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Assessment of Ameliorative Effects of Choline on Neurodegeneration in Aluminium Chloride Induced – Alzheimer's Disease Rat Model

Sonal G[a], Reshma K[a*], Sudha K[a], Mallika B[a], Neelam P[a], Amit M[b], Lakshmisha R[b], Vandana B[b], Vasavi K[a]

[a] Department of Biochemistry, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Karnataka, Manipal 576104, India

[b] Department of Anatomy, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Karnataka, Manipal 576104, India

Corresponding author: Dr Reshma Kumarchandra, Associate Professor, Department of Biochemistry, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Karnataka, Manipal 576104, India.

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KEYWORDS
Alzheimer's disease, Tau protein, Oxidative stress, Acetyl choline esterase, Choline, cognitive behaviour, Neuronal assay

ABSTRACT:
Introduction: Alzheimer's disease (AD), a common neurodegenerative disease of the elderly population is the most explored area of research in recent times owing to the dearth of successful treatment options for its reversal.

Objectives: To study the neuroprotective role of choline supplementation in comparison to AD therapeutic drug Donepezil in aluminium chloride induced Alzheimer's disease rat model.

Methods: In the present study Group I served as the normal control. An attempt was made to induce AD in experimental rats by orally treating them with AlCl3 at a dose of 300mg/kg body weight (Group II) and observing the changes in parameters associated with various established hypothesis of the disease. Group III animals were treated with the standard drug, Donepezil (0.5 mg/kg body wt.). Choline, was supplemented in AD induced rats which were then monitored for the same parameters to demonstrate its role as neuroprotective agent prior to and post AlCl3 supplementation (Groups V & IV) respectively. The parameters studied in the brain homogenate included markers of AD pathology namely tau protein and caspase8, markers of cholinergic hypothesis namely acetyl choline, markers of oxidative stress namely MDA, reduced GSH and NO and marker of cognitive behaviour, water maze test. Additionally neuronal assay of prefrontal cortex and hippocampus was performed.

Results: Indicated that choline supplemented group (post treated) showed a significant decrease in tau protein compared to AM model; pre and post choline treatment showed a significant increase in GSH ($p<0.05$) and a significant improvement in cognitive behaviour ($p<0.05$) without significant changes in other parameters. Mean number of neurons in the CA1 region increased in choline treated groups IV and V compared to Al Cl3 treated group (Group1) wherein a drastic degeneration of neurons was observed.

Conclusions: Choline seemingly ameliorates the oxidative stress by significantly improving GSH status and the cognitive behaviour, significantly reduces the tau protein content in the brain and reverses the neuronal damage in the prefrontal cortex and hippocampus of rats treated with aluminium chloride.

1. Introduction

Alzheimer's disease (AD), a neurodegenerative disorder usually seen in older individuals [1] is associated with progressive loss of memory, language, and other cognitive impairments in the aged population. Some of the psychological signs in AD include irritability, delusions, anxiety, lack of interest, depression, aggression, inability to learn new things and interfering with the daily activities [2].

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The hallmark of AD pathology includes the accumulation of extracellular amyloid-beta peptide (Aβ) a result of neuritic plaques in the brain tissue through an amyloidogenic pathway and the formation of neurofibrillary tangles (NFTs) containing phosphorylated Tau protein, thereby destroying the neurons. Brain inflammation also plays an important role in neurodegeneration [3].

Factors which are considered to be the underlying cause for AD include inflammation, head trauma, oxidative stress, proteasomal dysfunction, genetics, apoptosis and environmental factor such as aluminium (Al) toxicity. Oxidative stress in AD is characterized by an imbalance in the production of ROS and has been correlated with the development of AD [4].

AChE is the marker enzyme for the cholinergic activity that terminates and degrades the physiological action of ACh. Thus, cholinergic activity is considered as the main event in the neurochemical changes of AD [5].

Al is an abundantly existing neurotoxin, a high concentration of Al accumulation in brain regions such as the cortex and hippocampus results in apoptotic death of glial and neuron cells. Al impairs the enzymes that are involved in the synthesis of neurotransmitters thus affecting the ACh content. Synaptic transmission is reduced by Al by inhibiting the neurotransmitter receptors and voltage-gated Ca^{2+} resulting in the impairment of brain functions such as memory, learning, neurotoxicity, and neurodegeneration [6]. This compound therefore is widely being used in the development of an experimental rodent model of AD.

