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Research paper

Evaluation of the protective effect of *Myrtus communis* in scopolamine-induced Alzheimer model through cholinergic receptors☆

Asli Aykac[a,*], Dilek Ozbeyli[b], Murat Uncu[c], Büşra Ertaş[d], Olca Kılınc[e], Ali Şen[f], Oya Orun[e], Goksel Sener[d]

[a] Department of Biophysics, Faculty of Medicine, Near East University, Nicosia, Cyprus
[b] Vocational School of Health Services, Marmara University, Istanbul, Turkey
[c] Department of Clinical Biochemistry, Dr. Burhan Nalbantoglu State Hospital, Nicosia, Cyprus
[d] Department of Pharmacology, Faculty of Pharmacy, Marmara University, Istanbul, Turkey
[e] Department of Biophysics, Faculty of Medicine, Marmara University, Istanbul, Turkey
[f] Department of Pharmacognosy, Faculty of Medicine, Marmara University, Istanbul, Turkey

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ABSTRACT

Alzheimer's disease is a progressive neurodegenerative disorder causing common health problem with increasing age. Evidences show that the key symptoms of AD are mainly caused by cholinergic system dysfunction which has a role in cognitive disorders. Cholinergic pathways especially muscarinic receptors like M_1 subtype also have a major role in learning, memory, cognitive functions and emotional state. There is no available permanent treatment currently to cure AD or to change its progression. This study was designed to investigate the factors that play important role in pathogenesis of AD and to compare the effects of Galantamine treatment with effects of *Myrtus communis* treatment. The expression level of M_1, ACh, BDNF; AChE activity, GSH level, MDA and MPO activity and AChE gene expression were investigated in scopolamine-induced rat model. Results showed that, administration of MC significantly improves the SCOP-induced reduction of latency and object recognition time; increasing BDNF, M_1 and ACh receptor expression levels in the different brain regions. Additionally, MC showed an increased in AChE by enhancing GSH activity and reducing MDA level and MPO activity. In conclusion MC considered as a possible novel therapeutic approach that can be a valuable alternative way in the prevention and treatment of AD.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is characterized by loss of memory, diminished cognitive performance, reduced learning and intellectual abilities causing a common public health problem (Schliebs and Arendt, 2011; Azizi-Malekabadi et al., 2012). AD has a number of pathologies which mainly includes cholinergic transmission degeneration, neuro-inflammation, oxidative stress and decreased level of brain derived neurotrophic factor (BDNF) (Gil-Bea et al., 2011; Verri et al., 2012; Bassani et al., 2017; Heneka et al., 2015).

Evidences from the literature show that the key symptoms of AD are mainly caused by cholinergic system dysfunction which has a role in cognitive disorders (Schliebs and Arendt, 2011; Ozarowski et al., 2016; Davies and Maloney, 1976). It is known that acetylcholine (ACh) levels in synaptic cleft are regulated by Acetylcholinesterase (AChE) activity where AChE responds to many different insults including oxidative stress that plays important role in the progression of a variety of mental disorders. Therefore, this enzyme might be a target to treat AD (Klinkenberg et al., 2011). Cholinergic pathways involved in learning and memory functions are projected from the basal forebrain to the cortex, the hippocampus and the amygdale. Muscarinic receptors

Abbreviations: MDA, malondialdehyde; GSH, glutathione; MPO, myeloperoxidase; PMSF, phenylmethanesulfonyl fluoride; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; BSA, bovine serum albumin; NBT, nitroblue tetrazolium; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; BDNF, brain derived neurotrophic factor; mAChRs, muscarinic acetylcholine receptors; M_1, muscarinic acetylcholine receptor M_1 subtype; TBS, Tris Buffer Saline; TBS-T, Tris Buffer Saline 0.05% Tween-20; AD, Alzheimer's disease; ACh, acetylcholine; AChE, acetylcholinesterase; SCOP, scopolamine; GAL, Galantamine; MC, *Myrtus communis*; qRT-PCR, quantitative real-time PCR

☆ Declaration of interest: none

* Corresponding author at: Near East University, Faculty of Medicine, Department of Biophysics, 99138 Nicosia, Cyprus.
E-mail address: asli.aykac@neu.edu.tr (A. Aykac).

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(mAChRs) like M_1 subtype also have a major role in learning, memory, cognitive functions and emotional state (Klinkenberg et al., 2011; Deiana et al., 2011). It is known that mAChRs blockages cause to memory disorders. Scopolamine (SCOP) is a mAChRs antagonist that stimulates memory impairment and partially mimics AD and dementia (Schliebs and Arendt, 2011).

The AChE inhibitors especially Galantamine (GAL) that used in treatment of AD, nowadays, lost their effectiveness in time due to loss of efficacy. Therefore, there is no available permanent treatment currently to cure AD or to change its progression. In addition to these, anti-inflammation and anti-oxidant agents are well-known agents used in the adjuvant therapy of AD (Kumar and Singh, 2015; Jeon et al., 2017; Lu et al., 2018).

Myrtus communis (MC) determined to contain a various metabolites such as anthocyanins, essential oil, flavonoids and tannins (Tumen et al., 2012). Generally, all parts of MC are known to involve in treating diarrhea, peptic ulcers, hemorrhoids, inflammation, and bleeding; and besides known to have anti-inflammatory, analgesic, anti-oxidative, anti-mutagenic, anti-diabetic, anti-microbial, anti-parasitic effects and neuroprotection (Tumen et al., 2012; Alipour et al., 2014; Mahboubi, 2016). In previous studies it has been shown that MC has effect to increase the amount of GSH, a cellular antioxidant molecule, and to act AChE enzyme inhibition like GAL (Tumen et al., 2012).

In the light of these findings, the study was designed to investigate the factors that play important role in pathogenesis of AD and to compare the effects of GAL treatment with effects of MC treatment. Therefore, the molecular and biochemical assays; Western blotting (for the expression level of M_1 mAChR, ACh and BDNF), spectrophotometric analysis (for AChE activity, Glutathione (GSH), Malondialdehyde (MDA) and Myeloperoxidase (MPO) and qRT-PCR (for AChE gene expression) were comprehensively performed to identify the possible effects of MC on some underlying cholinergic mechanisms involved in scopolamine-induced rat amnesia model.

