

**Behavioral and Histopathological Improvement by Gallic Acid in Scopolamine-Induced Dementia:
Mitigation of Oxidative Stress, Inflammation, and Cholinergic Impairment**

Anas Sarhan*

Department of Internal Medicine, College of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia

Submit Date : 04 Nov. 2024

Revised Date : 22 Nov. 2024

Accept Date: 23 Nov. 2024

Keywords

- Scopolamine
- Gallic acid
- Oxidative stress
- Inflammation, memory loss
- Anticholinesterase enzyme

Abstract

Background: Gallic acid one of well-known natural phenolic compounds with antioxidant property that offers neuroprotective functions. Alzheimer's disease (AD) a neurodegenerative disease that results in dementia in elderly peoples. The aim of this study to investigate the ameliorative effect of gallic acid on scopolamine-induced memory loss and cognitive impairment. **Methods:** The rats were divided into four groups (n=6): Control group, Gallic acid group, Scopolamine group, Scopolamine+gallic acid group. Behavioral testes, including Y-maze, water maize, and passive avoidance testes, were conducted a half hour after-scopolamine injection, oxidative damage indicators such as SOD, MDA, CAT, GSH were measured. Also, EIISA measurements for inflammatory cytokines IL-1 β , TNF- α , IL-6, and AchE enzyme activity was performed in cortical tissues. Histological examination of cortical tissues was also performed. **Results:** Gallic acid significantly mitigated scopolamine-induced behavioral changes by decreasing entry latency and escape latency in water maize test. It also reduced lipid peroxidation (MDA), and cortical inflammatory markers (TNF- α , IL-1 β , IL-6,) and decreased AchE enzyme activity. Conversely, gallic acid significantly increased the spontaneous alternation%, and step-through latency in Y- maze and passive avoidance tests. Additionally, gallic acid elevated the level of antioxidant defense components (SOD, CAT, GSH) and increased the number of healthy neurons in cortical tissues. **Conclusions:** The finding of our study explored the novel neuroprotective effect of gallic acid against scopolamine-induced memory affection through its antioxidant, anti-inflammatory effects, and its inhibitory effect on AchE enzyme.

Introduction

Alzheimer's disease (AD) a worldwide disease, currently affecting over 26 million people. The number of individuals living with neurological diseases has doubled [1]. AD was considered the commonest form of dementia, leading to marked depression in cognitive and memory functions. By 2030, it is projected that 65.7 million individuals will complain from dementia expected to rise to 115.4 million by 2050 [2]. Various risk factors are associated with AD development and gradual diminishing in the cognitive function. Both environmental and genetic factors play a crucial role in the onset of AD [3,4]. However, the molecular mechanisms underlying the development of AD remain unknown.

Alzheimer's disease (AD) pathogenesis usually associated with different brain changes such as anatomical, neurochemical and histocellular changes [5]. These changes lead to a decline in neuronal activity, cortical neurons degeneration, synaptic disconnections, and impaired memory function. The prefrontal cortex and hippocampus, which are crucial for memory, are particularly affected by these pathological changes [6,7]. Oxidative stress is believed to play a crucial role in memory loss in AD [8]. Memory, a vital brain function, is preceded by learning [9,10]. Memory decline is linked to several etiological factors, including free radical production, aging, emotional variations, reduced cholinergic activity, increased oxidative stress, and neuroinflammatory responses. These factors contribute to conditions ranging from amnesia and dementia to more severe disorders like schizophrenia and Alzheimer's disease [11]. The cholinergic system is crucial for memory and learning. A key pathological feature

of memory impairment is the loss of cholinergic neurons and choline acetyltransferase activity decline in the hippocampus and cerebral cortex [12]. Scopolamine (Sco), a tropane alkaloid medication, disrupts cholinergic transmission by competitively antagonizing muscarinic acetylcholine receptors. This interference impacts short term memory and learning in human and mice [13]. Consequently, Sco administration in animals is used as an experimental model to study cognitive decline and memory loss [14]. As a muscarinic receptor antagonist, scopolamine targets the cholinergic system and affect the memory by enhancing oxidative insult [15-18]. Brain tissue, particularly the frontal cortex and hippocampus, is highly susceptible to oxidative stress due to its high oxygen consumption. These regions are crucial for cognition and memory, so damage here can insult a marked neurological effect [19]. The cholinergic pathways significantly influence the immune system, with systemic inflammatory responses being regulated by the anti-inflammatory and cholinergic pathways [20,22].

Gallic acid is a famous metabolite of plant that possesses a strong antioxidant character with a trihydroxybenzoic acid structure. Its phenolic structure allows several hydrogen atoms to easily delocalise free radicals, [23,24] which explains its powerful antioxidant effect. This capability enables gallic acid to protect organs and tissues from oxidative insult [23,25]. It is a common constituent in various herbal medicines and foods [26], known for increasing antioxidant enzymes and reducing inflammation in the brain [27,28]. Gallic acid has been shown to lower the risk of inflammation-related diseases, including,

cardiovascular disease [29,30], cancer [31], inflammation [32,33], liver diseases [34,35], and neurodegenerative diseases. This study aims to explore the neuroprotective effects of gallic acid against scopolamine-induced dementia and its underlying mechanisms.