Choline is an essential nutrient that produces acetylcholine, a neurotransmitter. It has an impact on brain functions that are involved in cognition. Choline has a role in maintaining cell membranes and myelination during the synthesis of phospholipid [7]. Choline supplementation, typically in developing rats modulates the signalling of hippocampal cholinergic neurons. Choline is, therefore, a vital component for the brain and is required for the functioning of the nervous system which includes muscle control, mood, and memory [8]. However, only 30% of choline is synthesised in the liver which makes it an essential dietary nutrient [9].

Dietary choline intake of less than 219mg/day was associated with dementia and less than 215mg/day was associated with AD in a study involving 3224 participants from the Framingham heart study [10].

Objectives

The present study attempts to exhibit the possible neuroprotective role of choline supplementation in comparison to AD therapeutic drug Donepezil in aluminium chloride induced Alzheimer's disease rat model.

Methods

This study was conducted at the Department of Biochemistry, Centre for Basic Sciences, Kasturba Medical College, Bejai, Mangalore. This study was conducted for a period of 3 months. All procedures were performed in compliance with relevant laws and institutional guidelines and have been approved by the Institutional Scientific committee and by the (KMC/MNG/IAEC/05-2019 on February 15, 2019) and Institutional Biosafety Committee.

Sample size:

A total of 24 Albino Wistar rats, 6 in each of the following groups were used for the study.

Group I: Control

Group II: AD group -Dementia induced through $AlCl_3$ (300 mg/kg body wt.)

Group III: $AlCl_3$ (300 mg/kg body wt.) + Donepezil (0.5 mg/kg body wt.)

Group IV: Post-treatment with Choline (1g/kg body wt.) - supplementation post $AlCl_3$ treatment

Group V: Prior-treatment with Choline (1g/kg body wt.) - supplementation prior to $AlCl_3$ treatment

Animal Profile:

Animal care and handling were carried out according to the CPCSEA guidelines. Adult (6 months old) Wistar rats were used in this study.

The animals were procured from the breeding colony of Kasturba Medical College Animal House, Mangalore. Prior approval of the Institutional Ethical committee was taken. Animals were housed in polypropylene cages with a paddy husk bedding at 25 ± 20°C temperature during

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the experiment. They received standard pellet and water ad libitum.

Induction of experimental dementia by AlCl3:

AlCl3 was chronically administered to animals at a dose of 300 mg/kg/day by oral route for 1 month to induce dementia.

Donepezil dosage:

0.5 mg/kg body weight. per day given for 15 days prior to AlCl3 treatment. The general dosage is 5 mg for 60 kg adult, thus the equivalent dosage for rats was 0.5 mg/kg [11].

Choline dosage:

1g/kg body wt. per day given for 15 days prior to AlCl3 treatment in the pre-treated group and for 15 days after AlCl3 treatment in the post- treated group.

Neuronal assay of prefrontal cortex and hippocampus:

Perfusion: Deeply anaesthetized (with ketamine) rats were secured on a dissection board, and the heart was exposed by opening the chest cavity. Perfusion was done with 100-150mL of 0.9% saline through the left ventricle at the rate of 1ml/min. Then 10% formalin was perfused at the same flow rate. Brain was removed by decapitating the animal and brains were kept in 10% formalin for 48hr (post fixation). Paraffin blocks were made in an embedding bath. Using rotary microtome, coronal sections of 6-7-µm thickness were taken in the dorsal hippocampus

Staining: Cresyl violet stain was used for staining the sections. In 100ml of distilled water 100mg of cresyl violet was dissolved. To attain the pH of 3.5-3.8, 0.5mL of 10% acetic acid was added.

Scoring: Under light microscopy (20X) quantitative analysis was done. In every section of dentate gyrus and prefrontal cortex, 50 X50 micron area was selected for counting.

In hippocampus (cornua amonis areas-CA1 250µm length area was chosen for quantifications. Nikon trinocular microscope (H600L) was used for screening. Imaging software NIS Elements Br version 4.30 was used to quantify the neurons as per Madhyastha et al. [12]

Collection of brain samples and estimation of biochemical parameters in brain homogenate:

Each rat was sacrificed under anaesthesia through cervical dislodgement. Brains were quickly excised, washed with saline, blotted with a piece of filter paper, and homogenized in an ice-cold phosphate buffer (pH 7.4) 10X and 1X for biochemical parameter and ELISA kits respectively to yield homogenate. The homogenates were centrifuged at 3000 rpm for 10 minutes. The supernatant of homogenates was used for biochemical estimations. The activity of acetylcholinesterase in the sample was estimated by Ellman's method [13]. Estimation of phosphorylated tau protein was performed using ELISA kit based on Sandwich-ELISA principle [14]. Estimation of caspase was performed using Abbkine Caspase 8 Assay Kit (Colorimetric) [15]. The total glutathione content was measured by the method described by Ellman et al. [16]. Nitric oxide in the brain homogenate was analysed by the method described by Griess et al [17]. The estimation of Malondialdehyde (MDA) was carried out by method published by Kei Satoh [18].

