Acta Pharm. Sci. Vol 63:(2), 2025
Original article
DOI: 10.23893/1307-2080.APS6318

Neuroprotective effects of aminophylline in LPS-induced Alzheimer's disease in rat model

Hebah Majed HAMOOD[1*], Adeeb A. AL-ZUBAIDY[2]

1 Al-Nahrain University, College of Pharmacy, Department of Pharmacology, Baghdad, Iraq
2 University of Warith Al-Anbiyaa, College of Medicine, Department of Pharmacology, Karbala, Iraq

ABSTRACT

This research aimed to evaluate the aminophylline neuroprotective benefits against Alzheimer's Disease -like characteristics caused by lipopolysaccharide (LPS) in forty rats divided into four groups: control group, induction group received lipopolysaccharide single dose once a day for seven days, third group received donepezil orally once daily for 21 days and fourth groups received aminophylline once daily for 21 days, and both third and fourth group received LPS once. The Barnes Maze behavioral parameters evaluated, inflammatory cytokines, and oxidative stress indicators were measured in brain tissue samples and the results showed that primary latency and primary error for the three phases of Barnes Maze behavioral test and the levels of studied inflammatory cytokines (IL-1b, IL-6, and TNFa) in addition to malondialdehyde (MDA) were reduced significantly in aminophylline group comparing with induction group while SOD1 levels increased significantly in aminophylline group comparing with induction group which indicate that aminophylline has neuroprotective effects via antioxidant and anti-inflammatory properties.

Keywords: neuroprotective effects, aminophylline, LPS-induced Alzheimer's disease

*Corresponding author: Hebah Majed HAMOOD
E-mail: hebarafal@yahoo.com
ORCIDs:
Hebah Majed HAMOOD: 0000-0001-6210-9595
Adeeb A. AL-ZUBAIDY: 0000-0002-5207-383X
(Received 12 Dec 2023, Accepted 7 May 2024)

INTRODUCTION

Alzheimer's disease (AD) is one of the most typical degenerative neurological conditions in the world, with symptoms including decreased cognitive function, abnormal behaviour, psychiatric difficulties, and memory loss[1]. The etiology of AD is unknown, but it is linked to several pathological manifestations, including accumulation of microtubule tau protein, cholinergic neuron death, abnormal amyloid beta (Aβ), oxidative stress caused by metals, and irregular acetylcholinesterase (AChE) activity[2]. Previous research demonstrated that Neurodegeneration considered one several pathological conditions that include also inflammation and cancer that related to lipid peroxides which are highly reactive elements, that participated in accelerating the chain reaction of reactive oxygen species (ROS) formation[3]. The degradation of lipid peroxide leads to producing 4-hydroxynonenal and malondialdehyde (MDA) which are commonly used for the quantification of lipid peroxidation in the obtained tissue samples[3,4]. Catalase and Superoxide dismutase (SOD) enzymes provide the first line protection against the tissue injury which is caused by the ROSs as they have the ability to scavenge free radical along with the other nutritional antioxidants[5]. SOD protects the cell from the oxidative damage by catalyzing the conversion of superoxide into oxygen and hydrogen peroxide[6]. In the cases of SOD activity elevation, the possibility of lipid peroxidation become reduced[5] and the estimation of SOD's activity and its relation with the lipid peroxidation could help in understanding the prognosis of the neuronal damage and to provide an optimum treatment regimen[7].

Microglial cells in the brain of patients with AD showed an enhanced Inflammatory process as they become activated and produce high levels of cytokines. In early stages, microglia cells when activated become able to phagocytize Aβ, but if the activation persist for an extended period, they loss this ability and the pro inflammatory cytokines they release such as are interleukin (IL)-1, IL-1, IL-6, and tumour necrosis factor (TNF) participate in propagation of pathological tau proteins and neuron damage[8].

The current treatment for AD involves usage of cholinesterase inhibitor (rivastigmine, galantamine, and donepezil) and memantine which is considered as N-methyl-D-aspartate (NMDA) antagonists[9]. Aminophylline which is a methylxanthine derivative is considered as a competitive non-selective phosphodiesterase inhibitor that inhibits several types of the phosphodiesterase (PDE) enzyme family, mostly the PDE4 isoforms, and activates protein kinase. Also, it increases intracellular concentrations of cAMP and cGMP, inhibits the synthesis of TNF-alpha and leukotriene, and decreases inflammation and innate immunity[10].