2. Materials and methods

2.1. Plant material and preparation of extract

Leaves of *Myrtus communis* subsp. *communis* were collected from the Turgutlu region of Manisa in 2010. Voucher specimens were deposited in the Herbarium (Mare No: 13006). About 100 g of dried and ground MC were extracted with 96% EtOH, using a Soxhlet apparatus. Then, the filtrate evaporated under a vacuum in a rotary evaporator at 40 °C until it got dry. The obtained extract was stored in a dark container in refrigerator (4 °C) until it is used.

2.2. Animals and conditions

An approval from the institutional ethical committee was obtained Marmara University (MU) Animal Care and Use Committee (MUHDEK approval no: 112.2016.mar) before the experiments were started. Wistar albino rats (n = 32), aged 12–14 weeks, weighing 250–300 g were used. Rats were supplied by the MU Experimental Animals Research and Implementation Centre (DEHAMER). Animals were housed in an air conditioned room with 12-h: 12-h light-dark cycles (lights on 7 a.m.), where the temperature (22 ± 2 °C) and humidity (50 ± 5%) were kept constant. Rats were fed with standard rat chow and tap water in their cages (60 × 40 × 40 cm).

2.3. Experimental design

After a week of habituation period, the rats were divided randomly into four groups. Each group consisted of 8 rats. All treatments were given daily, at the same time of the day.

Sham-operated (control) group: 0.1 mg/kg physiological saline solution was administered intra-peritoneally (i.p) for 14 days.

Scopolamine (SCOP) group: SCOP (Sigma-Aldrich, St. Louis, MO, USA) is a mAChRs antagonist. 1 mg/kg/i.p SCOP was administered for induced amnesia model for 14 days (Ghasemi et al., 2018).

SCOP + MC group: 1 mg/kg/i.p SCOP and then 100 mg/kg/p.o. MC were administered for 14 days. MC was dissolved in 0.9% saline solution. In previous studies, MC was used at different doses especially at 50 and 100 mg/kg (Sen et al., 2016; Jabri et al., 2015). Additionally, it has been found out that 100 mg/kg MC dose showed an effective anti-oxidant activity in our unpublished experiments. Therefore, we used 100 mg/kg dose for MC treatment.

SCOP + GAL group: Firstly, 1 mg/kg/i.p SCOP and then 1.5 mg/kg/i.p GAL were administered for 14 days. GAL (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.9% saline solution (vehicle).

2.4. Cognition tests

2.4.1. Passive avoidance memory test

Passive avoidance memory test is a fear-aggravated test for assessing memory as previously defined by Kameyama et al. (1986). Passive Avoidance test apparatus (Northel Passive Avoidance System, Istanbul) was consisted of a two-compartment dark/light shuttle box (each 20 × 20 × 20 cm) which is separated by a guillotine door. In addition to this, a conductor was available on the floor of the dark compartment for electric shocking. The test protocol has two stages as instruction and memory tests. In instruction test, each rat was located in the light compartment (with a 100 W bulb) and rats were allowed to habituate apparatus for 20 s. After habituating period, the guillotine door was opened and the rats were allowed to move freely into the dark compartment. When the rats put into the light compartment they are expected to enter the dark compartment due to their elemental tendencies for dark areas. After entering to the dark compartment, the door was automatically closed and an electrical foot shock (0.5 mA) was applied for 3 s. Twenty seconds after the foot shock rats were returned to their home cage. In memory test phase, each rat was retested with the same protocol without foot shock. As an indicator of the long-term memory, the latency time for entering the dark compartment was recorded. In memory phase of the test, the cut off time was used as 300 s and the long latencies indicated well retention memory while short latencies demonstrated impaired memory functions (Erşahin et al., 2010; Hosseinzadeh et al., 2013; Özbeyli et al., 2017).

2.4.2. Object recognition test

The object recognition tasks were used to investigate the short-term recognition memory functions. The test has 3 important stages as: familiarizing (adapted to the environment for 10 min), the sample object test and the novel object test (Utkan et al., 2012). After assigning each rat for 10 min to each box and objects (either 2 old or 1 old and 1 new), the rats were familiarized with the researcher and adapted to the environment before the test. Firstly, two identical (sample) objects were put against the center of the opposite wall with its back to the objects to prevent forced exploration of the objects. The exposure time of the sample object was recorded for 10 min by a video camera. Most remarkable point in this study is that there was no other stimulus in the environment that would distract the rat's attention. The apparatus and the objects were cleaned by 70% isopropyl alcohol and left to dry for 1 min before putting the next rat into the box. Then, for the Novel (new) object, the rats which completed the familiarizing step returned to a home cage. After an hour, one of the objects was changed to evaluate the short term memory of the rats. Then, the rats were located into the box where one old and one novel object were available with the same protocol as mentioned previously. Finally, for the evaluation, the results were analyzed by comparing the time spent with the old and the novel objects. Object recognition is reflected by spending more time in interacting with the novel than old object, and this gives variable results in difference score. The difference score (*sec*) was calculated by: time spent with the novel object − time spent with the sample object

(Bevins and Besheer, 2006).

2.5. Removal of tissue samples

The animals were sacrificed with thiopental sodium (50 mg/kg) (Pentothal[R], Abbott) 24 h after the last cognition test session. After rapid decapitation, the hippocampus, the frontal cortex and the amygdala complex were removed in accordance with the Rat Brain Atlas and were stored at −80 °C. Anteroposterior planes used for removing the cortex, the hippocampus and the amygdala which were located between 13.20 and 11.20 mm, 6.70–4.70 mm and 7.20–5.70 mm anterior to the interaural line rat brain respectively, and was accepted as 9.00 mm posterior to the tip of the brain (Paxinos and Watson, 1986).

2.6. The assay of glutathione, malondialdehyde, and myeloperoxidase

As it is described by Beuge and Aust (1978), in order to measure the GSH and MDA levels, the samples of tissue were homogenized with ice-cold 150 mM KCl. As described previously in our study (Beutler, 1975), GSH was determined according to Ellman method (Beutler, 1975; Aykac et al., 2018). After mixing samples, the absorbance of the resulting color was measured at 412 nm in the spectrophotometer. Firstly, thiobarbituric acid reactive substances solution was added on the homogenate and as described in more detailed in our previous study (Beutler, 1975), MDA levels were determined according to Beuge method whereas MPO activity in brain tissues was determined by Hillegeas method (Beuge and Aust, 1978; Hillegass et al., 1990).