Evaluation of cognitive behaviour of animals:

In order to monitor the animal behaviour in the different study groups, Morris water maze test was conducted which is based on special learning for rodents that relies on distal cues to navigate from start locations around the perimeter of an open swimming arena to locate a submerged escape platform.

A statistical package SPSS version 20 was used for the data analysis. The results were expressed as mean ± standard error of means (SEM). The data obtained from various groups was statistically analyzed using one-way ANOVA followed by Tukey's multiple range tests. $p<0.05$ was considered to be statistically significant.

2. Results

Biochemical investigations

All the biochemical and the cognitive parameters are depicted in table 1. There was an apparent increase in tau proteins in AlCl3 induced group and a significant decrease in the concentration of tau proteins (Figure 1) in the choline supplemented group (post treatment group) when compared to the Alzhiemer model (AM) ($p=0.002$).

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All the groups that were treated with AlCl3 (AM) showed a slight increase in brain AChE activity when compared to the control group but not to the value of significance. However, there was no significance in AChE in groups treated with choline. Caspase 8 significantly increased in AM compared to control. But observations were not significant in AM with pre and post choline treatment. In AM model groups there was a slight increase in MDA levels, implying lipid peroxidation but no significant decrease was observed in the supplemented groups. Choline supplementation has resulted in a significant increase in glutathione ($p<0.05$) in both the AM animal groups that were pretreated and post-treated with choline as compared to the AM group (Figure 2). Choline post treated group showed a much greater significance when compared to choline pretreated group. A significant increase in NO was observed in the AM group compared to control ($p<0.001$) which decreased significantly in the group post-treated with choline.

Cognitive parameter

The escape latency time (ELT) to locate the hidden platform in the water maze after training was noted and 3 trials were recorded and their average was used as an index for acquisition or learning. It was observed that in comparison to control animals there was significant increase in ELT for rats treated with AlCl3 indicating impairment in learning and memory. Administration of choline in AlCl3 treated rats showed a significant reduction in ELT (Figure 3).

Histological findings

Mean number of neurons in the CA1 region increased in choline treated groups IV and V compared to Al Cl3 treated group (Group1) (Table 2) wherein a drastic degeneration of neurons was observed (Figure 4).

Discussion

Hyperphosphorylation of tau proteins is the suggested hypothesis for the onset of AD. In our study, it was observed that there were apparently elevated levels of microtubule-binding phosphorylated tau protein in the hippocampus of the rat brain of AM. Choline pretreated group showed a significant decrease in the same when compared to AM. Earlier studies have reported that D gal/AlCl3 treatment has increased phosphorylated tau, Aβ, and AchE in rat brain [19]. Also, an increase in tau protein in AlCl3 treated albino rats was observed which decreased after treatment with resveratrol and tannic acid [20]. In a most recent study by Jessica et al, choline deficient diet induced amyloid plaque formation with associated increase in TNFα in 3xTg mice model while choline supplemented diet significantly decreased amyloid plaque and TNFα in APP/PS1 mice model [21]. Certain evidence states that there are other potentially triggering mechanisms that may commence earlier to tau formation such as mitochondrial dysfunction, vascular pathology, hypoxia, chronic neuroinflammation, oxidative stress, and insulin resistance [22]. Cholinergic hypothesis although is a hallmark of Alzheimer's etiology, certain reports state that cholinergic marker is less likely to be useful in the early diagnosis of AD [5]. The experimental results on rat hippocampal slice by Kar et al showed that under acute conditions, the uptake of choline was inhibited by amyloid peptides which in turn decreased the release of endogenous acetyl choline not showing any effects on AChE activity [23]. These reports are in agreement with our study findings.