The present research was aimed at investigating and evaluating the potential neuroprotective ability of aminophylline against AD-like characteristics caused by lipopolysaccharide in rats.

METHODOLOGY

Experimental groupings and treatments

A case-control study was conducted in the department of Pharmacology in the College of medicine, Al Nahrain University between June and December 2022 that include forty male rats (250 to 500 g in weight) of 3 to 4 months old were accommodated under standard laboratory conditions at a temperature of 20-22°C. Aminophylline vial (Radiant Pharma), lipopolysaccharide powder (LPS) (Sigma-Aldrich, USA), and donepezil (Pfizer) were dissolved in normal saline. The rats were split into four treatment groups and each group consist of ten rats as the following:

Group 1: which is consist of the control group.

Group 2 (induction group): received a dose of 250 g/kg of LPS i.p. once a day for 7 days, to induce AD-like characteristics[11].

Group 3: in this group the animals received a prophylactic oral treatment with donepezil in a dose of 0.5 mg/kg once a day[12], for 21 days, followed by LPS (250 g/kg i.p. once a day) and the same dosages of donepezil for another 7 days.

Group 4: administered prophylactic treatment with aminophylline (20 mg/kg i.p. once a day)[13], for 21 days, followed by LPS (250 g/kg i.p. once a day) and the same dosages of aminophylline for another 7 days.

After three days of treatment, a behavioural test, including cognitive testing utilizing the Barnes Maze, was performed.

Behavioural test using the Barnes Maze procedure

Barnes Maze was manufactured in Baghdad. The Barnes Maze was made up of a wood circular platform (122 cm diameter) with 21 similar and evenly spaced holes (10 cm wide) around it, one of which was connected to an escape box (35 cm x 12 cm x 12 cm), and the Maze was elevated 100 cm above the ground. Because all of the holes looked the same, the rats couldn't tell the escape hole apart from the others until they were right next to it. The buzzer positioned in the center of the Maze was to give sounds that encouraged the escape from the platform to stimulate an effective escape response. The Barnes Maze procedure was conducted in line with the method of Kuzmin et al.[14], with slight adjustments. The Barnes Maze procedure includes the following phases:

Habituation phase: To lessen anxiety, the animals had become accustomed to the platform and the escape box a day prior to the acquisition phase. This familiarization procedure was conducted without the use of a buzzer.

The acquisition phase: This phase started 24 hours following the Maze habituation phase. Three training sessions each day were included in the acquisition. Each training session lasted 180 seconds, with a 10-minute break after that, the animals were put back in the cage from which they came. Throughout the acquisition trials, the escape box and platform position were still fixed. In each experiment, the rats were placed in the platform middle, the buzzer was activated, and they were free to roam around the facility. The experiment ended 180 seconds once the animal had gotten to the escape box. Then, the hole was immediately closed for 30 seconds, and the animal was brought back to its initial cage. The animal was led there slowly and given 30 seconds to explore it if it failed to reach the escape box within 180 seconds. After each session, the outer layer of the facility and the escape box were sanitized by employing a 100% (w/v) solution of ethanol for removing smell cues. Primary errors and primary latency were captured as parameters. Primary latency was defined as the time it took the rat to make first contact with the escape box. The primary error was the number of holes that were explored before coming into contact with the escape box[14].

Probe trial: After 24 hours of acquisition, a probe trial commenced. This phase was distinguished by a duration of 90 seconds to assess spatial memory, and in this trial, the escape box was shut[15]. Therefore, the animals were permitted to look around the Maze and the escape box, and neighbouring holes. The primary errors and primary latency also were recorded.

Reversal learning: Three reversal trials were held an hour following the conclusion of the probe experiment (180 seconds for each trial). Except for the escape hole being rotated 180°, the reversal learning experiment was similar to the acquisition experiment. As a result, despite the acquired spatial clues, the animal was unable to escape the Maze and was forced to remember the new position of the hole. The primary errors and primary latency required to get to the escape box were then calculated. The animals were anesthetized by inhaling diethyl ether after completing all phases of the behavioural test[14].