2.7. Tissue preparation and immunoblotting

For the molecular analysis of the tissues, the frozen tissues were weighed and homogenized in ice-cold 20 mM Tris-HCl buffer containing 1 mM EDTA, protease inhibitors, 10% glycerol, 50 mM NaCl and 2 mM DTT which were added into the tissues and homogenized for 90 s. After, the homogenates were centrifuged at 2000 ×*g* for 10 min. Pellets were incubated with 50 mM Tris-HCl, 5% glycerol, 1 mM EDTA, 5 mM DTT, protease inhibitors and 0.05% Triton X-100 for 90 min vortexing. All these operations are done at +4 °C. The protein content of the samples was determined by the Lowry method (Lowry et al., 1951). All extractions were used to determine mAChR M_1, BDNF and AChE expression levels. Samples including 50 μg (for the frontal cortex) and 100 μg (for the hippocampus and the amygdale) protein were run on gel electrophoresis and transferred onto nitrocellulose membranes for 90 min at 80 V. The membranes were blocked with Tris containing 1% BSA, 0.05% Tween-20 (TBS-T). After blocking, the membranes were incubated over night with polyclonal primary antibodies [M_1 (1:100), BDNF (1:200) or AChE (1:100)] at +4 °C. Immunoblots were standardized according to expression of β-actin (1:200) for all the membranes. The membranes were incubated with anti-goat IgG secondary antibodies (1:1000) for 1 h at 20 °C and then washed with TBS-T (Aykac et al., 2018; Aykac et al., 2012). The densitometric analysis was carried out with Bio-Rad Molecular Analyst software (free edition, www.totallab.com). Molecular weights for M_1, BDNF, ACh and β-actin are 52 kDa, 14 kDa, 72 kDa and 43 kDa, respectively.

2.8. Acetylcholinesterase activity

Acetylcholinesterase activity was performed according to procedure followed by Ozarowski et al. (2015). The activity of AChE in homogenate brain tissues were expressed as μmol/min/mg protein by using spectrophotometric method (λ = 412 nm) (Ozarowski et al., 2016).

2.9. Total RNA isolation and reverse transcription reaction

Total RNA isolation from different brain regions were extracted using RNeasy Plus Mini Kit (Qiagen) according to manufacturer's protocol. The concentration of the integrity of RNA was evaluated by measuring the absorbance at 260 and 280 nm using Synergy H1 Multi-Mode Reader, Take 3 Micro-Volume Plates. 50 ng of RNA samples were stored at −80 °C and the 1 μg of total RNA from all samples were used as template for the reverse transcription into cDNA by using SensiFAST cDNA Synthesis Kit (Bioline) (Ozarowski et al., 2016).

2.10. Quantitative real-time PCR mRNA quantification

Obtained cDNA samples were used for the quantitative real-time PCR (qRT-PCR). The AChE gene expression level was analyzed in a volume of 10 μL reaction mixture by using relative quantification methodology with a LightCycler TM Instrument (Roche, Germany) and a LightCycler® FastStart DNA Master SYBR Green I kit (Roche Applied Science). AChE primers used were: ACHE-F, 5′-CAGCAATACGTGAGC CTGAA and ACHE-R, 5′-TTCCAGTGCAGCATGTAGGA. A β-actin gene was used as a house-keeping internal standard gene for normalization of qRT-PCR. β-Actin primers were: BACT-F, 5′-CCAACCGCGAGAAGA TGA and BACT-R, 5′-CCAGAGGCGTACAGGGATAG. All primers sequences were designed by using the Oligo 6.0 software (National Biosciences) and pre-verified by assessment of a single PCR product on agarose gel and by a single temperature melting curve analysis of each cDNA amplification product. The data were performed using LightCycler Run 4.5 software (Roche Applied Science) and each PCR run included a non-template control to detect potential contamination of reagents. Calculations were performed using delta Ct method, where relative fold change between control and treated groups were compared for genes of interest and normalized relative to changes in the housekeeping gene β-actin (Ozarowski et al., 2016).

2.11. Statistical analysis

For statistical analysis GraphPad software (Prism 3.0; GraphPad Software, San Diego, CA, USA) was used. All data were expressed means ± standard error of the mean SEM. The groups of data were compared with analysis repeated measures ANOVA followed by *Bonferroni* multiple comparison post hoc tests. The values of $p < 0.05$ were considered as a statistical significant difference.

3. Results

3.1. Cognition tests

3.1.1. Passive avoidance task

After using a passive avoidance task, analysis revealed significant differences in long-term memory when comparison made between the control group and SCOP group ($p < 0.01$, Fig. 1A). In treatment groups, latency time of passive avoidance task score were reversed when compared to SCOP groups (SCOP versus SCOP + MC, $p < 0.05$ and SCOP versus SCOP + GAL, $p < 0.05$).

3.1.2. Object recognition test

The results of the object recognition test showed that the implementation of SCOP significantly decreased the short-term memory compared to all groups (Control, SCOP + MC and SCOP + GAL groups, $p < 0.05$–0.001; Fig. 1B).

3.2. The results of GSH, MDA and MPO

In accordance with these findings, the level of GSH in the SCOP group was significantly decreased ($p < 0.001$) whereas in SCOP + MC and SCOP + GAL, decreased GSH stores were moderately recovered with these molecules ($p < 0.05$; Fig. 2A). When the MDA levels were examined, a significant increase was found in the brain tissues of SCOP group compared to the control group ($p < 0.01$). The administration of