A significant increase in the levels of caspase in the hippocampus of Al Cl3 induced AD was observed in our study compared to controls ($p<0.05$). Caspases are markers of apoptosis that lead to the death of damaged cells by initiating certain signaling pathways, whenever a tissue is challenged with some trauma. Chronic administration of Aluminium is found to produce oxidative stress, cholinergic dysfunction, and cognitive impairment in the rat brain [24] which is in agreement with our findings. Neuronal damage that has occurred in these rats might have been mediated through oxidative stress although apoptotic marker doesn't appear to be remarkably changed.

There are numerous studies that indicate a significant increase in MDA in different rat models of AD [25] and flavonoids supplementation significantly decreasing the same showing neuroprotection. Our study has not shown significant changes in MDA levels in any of the study groups. Perhaps better models of AD-like Aβ induction or transgenic mouse model would have yielded the desired results.

There were no significant changes in GSH between controls and AD in the present study. But choline supplementation has resulted in a significant increase in glutathione ($p<0.05$) in both the AD animal groups that

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were pretreated and post-treated with choline as compared to the AD group. Oxidative damage in DNA leading to apurinic and apyrimidinic sites in DNA was found in choline-deprived rats as stated by Powell et. al [26] emphasizing the role of choline in improving the antioxidant status. Reduced GSH forms an important part of the endogenous antioxidant redox potential in the neuronal cell [27]. Moreover, methyl groups of choline are utilized in transmethylation reactions that generate homocysteine which is a precursor for cysteine and glutathione [28]. This perhaps is the plausible explanation for the elevation of GSH in AD rats pre-treated and post-treated with choline in our study.

A significant increase in NO was observed in the AD group compared to controls ($p<0.001$) which decreased significantly in the group post-treated with choline. Studies on AD rats have shown either a significant increase [29], a significance decrease [30], or no significant change [31] in NOS levels in AD animals as compared to controls, although very few studies are available on nitric oxide levels as such. NO is a much-debated molecule in neuronal pathology due to its dual role of being either neuroprotective or neurotoxic. Further NO, a freely diffusible molecule across membranes, has a very short half-life of < 3 sec and immediately gets converted to peroxynitrite which has a half-life of < 1 sec. Therefore measurement of this molecule poses to be a challenge. Neurotoxic effects could be attributed to the formation of peroxynitrite produced on the reaction of NO with superoxide anion in the brain initiating damaging effects like lipid peroxidation and damaging effects on proteins by nitration of tyrosine residues and nitrosylation of cysteine residues, interfering in their functions which have been identified as markers of AD. Therefore, a rise in NO in the AD group in our study could be due to the nitrosative stress. On the contrary, NO could be neuroprotective due to its excitation of presynaptic neurons enhancing the release of neurotransmitters in the brain [32]. Choline supplementation post AD has apparently reduced the levels of NO implicating the role of choline in combating nitrosative stress.

There was a significant reduction in the time taken by AD rats to reach the target in the Moris water Maze test as compared to controls, which improved significantly in both AD groups that were pre-treated and post-treated with choline. Certain reports support our study where improvement in spatial memory and learning of AD rats were observed by the supplementation of flavonoids like hesperidin, quercetin, anthocyanins, rutin [25].

As reported in one of the studies, 3xTg mouse model is the most suitable one for studying Alzheimer's disease as there is a sequential progression of the disease starting with the production of Aβ in the neurons in the first three months after which plaque development occurs in the cortex and hippocampus within 6 months and finally culminating in the formation of neurofibrillary tangles initially in the CA1 region of hippocampus area followed by cortex[33].Additionally in our rat model we have found a considerable damage to neurons with Al Cl3 and a good improvement in the number and anatomy of the neurons in choline supplemented groups Rodent models are good models for studying the effects of AD due to cholinergic neuron loss and lesioning of cholinergic neurons mimicking the cognitive decline [34].

Conclusion

In the present study, the drug Donepezil apparently seems to be mediating its action by decreasing Tau protein and caspase 8 probably preventing neuronal apoptosis, as well as inhibiting acetyl choline esterase activity supporting cholinergic hypothesis, without much role in ameliorating oxidative stress, On the contrary choline seemingly improves the oxidative stress by significantly enhancing GSH status without being involved in other mechanisms implicated in AD.