Sample collection and biochemical analysis

The animals were killed, and their brains were promptly rinsed in cold phosphate-buffered saline (0.02 mol/L, pH 7.2-7.4)[16]. The right hemisphere of the brain was washed with BSP and homogenized. The homogenized tissue was

utilized to evaluate oxidative stress indices which include malondialdehyde (MDA) and superoxide dismutase 1 (SOD1) and cytokines of inflammation that include tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) that estimated by using ELISA according to the manufacturer's (Elabscience) recommendations.

Statistical analysis

The data of the study are presented as mean ± standard deviation. To compare the means of two groups, the unpaired t-test was utilized, and analysis of variance (ANOVA) with a post-hoc Tukey test was employed to compare the means of three or more groups. The data were analyzed employing the Statistical Package for Social Sciences (SPSS) version 23. ($p<0.05$) or ($p\leq0.001$) were regarded as significant or highly significant, respectively[17].

RESULTS and DISCUSSION

Behavioural pattern of the experimental rats

In the results obtained in the current work, the averages for primary latency and primary error for the three phases (acquisition, probe, and reversal) were increased significantly ($p<0.001$) in the induction group in relation to the control group. On the other hand, the parameters evaluated showed to be decreased significantly ($p<0.001$) in rats receiving either donepezil or aminophylline in contrast to the induction group and these averages demonstrated values comparable to that of control group which indicated by the non-significant ($p>0.05$) different in these parameters between groups receiving either donepezil or aminophylline and those of controls as illustrated in Table 1.

Table 1. Effects of aminophylline on rats in the Barnes maze test

Group		Control	Induction (LPS)	Donepezil	Aminophylline
Acquisition Phase	Primary Latency	99.14 ± 23.86	155.72 ± 13.99##	99.33 ± 24.0**	109.48 ± 17.94**
	Primary Error	18.76 ± 4.85	28.43 ± 3.55##	18.76 ± 4.85**	17.57 ± 3.74**
Probe Phase	Primary Latency	27.0 ± 11.62	69.29 ± 23.01##	28.57 ± 11.1**	44.14 ± 18.56**
	Primary Error	7.43 ± 5.13	31.29 ± 7.87##	7.43 ± 5.13**	9.29 ± 2.69**
Reversal Phase	Primary Latency	80.05 ± 29.39	159.76 ± 10.77##	80.19 ± 29.52**	135.33 ± 12.93**
	Primary Error	10.14 ± 3.17	27.62 ± 7.56##	10.19 ± 3.18**	16.33 ± 7.27**

##: Highly statistically significant ($p\leq0.001$) compared to control group; **: Highly statistically significant ($p\leq0.001$) compared to induction LPS group.

Inflammatory cytokine levels in the experimental rats

In the induction group, TNFa, IL-6, and IL-1b levels showed to be increased significantly ($p<0.05$) in a comparison with those levels in the control group. Meanwhile, the TNFa, IL-6, and IL-1b levels were significantly ($p<0.05$) reduced in rats receiving donepezil or aminophylline when compared to the induction group. The levels of these pro-inflammatory markers in groups administered donepezil or aminophylline were non-significantly differ from those of controls as illustrated in Table 2.

Table 2. Effects of aminophylline on inflammatory cytokines

Group	Control	Induction (LPS)	Donepezil	Aminophylline
IL-1b (pg/ml)	78.26 ± 32.95	177.51 ± 64.02##	91.57 ± 36.19**	98.83 ± 22.73**
IL-6 (pg/ml)	52.47 ± 9.5	144. 23 ± 64.09##	57.02 ± 9.82**	69.27 ± 12.53**
TNF-a (pg/ml)	110.92 ± 5.71	316.10 ± 30.178##	123.75 ± 6.88**	134.44 ± 7.83**

##: Highly statistically significant ($p\leq0.001$) compared to control group; **: Highly statistically significant ($p\leq0.001$) compared to induction LPS group.

