On the 15th day, all of the groups were injected bilaterally intrahippocampal of Aß (5 µg/µL), except controls that injected normal saline as a vehicle. Aß was injected into the CA1 region of the hippocampus at 5 µg/µL once on the surgery day. The dose of Aß was selected on the basis of previous studies (19,20) and a pilot study in our laboratory. Betaine was administered at doses of 5, 10, and 15 mg/kg orally once daily for 14 consecutive days according to our report (10) and a previous study (6), before the administration of Aß(Fig. 1).

Surgery and intra-CA1 administration of Aß

Betaine treatment was stopped on the 14th day and the animals were anesthetized by intraperitoneal injection of xylazine (5 mg/kg, i.p.) and ketamine (100 mg/kg, i.p.) (Merck Company, Germany) for stereotaxic surgery. Rats were placed on a stereotaxic frame (ST3000 Company, Iran) and two stainless steel guide cannulas (21-gauge) were inserted into the skull 1 mm above the CA1 region of the hippocampus (Fig. 2). The coordinates for the CA1 were AP: 3.3 mm posterior to bregma, L: ±2 mm lateral to the midline and DV = 2.8 mm ventral from the skull surface according to the atlas of Paxinos and Watson (21).

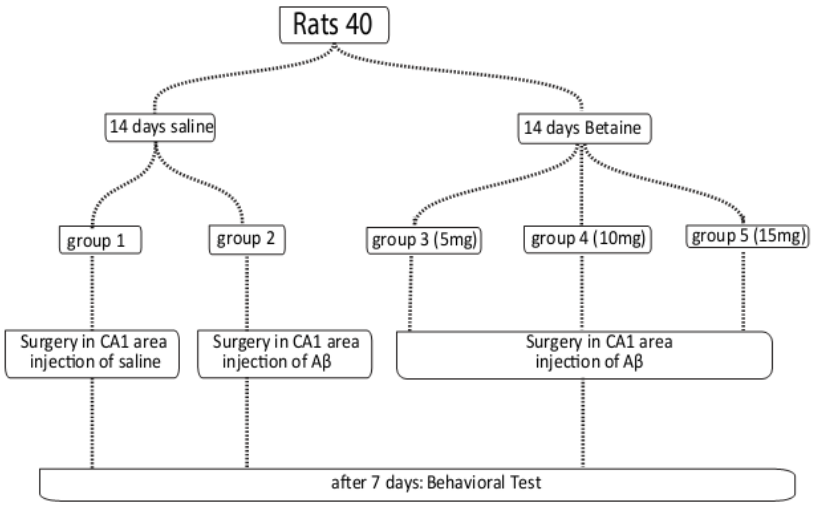

Fig. 1. The experimental protocols for the betaine effect on oxidative stress and cognitive dysfunction in an amyloid β-induced rat model of Alzheimer's disease.

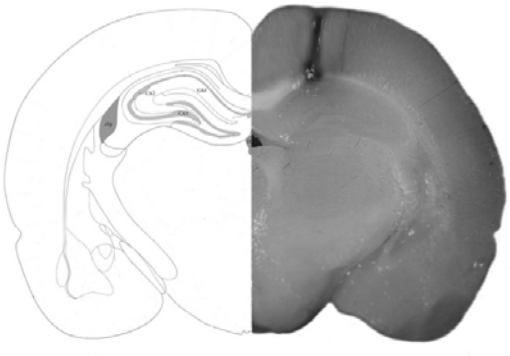

Fig. 2. The coronal sections of the rat brain show the approximate location of the CA1 injection sites in the experiments.

Fig. 3. Passive avoidance apparatus.

The intra-CA1 injection was carried out through 27-gauge needles attached to the 5-µL Hamilton micro syringes *via* a polyethylene tube. One µL of Aß or saline was injected into the right and left CA1 hippocampus (0.5 µL each side). The injection time was 2 min and the needles were kept in place for at least 1 min after injection to allow for diffusion of the solution away from the needle tip. At the end of the surgery, the rats were returned to their home cages and allowed 7 days for the recovery period.