2. Material and Methods

2.1. Experimental animals

The study utilized 24 male Wistar rats, each 180 and 220grams. The rats were housed in groups of four per metal cage, with veterinary supervision. They were maintained at a 20°C temperature and 50% humidity, under a 12-hour day/night cycle. The rats had unrestricted access standard diet and water.

2.2. Experimental design and specimen collection

After a two-week acclimatization period, the rats were divided into four groups of six. Control group received 0.9% normal saline orally. Gallic acid group: rats of this group was given 20 mg/kg/day of gallic acid dissolved in distilled water through gastric gavage for two weeks [36]. Scopolamine group: rats of this group was received 20 mg/kg of scopolamine hydrobromide liquified in saline via intraperitoneal injection [37]. The Scopolamine+gallic acid group, which was also subjected to scopolamine after two weeks intake of 20 mg/kg/day of gallic acid.

Passive avoidance, Y-maze and water maze tests were conducted 30 minutes after the scopolamine injection. After that, the rats were euthanized by decapitation, and their brains were removed. Each brain was divided at the midline into two cerebral hemisphere's. To prepare brain homogenate one of the two hemisphere was homogenised in ice-cold mM phosphate buffer, which was utilized to

estimate levels of lipid peroxidation marker (MDA), GSH, SOD, CAT, IL-1 β , IL-6, and TNF- α , in addition to AchE activity. The other hemisphere was preserved in 10% formalin for histopathological examination.

2.3. Behavioral assessment

2.3.1 Y-Maze Test

The Y-shaped device, constructed from white plastic, featured three passages, each measuring 16 cm in height, 43 cm in length, and 10 cm in width. It was designated as regions A, B, and C. Rats were located at the beginning of one arm, number of arm entries and their sequence were recorded over a one minute. The evaluation focused on the time of the numbers that the rats entered in the three arms (e.g., ABC or ACB), excluding repeated entries like ABB or ACC, to assess their capability to change their behaviors [38]. The examiner recognized the basic conditions for evaluating memory abilities and learning, ensuring the test method's rationality, accuracy, and reproducibility.

2.3.2 Water Maze Test

The water maze test utilized a rectangle tank with 42 cm in length, 28 cm in width, and 20 cm in height. With water at 22 \pm 2 °C temperature. A test platform was positioned at the end of the pool one centimeter above the water. Rats were allowed to swim for one minute, and the time taken to reach the platform was documented as the escape time [39].

2.3.3 Passive Avoidance Test

The passive avoidance test leverages rat's preference for dark sites. The education box is classified into a dark area, equipped with an electric shock source on the ground, and a light area not with electric shock, separated by a

guillotine door. When placed in the dark zone and subjected to repeated shocks (0.3 mA for 15 seconds), the rats move to the lighted area across the door of the guillotine. Long term memory is assessed by recording the step through latency time, which is the time the mice take to remember the electric shock and stay in the bright area. [40].

2.4. Detection of AChE activity in the cortical supernatant

Following the procedures outlined by Ellman et al. [41] and Gorun et al. [42], acetylcholinesterase activity in the cortical homogenate was measured using a Rat AChE ELISA kit (Cat# E-EL-R0355). According to the manufacturer's instructions.

2.5. Histological examination

The coronal sections of the prefrontal cortex (PFC) tissue were processed by the use of automated tissue processor. The brain tissues were fixed in paraffin, and the renal tissue blocks were serially sliced at 5 μm by a Leica rotary microtome. These sections were then overlapped on glass slides with DPX and stained with hematoxylin and eosin (H&E) to reveal the tissue histology.

2.6. Estimation of oxidative markers in cortical homogenates

Malondialdehyde (MDA), GSH, SOD, and CAT levels were measured in cortical homogenates using commercial kits bought from Biodiagnostic company in Cairo, Egypt. According to the manufacturer's rules.

2.7. Assessment of IL-6, IL-1 β , and TNF- α levels in the cortical tissue homogenates

The levels of IL-6 (Cat# ERA32RB), IL-1 β (Cat# ERIL1B), and TNF- α (Cat# KRC3011) were measured using rats ELISA kits following the protocols of the factory.

2.8. Statistical analysis

Data of this study were analyzed using GraphPad Prism 8.4.3. Statistical significance was determined using one-way ANOVA followed by Tukey's test. A p-value of <0.05 (*) was considered significant. Results were expressed as mean \pm standard deviation.

3. Results

3.1. Effect of gallic acid on scopolamine-induced behavioral changes.

Y-maze test was used to evaluate cognitive function. As shown in Figure 1A, scopolamine significantly $p \leq 0.05$ decreased spontaneous alternation percentage compared to control groups, on the contrast, oral intake of gallic acid with scopolamine significantly $p \leq 0.05$ increased the spontaneous alternation %. Additionally, water maze test to assessed spatial learning and cognitive functions, as shown in Figure 1B, C the time of entry latency and escape latency per second significantly $p \leq 0.05$ increased in the scopolamine group when compared to control groups. Controversy, in the Scopolamine +gallic acid group, there was a significant $p \leq 0.05$ decrease in entry latency and escape latency times compared to scopolamine group. Furthermore, the examination of long term by passive avoidance test, discussed in Figure 1D, showed that the scopolamine group had a significant $p \leq 0.05$ decrease in step-through latency time compared to control rats. Fortunately, coadministration with gallic acid significantly $p \leq 0.05$ increased the time of step-through latency in comparison to scopolamine group. From all of the above behavioral tests' results, we can conclude that gallic acid administration prevents scopolamine-induced amnesic and cognitive changes.