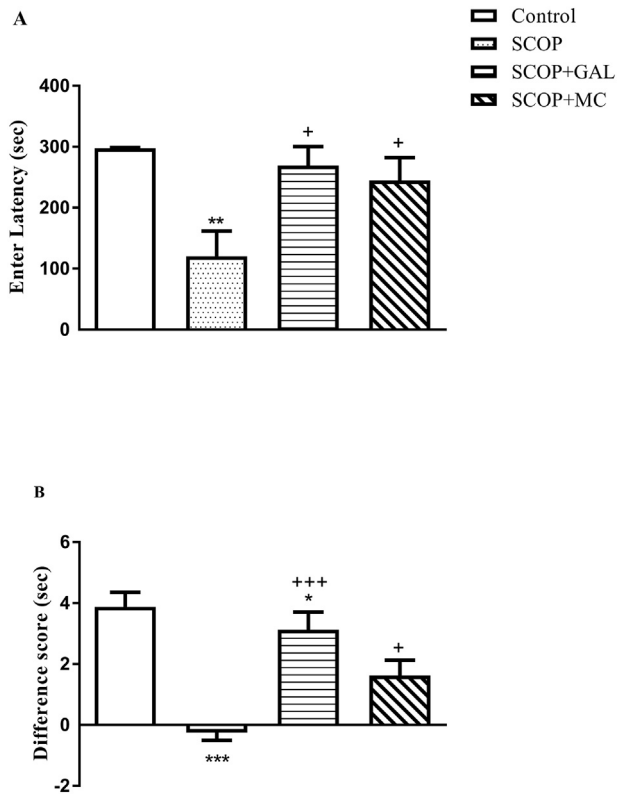


Fig. 1. Analysis of A) enters latency (sec) and B) object recognition tests of rats in control, SCOP, SCOP + GAL and SCOP + MC treated groups. Difference score (sec): Time spent with the novel object − time spent with the sample object. *, $p < 0.05$, **, $p < 0.01$; ***, $p < 0.001$ represents comparison with control group; +, $p < 0.05$ and +++, $p < 0.001$ represents comparison with SCOP group.

MC and GAL were found to reduce this elevation in MDA levels ($p < 0.05$ in both groups; Fig. 2B). Furthermore, the administration of SCOP significantly increased the neutrophil infiltration and the associated MPO values in brain tissue compared to the control group ($p < 0.001$) while MPO activity was significantly reduced in the SCOP + MC and SCOP + GAL groups ($p < 0.01$–0.001; Fig. 2C).

3.3. Immunoblotting analysis in different brain regions

In this study BDNF, ACh, and M_1 mAChR expressions were evaluated by Western blotting in order to determine possible effects of treatment on receptor expressions. The membranes and representative immunoblots of the different brain tissues (the amygdala complex, the hippocampus and the cortex) are illustrated in Fig. 3. In amygdala complex, the expression levels of BDNF, M_1 and ACh receptors significantly decreased in SCOP groups compared to the control group ($p < 0.05$–0.01; Table 1). The expression levels of BDNF, M_1 and ACh receptors in the SCOP + GAL and the SCOP + MC significantly increased compared to SCOP group (for SCOP + GAL $p < 0.05$, $p < 0.01$; for SCOP + MC $p < 0.05$, $p < 0.001$, respectively). The expression levels of BDNF, M_1 and ACh receptors significantly decreased with the administration of SCOP compared to the control group ($p < 0.01$, $p < 0.001$ and $p < 0.01$ respectively) in the hippocampus. The expression levels of M_1 and ACh receptors in the SCOP + GAL and the SCOP + MC significantly increased (for SCOP + GAL $p < 0.001$ and $p < 0.01$ and for SCOP + MC $p < 0.001$ and $p < 0.001$ respectively) whereas the level of BDNF expression did not change in SCOP + GAL compared to the SCOP group (Table 1). In the cortex, there is no finding that the administration of GAL has any effects on the level of BDNF, M_1 and ACh receptors expression. It is

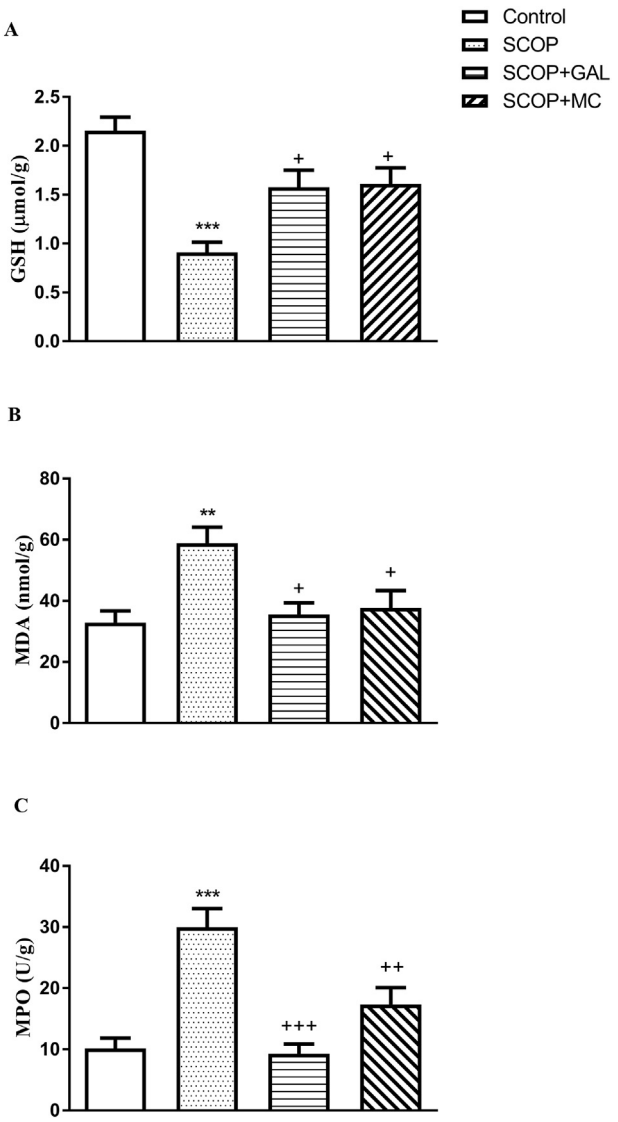


Fig. 2. A) Glutathione (GSH), B) malondialdehyde (MDA) levels and C) myeloperoxidase (MPO) activity in the brain tissue of rats, which were control, SCOP, SCOP + GAL and SCOP + MC treated groups. Each group consisted of 8 animals (n = 8). *, $p < 0.05$, **, $p < 0.01$; ***, $p < 0.001$ represents comparison with control group; +, $p < 0.05$; ++, $p < 0.01$ and +++, $p < 0.001$ represents comparison with SCOP group.

found out that the levels of BDNF, M_1 and ACh receptor expression significantly decreased in SCOP group when compared to the control group ($p < 0.01$). The levels of M_1 and Ach receptors expression significantly increased in the group of SCOP + MC compared to the SCOP group ($p < 0.05$, Table 1).