Inhibitory avoidance apparatus

The inhibitory avoidance apparatus (Medicine Teb Company, Iran) was used to evaluate memory (Fig. 3). The apparatus consisted of two light and dark chambers which were separated by a guillotine door. The walls and floor of the light compartment consisted of white opaque resin. The floor of dark chambers consisted of stainless-steel rods of 2.5 mm diameter. Intermittent electric foot shocks (50 Hz, 3 s, 1 mA) were delivered to the grid floor of the dark compartment using an insulated stimulator.

Inhibitory avoidance test procedure

Seven days after surgery the behavioral test was performed. Inhibitory avoidance test consists of 2 phases: training and memory tests. On the training day, rats were transported to the experimental room and allowed 1 h to habituate to the environment before the training. Then, each rat was placed in the light chamber of the apparatus. After 5 s, the guillotine door was

opened and the animal was allowed to enter the dark chamber. The latency of the animal crossing into the dark chamber was recorded. The rats which avoided more than 120 s to enter the dark chamber were eliminated from the experiment (less than 1%). Once the animal crossed to the dark chamber, the door was closed and the rat was given 1 mA electrical shock for 3 s. After 20 s, the animal was returned to its home cage. After 2 min, the training was repeated, if the rat did not enter the dark chamber during 120 s (22), the successful acquisition was recorded. Otherwise, in the case of entrance into the dark chamber before 120 s, the animal received the same shock again. The training phase was performed with a maximum of 3 trials. The testing phase was performed 24 h later similar to the training phase except that the animals did not receive a shock. The step-through latency was recorded up to 300 s (23).

Histology

In a pilot trial, four rats were implanted in CA1 regions (as mentioned in the "*Experimental design"* section). After the recovery period (one week), 0.5 µL of 4% methylene blue was injected into the CA1 regions. The brains were extracted and stored in 10% formaldehyde for 7 days. The brains were sectioned and the needle tip location was determined according to a rat brain atlas (Paxinos and Watson, 2007).

Biochemical tests

Tissue preparation for biochemical analysis

After behavioral tests, each animal was decapitated upon light ether anesthesia and after killing their hippocampi were removed. The hippocampi were thawed and homogenized in a cold phosphate buffer (0.1 M, 5 mol/L EDTA, pH 7.4) and then centrifuged at 2000 *g* for 5 min. We used supernatants for the measurement of MDA concentration as lipid peroxidation markers, protein concentration, and antioxidant enzyme activities.

Quantificationof GSH content

The GSH content was measured using a GSH kit based on its manufacturer's instructions. GSH content was evaluated by ELISA reader (Biotek, USA) against blank at 405 nm and expressed as (nmol/mg protein).

Assay of GPx activity

GPx activity was measured with a GPx detection kit according to the manufacturer's instructions. The decrease in absorbance was measured spectrophotometrically against a blank at 340 nm ELISA reader. One unit (U) of GPx was defined as 1 µmol of oxidized NADPH per min per mg of tissue protein expressed as milliunit per mg of tissue protein (mU/mg protein).

Measurement of MDA concentration

MDA concentrations were measured in homogenate tissue with the MDA kit, as stated in the manufacturer's instructions. The results were expressed as nmol MDA per mg of tissue protein (nmol/mg protein)

Assay of CAT activity

CAT activity was measured using a CAT kit along with the manufacturer's instructions. The reaction was initiated by the addition of hydrogen peroxide (H_2O_2) and the change in the absorbance was followed at 495 nm. One unit of activity was defined as the conversion of 1 µmol H_2O_2 to product per min. The CAT activity was expressed as mU/mg protein.

Statistical analysis

The results were expressed as means ± SEM. One-way ANOVA was used to analyze both behavioral and biochemical estimations data. GraphPad Prism version 9 was used to analyze the results (GraphPad Software, San Diego, CA, USA). Post-hoc comparisons between groups were made using an LSD test. $P< 0.05$ was considered statistically significant.

