Bulletin of Faculty of Pharmacy, Cairo University (2014) **52**, 15–25

Cairo University

Faculty of Pharmacy

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Bulletin of Faculty of Pharmacy, Cairo University

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ORIGINAL ARTICLE

Naringenin protects against scopolamine-induced dementia in rats

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Received 7 September 2013; accepted 1 November 2013
Available online 28 November 2013

KEYWORDS

Dementia;
Scopolamine;
Naringenin;
Oxidative stress;
Acetylcholinesterase

Abstract *Background:* Alzheimer's disease (AD), the most common cause of progressive dementia in the elderly population, is a chronic neurodegenerative disorder that leads to disturbances of cognitive functions. Although the primary cause of AD remains unclear, brain acetylcholine deficiency and oxidative stress are principal pathogenic factors.

Aim of the study: The present study was constructed to investigate the anti-amnestic effect of naringenin on scopolamine-induced behavioral and neurochemical changes in rats.

Methods: Naringenin (50 and 100 mg/kg) and donepezil (2.5 mg/kg) were orally administered for 7 successive days. Dementia was induced at the end of the treatment period by a single injection of scopolamine (20 mg/kg; i.p.). Conditioned avoidance and Y-maze tests were conducted 30 min thereafter then rats were sacrificed and brain homogenates were used for the estimation of noradrenaline, dopamine, serotonin and γ-amino butyric acid contents along with acetylcholinesterase activity. In addition, certain inflammatory and oxidative stress markers as well as histopathologic studies were performed.

Results: Scopolamine resulted in memory impairment that was coupled by alterations in the estimated neurotransmitters and acetylcholinesterase activity as well as increased brain oxidative stress. Pretreatment of rats with naringenin in both doses mitigated scopolamine-induced behavioral, neurochemical and histological changes in a manner comparable to donepezil.

Conclusions: The observed anti-amnestic effect of naringenin makes it a promising candidate for clinical trials in patients with cognitive impairment.

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Peer review under responsibility of Faculty of Pharmacy, Cairo University.

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1. Introduction

Alzheimer's disease (AD), the most common form of senile dementia, is characterized by memory loss accompanied by degeneration of basal forebrain cortical cholinergic neurons.[1,2] The pathogenesis of AD is multifactorial and includes degeneration of cholinergic neurons, abnormal phosphorylation of the protein tau, oxidative stress, and altered protein processing resulting in abnormal β-amyloid peptide (Aβ) accumulation.[1–3]

1110-0931 http://dx.doi.org/10.1016/j.bfopcu.2013.11.001

Scopolamine, an anticholinergic drug, causes amnesia in humans and also impairs learning in animals.[4] Hence, it is widely utilized as a model simulating human dementia in general and AD in particular.[5,6]

Treatment of dementia of Alzheimer type is usually done using acetylcholinesterase (AchE) inhibitors as donepezil and memantine, an N-methyl-D-aspartate receptor antagonist.[1,7] The present therapies produce modest symptomatic improvements in patients; hence, searching for alternative or adjuvant anti-amnestic therapies is mandatory.

Naringenin is a flavanone found abundantly in citrus fruits. It possesses a plethora of pharmacological effects including antioxidant,[8,9] anti-inflammatory,[10,11] immune-modulatory[12,13] and neuroprotective properties[14,15] as well as peroxisome proliferator-activated receptor activation[16] and nuclear factor-kappaB (NF-κB) inhibition.[15]

Accordingly, the present study was performed to investigate the possible modulatory effect of naringenin on scopolamine-induced dementia and to compare such effects with donepezil, a commonly used agent in dementia and AD. The study design included evaluating effects of naringenin on some of the underlying mechanisms involved in AD progression.

2. Materials and methods

2.1. Animals

Adult male albino Wistar rats, weighing 150–200 g each, were used in the present study. Animals were allocated in groups and were allowed to accommodate for one week in the animal house at Faculty of Pharmacy, Cairo University, before subjecting them to experimentation. They were provided with a standard pellet diet and were given water ad libitum. The animals were kept at a temperature of 22 ± 3 °C and a 12 h light/dark cycle as well as a constant relative humidity throughout the experimental period. The study was approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University, Egypt.

2.2. Drugs and chemicals

Scopolamine hydrobromide was purchased from Sigma–Aldrich (MO, USA). It was dissolved in saline and i.p. administered in a dose of 20 mg/kg.[17] Donepezil hydrochloride was purchased from Pfizer (Cairo, Egypt). It was dissolved in saline and administered p.o. in a dose of 2.5 mg/kg.[18] Naringenin was purchased from Sigma–Aldrich (MO, USA). It was suspended in 1% Tween 80 and administered p.o. in doses of 50 and 100 mg/kg.[19] Reagent kits for tumor necrosis factor-alpha (TNF-α) and AchE were purchased from R&D Systems (MN, USA) and Quimica Clinica Aplicada (S.A., Spain), respectively.

2.3. Experimental design

Animals were classified into five groups (10 rats each). Treatments were given p.o. for seven successive days. One hour after the last dose of test agents, all animals were i.p. injected with scopolamine hydrobromide (20 mg/kg) except the first group (control group). Animals were treated according to the following scheme: groups I and II received 1% Tween 80; meanwhile groups III and IV received donepezil hydrochloride (2.5 mg/kg) or naringenin (50 and 100 mg/kg), respectively. Conditioned avoidance and Y-maze spontaneous alternation tests were conducted 30 min after scopolamine injection. Immediately after performing the behavioral tests, rats were sacrificed by decapitation, brains were rapidly isolated. Each brain was dissected through the midline into two hemispheres. Each hemisphere was weighed. One of the two hemispheres was homogenized in ice-cold acidified butanol to obtain 10% homogenate that was used for the determination of brain dopamine (DA), norepinephrine (NE) and serotonin (5-HT) contents. The other hemisphere was homogenized in ice-cold 50 mM phosphate buffer (pH 7.4) to prepare 10% homogenate that was used for the estimation of gamma aminobutyric acid (GABA), lipid peroxides, reduced glutathione (GSH), nitric oxide (NO) and TNF-α contents as well as AchE activity. Finally, brains of 2–3 rats from each group were preserved in 10% formalin and kept for histopathologic examination.