3.4. Acetylcholinesterase activity

The activity level of AChE showed variations in all groups at different rat brain regions ($p < 0.05$). Analyses of the hippocampus, amygdala complex and cortex showed that the administration of SCOP was increased the level of AChE activity in SCOP group compared to the control group ($p < 0.001$). The treatment of MC and GAL were decreased AChE activity in the hippocampus, the amygdala complex and the cortex ($p < 0.001$, Table 2).

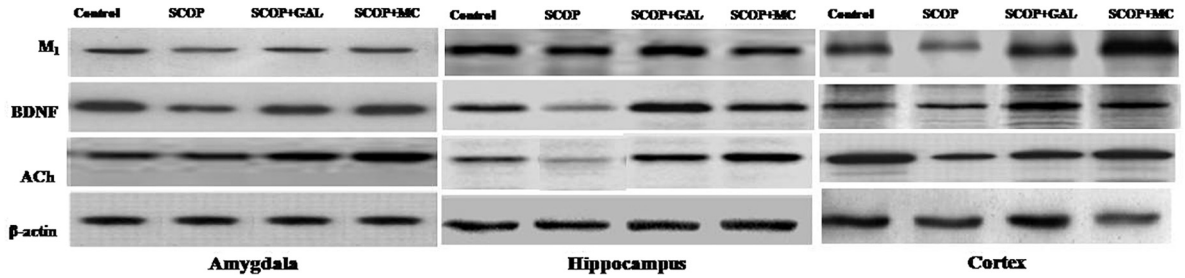


Fig. 3. The representative photographs of membranes collected from immunoblotting experiments for SCOP-induced rat model treated with MC or GAL. For each group n = 4; and the analyses were performed as duplicate. β-Actin was used to normalize the amount of protein loaded in each lane. Molecular weights for M_1, BDNF, ACh and β-actin are 52 kDa, 14 kDa, 72 kDa and 43 kDa, respectively.

3.5. The analysis of AChE-mRNA level

The results of the mRNA expression levels of AChE gene exposing to SCOP and treating with MC and GAL were represented in Table 2. For all brain regions, the results revealed that SCOP administration caused a significant increase in AChE-mRNA level when compared to the control rats ($p < 0.001$). However, treatment with MC and GAL prevented an increase in AChE-mRNA expression level when compared to the SCOP group ($p < 0.01$–0.001; in all regions).

4. Discussion

Alzheimer's disease is a very severe progressive neurodegenerative disorder that leads to death with increasing of aging population and lifetime expectancy, and it is very crucial to find novel drugs that can effectively cure AD. Cholinergic hypo-function, oxidative stress, and neuro-inflammation are one of the main characteristics of AD. As many studies have shown that SCOP can impair the ability of learning and memory increased oxidative stress and inflammation in humans and animal, SCOP-induced AD model became widely used model to study cognitive deficiency and screen for anti-AD drugs (Li et al., 2018). In the current study, the changes of cholinergic system parameters and oxidative stress markers were analyzed in order to further clarify the underlying mechanism of improving learning and memory of MC and GAL in the SCOP-induced rat for the first time. Our study has shown that SCOP significantly diminished learning and memory in passive avoidance and novel object recognition tests. According to the results, it can be concluded that administration of MC ameliorated short and long term memory in SCOP model and SCOP treatment decreased latency time of passive avoidance task score, indicating a decline in long term memory. These results support the previous data showing the decreased short and long term memory in SCOP model (Li et al., 2018; Wang et al., 2014). The novel object recognition test evaluates recognition memory whereas the passive avoidance task assesses reference memory and emotional memory which is related to the amygdala (Özbeyli et al., 2017; Wang et al., 2014). In behavioral tests, treatment with MC was shown to prevent SCOP-induced cognitive impairment. In addition, the passive avoidance test is used in our study, that is a fear-conditional test using learning and memory alterations in rats following SCOP administration and the treatment of GAL and MC also have ameliorative effect in the hippocampus, the cortex and the amygdale. In literature, there is no data about the effect of MC on memory functions in AD or in any neurodegenerative disorders, thus for the first time, this current study reported the beneficial effects of MC in SCOP-induced cognitive impairment.

Previous studies have established increased MDA levels, MPO activity and reduced GSH levels in rat brain with AD (Özbeyli et al., 2017; Singh et al., 2010). Recently, it has been shown that SCOP-induced memory impairment is associated with an oxidative stress status in the brain (Fan et al., 2005; Rahimzadegan and Soodi, 2018; Budzynska et al., 2015; Hancianu et al., 2013). In accordance with previous studies, we found out that SCOP significantly increased the MDA level and decreased GSH level in brain tissues. Our results demonstrated that MDA level and MPO activity of brain tissue reduce via MC and GAL. Furthermore, diminished GSH level of brain via SCOP ameliorated with MC and GAL. Although there is no data in the literature about the effects of MC in SCOP model, MC found to inhibit lipid peroxidation and inflammation by reducing MDA levels and MPO activity via increasing GSH in rat (Sen et al., 2016; Rossi et al., 2009; Maxia et al., 2011; Ozkol et al., 2013; Mhatre et al., 2004). Taken together, our results suggest that anti-oxidant and anti-inflammatory activities of the MC may be mediated neuro-protective effects against SCOP-induced oxidative

Table 1
The expression levels of BDNF, M_1 and ACh after MC and GAL treatment in SCOP-induced rat model in all brain tissues. For each group n = 4 and the analyses were performed as duplicate. *, $p < 0.05$; **, $p < 0.01$ represents comparison with control group; $^{+}$, $p < 0.05$; $^{++}$, $p < 0.01$ and $^{+++}$, $p < 0.001$ represents comparison with SCOP group.

	Control	SCOP	SCOP + GAL	SCOP + MC
	Relative density of BDNF/β-actin			
Amygdala	1.47 ± 0.07	1.22 ± 0.04*	1.42 ± 0.07^{+}	1.46 ± 0.05^{+}
Hippocampus	1.34 ± 0.02	1.11 ± 0.05	1.19 ± 0.05	1.29 ± 0.05
Cortex	1.64 ± 0.01	1.52 ± 0.01*	1.58 ± 0.03	1.57 ± 0.05
	Relative density of ACH/β-actin			
Amygdala	1.31 ± 0.08	0.77 ± 0.09**	1.23 ± 0.06^{+}	1.40 ± 0.06^{+}
Hippocampus	1.78 ± 0.08	1.49 ± 0.07	1.76 ± 0.1	1.92 ± 0.04
Cortex	1.41 ± 0.04	1.17 ± 0.05*	1.36 ± 0.05	1.38 ± 0.13^{+}
	Relative density of M_1/β-actin			
Amygdala	1.32 ± 0.09	1.09 ± 0.07**	1.26 ± 0.04^{++}	1.25 ± 0.05^{+++}
Hippocampus	1.67 ± 0.03	1.20 ± 0.15	1.56 ± 0.05	1.65 ± 0.04
Cortex	1.14 ± 0.02	0.95 ± 0.03**	1.05 ± 0.04	1.10 ± 0.04^{+}

SCOP: scopolamine, GAL: Galantamine, MC: *Myrtus communis*, AChE: acetylcholinesterase.