RESULTS

Betaine improved Aß-induced inhibitory avoidance memory deficits

Figure 4shows the effect of intra-hippocampal injection of Aß on memory performance in avoidance tasks. Data analysis showed that Aß significantly lowered latencies to enter the dark compartment in comparison with the control group. Betaine treatment dose-dependently increased latency relative compared to the Aß-injected group indicating improved memory deficit.

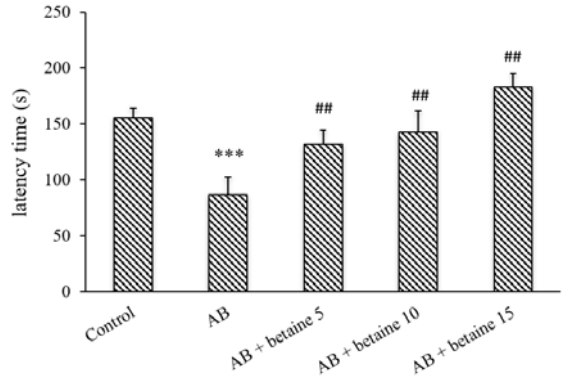

Fig. 4. Effect of betaine on memory deficit induced by Aß injection in inhibitory avoidance task. Betaine at 5, 10, and 15 mg/kg significantly increased step-through latency in Aß-injected rats. Data are presenting the mean ± SEM, n = 8. ***P< 0.001 indicates significant differences in comparison with the control group; $^{\#\#}P$ < 0.01 versus Aß group. Aß, Amyloid beta.

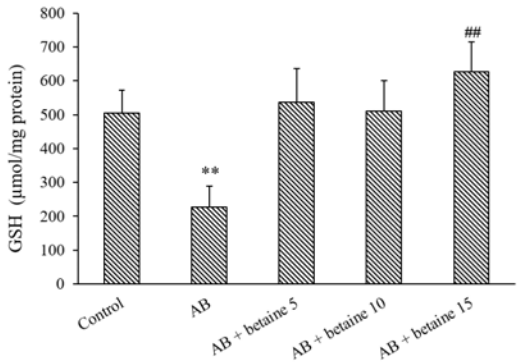

Fig. 5. Effect of betaine at 5, 10, and 15 mg/kg on GSH concentration in the hippocampus of Aß-injected rats. Data are expressed as mean ± SEM, n = 8. **P< 0.01 indicates significant differences in comparison with the control group; $^{\#\#}P$ < 0.01 versus Aß group. Aß, Amyloid beta; GSH, glutathione.

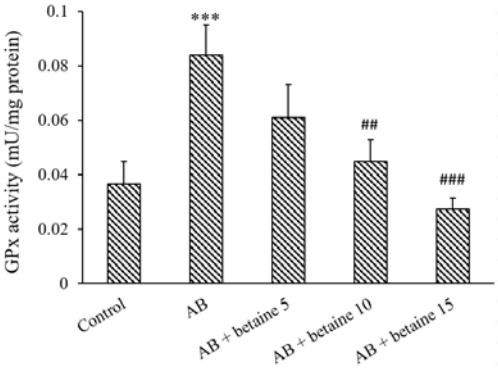

Fig. 6. Effect of betaine at 5, 10, and 15 mg/kg on GPx activity in the hippocampus of Aß-injected rats. Data represent mean ± SEM, n = 8. ***P< 0.001 indicates significant differences in comparison with the control group; $^{\#\#}P$ < 0.01 and $^{\#\#}P$ < 0.001 versus Aß group. Aß, Amyloid beta; GPx, glutathione peroxidase.

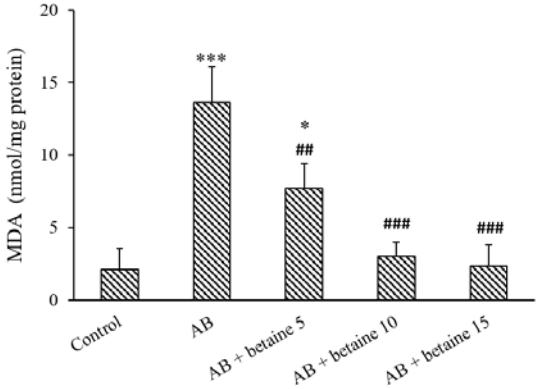

Fig. 7. Effect of betaine at 5, 10, and 15 mg/kg on MDA concentrations in the hippocampus of Aß-injected rats. Data are expressed as mean ± SEM, n = 8. ***P< 0.001 indicates significant differences in comparison with the control group; $^{\#\#}P$ < 0.01 versus Aß group. Aß, Amyloid beta; MDA, malondialdehyde.