2.4. Conditioned avoidance test

The test was carried out using a shuttle box (Reflex-16, Columbus Instruments, USA) as described by Hinrichs et al.[20] The shuttle box consisted of two grid floor compartments ($29 \times 29 \times 26$ cm) separated by a non-transparent partition with a single opening (8×4.5 cm). Light (12 W) attached to the ceiling of the shuttle box was used as conditioned stimuli; whereas, the unconditioned stimulus was a foot shock (0.8 mA) delivered through the metallic grid floor. Rats were trained for three days prior to conduction of the experiment by subjecting them to the conditioned stimulus (light) followed by the unconditioned one (electric shock). After injection of scopolamine, each rat was placed in the shuttle box and allowed to adapt for 3 min. Following adaptation, the conditioned stimulus was presented for 10 s prior to the unconditioned stimulus. If the rat crossed to the opposite compartment during 10 s of conditioned stimulus, the electric shock was avoided, otherwise failure of avoidance was recorded.

2.5. Y-maze spontaneous alternation test

The Y-maze test was performed as described by Wall and Messier.[21] The maze was made of 3 identical arms, 40 cm long, 35 cm high and 12 cm wide, positioned at equal angles and labeled A, B, and C. Rats were placed at the end of one arm and allowed to move freely through the maze during a 5 min session. Spontaneous alternation was examined by visually recording the pattern of entrance into each arm in the maze for each rat. Arm entry was considered to be complete when the hind paws of the rat were completely placed in the arm. Alternation was defined as successive entries into the three arms on overlapping triplet set (i.e., ABC, BCA. . ...). Accordingly, the spontaneous alternation performance (SAP) score, spontaneous alternation percentage (SAP %) and total arm entries (TAE) were calculated.

2.6. Estimation of brain dopamine, noradrenaline and serotonin contents

Determinations of brain DA, NE and 5-HT contents were carried out according to the method described by Ciarlone[22] and expressed as μg/g wet tissue. The method is based on a fluorometric assay in which a fluorescent product results from the reaction with a mixture of alkaline sulfite and iodine solution (in case of DA and NE) and the reaction with ortho-phthalaldehyde solution (in case of 5-HT). The fluorescence of DA was read at excitation 320 nm and emission 375 nm; whereas that of NE was read at excitation 380 nm and emission 480 nm. Regarding 5-HT, its fluorescence was read at excitation 355 nm and emission 470 nm using Shimadzu spectrophotofluorometer (RF-510, Japan). The results were expressed as μg/g wet tissue.

2.7. Estimation of brain lipid peroxides content

Lipid peroxides were estimated as thiobarbituric acid reactive substances (TBARS) using malondialdehyde (MDA) as a standard according to the thiobarbituric acid reaction of Mihara and Uchiyama.[23] To brain homogenates, orthophosphoric acid (1%) and thiobarbituric acid (0.6%) were added, mixtures were boiled for 45 min at 100 °C, then cooled. The colored product was extracted by n-butanol. The absorbance of the organic layer was read at 535 and 520 nm and the difference in absorbance was calculated as lipid peroxides level expressed as TBARS (nmol/g wet tissue).

2.8. Estimation of brain reduced glutathione content

The method for the assessment of GSH (mg/g wet tissue) in the brain homogenates was based on that of Beutler et al.[24] Homogenates were deproteinized with 5-sulfuosalicylic acid (10%) for 30 min at 4 °C then centrifuged at 3000*g* for 15 min at 4 °C. An aliquot of the acid soluble supernatant was diluted with phosphate buffer (0.3 M, pH = 7.7) and 5,5′-dithiobis-2-nitrobenzoic acid (1 mM) was added to the samples. The optical density of the produced colored product was determined at 412 nm.

2.9. Estimation of brain nitric oxide content

NO in brain homogenates was determined as total nitrate/nitrite (NO_x) using Griess reagent,[25] after reduction of nitrate to nitrite by vanadium trichloride, and expressed as μmol/g wet tissue. Griess reaction entails formation of a chromophore from the diazotization of sulfanilamide by acidic nitrite followed by coupling with N-(1-naphthyl) ethylenediamine to form a colored azo derivative that can be measured spectrophotometrically at 540 nm.

2.10. Estimation of brain GABA content

Determination of brain GABA content was carried out according to the method described by Sutton and Simmonds.[26] The method depends on the formation of a fluorescent product from the reaction between GABA and ninhydrin at alkaline pH in the presence of glutamate. The resultant fluorescence is read at wavelengths of 380 nm for excitation and 450 nm for emission using Shimadzu spectrophotofluorometer (RF-510, Japan) and the concentration of GABA was expressed as μg/g wet tissue.

2.11. Estimation of brain content of tumor necrosis factor-alpha and acetylcholinesterase activity

Brain TNF-α content was assessed using ELISA kits and the results were expressed as pg/g wet tissue. AchE activity (U/g wet tissue) was measured according to the method of den Blaauwen et al.[27] using a colorimetric reagent kit. All the procedures of the used kits were performed following the manufacturer's instruction manual.

2.12. Histopathologic examination of brain tissues

Histopathologic assessment was performed on the brains of 2–3 rats randomly selected from each group. Brains were immediately fixed in 10% phosphate buffered formaldehyde, embedded in paraffin, and 5 μm longitudinal sections were performed. The sections were stained with hematoxylin and eosin (H&E) and examined microscopically.