Table 2
The level of expression and the activity of AChE in SCOP-induced rat model after MC and GAL treatment in all brain tissues. Data are shown as mean ± SEM (n = 8 rats per group). **, $p < 0.01$; ***, $p < 0.001$ in comparison with control group; $^{+}$, $p < 0.05$; $^{++}$, $p < 0.01$ and $^{+++}$, $p < 0.001$ in comparison with SCOP group. SCOP: scopolamine, GAL: Galantamine, MC: *Myrtus communis*, AChE: acetylcholinesterase.

	AChE activity (nmol/min/mg protein)				AChE-mRNA expression level (%)			
Brain Regions	Groups							
Amygdala	Control	SCOP	SCOP + GAL	SCOP + MC	Control	SCOP	SCOP + GAL	SCOP + MC
	94 ± 21	339 ± 28***	136 ± 27$^{+++,+}$	124 ± 37^{+++}	103 ± 12	160 ± 13***	121 ± 18^{+++}	110 ± 13^{+++}
Hippocampus	Control	SCOP	SCOP + GAL	SCOP + MC	Control	SCOP	SCOP + GAL	SCOP + MC
	104 ± 25	422 ± 63***	108 ± 19^{+++}	115 ± 25^{+++}	102 ± 12	155 ± 13***	116 ± 17^{++}	118 ± 27^{++}
Cortex	Control	SCOP	SCOP + GAL	SCOP + MC	Control	SCOP	SCOP + GAL	SCOP + MC
	101 ± 19	271 ± 34***	107 ± 18^{+++}	114 ± 10^{+++}	100 ± 17	144 ± 18***	100 ± 6^{+++}	101 ± 20^{+++}

SCOP: scopolamine, GAL: Galantamine, MC: *Myrtus communis*, AChE: acetylcholinesterase.

stress in the rat brain. The GAL, which is used in the treatment of AD patients due to its beneficial effects on cognitive function and inhibition of glutathione system disorders and due to the neuronal damage-inhibiting property, acts as an AChE inhibitor enhancing the sensitivity of cholinergic receptors and neurotransmitter release (Melo et al., 2009). Our data suggest that GAL prevents GSH levels from being reduced by SCOP. Clinical and experimental studies report that loss of cholinergic function causes significant and severe learning disabilities (Melo et al., 2009; Yoo et al., 2013). Similarly, in our study, we observed a significant reduction in hippocampal-dependent spatial memory in object recognition and passive avoidance tests by applying SCOP as a mAChRs blocker. Our results have shown that the implementation of SCOP significantly reduces the acquisition of short-term memory, and the application of MC or GAL significantly improves short term memory acquisition in behavioral tests. As there is no difference between the GAL and MC, this suggests the idea that the MC used for treatment may be as effective as the GAL.

It is generally known that, AD is associated with insufficient levels of various neurotransmitters. Previous studies have revealed that SCOP alone showed a significant decrease in cholinergic system reactivity, as indicated by a reduced ACh level and an elevated AChE activity (Ozarowski et al., 2015). Hippocampal cholinergic system profoundly influences the hippocampal-dependent learning and memory. In the results of our study, we found that the SCOP application significantly increased the both activity and the levels of AChE mRNA level whereas with MC or GAL application those effects were reduced in all tissues. A study was observed that SCOP diminished AChE mRNA level in the frontal cortex and the hippocampus by 50% and 45% (Ozarowski et al., 2016). Similar to other studies, compared to the control group, it has been found out that the AChE mRNA level was 4 times higher in the hippocampus; 2.7 times higher in the frontal cortex and 3.6 times higher in the amygdala in the SCOP group. In addition to this, SCOP + MC showed a significant reduction of AChE activity in the hippocampus by 27%, in the cortex by 42% and in the amygdala by 40%, when compared to the control rats. Decreased antioxidant and increased inflammatory parameters via SCOP were improved by MC in brain tissue. Moreover, increased AChE activity observed with SCOP model were lightened with MC. The inconsistency between the activity and mRNA expression may be because of the location of the AChE enzyme and the prevalence of its different molecular forms.

Brain derived neurotrophic factor, significantly increases neurogenesis, and its receptors are involved in the survival, maintenance of cholinergic neurons. Broad evidences from the literature suggest that BDNF may have a vital role in AD (Melo et al., 2009). In accordance with the literature, the administration of SCOP significantly decreased the BDNF protein levels in the amygdale, the hippocampus and the cortex. Similar to the previous studies, it has been found out that central cholinergic deficiency caused by a lesion or GAL decreases the level of different brain regions of BDNF protein expression (Melo et al., 2009). These studies have demonstrated that cholinergic modulation of BDNF expression in hippocampal neuronal culture is muscarinic-dependent (Gil-Bea et al., 2011). Animals were treated with a competitive and reversible AChE inhibitor known as GAL which finally leads to an increased ACh release at synapses (Woodruff-Pak et al., 2001). GAL treatment in rats had shown to improve acquisition performance in Morris water maze together with BDNF levels, providing further support to the role of cholinergic system on BDNF mediated modulation of memory formation. According to this study, BDNF protein expression levels were reported to increase in hippocampus in GAL administered rats (Golime et al., 2018). In our study, MC had rising effect on a BDNF receptor levels in the brain tissues. As there is no difference between the GAL and the MC in the expression level of BDNF, MC treatment might be effective as the GAL.