Antioxidant activities estimation

Reduced GSH

Figure 5 shows the effect of Aß and betaine + Aß treatment on GSH levels in the hippocampus of treated animals. Levels of GSH were significantly lowered in the Aß-injected group as compared to the control group. Also, supplementation with betaine significantly prevented the decrease in GSH levels induced by Aß.

GPx activity

The GPx activity was significantly higher in the Aß-injected rats as compared to the control group. Furthermore, pretreatment with betaineresulted in a significant decrease in GPx activity when compared to the Aß-injected group (Fig. 6).

MDA concentration

As shown in Fig. 7, the MDA level was measured in the hippocampus of Aß and Aß / Aß + betaine-treated groups. The analysis indicated that there was an increase in MDA level in the hippocampus of the Aß-injected group as compared to the control group. Moreover, pre-treatment with betaine supplements caused a significant reduction of MDA levels in comparison with the Aß-injected group.

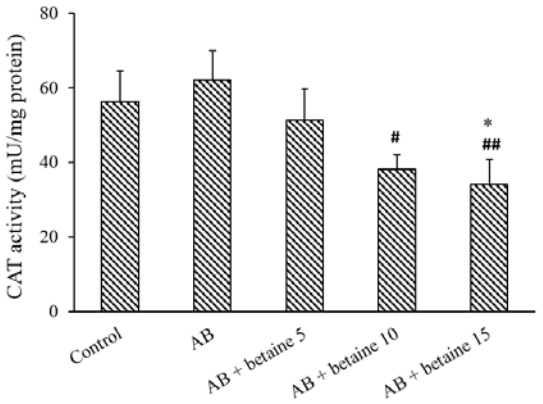

Fig. 8. Effect of betaine at 5, 10, and 15 mg/kg on CAT activity in the hippocampus of Aß-injected rats. Data are presented as mean ± SEM, n = 8. $^{*}P< 0.05$ indicates significant differences in comparison with the control group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus Aß group. Aß, Amyloid beta; CAT, catalase.

CAT activity

Figure 8 shows the effect of Aß and betaine/Aß co-treatment on CAT activity in the hippocampus. The analysis revealed that there was a statistically significant difference in CAT activity between the control group and rats treated with betaine at 15 mg/kg.

DISCUSSION

It has been well-documented that betaine enhances antioxidant defenses (24). Hence, in the present research, we investigated betaine's effects on oxidative stress and memory impairment induced by Aß administration. In agreement with the other literature, our data showed that intra-CA1 Aß injection decreased step-through latency in avoidance tasks and impaired memory performance. A previous study indicated that Aß impairs spatial memory *via* interference with hippocampus function(23). The results from another study also indicated that injection of Aß peptide into the CA1 region of the hippocampus caused cognitive impairments in working memory tasks (24). In the present study, oral treatment of betaine, for 14 consecutive days significantly improved learning performance in the Aß-injected rats as can be seen from the significant increase in latency times. In contrast, the lipid peroxidation marker (MDA) in Aß-injected rats increased when compared with the control group indicating Aß induced oxidative stress in accordance with a previous study (4).

It seems that oxidative damage is one of the principal pathological characteristics of AD (24). Many of the previous studies, demonstrated that Aß peptide induces oxidative damage (25). Multiple mechanisms have been hypothesized for Aß peptide-induced oxidative damage and consequently memory deficits. Firstly, Aß peptides directly increase reactive oxygen species (ROS) production in the neurons and glial cells (25). Secondly, Aß inserted into neuronal membranes triggers a series of toxic events that induces lipid peroxidation (4). In the plasma membrane at the site of synapses, lipid peroxidation alters dendritic spines, signaling pathways, and receptor trafficking leading to synaptic dysfunction (25). Aß peptides also increase N-methyl-D-aspartate (NMDA) receptor-mediated calcium influx leading to excitotoxicity and oxidative damage (26-28). These pieces of evidence support that inhibition of Aß-mediated oxidative stress could be a suitable option to decrease the rate of AD progression. In this regard, the use of dietary antioxidants such as betaine as a natural antioxidant agent is being considered.