2.13. Statistical analysis

Data were expressed as means ± S.E.M., and comparisons between means were carried out using one way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test. Results of the behavioral experiments were analyzed using Kruskal–Wallis non-parametric one way ANOVA followed by Dunn's multiple comparisons test. A probability level of less than 0.05 was accepted as being significant in all types of statistical tests.

3. Results

Scopolamine-induced dementia significantly reduced the conditioned avoidance response (CAR) by 32.32% and increased unconditioned avoidance response (UAR) by 3.3-folds as compared to the normal group. Pretreatment with donepezil significantly increased CAR by 32.51% and decreased UAR by 53.82%; meanwhile, naringenin (50 mg/kg) increased CAR by 38.98% and decreased UAR by 66.80% when compared to scopolamine control group (Table 1).

In Y-Maize test, scopolamine significantly decreased SAP % by 37.12% as compared to the normal control group. Pretreatment of rats with donepezil or naringenin (50 mg/kg) increased SAP % by 59.69% and 54.41%, respectively as compared to scopolamine control group (Table 2).

Scopolamine administration increased brain AchE activity by 73.72% as compared with the normal control group. Administration of donepezil or naringenin (50 and 100 mg/kg) decreased scopolamine-induced elevation in AchE activity by 44.44%, 49.10% and 34.24%, respectively as compared to scopolamine control group (Fig. 1A).

In the same context, scopolamine reduced brain GABA content by 21.1% as compared to the normal control group. Treatment with donepezil increased GABA content by 27.27% as compared to scopolamine control group. On the other hand, administration of naringenin did not prevent scopolamine-induced reduction in brain GABA content (Fig. 1B).

Table 1 Effect of naringenin on the active avoidance learning in scopolamine-induced demented rats.

Groups	Parameters	
	% CAR	% UAR
Control (1% Tween 80)	91.43 ± 2.10	10.00 ± 1.83
Scopolamine (20 mg/kg)	61.88 ± 3.65*	33.13 ± 2.49*
Donepezil (2.5 mg/kg)	82.00 ± 4.64@	15.30 ± 3.28@
Naringenin (50 mg/kg)	86.00 ± 3.67@	11.00 ± 2.45@
Naringenin (100 mg/kg)	70.00 ± 3.54*	23.75 ± 4.73*

Donepezil and naringenin were orally administered for 7 successive days. Dementia was induced on the 7th day by single i.p. injection of scopolamine 1 h after the last dose of test agents. Conditioned avoidance test was performed 30 min after scopolamine injection in trained rats. Each value represents the mean percentage of conditioned (CAR) and unconditioned (UAR) avoidances for each group (8 rats) ± S.E. Statistical analysis was carried out by non-parametric One-Way ANOVA followed by Dunn's multiple comparisons test.

* $p < 0.05$ vs. control.

@ $p < 0.05$ vs. scopolamine.

Table 2 Effect of naringenin on Y-maze behavioral test in scopolamine-induced demented rats.

Groups	Parameters		
	SAP %	TAE	SAP
Control (1% Tween 80)	28.88 ± 0.66	16.67 ± 2.30	4.63 ± 0.68
Scopolamine (20 mg/kg)	18.16 ± 1.45*	15.27 ± 1.66	2.64 ± 0.51
Donepezil (2.5 mg/kg)	29.00 ± 1.56@	14.33 ± 1.54	3.33 ± 0.42
Naringenin (50 mg/kg)	28.04 ± 1.78@	18.00 ± 4.97	5.00 ± 0.71
Naringenin (100 mg/kg)	24.82 ± 1.61	15.50 ± 5.25	3.50 ± 1.61

Donepezil and naringenin were orally administered for 7 successive days. Dementia was induced on the 7th day by single i.p. injection of scopolamine 1 h after the last dose of test agents. Y-maze behavioral test was performed 1 h after scopolamine injection. Total arm entries (TAE) and spontaneous alternation performance (SAP) were measured during 5-min session and % SAP was calculated.

Data are expressed as mean ± S.E.M of 8 animals. Statistical analysis was carried out by non-parametric One-Way ANOVA followed by Dunn's multiple comparisons test.

* $p < 0.05$ vs. control.

@ $p < 0.05$ vs. scopolamine.

Scopolamine-induced dementia significantly increased brain contents of 5-HT, NA and DA by 38.56%, 33.75% and 32.98%, respectively as compared with the normal group. Donepezil decreased the elevated 5-HT, NE and DA brain contents by 16.04%, 36.16% and 23.98%, respectively as compared with scopolamine control group (Fig. 2A–C). Likewise, pretreatment of rats with naringenin (50 mg/kg) caused a significant decrease in the elevated brain 5-HT and NE contents by 33.02% and 22.43%, respectively as compared to that of scopolamine control animals; meanwhile, naringenin (100 mg/kg) significantly decreased the elevated 5-HT and NE brain contents by 34.43% and 28.97%, respectively as compared with scopolamine control group (Fig. 2A and B).

In the present study, administration of scopolamine significantly increased brain TBARS content by 43.80% as compared to that of the normal group. Donepezil reduced scopolamine-induced elevation of TBARS by 22.81% as compared to scopolamine control group; meanwhile, pretreatment with naringenin (50 and 100 mg/kg) normalized the elevated TBARS content as compared with scopolamine control group (Fig. 3A).

Scopolamine-induced dementia resulted in decreased brain GSH content by 43.94% as compared to the normal group. Pretreatment with donepezil or naringenin (50 and 100 mg/kg) increased brain GSH content by 47.25%, 78.00% and 97.80%, respectively as compared to scopolamine control group (Fig. 3B).

