Effect of coenzyme Q10 and/or epigallocatechin gallate on memantine-treated amnesia model in rats

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Background
Alzheimer's dementia is a progressive, fatal neurodegenerative disease that manifests as a disintegration of perception and memory.
Objectives
The current study evaluated the possible therapeutic effect of coenzyme Q10 (CoQ10) and/or epigallocatechin-3-gallate (EGCG) combined with memantine on scopolamine-induced amnesia in rats by evaluating the behavioral, biochemical, and histopathological changes.
Materials and methods
Rats were randomly allocated to 11 groups, each of which contained 16 rats. Six rats were used for biochemical tests, while ten rats were used for behavioral and histological examinations. Two behavioral assessments were conducted: an object-recognition test and a conditioned-avoidance test. The dopamine (DA) content of brain tissues was determined, as well as oxidative stress markers, such as superoxide dismutase, lipid peroxide end product malondialdehyde, and reduced glutathione. Besides, the activity of acetylcholine esterase (AchE), total antioxidant capacity, and inflammatory markers, such as tumor necrosis factor-alpha and interleukin-one beta, were determined in serum. Furthermore, histological examinations of whole-brain tissues were made.
Results
Scopolamine-treated rats were administered memantine at a dose of 20 mg/kg, coenzyme Q10 at a dose of 10 mg/kg, and EGCG at a dose of 10 mg/kg, individually or in combination, resulting in an enhancement of cognitive impairment in the condition-avoidance and object-recognition tests, as well as an improvement in all oxidative stress biomarkers, inflammatory biomarkers, and histological examination.
Conclusion
Rats were administered memantine and pretreated by the combination of CoQ10 and EGCG, resulting in potentiating the memantine action in scopolamine-induced amnesia in rats. The improvement in cognitive memory could be due to the synergistic effect of these drugs by decreasing AchE activity, DA level, anti-inflammatory, and antioxidant effects.

Keywords:
amnesia, coenzyme q10, epigallocatechin gallate, memantine

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Abbreviations: Acetylcholine esterase (AchE); epigallocatechin-3-gallate (EGCG); interleukin one-beta (IL-1β); malondialdehyde (MDA); reduced glutathione (GSH); superoxide dismutase (SOD); thiobarbituric acid-reactive substances (TBARS); total antioxidant capacity (TAC); tumor necrosis factor alpha (TNF-α).

Introduction

Dementia is a brain disorder characterized by the aggravation of various cerebrum capacities, including memory, considering, orientation, understanding, calculation, learning limit, language, and judgment. Intellectual capacity impairments are frequently accompanied by, and incidentally preceded by, a decline in enthusiastic control, social behavior, or inspiration [1]. Alzheimer's disease (AD) is the most well-known type of dementia, accounting for up to 75% of cases, either alone or in combination with other types of pathology (a condition referred to as "mixed dementia") [2]. AD is a progressive, fatal neurodegenerative disorder characterized by disintegration of perception and memory, progressive impairment of the ability to perform daily activities, and various neuropsychiatric and behavioral symptoms [3]. The abnormal deposition of insoluble "plaques" of a fibrous protein called amyloid and twisted fibers called "neurofibrillary

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tangles" in the brain results in brain changes. These strange plaques and tangles obstruct normal brain cell function. Besides, the neurotransmitter acetylcholine (Ach), which is necessary for learning and memory, is insufficient [4].

Although the cause of AD is unknown, there are three primary hypotheses based on the disease's hallmarks: the cholinergic theory, the amyloid theory, and the tau hypothesis. The amyloid cascade hypothesis and the amyloid-beta (Aβ) lethality theory have ruled research for a long time. The testimony of Aβ peptide in the cerebrum is viewed as a focal occasion in AD [5].

Moreover, mounting evidence indicates that oxidative stress plays a significant role in the onset and progression of AD. In AD patients' brains, oxidative stress is manifested by protein oxidation, lipid peroxidation, DNA oxidation, and the development of 3-nitrotyrosine [6].

The evidence for the involvement of inflammatory processes in the pathogenesis of AD has been reported. Moreover, the inflammation hypothesis has evolved. Indeed, the inflammatory response is still portrayed as a downstream effect of the gathered proteins (Aβ and tau) in this theory [7].

The cholinergic system is active in cognitive functioning, most notably in consideration, memory, and emotion. Studies have reported a loss of cholinergic neurons in patients with AD, decreased choline acetyltransferase activity, and Ach production [8].

Memantine is a noncompetitive glutamatergic N-methyl-D-aspartate (NMDA) receptor blocker that has been approved for the treatment of mild-to-severe AD. This medication mitigates the effects of pathologically elevated tonic glutamate levels [9].

Epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin-3-gallate (EGCG) are the four significant subordinates of green tea polyphenols, depending on their structural variations, where EGCG accounts for ~10% of the concentrate's dry weight [10]. EGCG has gained considerable attention as a potential therapeutic agent for preventing neurodegenerative [11], inflammatory [12], and malignant growth [13] diseases, owing to its beneficial effects on human health. EGCG has been shown to inhibit a variety of proinflammatory cytokine activities [14].

Coenzyme Q10 (CoQ10) is a critical component of cellular energy metabolism, where it is incorporated into the mitochondria's electron transport chain to promote ATP synthesis [15]. It is a potent antioxidant that protects against oxidative damage caused by free radicals, including lipid oxidation within the mitochondrial membrane [16].

Singh and Kumar uncovered that chronic treatment with CoQ10 in Aβ-treated animals significantly weakened an impairment of spatial learning and memory task implementation.

Acetylcholine esterase (AChE) activity and oxidative harm reestablished mitochondrial respiratory enzyme complex activities, and tumor necrosis factor alpha (TNF-α) level, recommending its antioxidant, mitochondrial reestablishing, and anti-inflammatory activity, when contrasted with Aβ-treated animals [17].

The purpose of this study is to determine whether CoQ10 or EGCG, or their combination, has a potentiating effect on memantine's therapeutic efficacy against scopolamine-induced amnesia in rats by evaluating the behavioral, biochemical, and histopathological changes.

Materials and methods

Drugs and reagents

All drugs and reagents were purchased from Sigma-Aldrich Chemical Co., St. Louis, Missouri, USA. EGCG was acquired with a clarity of more than or equal to 95%. EGCG was freshly prepared and administered intraperitoneally at a dose of 10 mg/kg (i.p.) in normal saline [18]. CoQ10 was dissolved in corn oil at a dose of 10 mg/kg (i.p.) [19]. Memantine hydrochloride was dissolved in normal saline at a dose of 20 mg/kg (i.p.) [20]. Scopolamine hydrobromide was dissolved in normal saline and given at a dose of 16 mg/kg (i.p.) to induce amnesia [21].