The beneficial effects of herbs and herbal production including betaine on cognitive function have been suggested in previous studies (29-32). Chai *et al.* indicated that betaine attenuates memory impairment in AD by preventing Aß accumulation (8). Moreover, it has been reported that the administration of betaine improved memory impairment induced by lipopolysaccharide*via* the action of betaine / GABA transporter 2 (33). Herein, we demonstrated that memory improving effect of betaine is also related to its antioxidant properties. It should be noted that the neuroprotective effects of betaine under oxidative stress conditions have been attributed to its antioxidant properties (10,11).

GSH is the non-enzymatic antioxidant in the brain that is capable of preventing damage to cellular components caused by free radicals and lipid peroxidation products (34). GSH reduces H_2O_2 and lipid peroxides in a reaction that is catalyzed by the GPx enzyme as a cofactor (35). Mandal *et al.* showed that GSH level decreases

in AD and is correlated with a decline in cognitive performance (36). Our data showed a reduction in hippocampal GSH level in Aß-injected rats that could be due to its consumption as a GPx cofactor which is in accordance with Mandal *et al.* findings. Interestingly, our data indicated that Aß increases GPx activity in the hippocampus of Aß-injected rats which is in contrast with a previous report that indicated a decline in GPx activity in the AD frontal cortex region (37). In the context of GPx activity in oxidative conditions, our previous study also showed that the GPx activity of the cerebellum increased in response to ethanol as an oxidative stress inducer (11). It seems increasing of GPx activity in the AD group is a compensatory mechanism to suppress oxidative stress, however, this elevation is unable to suppress it completely. It should be noted that similar to the studies on brain GPx levels there are contradictory reports about the plasma activity of GPx between AD and control groups (38). We also found that betaine treatment increased GSH levels in the Aß-injected group. Moreover, betaine scavenges ROS and decreases oxidative stress, subsequently preventing increased GPx activity in the level of mRNA as well as/or enzyme activity.

Lipid peroxidation, a process in which ROS attacks unsaturated lipids in the cellular membranes and produces a wide variety of oxidant products, is an important consequence of oxidative stress (39). The brain has high levels of unsaturated lipids making the brain more vulnerable to oxidative stress (40). Benseny-Cases *et al.*showed that Aß plaques always co-localized with oxidized lipids in tissue samples of brains affected by AD (41). MDA is an important product of lipid peroxidation implicated in the pathogenic cascade in AD (39). In agreement with these studies, we found that MDA levels increased in the hippocampus sample of Aß-treated rats indicating enhanced oxidative stress. In contrast, betaine lowered MDA concentration in the betaine-treated rats.

CAT as an antioxidant enzyme can neutralize H_2O_2 produced in response to Aß(42). It has been recognized that Aß binds to CAT in a dose-dependent manner leading to CAT inhibition and decreasing enzyme activity in AD (43). However, we observed no statistically significant differences in CAT activity between the control Aß-treated rats. On the other hand, it seems that there was a significant difference between control andbetaine-treated rats. One possible explanation for our present data could be insufficient concentrations of Aß that were unable to inhibit CAT activity, however, it is needed to be clarified in future studies.

CONCLUSION

We found that the attenuation of memory deficit by betaine is directly associated with the antioxidant properties of betaine. These results provide further support for the hypothesis that betaine could be considered a good candidate as an antioxidant agent for more research in an animal model of AD. However, the influence of betaine on hippocampal synaptic plasticity, acetylcholinesterase activity, and memory molecules such as cAMP response element-binding protein needs further investigation.

Conflict of interest statement

All authors declared no conflict of interest in that study.