In addition, scopolamine resulted in significant elevation in brain NO_x content by 44.8% as compared to the control group. Donepezil significantly decreased the elevated brain NO_x content by 38.98%; meanwhile naringenin in both dose levels used normalized the elevated NO_x content as compared to scopolamine control group (Fig. 4A).

In the current study, scopolamine-induced dementia was not associated with changes in brain content of TNF-α when compared to the normal group; however, pretreatment of rats with naringenin (50 and 100 mg/kg) significantly elevated the brain content of TNF-α by 26.77- and 19.85-folds, respectively as compared to scopolamine control group (Fig. 4B).

Examination of brain sections of control rats showed the normal structure of the cerebral cortex and hippocampus (Fig. 5A). On the other hand, brain sections of scopolamine-induced demented rats revealed severe congestion in the blood capillaries with perivascular edema in the cortex; meanwhile, the hippocampus showed encephalomalacia and edema in the tissue matrix with demyelination and neuronal degeneration (Fig. 5B).

Brains of rats pretreated with donepezil showed diffuse gliosis all over the cerebral cortex coupled by some pathological changes in the hippocampus in the form of shrinkage and swelling of some neuronal cells with vacuolation of cytoplasm (Fig. 5C).

Brain sections of rats pretreated with naringenin (50 mg/kg) revealed focal gliosis in the cerebral cortex and pyramidal cells

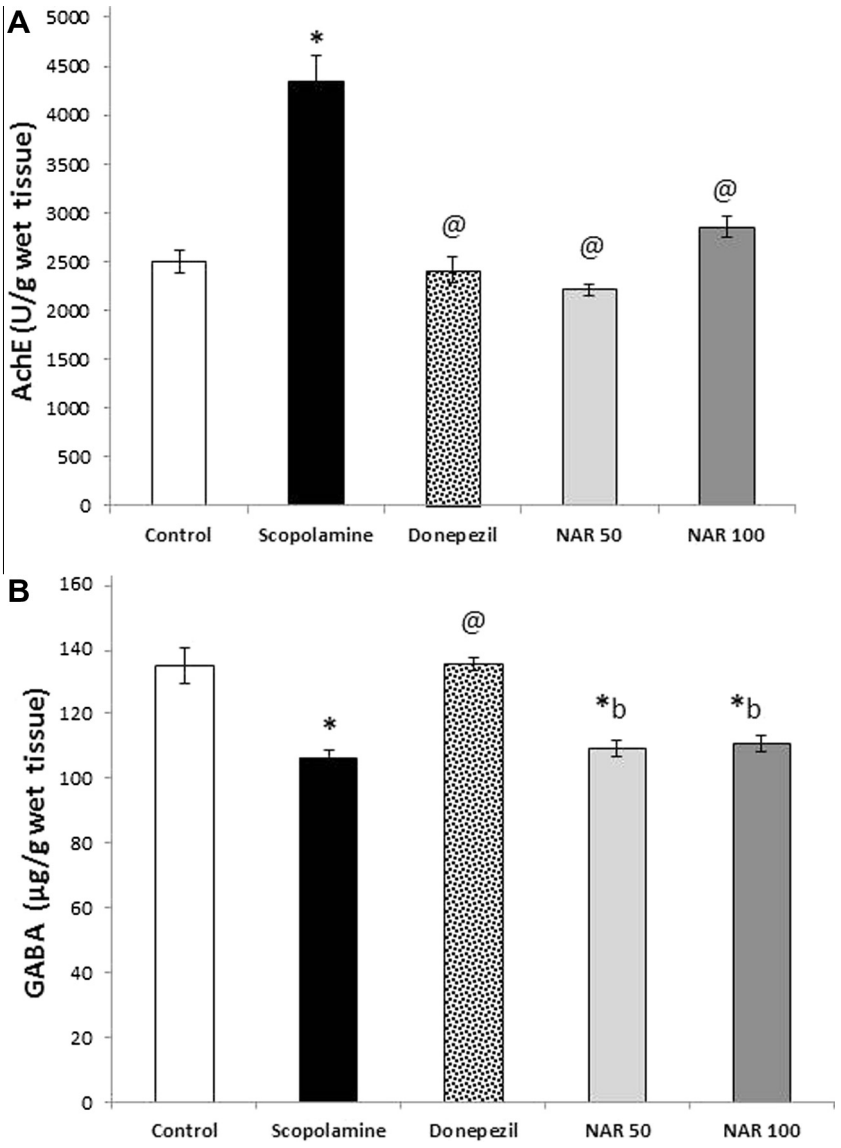

Figure 1 Effect of naringenin (NAR) on brain: (A) cholinesterase (AchE) activity and (B) γ-aminobutyric acid (GABA) content in scopolamine-induced demented rats. Donepezil (2.5 mg/kg) and NAR (50 and 100 mg/kg) were orally administered for 7 successive days. Dementia was induced on the 7th day by single i.p. injection of scopolamine (20 mg/kg) 1 h after the last dose of test agents. Each bar with vertical line represents the mean of 8 rats ± S.E. Statistical analysis was carried out using One-Way ANOVA followed by Tukey Kramer's multiple comparisons test; $^{*}p < 0.05$ vs. control, $^{@}p < 0.05$ vs. scopolamine; $^{b}p < 0.05$ vs. donepezil.

in the hippocampus separated from each other with irregular outline (Fig. 5D). Brain sections of rats pretreated with naringenin (100 mg/kg) showed the normal histological structure of the cerebral cortex; however, the hippocampus showed shrinkage in some pyramidal cells and vacuolated cytoplasm (Fig. 5E).