Animals

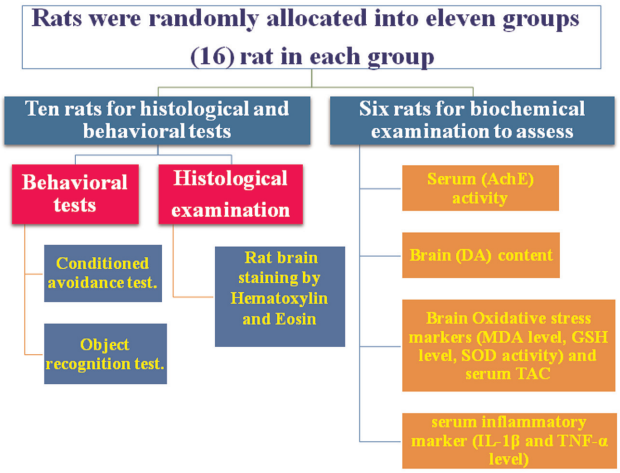
Male Sprague Dawley rats, weighing 200–250 g, were obtained from the animal house of El Nile Co. for pharmaceutical, El Amyria, Cairo, Egypt. The animals were kept at controlled ecological conditions with a constant temperature of 24±1°C and a 12/12-h light/dark cycle. They were acclimatized for 1 week before any trial strategies. They were acclimated for 1 week before any trial strategies and fed standard rat chow (El-Nasr, Abu Zaabal, Cairo, Egypt) that contained at least 20% protein, 5% fiber, 3.5% fat, 6.5% ash, and a vitamin blend; water was optional. The experimental

protocol utilized in this examination was endorsed by the Animal Ethics Committee No. 77/2016 of the Faculty of Pharmacy, Al-Azhar University, Egypt.

Experimental design

Rats were randomly allocated into 11 groups. Each group consists of 16 rats; 6 rats were utilized for biochemical examination and 10 rats for histological and behavioral tests. The work's design is summarized in Figure 1 and Table 1.

Figure 1



Design of the work.

Behavioral tests

Conditioned-avoidance test

This procedure was followed, as described by Arnt [22], with the modification made by Garofalo *et al.* [23]. Three days before the experiment, rats were trained by being exposed to a conditioned stimulus (electric bell) followed by an unconditioned stimulus (electric shock). The training consisted of pairing a 5-second auditory stimulus (Conditioned stimulus) with a 5-second foot shock. The number of trials post treatment to reach the safety area (i.e., to avoid the electric shock) during the first 5 s of the conditioned stimulus were recorded.

Object-recognition test

This procedure was carried out according to Ennaceur and Delacour [24]. Animals were exposed to 3 consecutive days of training in the apparatus without objects for 2 min/day. On the final day, 30 min after scopolamine administration, a session of two trials lasting 2 min each was permitted. In the "sample" trial (T1), two identical objects were placed in two opposite corners of the apparatus. A rat was placed in the apparatus and left to explore these two identical objects. After T1, the rat was placed back in its home cage, and a 1-hour intertrial interval was given. Subsequently, the "choice" trial (T2) was performed. In T2, a new object (N) was substituted for one of the

Table 1 Design of the investigation

Groups	First week	Second week	Third week	Behavioral and histological test day
1. Control	Corn oil 0.5 ml/200 g	Corn oil +normal saline 0.5 ml/200 g	Corn oil +normal saline	Normal saline
2. Scopolamine	Corn oil 0.5 ml/200 g	Corn oil + normal saline 0.5 ml/200 g	Corn oil + normal saline	Scopolamine (16 mg/kg, i.p.)
3. Memantine	Corn oil 0.5 ml/200 g	Corn oil + memantine (20 mg/kg, i.p.)	Corn oil + memantine (20 mg/kg, i.p.)	Normal saline
4. EGCG	Corn oil 0.5 ml/200 g	Corn oil +EGCG (10 mg/kg, i.p.)	Corn oil + EGCG (10 mg/kg, i.p.)	Normal saline
5. Coenzyme Q10	CoQ10 (10 mg/kg, i.p.) 0.5 ml/200 g	CoQ10 (10 mg/kg, i.p.)	CoQ10 (10 mg/kg, i.p.) + normal saline	Normal saline
6. Memantine +scopolamine	Corn oil 0.5 ml/200 g	Corn oil + memantine (20 mg/kg, i.p.)	Corn oil + memantine (20 mg/kg, i.p.)	Scopolamine (16 mg/kg, i.p.)
7. EGCG + scopolamine	Corn oil 0.5 ml/200 g	Corn oil + EGCG (10 mg/kg, i.p.)	Corn oil + EGCG (10 mg/kg, i.p.)	Scopolamine (16 mg/kg, i.p.)
8. Coenzyme Q10 +scopolamine	CoQ10 (10 mg/kg, i.p.) 0.5 ml/200 g	CoQ10 (10 mg/kg, i.p.) + normal saline	CoQ10 (10 mg/kg, i.p.) + normal saline	Scopolamine (16 mg/kg, i.p.)
9. Memantine + EGCG + scopolamine	Corn oil 0.5 ml/200 g	Corn oil + EGCG (10 mg/kg, i.p.) + memantine (20 mg/kg, i.p.)	Corn oil + EGCG (10 mg/kg, i.p.) + memantine (20 mg/kg, i.p.)	Scopolamine (16 mg/kg, i.p.)
10. Memantine +coenzyme Q10 +scopolamine	CoQ10 (10 mg/kg, i.p.) 0.5 ml/200 g	CoQ10 (10 mg/kg,i.p.) + memantine (20 mg/kg, i.p.)	CoQ10 (10 mg/kg, i.p.) + memantine (20 mg/kg, i.p.)	Scopolamine (16 mg/kg, i.p.)
11. Memantine +EGCG +coenzyme Q10 +scopolamine	CoQ10 (10 mg/kg, i.p.) 0.5 ml/200 g	CoQ10 (10 mg/kg, i.p.)+ memantine (20 mg/kg, I.P.) + EGCG (10 mg/kg, i.p.)	CoQ10 (10 mg/kg, i.p.) + memantine (20 mg/kg, I.P.) + EGCG (10 mg/kg, i.p.)	Scopolamine (16 mg/kg, i.p.)

objects presented in T1, and rats were then exposed to two distinct objects: the familiar (F) and the new one (N).

Exploration was defined as directing the nose toward an object at a maximum distance of 2 cm and/or touching the object with the nose. The total time spent exploring two identical objects in T1, the total time spent exploring two distinct objects, F and N in T2, and the discrimination between F and N in T2 was determined by comparing the total time spent exploring the F to the total time spent exploring the N. DI is the discrimination index and represents the difference in exploration time expressed as a percentage of the total time spent exploring the two objects in T2. DI was then calculated as DI=N–F/N+F.