Authors' contributions

F. Nazari-Serenjeh and M. Alirezaei contributed to the design of the study, analysis, and interpretation of the data; F. Alipourfard contributed to the data acquisition and writing manuscript; H. Shajiee and V. Hojati contributed to the interpretation of the data. The finalized article was approved by all authors

REFERENCES

1. DeTure MA, Dickson DW. The neuropathological diagnosis of Alzheimer's disease. MolNeurodegener. 2019;14(1):32,1-18. DOI: 10.1186/s13024-019-0333-5.
2. Wojsiat J, Zoltowska KM, Laskowska-Kaszub K, Wojda U. Oxidant/antioxidant imbalance in Alzheimer's disease: therapeutic and diagnostic prospects. Oxid Med Cell Longev. 2018;2018:6435861,1-16. DOI: 10.1155/2018/6435861.
3. Collin F, Cheignon C, Hureau C. Oxidative stress as a biomarker for Alzheimer's disease. Biomark Med. 2018;12(3):201-203. DOI: 10.2217/bmm-2017-0456.

4. Butterfield DA, Swomley AM, Sultana R. Amyloid β-peptide (1-42)-induced oxidative stress in Alzheimer disease: importance in disease pathogenesis and progression. Antioxid Redox Signal. 2013;19(8):823-835. DOI: 10.1089/ars.2012.5027.
5. Cai Z, Zhao B, Ratka A. Oxidative stress and β-amyloid protein in Alzheimer's disease. Neuromolecular Med. 2011;13(4):223-250. DOI: 10.1007/s12017-011-8155-9.
6. Cheignon C, Tomas M, Bonnefont-Rousselot D, Faller P, Hureau C, Collin F. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. Redox Biol. 2018;14:450-464. DOI: 10.1016/j.redox.2017.10.014.
7. Day CR, Kempson SA. Betaine chemistry, roles, and potential use in liver disease. BiochBiophysActa. 2016;1860(6):1098-1106. DOI: 10.1016/j.bbagen.2016.02.001.
8. Chai GS, Jiang X, Ni ZF, Ma ZW, Xie AJ, Cheng XS, *et al*. Betaine attenuates Alzheimer-like pathological changes and memory deficits induced by homocysteine. J Neurochem. 2013;124(3):388-396. DOI: 10.1111/jnc.12094.
9. Zhang CE, Tian Q, Wei W, Peng JH, Liu GP, Zhou XW, *et al*. Homocysteine induces tau phosphorylation by inactivating protein phosphatase 2A in rat hippocampus. Neurobiol Aging. 2008;29(11):1654-1665. DOI: 10.1016/j.neurobiolaging.2007.04.015.
10. Alirezaei M. Betaine protects cerebellum from oxidative stress following levodopa and benserazide administration in rats. Iran J Basic Med Sci. 2015;18(10):950-957. PMID: 26730328.
11. Alirezaei M, Jelodar G, Niknam P, Ghayemi Z, Nazifi S. Betaine prevents ethanol-induced oxidative stress and reduces total homocysteine in the rat cerebellum. J PhysiolBiochem. 2011;67(4):605-612. DOI: 10.1007/s13105-011-0107-1.
12. Kunisawa K, Kido K, Nakashima N, Matsukura T, Nabeshima T, Hiramatsu M. Betaine attenuates memory impairment after water-immersion restraint stress and is regulated by the GABAergic neuronal system in the hippocampus. Eur J Pharmacol. 2017;796:122-130. DOI: 10.1016/j.ejphar.2016.12.007.
13. Leiteritz A, Dilberger B, Wenzel U, Fitzenberger E. Betaine reduces β-amyloid-induced paralysis through activation of cystathionine-β-synthase in an Alzheimer model of *Caenorhabditiselegans*. Genes Nutr. 2018;13:21,1-7. DOI: 10.1186/s12263-018-0611-9.