4. Discussion

Scopolamine-induced dementia has been used extensively to evaluate potential therapeutic agents for treating AD.[5,6] Scopolamine influences the expression of several genes associated with muscarinic receptor signaling pathways, apoptosis, and cell differentiation in rat brain.[28] Hence, it causes profound memory impairment in animals and humans as degeneration and dysfunction of the cortical cholinergic neurons is closely associated with cognitive deficits in AD.[29]

Indeed in the current study, single i.p. injection of scopolamine was coupled by significant decrease in CAR in conditioned avoidance test and decreased SAP % of rats in Y-maze test coupled with increased brain AchE activity and marked histopathologic changes in the brain. Scopolamine-induced behavioral, neurochemical and histological changes were attenuated by pretreatment of rats with donepezil and naringenin. Similar results regarding the effects of scopolamine,[30–32] donepezil[30,31] and naringenin[19,33] were reported.

The loss of cholinergic innervation correlates well with the degree of dementia and the severity of the neuropathologic hallmarks of AD.[29] Therefore, naringenin through its inhibitory effect on AChE activity as observed in the present study

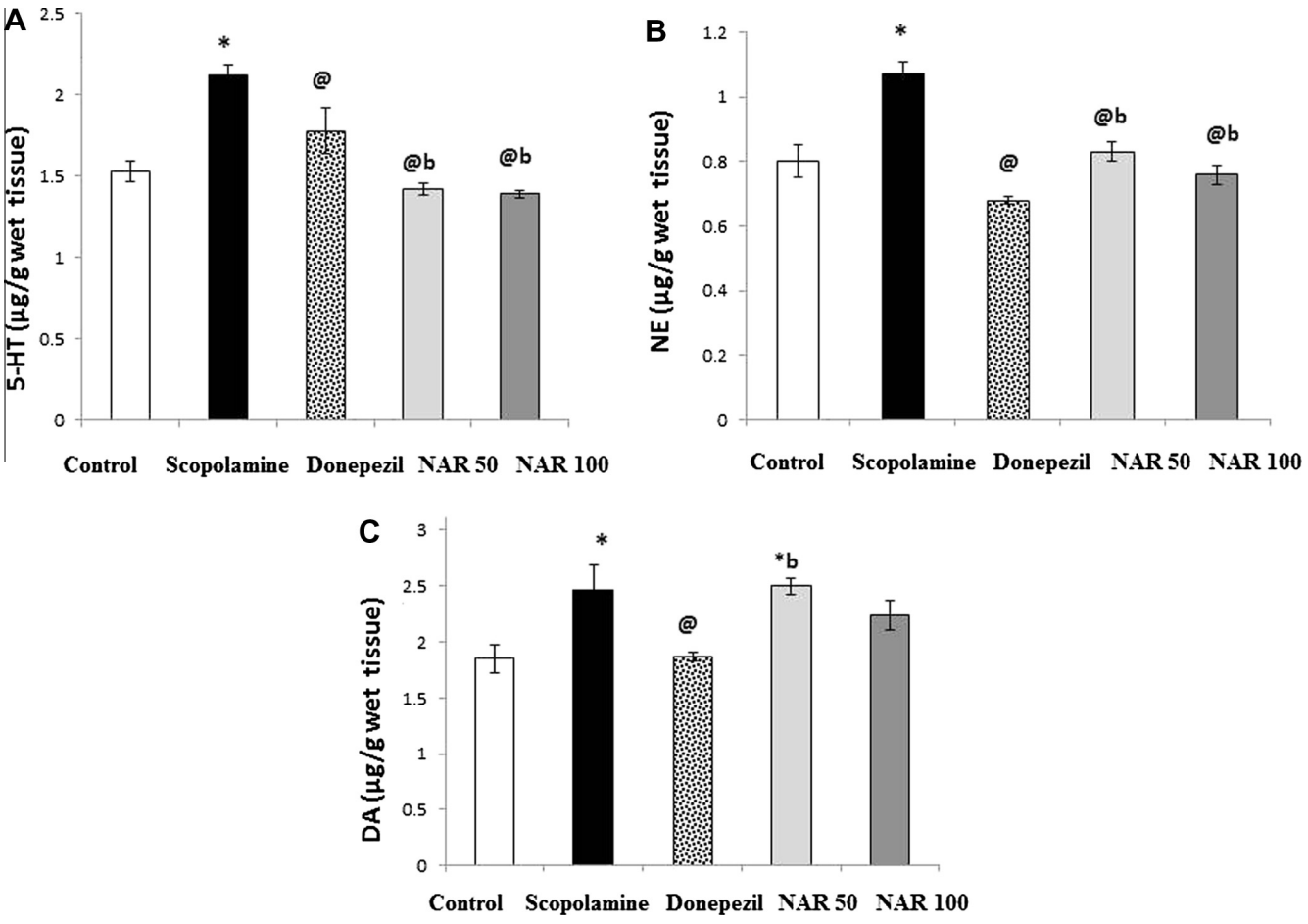

Figure 2 Effect of naringenin (NAR) on brain contents of: (A) serotonin (5-HT), (B) norepinephrine (NE) and (C) dopamine (DA) in scopolamine-induced demented rats. Donepezil (2.5 mg/kg) and NAR (50 and 100 mg/kg) were orally administered for 7 successive days. Dementia was induced on the 7th day by single i.p. injection of scopolamine (20 mg/kg) 1 h after the last dose of test agents. Each bar with vertical line represents the mean of 8 rats ± S.E. Statistical analysis was carried out using One-Way ANOVA followed by Tukey Kramer's multiple comparisons test; $^{*}p < 0.05$ vs. control, $^{@}p < 0.05$ vs. scopolamine; $^{b}p < 0.05$ vs. donepezil.

and reported by other investigators[8,19,33] could explain its observed anti-amnestic effects.