Biochemical estimation

Blood samples were drawn from the retro-orbital junction, where blood is drawn from the venous sinus [25] and centrifuged at 3000 rpm for 20 min. The serum was used to determine the total antioxidant capacity (TAC), AChE activity, and inflammatory markers. Brain tissues were rapidly excised, washed with saline, and homogenized to a 20% homogenate for the determination of lipid peroxidation as malondialdehyde (MDA), reduced glutathione (GSH) content, and superoxide dismutase (SOD) activity. Furthermore, the brain tissues were used to determine dopamine (DA) content. Generally, samples are stored at −80°C, until they are prepared for biochemical testing.

The serum AChE assay is a modified version of the method of Ellman *et al.* [26], in which thiocholine, produced by AChE, reacts with 5,5′-dithiobis (2-nitrobenzoic acid) to form a colorimetric (412 nm) product proportional to the AChE activity present. A unit of AChE is defined as the amount of enzyme required to catalyze the production of 1.0 mmol of thiocholine per minute at a pH of 7.5.

The brain DA content was determined using Ciarlone's method [27]. External standards for DA were prepared in 0.2-N acetic acid with a total volume of 1.6 ml per tube. All tubes were vortex-mixed for 30 s and then centrifuged at 2000 rpm for 5 min. After discarding the organic supernatant phase, 1 ml of the aqueous phase was transferred to a clean, dry test tube. All tubes (e.g., sample, internal standard, external standard, and reagent blank) (1 ml of 0.2-N acetic acid) were mixed with 0.2 ml of EDTA reagent. The solution was then re-mixed with 0.1 ml of 0.1-N iodine. In all, 0.2 ml of alkaline sulfite reagent was added and mixed 2 min later. The tubes were allowed to stand precisely for 2 min, followed by the addition of 0.2 ml of 5-N acetic acid and mixing. All tubes were placed in a boiling-water bath for 2 min, cooled under running tap water, and analyzed for DA fluorescence at 320- and 375-nm excitation and emission wavelengths, respectively.

Assessment of oxidative stress markers

In the brain, lipid peroxidation was determined by estimating the concentration of thiobarbituric acid-reactive substances, which were quantified as MDA. MDA is a decomposition product of the lipid peroxidation process and is thus used as a marker for this process. In an acidic medium at a temperature of 95°C for 30 min, thiobarbituric acid reacts with MDA to form a thiobarbituric acid-reactive product. The absorbance of the resulting pink product can be measured at 534 nm [28].

The concentration of nonprotein sulfhydryl compounds, which is indicative of GSH content in the brain, was determined using Ellman's method [29]. The method is based on reducing Ellman's reagent by the SH group in GSH to form an intense yellow product (5-thio-2-nitrobenzoic acid) with a colorimetric detection limit of 412 nm. Precipitation of protein thiols by trichloroacetic acid was carried out with a suitable precipitating solution before the addition of Ellman's reagent; the results were expressed as μmol/g tissue.

The assay for SOD is dependent on the brain SOD enzyme's ability to inhibit the reduction of nitroblue tetrazolium dye by phenazine methosulfate [30]. We determined the increase in absorbance at 560 nm over 5 min.

The serum TAC is determined by reacting the sample's antioxidants with a defined amount of exogenously supplied H_2O_2. Colorimetric determination of residual H_2O_2 is accomplished through an enzymatic reaction involving the conversion of 3,5-dichloro-2-hydroxybenzene sulfonate to a color product [31].

Assessment of inflammatory markers

Interleukin 1-beta (IL-1β) assay employs a quantitative sandwich ELISA RayBio® with a precoated polyclonal antibody specific for serum IL-1β. Pipette standards, controls, and samples into the wells, and the immobilized antibody binds to any rat IL-1β present. After washing, a polyclonal anti-rat IL-1β

antibody that has been biotinylated was added. After a second wash, avidin-HRP was added to create a sandwich of antibody–antigen–antibody. After repeating the washing step, a substrate solution was added, resulting in the formation of a blue color proportional to the amount of rat IL-1β in the sample. Stop buffer was then added to terminate the reaction, resulting in a change in color from blue to yellow. The wells were then read at 450 nm [32].

For determination of serum TNF-α by using a solid-phase sandwich ELISA Quantikine®, a monoclonal antibody specific for rat TNF-α coated on a 96-well plate was used. Standards and samples were added to the wells and incubated for TNF-α present to bind to the immobilized antibody. The wells were then washed, and a biotinylated polyclonal anti-rat TNF-α antibody was added. After a second wash, avidin-HRP was added, producing an antibody–antigen–antibody sandwich. After repeating the washing step, a substrate solution was added, which produces a blue color directly proportional to the rat TNF-α present in the sample. Stop buffer was then added to terminate the reaction, resulting in a color change from blue to yellow. The wells are then read at 450 nm [33].

Histopathological examination

After behavioral testing, rats were decapitated, their entire brains removed, and brain specimens were fixed in 10% buffered formalin for 24 h, then washed with tap water and dried with alcohol, cleared in xylene, and implanted in paraffin. For histopathological examination, seven sections of 3-μm thickness were cut and stained with hematoxylin and eosin. All histopathological handling and appraisal of the specimens were performed by an experienced observer who was unaware of the identity of the examined samples to avoid bias [34].

Statistical analysis

Data concerning conditioned-avoidance test, object-recognition test, and biochemical parameters were expressed as mean+SEM. Comparison between more than two groups in conditioned-avoidance test, object-recognition test, and biochemical parameters, was carried out using one-way analysis of variance (ANOVA) followed by the Tukey multiple-comparison test. Student's t-test was used to compare the exploration times of T1 versus T2 and the F and N objects in T2 within each group. All statistical analyses and graphs were created using the Graph Pad Prism (ISI, GraphPad Software, San Diego, California, USA) software (version 5). The threshold for statistical significance was set to $P<0.05$.

Results

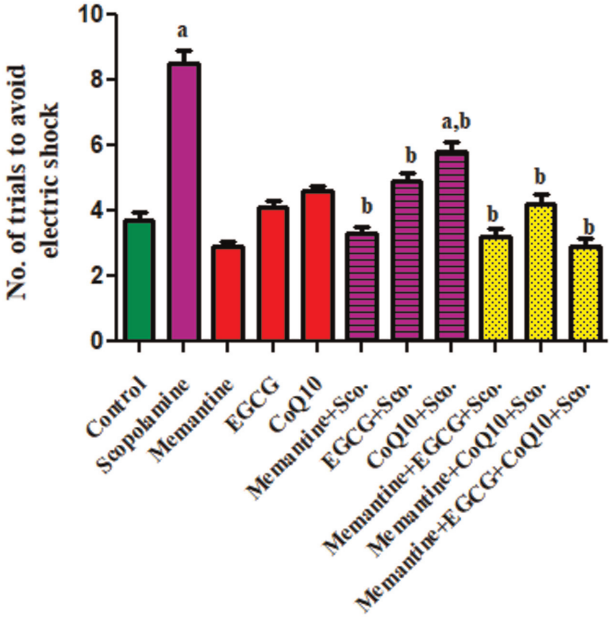
Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on behavioral performance in the conditioned-avoidance test in scopolamine-induced amnesia in rats

When rats were given scopolamine, the number of trials to avoid electric shock increased significantly, reaching ~129.7% compared with the control group. Rats were administered scopolamine and pretreated with memantine, showing a significant decrease in the number of trials to avoid electric shock reaching ~61.1% compared with scopolamine-treated rats. Scopolamine-treated rats pretreated with memantine and EGCG or CoQ10, or a combination of EGCG and CoQ10, demonstrated a significant reduction in the number of trials to avoid electric shock, reaching ~62.3%, 50.5%, and 65.8%, respectively, compared with scopolamine-treated rats. It may be concluded that the combination treatment produces the most significant results (Fig. 2).

Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on behavioral performance in object-recognition test in scopolamine-induced amnesia in rats

The control group's total exploration time in T1 was 14.26 s. In the scopolamine group, administering a single dose of scopolamine (16 mg/kg) 30 min before starting T1 had no significant effect on the total

Figure 2



Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on behavioral performance in the conditioned-avoidance test in scopolamine-induced amnesia in rats. Values are presented as mean±SEM (n=10). [a]$P<0.05$ versus control group, [b]$P<0.05$ versus scopolamine group using one-way ANOVA followed by Tukey–Kramer test for multiple comparisons.

exploration time in T1. Scopolamine-treated rats pretreated with memantine showed no significant difference in T1 compared with the control group. Rats administered scopolamine and pretreated with memantine revealed no significant difference from the control group in T1. Rats that were administered scopolamine and pretreated with memantine and EGCG or CoQ10, or a combination of EGCG and CoQ10, showed no significant difference from the control group in T1.

The total exploration time in T2 of control rats was 14.16 s, performed 1 h after T1. Administration of a single dose of scopolamine (16 mg/kg) 30 min before starting T1 had no significant effect on the total exploration time in T2. Moreover, rats given scopolamine and pretreated with memantine demonstrated no significant difference in T2 from the control group. Rats that were administered scopolamine and pretreated with memantine and EGCG or CoQ10, or a combination of EGCG and CoQ10, showed no significant difference from the control group in T2.

The exploration time of the new object (N) in T2 of the control group increased significantly by 65.4% compared with its correspondent exploration time of the familiar object (F). Scopolamine had no significant effect on the exploration time of (N) compared with its exploration time in (F), whereas rats given scopolamine and pretreated with memantine counteracted scopolamine-induced memory impairment, as evidenced by a significant increase in the exploration time of (N) of 47.17% to their performance in (F). Scopolamine was administered to rats, and pretreatment with memantine and EGCG or CoQ10, or a combination of EGCG and CoQ10, significantly increased the exploration time of (N) by 64.7%, 84.6%, and 81.3%, respectively, compared with their performance in (F).

The DI revealed that the control group discriminated against the new object (N) significantly better than the familiar one (F), as evidenced by a high positive (DI) value. In contrast, rats given scopolamine were unable to discriminate between them, as evidenced by a negative (DI) value. Rats pretreated with memantine completely reversed the scopolamine effect. Rats were administered scopolamine and pretreated with memantine and EGCG or CoQ10, or a combination of EGCG and CoQ10 showed similar behavior to the control group and discriminated significantly the new object (N) better than the familiar one (F), as evidenced by a high positive DI (Table 2).

Table 2 Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on behavioral performance in object-recognition test in scopolamine-induced amnesia in rats

Parameter treatment	Total exploration time in T1 (s)	Exploration times in T2(s)			Discrimination index
		Total	Familiar object	New object	
Control	14.26±0.88	14.16 ±0.80	5.33±0.39	8.82±0.46[c]	0.249±0.025
Scopolamine (Sco.)	11.45±0.47	11.35 ±0.72	5.79±0.40	5.55±0.34	–0.016±0.019[a]
Memantine (Mem.)	15.2±0.97	14.48 ±0.74	5.84±0.47	8.63±0.40[c]	0.199±0.034
Epigallocatechin-3-gallate (EGCG)	15.02±0.72	14.52 ±0.80	5.65±0.41	8.86±0.42[c]	0.227±0.021
Coenzyme Q10 (CoQ10)	14.9±0.81	14.14 ±0.88	5.15±0.53	8.98±0.37[c]	0.286±0.029
Scopolamine +memantine	14.65±0.60	14.00 ±0.75	5.66±0.31	8.33±0.56[c]	0.186±0.029[b]
Scopolamine +EGCG	12.77±0.57	13.22 ±0.84	5.16±0.35	8.04±0.50[c]	0.218±0.011[b]
Scopolamine +CoQ10	14.61±0.87	12.42 ±0.37	5.93±0.27	6.48±0.24	0.045±0.028[a]
Scopolamine +memantine +EGCG	14.80±1.07	14.65 ±1.19	5.53±0.51	9.11±0.71[c]	0.250±0.023[b]
Scopolamine +memantine +CoQ10	15.23±0.70	15.18 ±0.72	5.33±0.34	9.84±0.45[c]	0.298±0.022[b]
Scopolamine +memantine +EGCG +CoQ10	15.23±1.04	14.64 ±1.02	5.20±0.46	9.43±0.59[c]	0.297±0.023[b]

Values are presented as mean±SEM (n=10). [a]$P<0.05$ versus control group. [b]$P<0.05$ versus scopolamine group using one-way ANOVA followed by Tukey–Kramer test for multiple comparisons. [c]$P<0.05$ versus F group using Student's t-test.

Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on serum acetylcholine esterase (AchE) activity in scopolamine-induced amnesia in rats

When rats were given scopolamine, their AChE activity increased significantly, reaching ~174.2% compared with the control group. Scopolamine-treated rats were found to significantly decrease AChE activity, reaching ~59.8%, compared with the scopolamine-treated rats. Scopolamine-treated rats were pretreated with memantine and EGCG or CoQ10 or a combination of EGCG and CoQ10, which resulted in a significant decrease in AChE activity of ~70.3%, 62.3%, and 59.7%, respectively, compared with scopolamine-treated rats. Combination therapy has been shown to have the most beneficial effects (Fig. 3).

Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on brain dopamine (DA) level in scopolamine-induced amnesia in rats

Scopolamine administration resulted in a significant increase in DA levels, reaching ~119.1%, compared with the control group. Scopolamine-treated rats pretreated with memantine had a significant decrease in DA levels, reaching ~29.7%, compared with the scopolamine-treated rats. Scopolamine-treated rats pretreated with memantine and EGCG or CoQ10, or a combination of EGCG and CoQ10, demonstrated a significant reduction in DA levels of ~37.3%, 43.2%, and 45.5%, respectively, compared with the scopolamine-treated rats. The most significant results were obtained through combination treatments (Fig. 4).