14. Mc Carty MF, O'Keefe JH, DiNicolantonio JJ. A diet rich in taurine, cysteine, folate, B12 and betaine may lessen risk for Alzheimer's disease by boosting brain synthesis of hydrogen sulfide. Med Hypotheses. 2019;132:109356,1-5. DOI: 10.1016/j.mehy.2019.109356.
15. Kunisawa K, Kido K, Nakashima N, Matsukura T, Nabeshima T, Hiramatsu M. Betaine attenuates memory impairment after water-immersion restraint stress and is regulated by the GABAergic neuronal system in the hippocampus. Eur J Pharmacol. 2017;5:796,122-130. DOI: 10.1016/j.ejphar.2016.12.007.
16. Ibi D, Hirashima K, Kojima Y, Sumiya K, Kondo S, Yamamoto M, *et al*. Preventive effects of continuous betaine intake on cognitive impairment and aberrant gene expression in hippocampus of 3xTg mouse model of Alzheimer's disease. J Alzheimers Dis. 2021;79(2):639-652. DOI: 10.3233/JAD-200972.
17. Ibi D, Tsuchihashi A, Nomura T, Hiramatsu M. Involvement of GAT2/BGT-1 in the preventive effects of betaine on cognitive impairment and brain oxidative stress in amyloid β peptide-injected mice. Eur J Pharmacol. 2019;842:57-63. DOI: 10.1016/j.ejphar.2018.10.037.
18. Mueed Z, Mehta D, Kumar Rai P, A. Kamal M, Poddar NM. Cross-interplay between osmolytes and mTOR in Alzheimer's disease pathogenesis. Curr Pharm Des. 2020;26(37):4699-4711. DOI: 10.2174/1381612826666200518112355.
19. Geng Y, Li C, Liu J, Xing G, Zhou L, Dong M, *et al*. Beta-asarone improves cognitive function by suppressing neuronal apoptosis in the beta-amyloid hippocampus injection rats. Biol Pharm Bull. 2010;33(5):836-843. DOI: 10.1248/bpb.33.836.
20. Zheng M, Liu J, Ruan Z, Tian S, Ma Y, Zhu J, *et al*. Intrahippocampal injection of Aβ1-42 inhibits neurogenesis and down-regulates IFN-γ and NF-κB expression in hippocampus of adult mouse brain. Amyloid. 2013;20(1):13-20. DOI: 10.3109/13506129.2012.755122.
21. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th ed. Australia: Academic Press, Elsevier; 2006. pp. 115-118.
22. Yadegary A, Nazari-Serenjeh F, Darbandi N. Synergistic improvement effect of nicotine-ghrelin co-injection into the anterior ventral tegmental area on morphine-induced amnesia. Neuropeptides. 2020;80:102025,1-7. DOI: 10.1016/j.npep.2020.102025.
23. Pearson-Leary J, McNay EC. Intrahippocampal administration of amyloid-β 1-42 oligomers acutely impairs spatial working memory, insulin signaling, and hippocampal metabolism. J Alzheimers Dis. 2012;30(2):413-422. DOI: 10.3233/JAD-2012-112192.
24. Faucher P, Mons N, Micheau J, Louis C, Beracochea DJ. Hippocampal injections of oligomeric amyloid β-peptide (1-42) induce selective working memory deficits and long-lasting alterations of ERK signaling pathway. Front Aging Neurosci. 2016;7:245,1-15. DOI: 10.3389/fnagi.2015.00245.
25. Tönnies E, Trushina E. Oxidative stress, synaptic dysfunction, and Alzheimer's disease. J Alzheimers Dis. 2017;57(4):1105-1121. DOI: 10.3233/JAD-161088.
26. Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, *et al*. Regulation of NMDA receptor trafficking by amyloid-β. Nat Neurosci. 2005;8(8):1051-1058.