Since M_1 mAChRs have long been considered to be one of the most vital targets for restoring cholinergic signaling in memory and learning, experiments conducted on M_1 mAChR knockout mice showed that there is an increase in the progression of the AD (Verma et al., 2018). According to the literature, our results suggest that there is a reduction in M_1 receptor expression level in the SCOP group. It is known that the GAL that is used for treatment enhances the expression of the M_1 receptor. Additionally, in our study, GAL application increased M_1 receptor expression in accordance with the literature. Studies conducted so far have not evaluated the effect of MC on M_1 receptor expression. The results of our study showed that the effect of MC treatment was beneficial in recovering the decreased expression levels of M_1 in all examined brain regions.

5. Conclusion

Our results suggest that, administration of MC drastically improves the SCOP-induced reduction of spatial memory and increasing BDNF, M_1 and ACh receptor expression levels in the different brain regions. Besides, MC showed an elevation in cholinergic neurotransmission through suppressing AChE and increasing antioxidant defense by enhancing GSH activity and decreasing MDA level and MPO activity. MC with neurobiological activity which may be a possible novel therapeutic approach can be a valuable alternative way in the prevention and treatment of AD. Hence, the regulation of MC on cholinergic function, neurotransmitters and inflammation was as clear as GAL.

Declaration of interest

The authors report no conflict of interest in this work.

Transparency document

The Transparency document associated with this article can be found, in online version.

CRediT authorship contribution statement

Asli Aykac: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Dilek Ozbeyli:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Writing - original draft, Writing - review & editing. **Murat Uncu:** Data curation, Formal analysis, Writing - review & editing. **Büşra Ertaş:** Data curation, Formal analysis, Writing - review & editing. **Olca Kılınc:** Data curation, Formal analysis, Writing - review & editing. **Ali Şen:** Data curation, Formal analysis, Writing - review & editing. **Oya Orun:** Conceptualization, Data curation, Formal analysis, Methodology, Resources, Writing - review & editing. **Goksel Sener:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Writing - original draft, Writing - review & editing.

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References

Alipour, G., Dashti, S., Hosseinzadeh, H., 2014. Review of pharmacological effects of *Myrtus communis* L. and its active constituents. Phytother. Res. 28, 1125–1136. https://doi.org/10.1002/ptr.5122.

Aykac, A., Aydın, B., Cabadak, H., Goren, M.Z., 2012. The change in muscarinic receptor subtypes in different brain regions of rats treated with fluoxetine or propranolol in a model of post-traumatic stress disorder. Behav. Brain Res. 232, 124–129. https://doi.org/10.1016/j.bbr.2012.04.002.

Aykac, A., Karanlik, B., Sehirli, A.Ö., 2018. Protective effect of silk fibroin in burn injury in rat model. Gene 641, 287–291. https://doi.org/10.1016/j.gene.2017.

Azizi-Malekabadi, H., Hosseini, M., Soukhtanloo, M., Sadeghia, R., Fereidoni, M., Khodabandehloo, F., 2012. Different effects of scopolamine on learning, memory, and nitric oxide metabolite levels in hippocampal tissues of ovariectomized and Sham-operated rats. Arq. Neuropsiquiatr. 70, 447–452. https://doi.org/10.1590/S0004-282X2012000600012.

Bassani, T.B., Turnes, J.M., Moura, E.L.R., Bonato, J.M., Cóppola-Segovia, V., Zanata, S.M., Oliveira, R.M.M.W., Vital, M.A.B.F., 2017. Effects of curcumin on short-term spatial and recognition memory, adult neurogenesis and neuroinflammation in a streptozotocin-induced rat model of dementia of Alzheimer's type. Behav. Brain Res. 335, 41–54. https://doi.org/10.1016/j.bbr.2017.08.014.

Beuge, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods Enzymol. 53, 302–311. https://doi.org/10.1016/S0076-6879. ((78)52032-6).

Beutler, E., 1975. Glutathione in Red Blood Cell Metabolism, A Manual of Biochemical Methods. Grune & Stratton, New York, pp. 112–114.

Bevins, R., Besheer, J., 2006. Object recognition in rats and mice: a one-trial non-matching to sample learning task to study 'recognition memory'. Nat. Protoc. 1, 1306–1311. https://doi.org/10.1038/nprot.2006.205.

Budzynska, B., Boguszewska-Czubara, A., Kruk-Slomka, M., Skalicka-Wozniak, K., Michalak, A., Musik, I., Biala, G., 2015. Effects of imperatorin on scopolamine-induced cognitive impairment and oxidative stress in mice. Psychopharmacology 232, 931–942. https://doi.org/10.1186/s13065-016-0197-5.

Davies, P., Maloney, A.J.F., 1976. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet 308, 1403. https://doi.org/10.1016/S0140-6736(76)91936-X.

Deiana, S., Platt, B., Riedel, G., 2011. The cholinergic system and spatial learning. Behav. Brain Res. 221, 389–411. https://doi.org/10.1016/j.bbr.2010.11.036.

Erşahin, M., Toklu, H.Z., Erzik, C., Cetinel, S., Akakin, D., Velioğlu-Oğünç, A., Tetik, S., Ozdemir, Z.N., Sener, G., Yeğen, B.C., 2010. The anti-inflammatory and neuroprotective effects of ghrelin in subarachnoid hemorrhage-induced oxidative brain damage in rats. J. Neurotrauma 27, 1143–1155. https://doi.org/10.1089/neu.2009.1210.

Fan, Y., Hu, J., Li, J., Yang, Z., Xin, X., Wang, J., Ding, J., Geng, M., 2005. Effect of acidic oligosaccharide sugar chain on scopolamine-induced memory impairment in rats and its related mechanisms. Neurosci. Lett. 374, 222–226. https://doi.org/10.1016/j.neulet.2004.10.063.

Ghasemi, S., Moradzadeh, M., Hosseini, M., Beheshti, F., Sadeghnia, H.R., 2018. Beneficial effects of *Urtica dioica* on scopolamineinduced memory impairment in rats: protection against acetylcholinesterase activity and neuronal oxidative damage. Drug Chem. Toxicol. 1525–6014. https://doi.org/10.1080/01480545.2018.1463238.

Gil-Bea, F.J., Solas, M., Mateos, L., Winblad, B., Ramírez, M.J., Cedazo-Míminguez, A., 2011. Cholinergic hypofunction impairs memory acquisition possibly through hippocampal Arc and BDNF downregulation. Hippocampus 21, 999–1009. https://doi.org/10.1002/hipo.20812.

Golime, R., Palit, M., Acharya, J., Dubey, D.K., 2018. Neuroprotective effects of galantamine on nerve agent-induced neuroglial and biochemical changes. Neurotox. Res. 33, 738–748. https://doi.org/10.1007/s12640-017-9815-9.