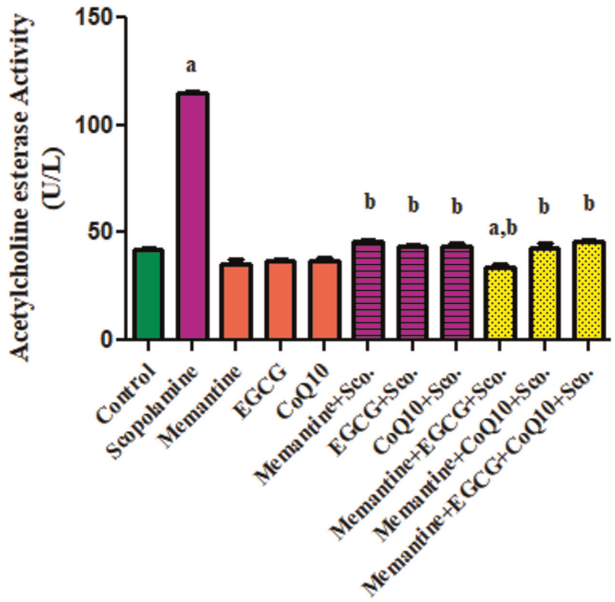
Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on brain malondialdehyde (MDA) level in scopolamine-induced amnesia in rats

When rats were given scopolamine, the MDA level increased significantly, reaching ~965.2%, compared with the control group. Scopolamine-treated rats pretreated with memantine demonstrated a significant reduction in MDA levels of ~41.4% compared with the scopolamine-treated rats. Scopolamine-treated rats pretreated with memantine and EGCG or CoQ10, or a combination of EGCG and CoQ10, demonstrated a significant reduction in MDA levels of ~69.9%, 63.7%, and 90%, respectively, compared with the scopolamine-treated rats. The combined treatment provided the most notable findings (Table 3).

Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on reduced glutathione (GSH) level in scopolamine-induced amnesia in rats

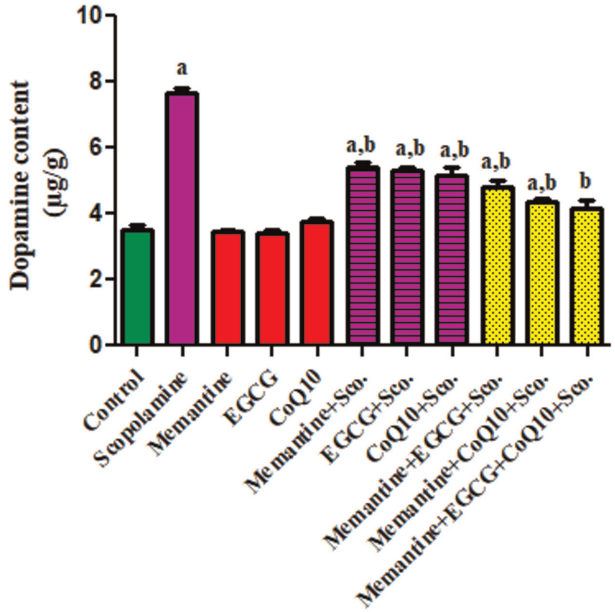
Scopolamine treatment resulted in a significant decrease in GSH levels in rats, reaching ~66.5%, compared with the control group. Scopolamine-treated rats pretreated

Figure 3



Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on serum acetylcholine esterase activity (AchE) in scopolamine-induced amnesia in rats. Values are presented as mean ±SEM (n=6). [a]P<0.05 versus control group, [b]P<0.05 versus scopolamine group using one-way ANOVA followed by Tukey–Kramer test for multiple comparisons.

Figure 4



Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on brain dopamine (DA) level in scopolamine-induced amnesia in rats. Values are presented as mean±SEM (n=6). [a]P<0.05 versus control group, [b]P<0.05 versus scopolamine group using one-way ANOVA followed by Tukey–Kramer test for multiple comparisons.

Table 3 Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on brain malondialdehyde (MDA) and reduced glutathione (GSH) levels, superoxide dismutase (SOD) enzyme activity, and serum total antioxidant (TAC) capacity in scopolamine-induced amnesia in rats

Groups	MDA (nmol/mg tissue)	GSH (mmol/g tissue)	SOD (U/g tissue)	TAC (mmol/l)
Control	1.072±0.0298	6.075±0.1331	2.575±0.1263	1.53±0.11
Scopolamine (Sco.)	11.42±0.4607[a]	2.032±0.0756[a]	0.218±0.0079[a]	0.42±0.10[a]
Memantine	1.308±0.1019	6.483±0.0721	2.883±0.1579	1.49±0.15
Epigallocatechin-3-gallate (EGCG)	1.347±0.1543	6.095±0.1137	2.935±0.1284	1.55±0.13
Coenzyme Q10 (CoQ10)	1.247±0.1540	5.840±0.2850	2.953±0.0768	1.74±0.14
Scopolamine +memantine	6.692±0.3267[a,b]	4.485±0.1501[a,b]	0.910±0.0254[a,b]	1.45±0.15[b]
Scopolamine +EGCG	5.833±0.1308[a,b]	3.573±0.1424[a,b]	0.903±0.0419[a,b]	1.33±0.10[a,b]
Scopolamine +CoQ10	8.083±0.1470[a,b]	3.445±0.1822[a,b]	0.953±0.0799[a,b]	0.80±0.12[a]
Scopolamine +Memantine +EGCG	3.433±0.4702[a,b]	4.938±0.1975[a,b]	1.378±0.1043[a,b]	1.70±0.08[b]
Scopolamine +memantine +CoQ10	4.142±0.4251[a,b]	5.287±0.1069[a,b]	2.257±0.1233[b]	1.52±0.15[b]
Scopolamine +memantine +EGCG +CoQ10	1.138±0.0407[b]	5.485±0.08282[b]	3.035±0.0842[b]	1.42±0.09[b]

Values are presented as mean±SEM (n=6). [a]$P<0.05$ versus control group. [b]$P<0.05$ versus scopolamine group using one-way ANOVA followed by Tukey–Kramer test for multiple comparisons.

with memantine had a significant increase in GSH levels, reaching ~120.7%, compared with scopolamine-treated rats. Scopolamine-treated rats were pretreated with memantine and EGCG or CoQ10, or a combination of EGCG and CoQ10, which resulted in a significant increase in GSH levels of ~143%, 160.1%, and 169.9%, respectively, compared with the scopolamine-treated rats. Combination therapy produced the most significant results (Table 3).

Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on superoxide dismutase enzyme (SOD) activity in scopolamine-induced amnesia in rats

When rats were given scopolamine, their SOD activity decreased significantly, reaching ~91.5%, compared with the control group. Scopolamine-treated rats pretreated with memantine showed a significant increase in SOD activity, reaching ~317.4%, compared with the scopolamine-treated rats. Scopolamine-treated rats pretreated with memantine and EGCG or CoQ10, or a combination of EGCG and CoQ10, showed a significant increase in SOD activity of ~532.1%, 935.3%, or 1292.2%, respectively, compared with the scopolamine-treated rats. Combination therapy has revealed the most significant results (Table 3).

Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on serum total antioxidant capacity (TAC) in scopolamine-induced amnesia in rats

Scopolamine-treated rats demonstrated a significant decrease in serum TAC of ~72.5% compared with the control group. Scopolamine-treated rats pretreated with memantine were found to significantly enhance TAC in serum, reaching ~245.2%, compared with the scopolamine-treated rats. Scopolamine-treated rats were pretreated with memantine and EGCG or CoQ10, or a combination of EGCG and CoQ10, which led to a significant increase in TAC in serum of ~261.9%, 238%, and 304.7%, respectively, compared with the scopolamine-treated rats. A tremendous increase has been observed in combination therapy (Table 3).

Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on serum interleukin-one beta (IL-1β) level in scopolamine-induced amnesia in rats

When rats were given scopolamine, the level of IL-1β increased significantly, reaching ~262.8% higher than in the control group. Rats given scopolamine and pretreated with memantine significantly reduced IL-1β levels, reaching ~35%, compared with rats given scopolamine alone. Scopolamine-treated rats pretreated with memantine and EGCG or CoQ10, or a combination of EGCG and CoQ10, demonstrated a significant reduction in IL-1β levels of ~52.6%, 57.4%, and 71.5%, respectively, compared with the scopolamine-treated rats. Combination therapy has been shown to have the most beneficial effects (Table 4).

Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on serum tumor necrosis factor-alpha (TNF-α) level in scopolamine-induced amnesia in rats

When rats were given scopolamine, their TNF-α level increased significantly, reaching ~311.3% higher than in the control group. Scopolamine-treated rats pretreated with memantine had a significant decrease in TNF-α level, reaching ~40.2%, compared with the scopolamine-treated rats. Scopolamine-treated rats pretreated with memantine and EGCG, CoQ10, or a combination of EGCG and CoQ10, showed a reduction in TNF-α level of ~63.2%, 53.3%, and 71.3%, respectively, compared with the scopolamine-treated

rats. Combination therapy has demonstrated the most promising results (Table 4).

Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on histopathological alterations of the brain in scopolamine-induced amnesia in rats

The results are presented as a photomicrograph in Figures 5–7. The longitudinal section (LS) from the brain of a rat in the control group showed the normal histological structure of the meninges (m), cerebral cortex (cc), and hippocampus (hp) (Fig. 5a and b). Brain LS from scopolamine-treated rats demonstrated congestion and hemorrhage in the meninges (h), congestion in cerebral cortical blood vessels (v), focal gliosis (g), and pyknosis in the cerebral cortex, neuronal degeneration, and pyknosis in hippocampus cells (hp) (Fig. 5c–e). LS from the brains of rats treated with memantine revealed the cerebral cortex (cc) and hippocampus (hp) to have normal histological structures (Fig. 5f and g).

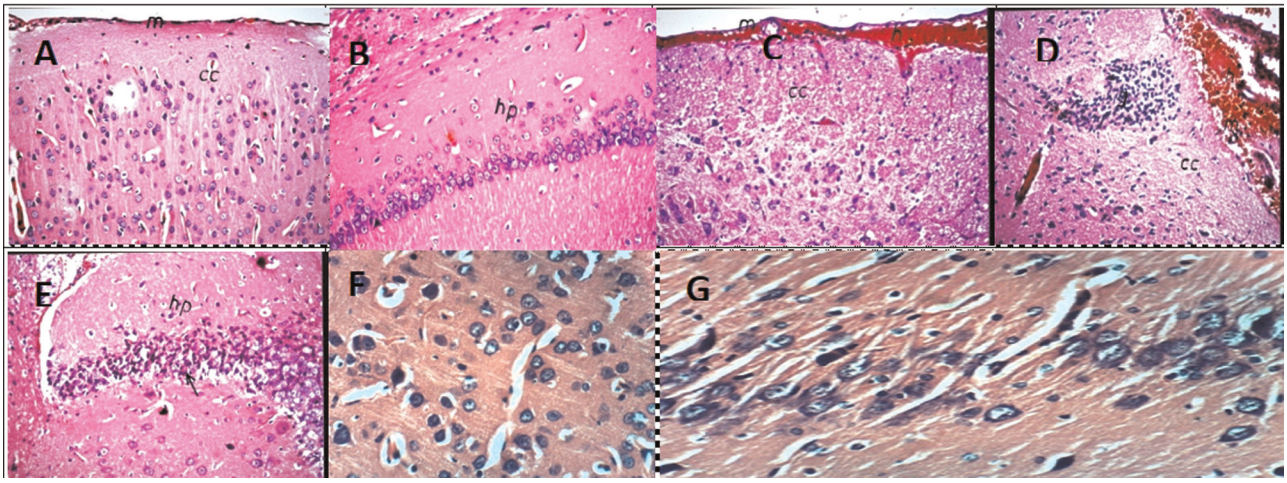
LS from the brain of rats treated with EGCG revealed the normal histological structure of the cerebral cortex (cc) and hippocampus (hp) (Fig. 6h and i). LS from the brain of rats treated with CoQ10 showed the normal histological structure of the cerebral cortex (cc) and hippocampus (hp) (Fig. 6j and k). LS from the brain of rats that received scopolamine and pretreated with memantine showed congestion and hemorrhage in meninges (h), pyknosis in the cerebral cortex (cc), and normal histological structure in hippocampus cells (hp) (Fig. 6l and m). The LS from the rats' brains receiving scopolamine and pretreated with EGCG displayed focal gliosis in the cerebral cortex (g) and normal histological structure in hippocampus cells (hp) (Fig. 6n and o).

Table 4 Effect of coenzyme Q10 and/or epigallocatechin-3-gallate combined with memantine on serum interleukin-one beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) levels in scopolamine-induced amnesia in rats

Groups	IL-1β (pg/ml)	TNF-α (pg/ml)
Control	32.30±0.736	34.08±2.070
Scopolamine (Sco.)	117.2±2.545[a]	140.2±5.394[a]
Memantine	30.85±1.010	36.68±2.861
Epigallocatechin-3-gallate (EGCG)	33.03±2.128	35.58±1.686
Coenzyme Q10 (CoQ10)	33.27±1.217	30.73±1.881
Scopolamine +memantine	76.17±1.428[a,b]	83.83±4.760[a,b]
Scopolamine + EGCG	76.35±3.583[a,b]	84.92±2.256[a,b]
Scopolamine + CoQ10	82.57±4.650[a,b]	91.47±4.902[a,b]
Scopolamine + memantine + EGCG	55.47±2.390[a,b]	51.50±3.673[a,b]
Scopolamine + memantine + CoQ10	49.90±1.791[a,b]	65.37±1.547[a,b]
Scopolamine + memantine + EGCG + CoQ10	33.37±1.642[b]	40.10±1.598[b]

Values are presented as mean±SEM (n=6). [a]$P<0.05$ versus control group. [b]$P<0.05$ versus scopolamine group using one-way ANOVA followed by Tukey–Kramer test for multiple comparisons.