DOI: 10.1038/nn1503.
27. Shankar GM, Bloodgood BL, Townsend M, Walsh DM, Selkoe DJ, Sabatini BL. Natural oligomers of the Alzheimer amyloid-β protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. J Neuro Sci. 2007;27(11):2866-2875. DOI: 10.1523/JNEUROSCI.4970-06.2007.
28. Bezprozvanny I, Mattson MP. Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. Trends Neurosci. 2008;31(9):454-463. DOI: 10.1016/j.tins.2008.06.005.
29. Huang B, Hu X, Hu J, Chen Z, Zhao H. Betaine alleviates cognitive deficits in diabetic rats via PI3K/Akt signaling pathway regulation. Dement GeriatrCognDisord. 2020;49(3):1-9. DOI: 10.1159/000508624.
30. Ohnishi T, Balan S, Toyoshima M, Maekawa M, Ohba H, Watanabe A, *et al*. Investigation of betaine as a novel psychotherapeutic for schizophrenia. E Bio Medicine. 2019;45:432-446. DOI: 10.1016/j.ebiom.2019.05.062.
31. Teymuori M, Yegdaneh A, Rabbani M. Effects of piper nigrum fruit and *Cinnamumzeylanicum* bark alcoholic extracts, alone and in combination, on scopolamine-induced memory impairment in mice. Res Pharm Sci. 2021;16(5):474-481. DOI: 10.4103/1735-5362.323914.
32. Momtazi-borojeni AA, Sadeghi-Aliabadi H, Rabbani M, Ghannadi A, Abdollahi E. Cognitive enhancing of pineapple extract and juice in scopolamine-induced amnesia in mice. Res Pharm Sci. 2017;12(3): 257-264. DOI: 10.4103/1735-5362.207198.
33. Miwa M, Tsuboi M, Noguchi Y, Enokishima A, Nabeshima T, Hiramatsu M. Effects of betaine on lipopolysaccharide-induced memory impairment in mice and the involvement of GABA transporter 2. J Neuroinflammation. 2011;8:153,1-13. DOI: 10.1186/1742-2094-8-153.
34. Peter C, Braidy N, Zarka M, Welch J, Bridge W. Therapeutic approaches to modulating glutathione levels as a pharmacological strategy in Alzheimer's disease. Curr Alzheimer Res. 2015;12(4):298-313. DOI: 10.2174/1567205012666150302160308.
35. Gu F, Chauhan V, Chauhan A. Glutathione redox imbalance in brain disorders. CurrOpinClinNutrMetab Care. 2015;18(1):89-95. DOI: 10.1097/MCO.0000000000000134.
36. Mandal PK, Saharan S, Tripathi M, Murari G. Brain glutathione levels-a novel biomarker for mild cognitive impairment and Alzheimer's disease. Biol Psychiatry. 2015;78(10):702-710. DOI: 10.1016/j.biopsych.2015.04.005.
37. Ansari MA, Scheff SW. Oxidative stress in the progression of Alzheimer disease in the frontal cortex. J NeuropatholExp Neurol. 2010;69(2): 155-167. DOI: 10.1097/NEN.0b013e3181cb5af4.
38. Chang YT, Chang WN, Tsai NW, Huang CC, Kung CT, Su YJ, *et al*. The roles of biomarkers of oxidative stress and antioxidant in Alzheimer's disease: a systematic review. Biomed Res Int. 2014;2014:182303,1-14. DOI: 10.1155/2014/182303.
39. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med cell Longev. 2014;2014:360438,1-31. DOI: 10.1155/2014/360438.
40. Reed TT. Lipid peroxidation and neurodegenerative disease. Free RadicBiol Med. 2011;51(7): 1302-1319. DOI: 10.1016/j.freeradbiomed.2011.06.027.
41. Benseny-Cases N, Klementieva O, Cotte M, Ferrer I, Cladera J. Microspectroscopy (μFTIR) reveals co-localization of lipid oxidation and amyloid plaques in human Alzheimer disease brains. Anal Chem. 2014;86(24):12047-12054. DOI: 10.1021/ac502667b.
42. Behl C, Davis J, Lesley R, Schubert D. Hydrogen peroxide mediates amyloid β protein toxicity. Cell. 1994;77(6):817-827. DOI: 10.1016/0092-8674(94)90131-7.
43. Milton NGN. Amyloid-beta binds catalase with high affinity and inhibits hydrogen peroxide breakdown. Biochem J. 1999;344:293-296. DOI: 10.1042/bj3440293.