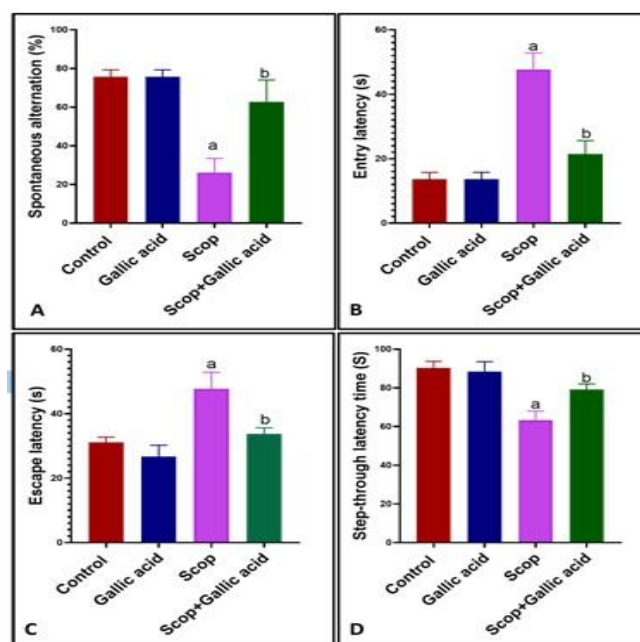


Fig. 1. Effect of scopolamine and gallic acid on behavioral testes (A) Y-maze test, (B) Water maze test, (C) Water maze test, (D) passive avoidance test. a indicates difference between Scop and control groups; b indicates difference between Scop+gallic acid and Scop groups. ($p \leq 0.05$).

3.2. Impact of gallic acid on scopolamine-induced cortical oxidative stress.

As shown in Figure 2, the scopolamine group showed a significant $p \leq 0.05$ decrease in the activity of CAT, SOD, GSH, CAT, and an increase in MDA compared to control groups. However, cotreatment with gallic acid significantly $p \leq 0.05$

depressed the MDA level, and increased SOD, GSH, CAT levels in comparison to scopolamine group. Meanwhile, there is no significance between control and gallic acid group. From all of the above data, it can be concluded that gallic acid exhibits strong antioxidant power against scopolamine-induced oxidative stress.

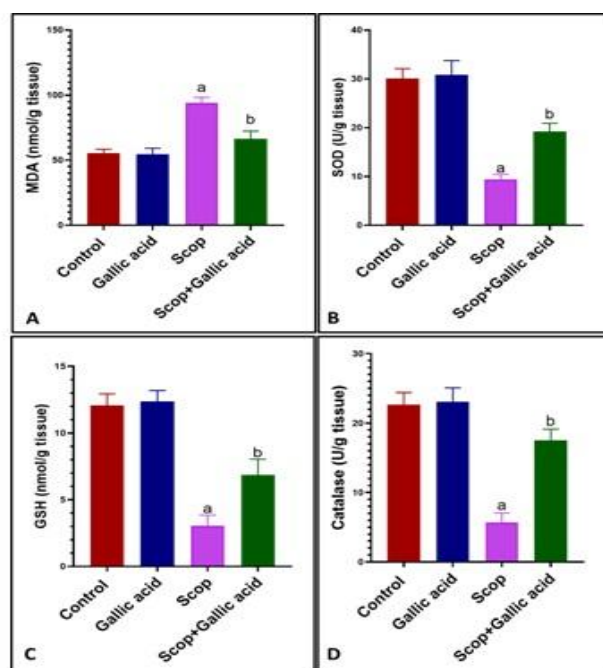


Fig. 2. Impact of scopolamine and gallic acid on oxidative stress markers in cortical tissues (A) MDA, (B) SOD, (C) GSH, (D) CAT. a Scop vs control groups; b Scop+gallic acid vs Scop groups. ($p \leq 0.05$).

3.3. The effect of scopolamine and gallic acid on inflammatory cytokines and acetylcholine esterase activity in cortical tissues

As shown in Figure 3, intraperitoneal injection of scopolamine significantly $p \leq 0.05$ increased the level of inflammatory mediators TNF- α , IL-1 β , IL-6, and AchE activity compared to control animals. On the opposite direction, oral intake of gallic acid with scopolamine in the fourth group

significantly $p \leq 0.05$ decreased the level of TNF- α , IL-1 β , IL-6, and AchE levels compared to scopolamine group. Together, these results indicate that gallic acid produces anti-inflammatory action against scopolamine-induced cortical inflammation and decreases the upregulatory effect of scopolamine on the acetylcholinesterase activity.