References

1. Mount C., Downton C., 2006.Alzheimer disease: progress or profit? Nat Med. 12(7),780-4. doi: 10.1038/nm0706-780.
2. Cummings JL., Doody R., Clark C., 2007.Disease-modifying therapies for Alzheimer disease: challenges to early intervention. Neurology. 69(16),1622-34. doi: 10.1212/01.wnl.0000295996.54210.69.
3. LaFerla FM., Oddo S., 2005. Alzheimer's disease: Abeta, tau and synaptic dysfunction. Trends Mol Med. 11(4),170-6. doi: 10.1016/j.molmed.2005.02.009.
4. Maheshwari S L., Murali R V., Balaji R., 2014.Aluminium induced cholinotoxicity in zebra fish brain-a sequel of oxidative stress. Int.J. Adv. Res. 2,322-335.

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JCHR (2024) 14(6), 1135-1141 | ISSN:2251-6727

5. Terry AV Jr., Buccafusco JJ., 2003. The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. J Pharmacol Exp Ther.306(3),821-7. doi: 10.1124/jpet.102.041616. Epub 2003 Jun 12.
6. Flaten TP., 2001.Aluminium as a risk factor in Alzheimer's disease, with emphasis on drinking water. Brain Res Bull. 55(2),187-96. doi: 10.1016/s0361-9230(01)00459-2.
7. Zeisel SH., 2000.Choline: needed for normal development of memory. J Am Coll Nutr. Oct19(5 Suppl),528S-531S. doi: 10.1080/07315724.2000.10718976.
8. Jiang X., West AA., Caudill MA., 2014.Maternal choline supplementation: a nutritional approach for improving offspring health? Trends Endocrinol Metab. 25(5),263-73. doi: 10.1016/j.tem.2014.02.001. Epub 2014 Mar 26.
9. Goh YQ., Cheam G., Wang Y., 2021.Understanding Choline Bioavailability and Utilization: First Step Toward Personalizing Choline Nutrition. J Agric Food Chem. 69(37),10774-10789. doi: 10.1021/acs.jafc.1c03077. Epub 2021 Aug 15.
10. Yuan J., Liu X., Liu C., Ang AF., Massaro J., Devine SA., Auerbach SH., Blusztajn JK., Au R., Jacques PF., 2022.Is dietary choline intake related to dementia and Alzheimer's disease risks? Results from the Framingham Heart Study. Am J Clin Nutr. 116(5),1201-1207. doi: 10.1093/ajcn/nqac193. Epub 2023 Feb 10.
11. Zhou Q., Nie S., Liu Z., 2016.Effect of Donepezil Hydrochloride on Cognitive Function Recovery of Rats with Alzheimer’s Disease. Med One. 1: e160023. https://doi.org/10.20900/mo.20160023
12. Madhyastha S., Somayaji SN., Rao MS., Nalini K., Bairy KL., 2002 Hippocampal brain amines in methotrexate-induced learning and memory deficit. Can J Physiol Pharmacol. 80(11),1076-84. doi: 10.1139/y02-135.
13. Ellman GL., Courtney KD., Andres V JR., Feather-Stone RM., 1961.A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 7,88-95. doi: 10.1016/0006-2952(61)90145-9.
14. Engvall E., Jonsson K., Perlmann P., 1971. Enzyme-linked immunosorbent assay. II. Quantitative assay of protein antigen, immunoglobulin G, by means of enzyme-labelled antigen and antibody-coated tubes. Biochim Biophys Acta. 251(3),427-34. doi: 10.1016/0005-2795(71)90132-2.
15. Caspase-3 Assay Kit. https://www.abbkine.com/datasheet/KTA3022-EN-Caspase-3%20Assay%20Kit%20(Colorimetric).pdf
16. Ellman GL., 1959 .Tissue sulfhydryl groups. Arch Biochem Biophys. 82(1),70-7. doi: 10.1016/0003-9861(59)90090-6.
17. Sun J., Zhang XJ., Broderick M., Fein H., 2003.Measurement of nitric oxide production in biological systems by using Griess reaction assay. Sensors. 3,276–284.doi: 10.3390/s30800276.
18. Satoh K., 1978.Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta. 90(1),37-43. doi: 10.1016/0009-8981(78)90081-5.
19. Zhang Y., Pi Z., Song F., Liu Z., 2016.Ginsenosides attenuate d-galactose- and AlCl3-inducedspatial memory impairment by restoring the dysfunction of the neurotransmitter systems in the rat model of Alzheimer's disease. J Ethnopharmacol. 194,188-195. doi: 10.1016/j.jep.2016.09.007.
20. Bhounsule A., Bhatt LK., 2024.Protective effect of resveratrol and tannic acid combination on aluminium chloride induced neurotoxicity in rats. Nutr Neurosci. 27(5),438-450. doi: 10.1080/1028415X.2023.2208908. Epub 2023 May 5.
21. Judd JM., Jasbi P., Winslow W., Serrano GE., Beach TG., Klein-Seetharaman J., Velazquez R., 2023.Inflammation and the pathological progression of Alzheimer's disease are associated with low circulating choline levels. Acta Neuropathol. 146(4),565-583. doi: 10.1007/s00401-023-02616-7. Epub 2023 Aug 7.
22. Nazem A, Sankowski R, Bacher M, Al-Abed Y. Rodent models of neuroinflammation for Alzheimer's disease. J Neuroinflammation. 2015 Apr 17;12:74. doi: 10.1186/s12974-015-0291-y.
23. Kar S., Issa AM., Seto D., Auld DS., Collier B., Quirion R., 1998.Amyloid beta-peptide inhibits high-affinity choline uptake and acetylcholine release in rat hippocampal slices. J Neurochem. 70(5),2179-87. doi: 10.1046/j.1471-4159.1998.70052179.x.