Levels of oxidative stress in experimental rats

In relation to the control group, the induction group revealed a significant increase ($p\leq0.001$) in MDA levels and a significant decrease ($p\leq0.001$) in SOD1 activity, whereas the levels of these oxidative stress markers in donepezil or aminophylline groups showed a significant decrease ($p\leq0.001$) in MDA levels with a significant increase ($p\leq0.05$) in SOD1 activity compared to the induction group as demonstrated in Table 3. It was also noticed that the levels of MDA and the activity of SOD1 in rats received donepezil or aminophylline were achieved levels somehow comparable to that of controls.

Table 3. Effects of aminophylline on oxidative stress parameters

Groups	Control	Induction (LPS)	Donepezil	Aminophylline
SOD1(ng/ml)	0.97 ± 0.13	0.70 ± 0.06##	0.83 ± 1.36**	0.81 ± 0.05**
MDA (ng/ml)	115.279 ± 83.9	241.37 ± 29##	122.17 ± 3.03**	131.96 ± 4.35**

##: Highly statistically significant ($p\leq0.001$) compared to control group; **: Highly statistically significant ($p\leq0.001$) compared to induction LPS group.

The pathogenesis of AD is complex, involving the accumulation of cerebral (beta-amyloid and hyperphosphorylated tau proteins), an inflammatory re-

sponse, and an increase in oxidative stress[18]. LPS was used to induce cognitive impairment in rats in this study because it induced memory loss and amyloidogenesis *in vivo* and *in vitro* due to systemic inflammation, by stimulating the generation and release of pro-inflammatory cytokines including TNFa, IL-6, and IL-1b[19]. Peripheral systemic infusion of LPS has been proven to cause oxidative stress as well as neuro-inflammation in the brain due to a rise in the Aβ level[20]. LPS treatment impaired both cognitive flexibility and spatial memory in the Barnes Maze test. Pre-treatment with cholinesterase inhibitors (donepezil) and aminophylline inhibited these LPS effects. Aminophylline has been demonstrated to have anti-inflammatory, anti-lipid peroxidation, and free radical scavenging characteristics[21]. Aminophylline has been reported to decrease levels of inflammatory cytokine's MDA and increase SOD in homogenized rat brains, an observation similar to the results of this study.

Many studies have shown that methyl-xanthine drugs have anti-inflammatory properties that act systemically as well as locally on airway inflammation[22,23]. By harvesting peripheral blood monocytes over 4 days, methyl-xanthine caused a progressive decrease in pro-inflammatory cytokine (TNFa, IL-8, IL-6, and IL-1) production (20% to 80%). This feature appears to be mediated by an epigenetic process that activates histone deacetylase-dependent gene switches, causing them to flip to a more anti-inflammatory phenotype. Thus, these impacts appear to be due to theophylline-induced macrophage re-direction, and other immune cells toward an anti-inflammatory state, which has been demonstrated to have a plethora of dose-dependent gene switches that regulate several cytokines[24]. As a non-selective adenosine receptor antagonist, aminophylline can antagonize adenosine receptors and inhibit the release and production of inflammatory factors by inhibiting phosphodiesterase activity[25]. Results obtained in the current study revealed that Aminophylline have a clear anti-inflammatory effect against a lipopolysaccharide (LPS)-induced inflammatory model which is consistent with recent study which demonstrated that aminophylline have reduced the permeability of the endothelial cell by down-regulating the related protein level in a lipopolysaccharide (LPS)-induced inflammatory model[26]. Aminophylline was discovered to effectively reduce lipid peroxidation in the rat brain, as evidenced by a considerable drop in MDA and pro-inflammatory cytokines generation as well as an increase in SOD activity in several parts of the rat brain, such as (cortex, cerebellum, midbrain, and basal ganglia). Aminophylline is a salt made up of two molecules of theophylline and one of ethylenediamine. Because of the ethylene diamine component, aminophylline at therapeutic concentrations is capable of antagonizing hypochlorous acid (HOCl). Some studies have found that low concentrations

of aminophylline are capable of effectively scavenging hydroxide radicals[27,28] because the anti-inflammatory and antioxidant activities of aminophylline improve cognitive flexibility spatial memory in the Barnes Maze task.

In conclusion, the findings in this study demonstrate that aminophylline enhances learning and memory in LPS-induced AD-like rats, implying that aminophylline has neuroprotective benefits via antioxidant and anti-inflammatory mechanisms.