Figure 5



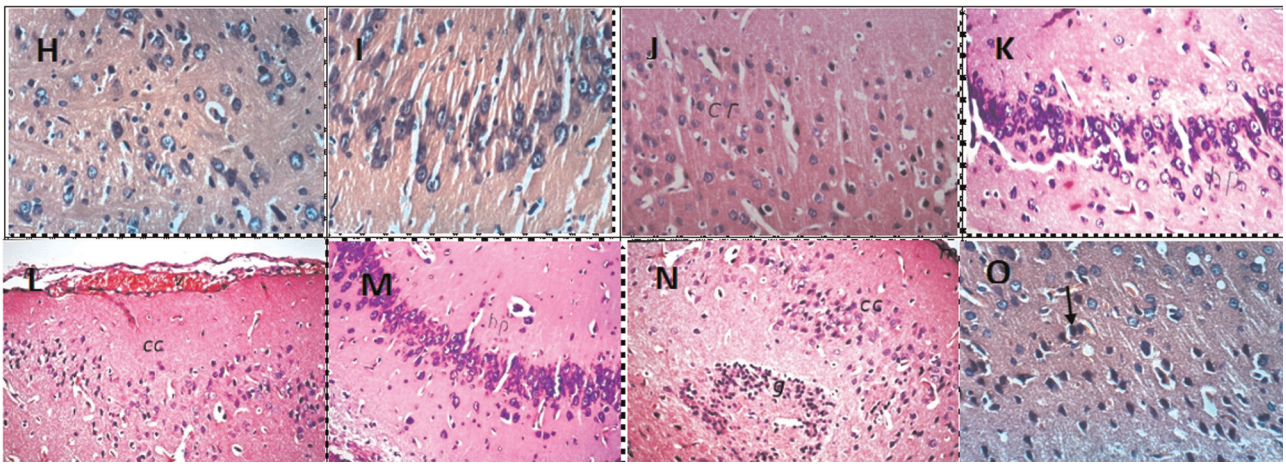
Representative photomicrographs of brain sections stained by H&E (×200). (A and B) Longitudinal section (LS) from the brain of a rat in the control group. (C–E) LS from the brain of a rat receiving scopolamine. (F and G) LS from the brain of rat treated with memantine.

The LS from the brain of a rat that received scopolamine and pretreated with CoQ10 exhibited pyknosis in the cerebral cortex (arrow) and neuronal degeneration with pyknosis in hippocampus cells (arrow) (Fig. 7p and q). Brain L.S. from scopolamine-treated rats pretreated with memantine and EGCG demonstrated focal gliosis in the cerebral cortex (g) and normal histological structure in hippocampus cells (hp) (Fig. 7r and s). Brain L.S. from scopolamine-treated rats pretreated with memantine and CoQ10 demonstrated pyknosis in the cerebral cortex (arrow) and normal histological structure in hippocampus cells (hp) (Fig. 7t and u). LS from the brain of a rat that received scopolamine and pretreated with memantine, EGCG, and CoQ10 showed small focal gliosis in the cerebral cortex (g) and normal histological structure in hippocampus cells (hp) (Fig. 7v and w). The severity of histopathological alterations in brain tissues of different experimental groups is shown in Table 5.

Discussion

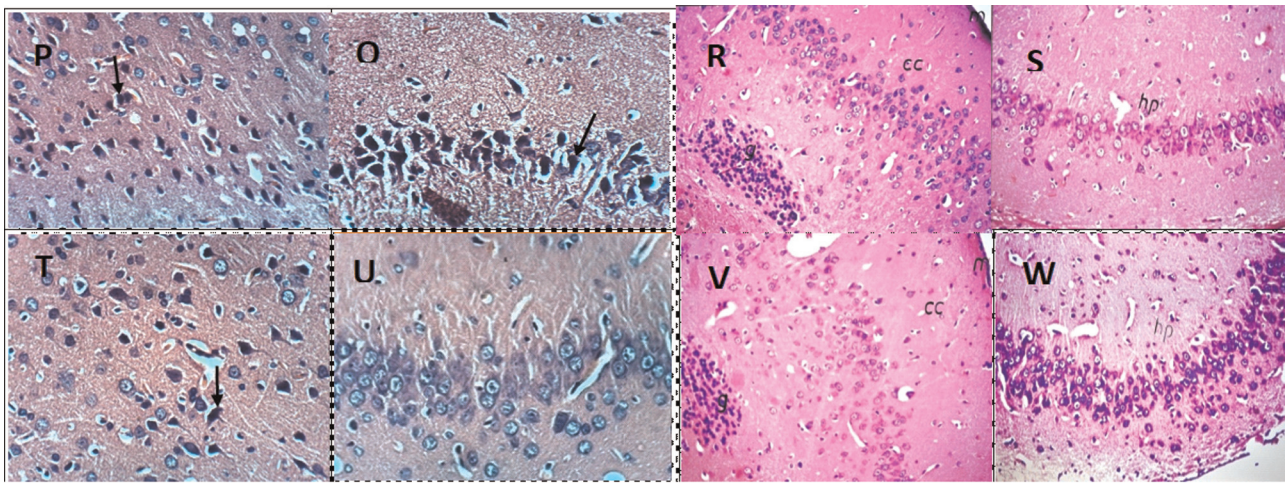
In the present study, a conditioned-avoidance test was conducted, in which scopolamine-treated rats pretreated with memantine revealed a significant

Figure 6



Representative photomicrographs of brain sections stained by H&E (×200). (H and I). Longitudinal section (LS) from the brain of rat treated with EGCG. (J and K) LS from the brain of a rat treated with CoQ10. (L and M) LS from the brain of a rat receiving scopolamine and pretreated with memantine. (N and O) LS from the brain of a rat receiving scopolamine and pretreated with EGCG.

Figure 7



Representative photomicrographs of brain sections stained by H&E (×200). (P and Q) LS from the brain of a rat receiving scopolamine and pretreated with CoQ10. (R and S) LS from the brain of a rat receiving scopolamine and pretreated with memantine and EGCG. (T and U) LS from the brain of a rat receiving scopolamine and pretreated with memantine and CoQ10. (V and W) LS from the brain of a rat receiving scopolamine and pretreated with memantine, EGCG, and CoQ10.