In the current experiments, scopolamine-induced pathological and behavioral changes were coupled by increased brain DA, NE and 5-HT contents that were prevented by pretreatment with donepezil; whereas naringenin protected against changes induced in NE and 5-HT but not DA. In a previous study, scopolamine ability to decrease DA turnover in hippocampus and frontal cortex parallel to its induced amnesic effects, measured by passive avoidance test, was reported.[34] Moreover, scopolamine-induced deficit in the radial-arm maze was counteracted by DA receptor blockers.[35]

Several lines of evidence from animal and clinical studies have also indicated the role of 5-HT and its receptors in different aspects of cognitive dysfunction. Yasuno et al.[36] demonstrated a dose-dependent decline in explicit verbal memory of healthy volunteers by administration of tandospirone, a 5-HT1A partial agonist. Indeed increased 5-HT1A receptor density correlated with the cognitive impairment observed in AD and provided the basis for the use of 5-HT1A antagonists in AD treatment.[37] Regarding NE, Szot and his colleagues demonstrated a significant increase in mRNA expression of the rate limiting enzyme for NE synthesis, tyrosine hydroxylase, in the locus ceruleus of AD subjects.[38]

The ability of naringenin and donepezil to ameliorate scopolamine-induced changes in brain neurotransmitters could be attributed to their observed effects on attenuating increased brain AchE activity and oxidative stress markers, the important hallmarks in dementia pathology.[29,39]

Scopolamine-induced dementia, in the present study, was coupled by reduced brain GABA content, an effect that was prevented by donepezil but not naringenin. Dysfunction of the GABAergic system is known to contribute in cognitive impairment in humans and the lower brain GABA content in patients with AD was reported.[40,41] Anderson et al.[42] reported that naringenin does not act as anxiolytic by modulation of the GABA-A receptor, which could explain the lack of naringenin effects on GABA brain content, in the present study.

Brain damage due to oxidative stress can induce impairment in learning and memory abilities.[39] In the same context, several investigators reported that scopolamine-induced amnesia and memory impairment in rats were associated with elevated brain lipid peroxides and reduced brain stores of

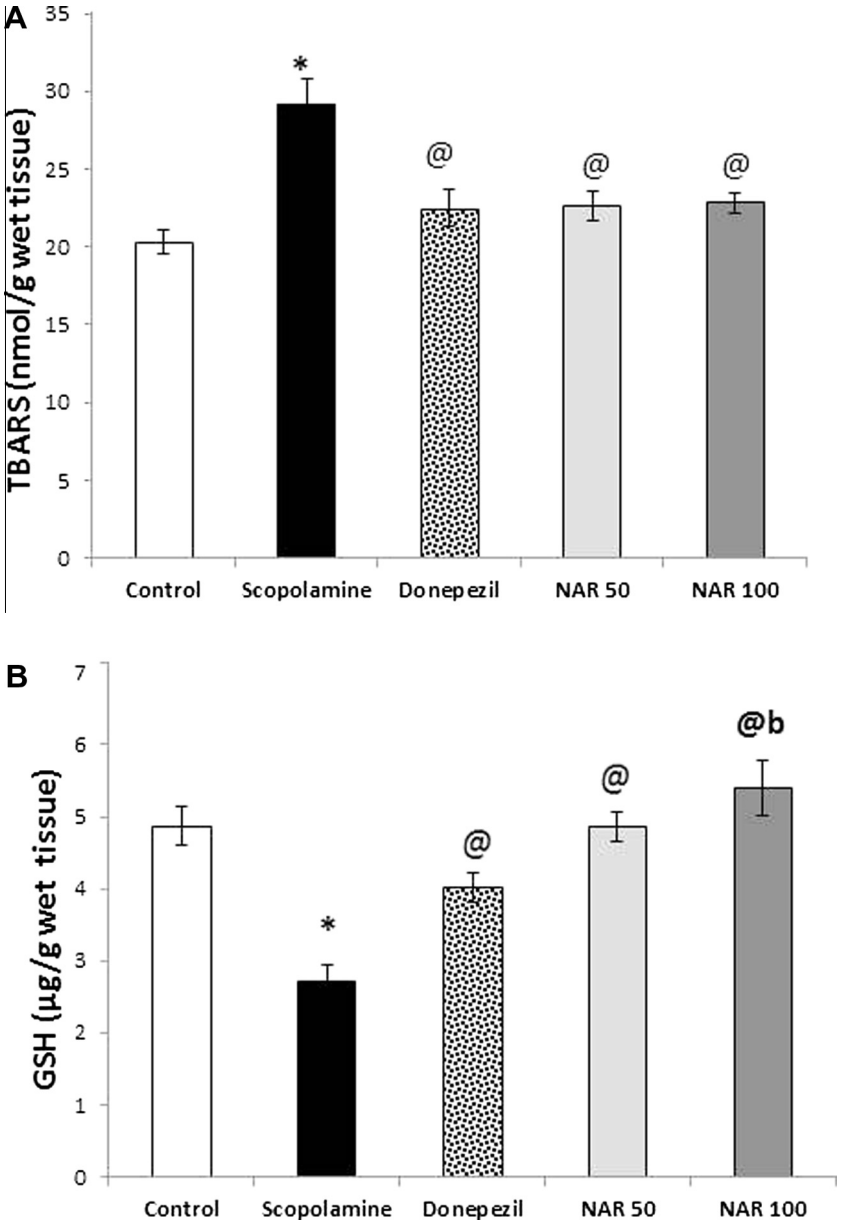

Figure 3 Effect of naringenin (NAR) on brain contents of: (A) thiobarbituric acid reactive substances (TBARS) and (B) reduced glutathione (GSH) in scopolamine-induced demented rats. Donepezil (2.5 mg/kg) and NAR (50 and 100 mg/kg) were orally administered for 7 successive days. Dementia was induced on the 7th day by single i.p. injection of scopolamine (20 mg/kg) 1 h after the last dose of test agents. Each bar with vertical line represents the mean of 8 rats ± S.E. Statistical analysis was carried out using One-Way ANOVA followed by Tukey Kramer's multiple comparisons test; $^{*}p < 0.05$ vs. control, $^{@}p < 0.05$ vs. scopolamine; $^{b}p < 0.05$ vs. donepezil.

antioxidants.[43–45] This was apparent in the present experiments by increased brain lipid peroxidation parallel to reduced GSH stores by scopolamine.