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24. Kumar A., Dogra S., Prakash A., 2009.Corrigendum to Protective effect of curcumin (Curcuma longa), against aluminium toxicity: Possible behavioral and biochemical alterations in rats Behav. Brain Res. 205 , 384-390. Behav Brain Res. 2020 Feb 17;380:112415. doi: 10.1016/j.bbr.2019.112415. Epub 2019 Dec 16. Erratum for: Behav Brain Res. 2009 Dec 28;205(2):384-90. doi: 10.1016/j.bbr.2009.07.012.
25. de Andrade Teles RB., Diniz TC., Costa Pinto TC., de Oliveira Júnior RG., Gama E Silva M., de Lavor ÉM., 2018.Flavonoids as Therapeutic Agents in Alzheimer's and Parkinson's Diseases: A Systematic Review of Preclinical Evidences. Oxid Med Cell Longev. 10,7043213. doi: 10.1155/2018/7043213.
26. Powell CL., Kosyk O., Bradford BU., Parker JS., Lobenhofer EK., Denda A., Fumiyuki U., Dai N., Ivan R.,2005. Temporal correlation of pathology and DNA damage with gene expression in a choline-deficient model of rat liver injury. Hepatology. 42(5),1137-47. doi: 10.1002/hep.20910.
27. Gella A., Durany N., 2009. Oxidative stress in Alzheimer disease. Cell Adh Migr. 3(1),88-93. doi: 10.4161/cam.3.1.7402. Epub 2009 Jan 13.
28. Abu Ahmad N., Raizman M., Weizmann N., Wasek B., Arning E., Bottiglieri T., Oren T. , 2019.Betaine attenuates pathology by stimulating lipid oxidation in liver and regulating phospholipid metabolism in brain of methionine-choline-deficient rats. FASEB J. 33(8),9334-9349. doi: 10.1096/fj.201802683R.
29. Dorheim MA., Tracey WR., Pollock JS., Grammas P., 1994.Nitric oxide synthase activity is elevated in brain microvessels in Alzheimer's disease. Biochem Biophys Res Commun. 205(1):659-65. doi: 10.1006/bbrc.1994.2716.
30. Gargiulo L., Bermejo M., and Liras A., 2000.Reduced neuronal nitric oxide synthetase and C protein kinase levels in Alzheimer’s disease. Revista de Neurologia. 30(4),301–303.
31. Hyman BT., Marzloff K., Wenniger JJ., Dawson TM., Bredt DS., Snyder SH., 1992.Relative sparing of nitric oxide synthase-containing neurons in the hippocampal formation in Alzheimer's disease. Ann Neurol. 32(6),818-20. doi: 10.1002/ana.410320618.
32. Balez R., Ooi L., 2016.Getting to NO Alzheimer's Disease: Neuroprotection versus Neurotoxicity Mediated by Nitric Oxide. Oxid Med Cell Longev. 2016,3806157. doi: 10.1155/2016/3806157. Epub 2015 Nov 30.
33. Drummond E., Wisniewski T., 2017.Alzheimer's disease: experimental models and reality. Acta Neuropathol. 133(2),155-175. doi: 10.1007/s00401-016-1662-x. Epub 2016 Dec 26.
34. Collins HM., Greenfield S., 2024.Rodent Models of Alzheimer's Disease: Past Misconceptions and Future Prospects. Int J Mol Sci. 25(11),6222. doi: 10.3390/ijms25116222.