Table 5 Histological examinations in different brain regions

Brain region	Exp. groups histopathological alterations	Group one (control) group three (Memantine) group four (EGCG) group five (CoQ10)	Group two (Scopolamine)	Group six (Scopolamine + Memantine)	Group seven (Scopolamine + EGCG)	Group eight (Scopolamine + CoQ10)	Group nine (Scopolamine + Memantine + EGCG)	Group ten (Scopolamine + Memantine + CoQ10)	Group eleven (Scopolamine + Memantine + EGCG + CoQ10)
Cerebral Cortex	(a) Nuclear pyknosis and degeneration in neurons	–	+++	+	–	++	–	+	–
	(b) Gliosis	–	+++	–	++	–	++	–	+
Hippocampus Subiculum	Nuclear pyknosis and degeneration in neurons	–	++	–	–	++	–	–	–
Foscia dentats	Nuclear pyknosis and degeneration in neurons	–	++	–	–	++	–	–	–
Hilus	Nuclear pyknosis and degeneration in neurons	–	+	–	–	+	–	–	–

Degree: +++ severe; ++ moderate; + mild; –absent.

reduction in the number of trials required to avoid electric shock, reaching ~61.1%, compared with the rats provided scopolamine. Rats were given scopolamine and pretreated with memantine, and then separated into new and familiar items for the object-recognition test. These findings corroborate those of a previous study [35].

The results of rats administered scopolamine and pretreated with EGCG agreed with the study by Ali *et al.* [36]. The present findings mirror those of a previous study, demonstrating that CoQ10 may improve scopolamine-caused spatial performance defects [37].

In the current study, scopolamine-treated rats pretreated with memantine and EGCG or CoQ10, or a combination of EGCG and CoQ10, resulted in a significant decrease in AchE activity and DA levels, compared with rats that received scopolamine. In terms of AchE activity, the results of scopolamine-treated rats pretreated with memantine are consistent with the findings of Ihalainen *et al.* [38]. It has been revealed that memantine can also increase Ach release, despite being an antagonist of the NMDA receptor [38].

Regarding the AchE action, the findings of rats administered scopolamine and pretreated with EGCG are consistent with those of Biasibetti *et al.* [39]. It has been expressed that green tea (-) EGCG reverses oxidative stress and diminishes AchE activity in a streptozotocin-induced dementia model [39]. Concerning AchE activity, the results of rats that received scopolamine and pretreated with CoQ10 are in concordance with those of previous studies [17,40]. It has been shown that chronic treatment with CoQ10 in Aβ- (1–42) treated rats significantly attenuated impairment of AChE activities.

According to the findings, rats that received scopolamine pretreated with memantine indicated a significant decline in DA content than the rats administered scopolamine. These results support previous research, which proposed that memantine showed a powerful defensive impact on dopaminergic neurons in experimental Parkinson's disease models [41].

For DA level, the findings of rats given scopolamine and pretreated with EGCG are in concordance with those of Al-Amri *et al.* [42]. In contrast, the results of rats that received scopolamine and pretreated with CoQ10 match those of Motawi *et al.* [43].

Scopolamine-treated rats pretreated with memantine and EGCG or CoQ10, or a combination of EGCG and CoQ10, demonstrated significant increases in GSH, SOD, and TAC, and a notable decrease in MDA compared with scopolamine-treated rats. Concerning the oxidative stress markers, the results of the rats administered scopolamine and pretreated with memantine are in line with those of previous studies [44]. Furthermore, the results of rats given scopolamine and pretreated with EGCG are in congruity with Dragicevic *et al.* [45]. The results recommend that EGCG has a powerful neuroprotective impact through antioxidative and antiapoptotic mechanisms. Yin *et al.* demonstrated that supplementation with EGCG increased GSH and SOD levels and decreased (MDA) levels following lead intoxication [46].

Concerning oxidative stress, the findings of rats administered scopolamine and pretreated with CoQ10 support previous research [47]. It has been demonstrated that using CoQ10 reduces neuronal degeneration, secondary brain damage, and ischemia caused by oxidative stress in rats with traumatic brain injury.

Rats administered scopolamine and pretreated with memantine and EGCG or CoQ10, or a mix of EGCG and CoQ10, demonstrated a significant decline in IL-1β and TNF-α levels compared with scopolamine-treated rats. Regarding inflammatory markers, the findings of rats receiving scopolamine and pretreated with memantine seem consistent with other research, which found that memantine treatment upgraded functional recovery and associated with both anti-inflammatory and antiapoptotic effects [48]. Nyakas *et al.* [49] established a link between the cholinergic system and inflammation by showing that memantine protected neocortical cholinergic filaments, weakened microglial enactment around intracerebral injury sites, and improved cognition and memory in Aβ42-infused rats with impaired learning and loss of cholinergic innervation of the neocortex.Concerning inflammatory markers, the results of rats given scopolamine and pretreated with EGCG corroborate those of Rameshrad *et al.* [50], who showed that EGCG treatment suppressed Aβ-prompted inflammatory reaction of microglia by hindering the expression of TNF-α, IL-1β, and IL-6.

Moreover, the results of rats administered scopolamine and pretreated with CoQ10 agree with those of Singh *et al.* [17]. They stated that chronic treatment with CoQ10 significantly decreased TNF-α levels in Aβ- (1–42) treated animals, implying its anti-inflammatory activity.

The histopathological examination of rats that received scopolamine and pretreated with memantine is in concordance with that of Rajagopal *et al.* [51]. They demonstrated that memantine protected Sprague–Dawley rats from neurodegeneration induced by monosodium L-glutamate.

The histopathological examination of the brain of rats administered scopolamine and pretreated with EGCG is in accordance with Gu *et al.* [52]. They demonstrated that chronic EGCG treatment significantly reduced histopathological variations in hippocampal regions in rats suffering from chronic unpredictable mild stress-induced cognitive impairment. The histological examinations of the brain of rats administered scopolamine and pretreated with CoQ10 are as per the study by Kalayci *et al.* [47].

The present study demonstrated that when rats were given scopolamine and pretreated with EGCG or CoQ10 combined with memantine, the results were superior behaviorally, biochemically, and histologically, compared with rats receiving a single medication in combination with memantine.

These findings corroborate Youdim and Buccafusco [53], who asserted that new therapeutic strategies should be explicitly designed to target various neural and biochemical targets for the treatment of cognitive impairment, motor dysfunction, depression, and neurodegeneration. These bi- or multiutilitarian combinations may provide greater symptomatic adequacy and utility as neuroprotective disease-modifying agents.

Conclusion

Rats given scopolamine and pretreated with memantine in the combination of EGCG and CoQ10 showed better protective effects than those administered scopolamine and pretreated with memantine. These findings suggest that scopolamine protects rats from neurodegenerative effects by decreasing AchE activity, maintaining DA homeostasis, and decreasing oxidative stress and inflammation in the rat brain.

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Conflicts of interest

There are no conflicts of interest.

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