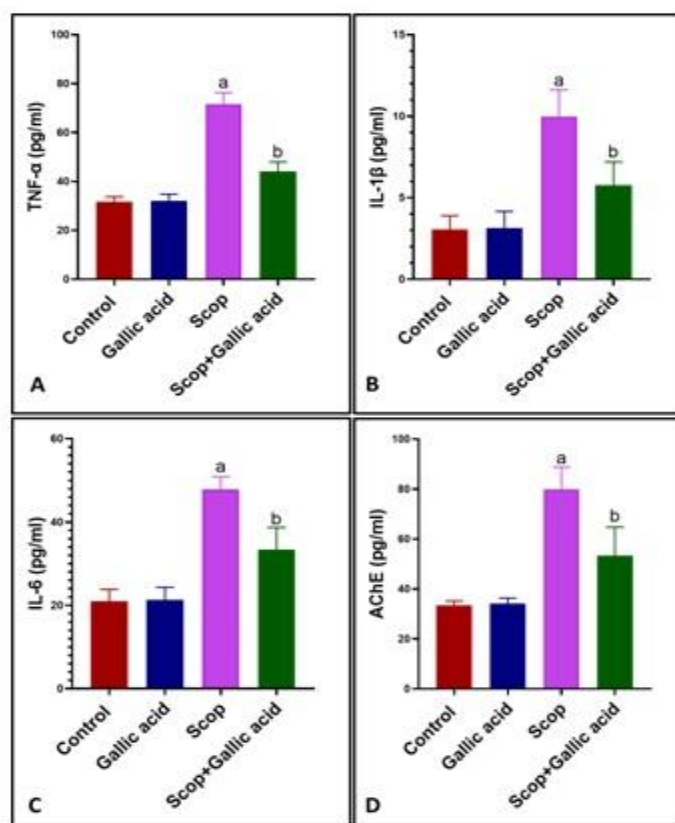


Fig. 3. Impact of scopolamine and gallic acid on proinflammatory cytokines (A) TNF- α , (B) IL-1 β , (C) IL-6, and acetylcholine esterase (D) AChE in cortical tissues. a Scop significant to control groups; b Scop+gallic acid significant Scop groups. ($p \leq 0.05$).

3.4. Histological examination of cortical tissue in different experimental groups.

H&E examination of cortical tissues from the control and gallic acid groups showed a normal picture of neurons and surrounding blood vessels (Fig. 4A-D), in contrast, the group of scopolamine showed a feature of cortical degeneration including a decrease in the number of neuronal

cells, perineural oedema and neurophagia [Fig. 4E,F]. However, cortical sections from Scopolamine+gallic acid group showed a clear improvement in the cortical tissue, with an increase in the number of neurocytes and a decrease in the previous observed changes [Fig. 4G,H].

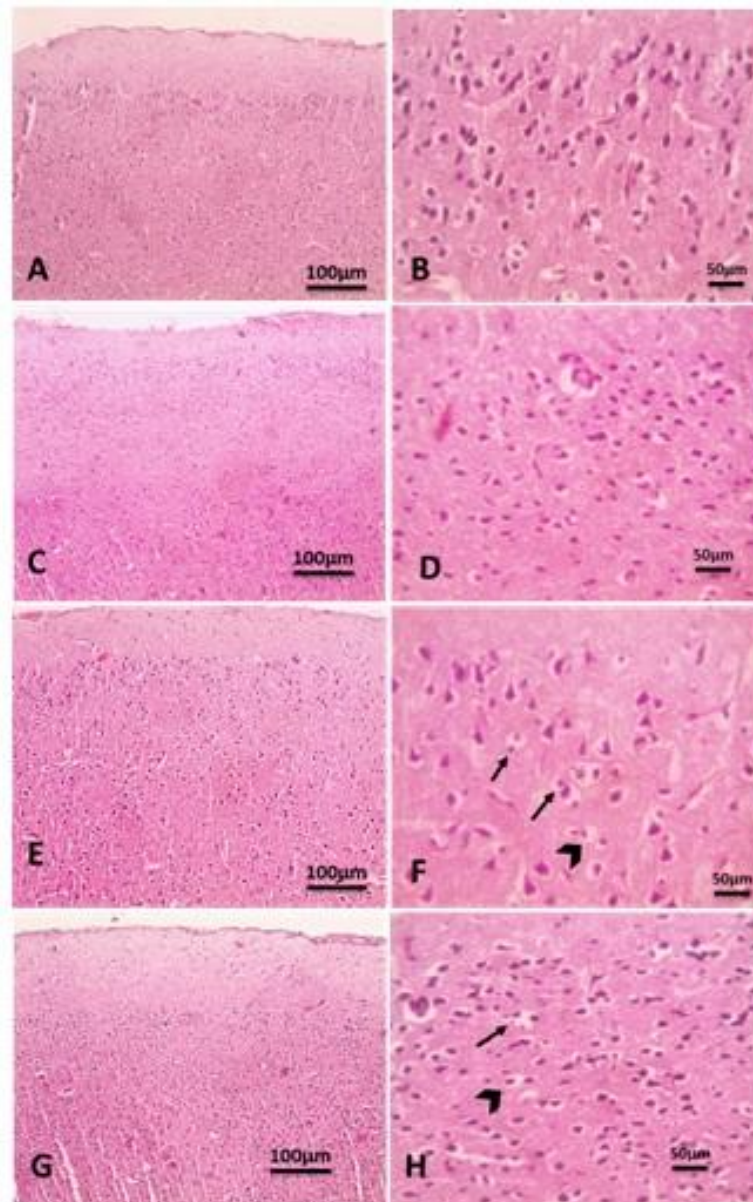


Fig. 4: H&E cortical sections showing normal neurons and blood vessels in control and gallic acid groups (A-D). Cerebral cortical sections from scop group showing decreased neuronal numbers, marked perineuronal edema (arrowheads) and neurophagia (black arrows) (E,F). Cerebral cortical sections from Scop+gallic acid group showing increased neuronal numbers (black arrow) and markedly decreased perineuronal edema (arrowheads) and neurophagia (black arrows) (G,H). Magnifications X: 100 bar 100 and 400 bar 50.