STATEMENT OF ETHICS

The study received approval from the "Institute Review Board (IRB) of Al-Nahrain University" in October, 2022 (142/2022).

CONFLICT OF INTEREST STATEMENT

Authors declared no conflict of interest.

AUTHOR CONTRIBUTIONS

Design – AL-Zubaidy AA; Acquisition of data – Hamood HM; Analysis of data – Hamood HM; Drafting of the manuscript – Hamood HM; Critical revision of the manuscript – AL-Zubaidy AA; Statistical analysis – Hamood HM, AL-Zubaidy AA; Technical or financial support – Hamood HM, AL-Zubaidy AA; Supervision – AL-Zubaidy AA.

FUNDING SOURCES

This work was not supported or funded by any drug company.

ACKNOWLEDGMENTS

Special appreciation to College of Pharmacy Al-Nahrain University for providing services and facilities for this research.

REFERENCES

1. Knopman DS, Amieva H, Petersen RC, Chételat G, Holtzman DM, Hyman BT, et al. Alzheimer disease. Nat Rev Dis Primers, 2021;7(1):33. Doi: 10.1038/s41572-021-00269-y

2. Morroni F, Sita G, Graziosi A, Ravegnini G, Molteni R, Paladini MS, et al. PQM130, a novel feruloyl-donepezil hybrid compound, effectively ameliorates the cognitive impairments and pathology in a mouse model of Alzheimer's disease. Front Pharmacol, 2019;10:658. Doi: 10.3389/fphar.2019.00658

3. Gaschler MM, Stockwell B. Lipid peroxidation in cell death. Biochem Biophys Res Commun, 2017;482(3):419-425. Doi: 10.1016/j.bbrc.2016.10.086

4. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signalling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev, 2014;2014:360438. Doi: 10.1155/2014/360438

5. Abdel-Hafez HZ, Mohamed EE, Abd-Elghany AA. Tissue and blood superoxide dismutase activity and malondialdehyde level in leprosy. J Eur Acad Dermatol Venereol, 2010;24(6):704-708. Doi: 10.1111/j.1468-3083.2009.03496.x

6. Coimbra-Costa D, Alva N, Duran M, Carbonell T, Rama R. Oxidative stress and apoptosis after acute respiratory hypoxia and reoxygenation in rat brain. Redox Biol, 2017;12:216-225. Doi: 10.1016/j.redox.2017.02.014

7. Mohan S, Gobinath T, Salomy A, Nisha M, Kandasamy M, Essa MM, et al. Biophysical interaction of resveratrol with sirtuin pathway: significance in Alzheimer's disease. Front Biosci (Landmark Ed), 2018;23:1380-1390. Doi: 10.2741/4650

8. Babić Leko M, Nikolac Perković M, Klepac N, Štrac DŠ, Borovečki F, et al. IL-1β, IL-6, IL-10, and TNFα single nucleotide polymorphisms in human influence the susceptibility to Alzheimer's disease pathology. J Alzheimers Dis, 2020;75(3):1029-1047. Doi: 10.3233/JAD-200056

9. Ghumatkar PJ, Patil SP, Jain PD, Tambe RM, Sathaye S. Nootropic, neuroprotective and neurotrophic effects of phloretin in scopolamine induced amnesia in mice. Pharmacol Biochem Behav, 2015;135:182-191. Doi: 10.1016/j.pbb.2015.06.005

10. Deree J, Martins JO, Melbostad H, Loomis WH, Coimbra R. Insights into the regulation of TNF-alpha production in human mononuclear cells: the effects of non-specific phosphodiesterase inhibition. Clinics, 2008;63(3):321-328. Doi: 10.1590/s1807-59322008000300006

11. Zhu B, Wang ZG, Ding J, Liu N, Wang DM, Ding LC, et al. Chronic lipopolysaccharide exposure induces cognitive dysfunction without affecting BDNF expression in the rat hippocampus. Exp Ther Med, 2014;7(3):750-754. Doi: 10.3892/etm.2014.1479

12. Zhou Q, Nie S, Liu Z. Effect of Donepezil hydrochloride on cognitive function recovery of rats with Alzheimer's disease. Med One, 2016;1:e160023.