The observed neuroprotective effect of naringenin or donepezil in the present study was paralleled by decreased brain lipid peroxidation and increased GSH stores. Naringenin antioxidant properties have been previously reported.[8,9] Naringenin can chelate ferrous ions and decrease the formation of hydroxyl radical via inhibition of iron-dependent Fenton reaction.[46] The antioxidant properties of naringenin reside in the presence of 4′-hydroxyl group in its structure which possesses electron donating properties thereby protecting membranes from the free radical attack.[47]

In the present study, scopolamine-induced dementia was associated by increased brain NO that was prevented by donepezil and naringenin. AD lesions were reported to display several hallmarks of oxidative and nitrosative injury, including nitration of protein tyrosine residues which indicates the vicinal production of peroxynitrite from NO and superoxide anion.[48,49]

Raza et al.[15] demonstrated the ability of naringenin to reduce brain NO content in an experimental model of stroke by suppressing NF-κB-mediated neuroinflammation. Suppression of iNOS expression by naringenin was also reported in different models of inflammation.[10,11] Hence, we can assume that naringenin may exert part of its observed neuroprotective

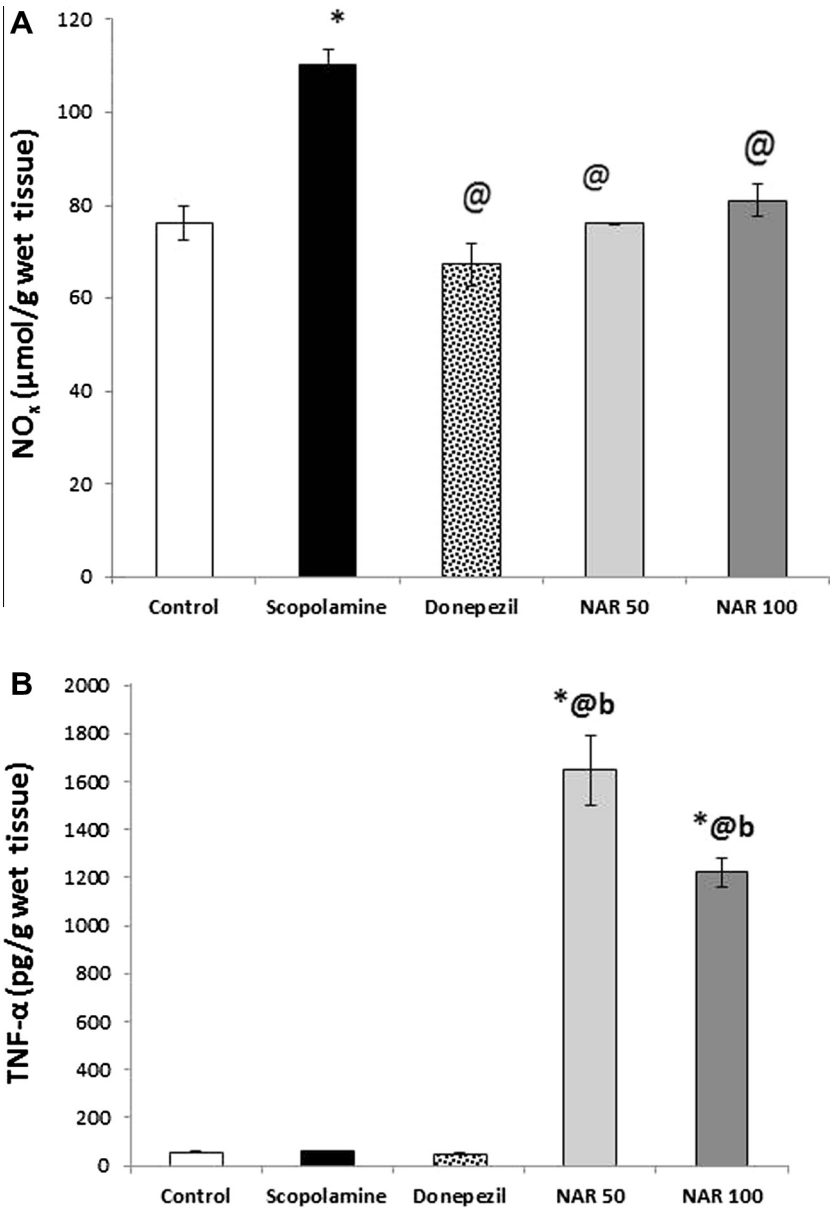

Figure 4 Effect of naringenin (NAR) on brain contents of: (A) total nitrate/nitrite (NO_x) and (B) tumor necrosis factor-alpha (TNF-α) in scopolamine-induced demented rats. Donepezil (2.5 mg/kg) and naringenin (50 and 100 mg/kg) were orally administered for 7 successive days. Dementia was induced on the 7th day by single i.p. injection of scopolamine (20 mg/kg) 1 h after the last dose of test agents. Each bar with vertical line represents the mean of 8 rats ± S.E. Statistical analysis was carried out using One-Way ANOVA followed by Tukey Kramer's multiple comparisons test; $^{*}p < 0.05$ vs. control, $^{@}p < 0.05$ vs. scopolamine; $^{b}p < 0.05$ vs. donepezil.