Discussion

The main findings of our study is to explore the neuroprotective effect of gallic acid against scopolamine-induced amnesic changes. These changes were evidenced by a decrease in spontaneous alternation percentage in Y-maze test, entry and escape latency in water maze test, and

increase in the time of step-through latency in passive avoidance test. The anti-amnesic effect of gallic acid is attributed to its upregulation to antioxidant enzymes production, reduction in lipid peroxidation and inflammatory markers, and activity of acetylcholine esterase enzyme.

Alzheimer's disease is a well-known neurodegenerative disorder manifested by reduced cognitive functions, memory loss, and altered behavior [43]. It is also considered the principal cause of dementia in elderly individuals [44].

In this study, we have performed three behavioral tests to examine learning, short- and long-term memory, and training memory process which affected by Alzheimer's disease. All these behavioral tests were impacted by the administration of scopolamine, which resulted in a significant decline in spontaneous alternation % and step through time, and an increase in entry and escape latency in relation to control group. This findings in are consistent with the previous works done by Park et al. [45], and Hafez et al. [46], and Choi et al. [47], who documented the affection of learning and memory by scopolamine injection. Additionally, the decline in cognitive function was supported by histopathological changes in prefrontal cortex, evidenced by neuronal degeneration and a decrease in neuronal number.

However, our study found that gallic acid intake alongside scopolamine improves spatial learning and memory function in behavioral tests, evidenced by histological clearance, showing a decrease in damaged neurons and an increase in healthy ones. This data concurrent with the previous study by Jafaripour et al. [48]. Which documented the protective effect of gallic acid on cognitive function and anxiety-like behaviors in induced hepatic encephalopathy via bile duct ligation. Also, Meftahi and Aboutaleb, [49] reported the ameliorative effect of gallic acid on stress-enhanced impairment in spatial learning and memory, and anxiety like behavior, as evaluated by (MWM) and Elevated-plus maze tests. They

also noted a reduction in neuronal loss in the prefrontal cortex and hippocampus.

One of the important mechanisms involved in the pathogenesis of Alzheimer's diseases is the oxidative stress [50]. MDA is a famous indicator for membranous lipid peroxidation [51]. Meanwhile, SOD, GSH, and CAT are considered the intrinsic enzymatic and non-enzymatic antioxidant system responsible for scavenging free radicals resulting from oxidative stress [52]

In this study, scopolamine injection exhibited a strong oxidative stress response, characterized by elevated MDA levels and a significant decrease in MDA, SOD, GSH levels. This data parallel to the study by Hosseini et al. [53], and Samir et al. [54]. Which documented sever oxidative stress in scopolamine-induced dementia. in contrast, gallic acid administration elevated antioxidant enzymes and decreased MDA levels, in consistency with the previous work done by Mansouri et al, [55], who reported that gallic acid combats streptozotocin-induced memory deficit by increasing the activity of SOD, CAT, GPx in cortical and hippocampal tissues.

The central cholinergic system is a principal pathway involved in cognitive function and memory. The fist pathological event in Alzheimer's disease is the decline in the cholinergic activity [56]. Damage to cholinergic neurons resulted in change in acetylcholine synthesis, which deteriorates memory function [46]. This finding aligns with results of Palle and Neerati, [57], who showed that scopolamine intraperitoneal injection significantly increased the activity of AchE enzyme in cortical tissues, leading to defect in cholinergic neurons, and neurochemical degeneration.

Neuroinflammation is another mechanism was involved in Alzheimer's disease pathology [58] Proinflammatory mediators TNF- α , IL1, and IL4 has been associated with the progression of Alzheimer's disease. The results of our work showed a marked increase in inflammatory markers TNF- α , IL-1 β , IL-6 in the scopolamine group, consistent with previous findings [59]. On the controversy, coadministration of gallic acid significantly reduced the release in inflammatory mediators. This is supported by the work of Wen et al, [60], which reported that gallic acid markedly decreased inflammatory cytokines in a rat model of depression and visceral pain. The antioxidant, ant-inflammatory properties of gallic acid, along with its inhibitory effect on

AchE enzyme activity, explain its ameliorative effect on the histological structure of the brain tissue, thereby improving the behavioral changes induced in scopolamine treated rats.

Conclusions

The results of our work confirmed that scopolamine induced cognitive impairment and amnesia in an experimental rat model, which was ameliorated by gallic acid. This neuroprotective function of gallic acid is regarded to its antioxidant and anti-inflammatory abilities, as well as its ability to improve cholinergic neurons by inhibiting AchE enzyme activity. Based on these results, we propose that gallic acid could be considered for clinical use against scopolamine-induced Alzheimer's disease.