13. Al-Thamir SN, Oubaid EN. Protective effect of aminophylline on doxorubicin-induced cardiotoxicity in rats. Med j Babylon, 2013;10(1):219-228.

14. Kuzmin A, Liljequist S, Meis J, Chefer V, Shippenberg T, Bakalkin G. Repeated moderate-dose ethanol bouts impair cognitive function in Wistar rats. Addict Biol, 2012;17(1):132-140. Doi: 10.1111/j.1369-1600.2010.00224.x

15. Patil SS, Sunyer B, Höger H, Lubec G. Evaluation of spatial memory of C57BL/6J and CD1 mice in the Barnes maze, the Multiple T-maze and in the Morris water maze. Behav Brain Res, 2009;198(1):58-68. Doi: 10.1016/j.bbr.2008.10.029

16. Bhattacharyya D, Pandit S, Mukherjee R, Das N, Sur TK. Hepatoprotective effect of himoliv, a polyherbal formulation in rats. Indian J Physiol Pharmacol, 2003;47(4):435-440.

17. Goodman SN. P values, hypothesis tests, and likelihood: implications for epidemiology of a neglected historical debate. Am J Epidemiol, 1993;137(5):485-496. Doi: 10.1093/oxfordjournals.aje.a116700

18. Mirzaei F, Khazaei M, Komaki A, Amiri I, Jalili C. Multitarget effects of coconut oil (virgin type) on Aβ-induced Alzheimer's disease animal model. Arch Neurosci, 2019;6(2):e85715. Doi: 10.5812/ans.85715

19. Jeong YH, Park JS, Kim DH, Kang JL, Kim HS. Anti-inflammatory mechanism of lonchocarpine in LPS- or poly(I:C)-induced neuroinflammation. Pharmacol Res, 2017;119:431-442. Doi: 10.1016/j.phrs.2017.02.027

20. Ho YH, Lin YT, Wu CW, Chao YM, Chang AY, Chan JY. Peripheral inflammation increases seizure susceptibility via the induction of neuroinflammation and oxidative stress in the hippocampus. J Biomed Sci, 2015;22(1):46. Doi: 10.1186/s12929-015-0157-8

21. Barnes PJ. Theophylline: new perspectives for an old drug. Am J Respir Crit Care Med, 2003;167(6):813-818. Doi: 10.1164/rccm.200210-1142pp

22. Monteiro JP, Alves MG, Oliveira PF, Silva BM. Structure-bioactivity relationships of methylxanthines: trying to make sense of all the promises and the drawbacks. Molecules, 2016;21(8):974. Doi: 10.3390/molecules21080974

23. Janitschke D, Lauer AA, Bachmann CM, Grimm HS, Hartmann T, Grimm MOW. Methylxanthines and neurodegenerative diseases: an update. Nutrients, 2021;13(3):803. Doi: 10.3390/nu13030803

24. Allen SC, Tiwari D. Theophylline as a systemic anti-inflammatory agent: the need for its revival as a possible adjunctive treatment for 'inflammaging'. Biol Eng Med, 2019;4(1):1-3. Doi: 10.15761/BEM.1000162

25. Zhang R, Liu H, Dai D, Ding X, Wang D, Wang Y, et al. Adjunctive sepsis therapy with aminophylline (STAP): a randomized controlled trial. Chin Med J, 2022;135(23):2843-2850. Doi: 10.1097/CM9.0000000000002282

26. Chen Q, Zhou X, Hou R, Zhou Z, Wang Z, Chen Y, et al. Aminophylline modulates the permeability of endothelial cells via the Slit2-Robo4 pathway in lipopolysaccharide-induced inflammation. Exp Ther Med, 2021;22:1042. Doi: 10.3892/etm.2021.10474

27. Pal M, Datta S, Mandol M. Antioxidant property of aminophylline in rat brain. Al Ameen J Med Sci, 2015;8(1):22-27.

28. Aldafaay AAA, Abdulamir HA, Abdulhussain HA, Badry AS, Abdulsada AK. The use of urinary α-amylase level in a diagnosis of chronic renal failure. Res J Pharm Technol, 2021;14(3):1597-1600. Doi: 10.5958/0974-360X.2021.00283.3