effect by its anti-inflammatory properties based on the strong link between inflammation and AD pathophysiology.[7]

Scopolamine-induced dementia was not coupled by changes in brain TNF-α; meanwhile naringenin in both dose levels markedly increased brain content of TNF-α. The dual role played by TNF-α in brain pathology was previously reviewed revealing significant beneficial effects predominantly mediated by TNFR2 receptor; whereas TNFR1 promotes neurotoxicity both directly and indirectly.[50,51] In the same context, several investigators reported a neuroprotective effect of TNF-α in certain brain insults.[52,53] Hence the effect of naringenin on brain content of TNF-α could be regarded as part of its neuroprotective theme that was apparent by cognitive enhancement, reduced oxidative and nitrosative stress as well as reduced brain AchE activity. Naringenin may also increase brain content of TNF-α by virtue of its immune-modulating properties[12,13] owing to the important role of TNF-α and inflammation in the immune response.

In conclusion, the prevalence of AD and dementia has increased significantly nowadays. As none of the available medications appears to be able to stop the disease progression, there is enormous medical need for the development of novel therapeutic strategies that target the underlying pathogenic mechanisms in AD. Keeping in mind that naringenin has broad cytoprotective properties as observed in the current study; its use might slow the progression of the multifactorial AD.

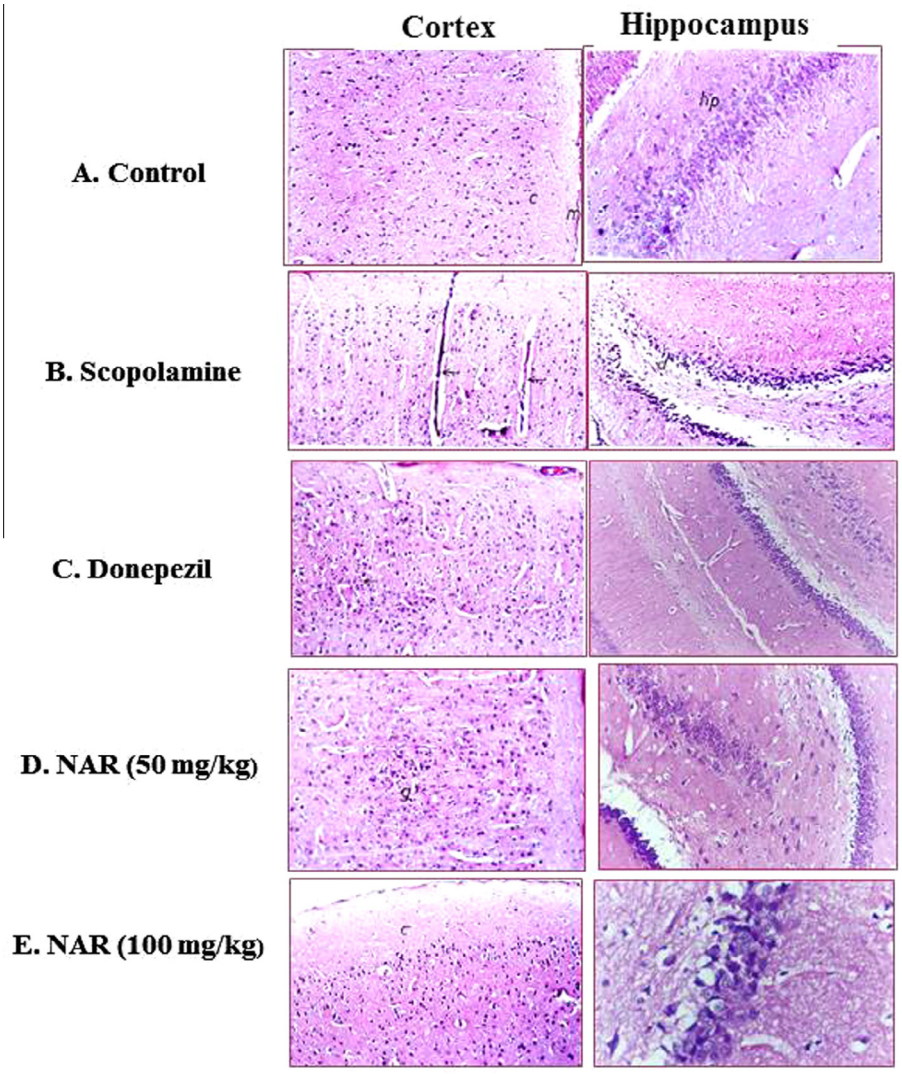

Figure 5 Photomicrograph of brain sections of: (A) control rat showing the normal histological structure of the meninges (m), cerebral cortex (c) and hippocampus (hp); (B) scopolamine-induced demented rat showing severe congestion in the blood capillaries with perivascular edema (arrow) in the cerebral cortex together with edema and encephalomalacia in the hippocampus (d); (C) scopolamine-induced demented rat treated with donepezil showing diffuse gliosis in the cerebral cortex and shrinkage with pyknotic nuclei in some pyramidal cells in the hippocampus; (D) scopolamine-induced demented rat treated with naringenin (50 mg/kg) showing focal gliosis (g) in the cerebral cortex and pyramidal cells in the hippocampus separated away from each other with irregular outline; (E) scopolamine-induced demented rat treated with naringenin (100 mg/kg) showing the normal histologic structure of the cerebral cortex in addition to shrinkage with pyknotic nuclei in some pyramidal cells and vacuolated cytoplasm in the hippocampus. (H&E × 40, 60, 150, 600).

5. Conflict of interest

None.

Acknowledgment

The authors are very grateful to Dr. Adel M. Bakeer, Professor of pathology, Faculty of Veterinary Medicine, Cairo University, for examining and interpreting histopathologic aspects of this study.

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