List of abbreviations

AD	Alzheimer's disease
AchE	acetylcholine esterase
Sco	Scopolamine
MDA	Malonaldehyde
SOD	Superoxide dismutase
CAT	Catalase
GSH	Reduced glutathione
IL-1 β	Interleukin-1 beta
TNF- α	Tumor necrosis factor-alpha
IL-6	Interleukin-6

References

1. **Birla H, Keswani C, Singh SS, Zahra W, Dilynashin H, Rathore AS, et al.** Unraveling the neuroprotective effect of *tinospora cordifolia* in a parkinsonian mouse model through the proteomics approach. *ACS Chem Neurosci* 2021;12:4319–4335. doi: 10.1021/acchemneuro.1c00481.
2. **Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP.** The global prevalence of dementia: A systematic review and meta-analysis. *Alzheimer's Dement* 2013;9:63–75. doi: 10.1016/j.jalz.2012.11.007.
3. **Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, et al.** Neuroinflammation in Alzheimer's disease. *Lancet Neurol* 2015;14:388–405. doi: 10.1016/S1474-4422(15)70016-5.
4. **Onyango IG, Jauregui GV, Čarná M, Bennett JP, Stokin GB.** Neuroinflammation in Alzheimer's disease. *Biomedicines* 2021;14. doi: 10.1016/S1474-4422(15)70016-5.

5. **Boisvert MM, Erikson GA, Shokhirev MN, Allen NJ.** The aging astrocyte transcriptome from multiple regions of the mouse brain. *Cell Rep* 2018;22(1):269–285.
6. **Huang WJ, Zhang X, Chen WW.** Role of oxidative stress in Alzheimer's disease. *Biomed Rep* 2016;4(5):519–522.
7. **Assefa BT, Gebre AK, Altaye BM.** Reactive Astrocytes as Drug Target in Alzheimer's Disease. *Biomed Res Int* 2018;4160247. doi: 10.1155/2018/4160247.
8. **Pohanka M.** Alzheimer's disease and oxidative stress: a review. *Curr Med Chem* 2014;21(3):356–364.
9. **Farahmandfar M, Naghdi N, Karimian SM, Kadivar M, Zarrindast MR.** Amnesia induced by morphine in spatial memory retrieval inhibited in morphine-sensitized rats. *Eur J Pharmacol* 2012;683:132–139.
10. **Johansen JP, Cain CK, Ostroff LE, LeDoux JE.** Molecular mechanisms of fear learning and memory. *Cells* 2011;147(3):509–524.
11. **Alikatte KL, Akondi BR, Yerragunta VG, Veerareddy PR, Palle S.** Antiamnesic activity of *Syzygiumcumini* against scopolamine-induced spatial memory impairments in rats. *Brain Dev* 2012;34:844–851.
12. **Gupta J, Kulshreshtha M.** Memory impairment concerning Alzheimer's disease: an update. *Int J Nutr Pharmacol Neurol Dis* 2017;7:45–53.
13. **Iversen SD.** Behavioural evaluation of cholinergic drugs. *Life Sci* 1997;60:1145–1152. doi: 10.1016/s0024-3205(97)00059-3.
14. **Bartus RT, Dean RL 3rd, Beer B, Lippa AS.** The cholinergic hypothesis of geriatric memory dysfunction. *Science* 1982;217:408–414. doi: 10.1126/science.7046051.
15. **Seifar F, Khalili M, Khaledyan H, et al.** α -Lipoic acid, functional fatty acid, as a novel therapeutic alternative for central nervous system diseases: a review. *Nutr Neurosci* 2019;22(5):306–316.
16. **Goverdhan P, Sravanthi A, Mamatha T.** Neuroprotective effects of meloxicam and selegiline in scopolamine-induced cognitive impairment and oxidative stress. *Int J Alzheimers Dis* 2012. doi: 10.1155/2012/974013.
17. **Jafariana S, King-Hwa L, Zurina H, Perimal-Lewis L, Sulaiman MR, Perimal EK.** Effect of zerumbone on scopolamine-induced memory impairment and anxiety-like behaviors in rats. *Alzheimers Dement Transl Res Clin Interv* 2019;5:637–643.
18. **Nigam A, Kulshreshtha M, Panjwani D.** Pharmacological evaluation of *Hibiscus Abelmoschus* against scopolamine-induced amnesia and cognitive impairment in mice. *Adv Hum Biol* 2019;9:116–123.
19. **Karim N, Khan I, Abdelhalim A, Abdel-Halim H, Hanrahan JR.** Molecular docking and anti-amnesic effects of nepitrin isolated from *Rosmarinus officinalis* on scopolamine-induced memory impairment in mice. *Biomed Pharmacother* 2017;96:700–709.

20. **Hoover DB.** Cholinergic modulation of the immune system presents new approaches for treating inflammation. *Pharmacol Ther* 2017;179:1–16. doi: 10.1016/j.pharmthera.2017.05.002.
21. **Tata AM, Velluto L, D'Angelo C, Reale M.** Cholinergic system dysfunction and neurodegenerative diseases: cause or effect? *CNS Neurol Disord Drug Targets* 2014;13:1294–1303. doi: 10.2174/1871527313666140917121132.
22. **Kilimann I, et al.** Parallel atrophy of cortex and basal forebrain cholinergic system in mild cognitive impairment. *Cereb Cortex* 2017;27:1841–1848. doi: 10.1093/cercor/bhw019.
23. **Nayeem N, Asdaq SMB, Salem H, Ahel-Alfgy S.** Gallic acid: A promising lead molecule for drug development. *J Appl Pharm* 2016;8(2):213–218. doi: 10.4172/1920-4159.1000213.
24. **Kahkeshani N, et al.** Pharmacological effects of gallic acid in health and disease: A mechanistic review. *Iran J Basic Med Sci* 2019;22(3):225–237. doi: 10.22038/ijbms.2019.32806.7897.
25. **Gao J, Hu J, Hu D, Yang X.** A role of gallic acid in oxidative damage diseases. *Nat Prod Commun* 2019. doi: 10.1177/1934578X19874174.
26. **Wu XC, Yu BT, Hou AL, Hu TT, Lu GQ.** Study on stability of gallic acid. *Med J Natl Def Forces Southwest China* 2006;16:484–485.
27. **Mansouri MT, et al.** Neuroprotective effects of oral gallic acid against oxidative stress induced by 6-hydroxydopamine in rats. *Food Chem* 2013;138:1028–1033.
28. **Sarkaki A, et al.** Gallic acid improved behavior, brain electrophysiology, and inflammation in a rat model of traumatic brain injury. *Can J Physiol Pharmacol* 2015;93:687–694.
29. **Priscilla DH, Prince PSM.** Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. *Chem Biol Interact* 2009;179(2–3):118–124.
30. **Patel SS, Goyal RK.** Cardioprotective effects of gallic acid in diabetes-induced myocardial dysfunction in rats. *Pharmacogn Res* 2011;3(4):239. doi: 10.4103/0974-8490.89743.
31. **Ohno Y, et al.** Induction of apoptosis by gallic acid in lung cancer cells. *Anticancer Drugs* 1999;10(9):845–851.
32. **Kim MJ, et al.** Gallic acid, a histone acetyltransferase inhibitor, suppresses β -amyloid neurotoxicity by inhibiting microglial mediated neuroinflammation. *Mol Nutr Food Res* 2011;55(12):1798–1808.
33. **Kroes BV, Van den Berg A, Van Ufford HQ, Van Dijk H, Labadie R.** Anti-inflammatory activity of gallic acid. *Planta Med* 1992;58(6):499–504. doi: 10.1055/s-2006-961535.
34. **Rasool MK, et al.** Hepatoprotective and antioxidant effects of gallic acid in paracetamol-induced liver damage in mice. *J Pharm Pharmacol* 2010;62(5):638–643.

35. **Ohno T, Inoue M, Ogihara Y.** Cytotoxic activity of gallic acid against liver metastasis of mastocytoma cells P-815. *Anticancer Res* 2001;21(6A):3875–3880.
36. **Ola-Davies OE, Olukole SG.** Gallic acid protects against bisphenol A-induced alterations in the cardiorenal system of Wistar rats through the antioxidant defense mechanism. *Biomed Pharmacother* 2018;107:1786–1794.
37. **Zaki HF, Abd-El-Fattah MA, Attia AS.** Naringenin protects against scopolamine-induced dementia in rats. *Bull Fac Pharm Cairo Univ* 2014;52:15–25. doi: 10.1016/j.bfopcu.2013.11.001.
38. **Kwon SH, Lee HK, Kim JA, Hong SI, Kim HC, Jo TH, Park YI, Lee CK, Kim YB, Lee SY, et al.** Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice. *Eur J Pharmacol* 2010;649:210–217. doi: 10.1016/j.ejphar.2010.09.034.
39. **Fukada MTH, Francoline-Silva AL, Almedia SS.** Early postnatal protein malnutrition affects learning and memory in the distal but not in the proximal cue version of the Morris water maze. *Behav Brain Res* 2002;133:271–277. doi: 10.1016/S0166-4328(02)00014-5.
40. **Alberini CM, Travaglia A.** Infantile amnesia: A critical period of learning to learn and remember. *J Neurosci* 2017;37:5783–5795.
41. **Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM.** A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961;7:88–95. doi: 10.1016/0006-2952(61)90145-9.
42. **Gorun V, Proinov I, Baltescu V, Balaban G, Barzu O.** Modified Ellman procedure for assay of cholinesterases in crude enzymatic preparations. *Anal Biochem* 1978;86:324–326. doi: 10.1016/0003-2697(78)90350-0.
43. **Simunkova M, Alwasel SH, Alhazza IM, Jomova K, Kollar V, Rusko M, et al.** Management of oxidative stress and other pathologies in Alzheimer's disease. *Arch Toxicol* 2019;93:2491–2513. doi: 10.1007/s00204-019-02538-y.
44. **Tang KS.** The cellular and molecular processes associated with scopolamine-induced memory deficit: a model of Alzheimer's biomarkers. *Life Sci* 2019;233:116695. doi: 10.1016/j.lfs.2019.116695.
45. **Park HR, Lee H, Park H, Cho WK, Ma JY.** Fermented Sipjeondaebo-tang alleviates memory deficits and loss of hippocampal neurogenesis in scopolamine-induced amnesia in mice. *Sci Rep* 2016;6:22405. doi: 10.1038/srep22405.
46. **Hafez HS, Ghareeb DA, Saleh SR, Abady MM, El Demellawy MA, Hussien H, et al.** Neuroprotective effect of ipriflavone against scopolamine-induced memory impairment in rats. *Psychopharmacology* 2017;234:3037–3053. doi: 10.1007/s00213-017-4690-x.

47. **Choi JH, Lee EB, Jang HH, Cha YS, Park YS, Lee SH.** Allium hookeri Extracts Improve Scopolamine-Induced Cognitive Impairment via Activation of the Cholinergic System and Anti-Neuroinflammation in Mice. *Nutrients* 2021;13(8):2890. doi: 10.3390/nu13082890.
48. **Jafaripour L, Esmailpour K, Maneshian M, Bashiri H, Rajizadeh MA, Ahmadvand H, Asadi-Shekaari M.** The effect of gallic acid on memory and anxiety-like behaviors in rats with bile duct ligation-induced hepatic encephalopathy: Role of AMPK pathway. *Avicenna J Phytomed* 2022;12(4):425–438. doi: 10.22038/AJP.2022.19720.
49. **Meftahi GH, Aboutaleb N.** Gallic acid ameliorates behavioral dysfunction, oxidative damage, and neuronal loss in the prefrontal cortex and hippocampus in stressed rats. *J Chem Neuroanat* 2023;134:102364. doi: 10.1016/j.jchemneu.2023.102364.
50. **Cassidy L, Fernandez F, Johnson JB, Naiker M, Owoola AG, Broszczak DA.** Oxidative stress in Alzheimer's disease: a review on emergent natural polyphenolic therapeutics. *Complement Ther Med* 2020;49:102294. doi: 10.1016/j.ctim.2019.102294.
51. **Zarrouk A, Hammouda S, Ghzaïel I, Hammami S, Khamlaoui W, Ahmed SH, et al.** Association between oxidative stress and altered cholesterol metabolism in Alzheimer's disease patients. *Curr Alzheimer Res* 2020;17:823–834. doi: 10.2174/1567205017666201203123046.
52. **Kong D, Yan Y, He XY, Yang H, Liang B, Wang J, et al.** Effects of resveratrol on the mechanisms of antioxidants and estrogen in Alzheimer's disease. *Biomed Res Int* 2019;2019:8983752. doi: 10.1155/2019/8983752.
53. **Hosseini MJ, Mahmoodi N, Eskandari J, Bijani S, Yazdinezhad AR, Anoush M.** Protective effects of Vinca herbaceous extract against scopolamine-induced behavioral disturbances and brain oxidative stress in rats. *Heliyon* 2022;8:e09295. doi: 10.1016/j.heliyon.2022.e09295.
54. **Samir SM, Hassan HM, Elmowafy R, ElNashar EM, Alghamdi MA, AlSheikh MH, Al-Zahrani NS, Alasiri FM, Elhadidy MG.** Neuroprotective effect of ranolazine improves behavioral discrepancies in a rat model of scopolamine-induced dementia. *Front Neurosci* 2024;17:1267675. doi: 10.3389/fnins.2023.1267675.
55. **Mansouri MT, Naghizadeh B, Ghorbanzadeh B, Farbood Y, Sarkaki A, Bavarsad K.** Gallic acid prevents memory deficits and oxidative stress induced by intracerebroventricular injection of streptozotocin in rats. *Pharmacol Biochem Behav* 2013;111:90–96. doi: 10.1016/j.pbb.2013.09.002.
56. **Blake MG, Krawczyk MC, Baratti CM, Boccia MM.** Neuropharmacology of memory consolidation and reconsolidation: insights on central cholinergic mechanisms. *J Physiol Paris* 2014;108:286–291. doi: 10.1016/j.jphysparis.2014.04.005.

-
57. **Palle S, Neerati P.** Quercetin nanoparticles attenuates scopolamine induced spatial memory deficits and pathological damages in rats. *Bull Fac Pharm Cairo Univ* 2017;55:101–106. doi: 10.1016/j.bfopcu.2016.10.004.
58. **Cho YS, Kim SK, Ahn CB, Je JY.** Inhibition of acetylcholinesterase by gallic acid-grafted-chitosans. *CarbohydrPolym* 2011;84(1):690–693.
59. **Demirci K, Nazıroğlu M, Övey İS, Balaban H.** Selenium attenuates apoptosis, inflammation and oxidative stress in the blood and brain of aged rats with scopolamine-induced dementia. *Metab Brain Dis* 2017;32:321–329. doi: 10.1007/s11011-016-9903-1.
60. **Wen L, Tang L, Zhang M, Wang C, Li S, Wen Y, Tu H, Tian H, Wei J, Liang P, Yang C, Li G, Gao Y.** Gallic Acid Alleviates Visceral Pain and Depression via Inhibition of P2X7 Receptor. *Int J Mol Sci* 2022;23(11):6159. doi: 10.3390/ijms23116159.