Hancianu, M., Cioanca, O., Mihasana, M., Hritcu, L., 2013. Neuroprotective effects of inhaled lavender oil on scopolamine-induced dementia via anti-oxidative activities in rats. Phytomedicine 20, 446–452. https://doi.org/10.1016/j.phymed.2012.12.005.

Heneka, M.T., Carson, M.J., El Khoury, J., Landreth, G.E., Brosseron, F., Feinstein, D.L., Jacobs, A.H., Wyss-Coray, T., Vitorica, J., Ransohoff, R.M., Herrup, K., Frautschy, S.A., Finsen, B., Brown, G.C., Verkhratsky, A., Yamanaka, K., Koistinaho, J., Latz, E., Halle, A., Petzold, G.C., Town, T., Morgan, D., Shinohara, M.L., Perry, V.H., Holmes, C., Bazan, N.G., Brooks, D.J., Hunot, S., Joseph, B., Deigendesch, N., Garaschuk, O., Boddeke, E., Dinarello, C.A., Breitner, J.C., Cole, G.M., Golenbock, D.T., Kummer, M.P., 2015. Neuroinflammation in Alzheimer's disease. Lancet Neurol. 14, 388–405. https://doi.org/10.1016/S1474-4422(15)70016-5.

Hillegass, L.M., Griswold, D.E., Brickson, B., Albrightson-Winslow, C., 1990. Assessment of myeloperoxidase activity in whole rat kidney. J. Pharmacol. Meth. 24, 285–295.

Hosseinzadeh, S., Dabidi-Roshan, V., Pourasghar, M., 2013. Effects of intermittent aerobic training on passive avoidance test (shuttle box) and stress markers in the dorsal hippocampus of wistar rats exposed to administration of homocysteine. Iran. J. Psychiatry. Behav. Sci. 7, 37–44.

Jabri, M.A., Tounsi, H., Rtibi, K., Marzouki, L., Sakly, M., Sebai, H., 2015. Ameliorative and antioxidant effects of myrtle berry seed (*Myrtus communis*) extract during reflux-induced esophagitis in rats. Pharm. Biol. 54 (9), 1575–1585. https://doi.org/10.3109/13880209.2015.1107748.

Jeon, S.J., Kim, B., Park, H.J., Zhang, J.B., Kwon, Y., Kim, D.H., 2017. The ameliorating effect of 1-palmitoyl-2-linoleoyl-3-acetylglycerol on scopolamine-induced memory impairment via acetylcholinesterase inhibition and LTP activation. Behav. Brain Res. 324, 58–65. https://doi.org/10.1016/j.bbr.2017.01.040.

Kameyama, T., Nabeshima, T., Kozawa, T., 1986. Step-down-type passive avoidance- and escape-learning method. Suitability for experimental amnesia models. J. Pharmacol. Meth. 16, 39–52. https://doi.org/10.1016/0160-5402(86)90027-6.

Klinkenberg, I., Sambeth, A., Blokland, A., 2011. Acetylcholine and attention. Behav. Brain Res. 221, 430–442. https://doi.org/10.1016/j.bbr.2010.11.033.

Kumar, A., Singh, A., 2015. A review on Alzheimer's disease pathophysiology and its management: an update. Pharmacol. Rep. 67, 195–203. https://doi.org/10.1016/j.pharep.2014.09.004.

Li, S.P., Wang, Y.W., Qi, S.L., Zhang, Y.P., Deng, G., Ding, W.Z., Ma, C., Lin, Q.Y., Guan, H.D., Liu, W., Cheng, X.M., Wang, C.H., 2018. Analogous β-carboline alkaloids harmaline and harmine ameliorate scopolamine-induced cognition dysfunction by attenuating acetylcholinesterase activity, oxidative stress, and inflammation in mice. Front. Pharmacol. 9, 346–362. https://doi.org/10.3389/fphar.2018.00346.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin-Phenol reagents. J. Biol. Ther. 191, 33–43.

Lu, C., Dong, L.M., Lv, J.W., Wang, Y., Fan, B., Wang, F., 2018. F, 20(S)-protopanaxadiol (PPD) alleviates scopolamine-induced memory impairment via regulation of cholinergic and antioxidant systems, and expression of Egr-1, c-Fos and c-Jun in mice. Chem. Biol. Interact. 279, 64–72. https://doi.org/10.1016/j.cbi.2017.11.008.

Mahboubi, M., 2016. *Myrtus communis* L. and its application in treatment of recurrent aphthous stomatitis. J. Ethnopharmacol. 193, 481–489. https://doi.org/10.1016/j.jep.2016.09.054.

Maxia, A., Frau, M.A., Falconieri, D., Karchuli, M.S., Kasture, S., 2011. Essential oil of *Myrtus communis* inhibits inflammation in rats by reducing serum IL-6 and TNF-alpha. Nat. Prod. Commun. 6, 1545–1548.

Melo, J.B., Sousa, C., Garçao, P., Oliveira, C.R., Agosthinho, P., 2009. Galantamine protects against oxidative stress induced by amyloid-beta peptide in cortical neurons. Eur. J. Neurosci. 29, 455–464. https://doi.org/10.1111/j.1460-9568.2009.06612.x.

Mhatre, M., Floyd, R.A., Hensley, K., 2004. Oxidative stress and neuroinflammation in Alzheimer's disease and amyotrophic lateral sclerosis: common links and potential therapeutic targets. J. Alzheimers Dis. 6, 147–157.

Ozarowski, M., Thiem, B., Mikolajczak, P.L., Piasecka, A., Kachlicki, P., Szulc, M., Kaminska, E., Bogacz, A., Kujawski, R., Bartkowiak-Wieczorek, J., Kujawska, M., Jodynis-Liebert, J., Budzianowski, J., Kędziora, I., Seremak-Mrozikiewicz, A., Czerny, B., Bobkiewicz-Kozłowska, T., 2015. Improvement in long-term memory following chronic administration of *Eryngium planum* root extract in scopolamine model: behavioral and molecular study. Evid. Based Complement. Alternat. Med. 2015, 145140–145153. https://doi.org/10.1155/2015/145140.

Ozarowski, M., Mikolajczak, P.L., Piasecka, A., Kachlicki, P., Kujawski, R., Bogacz, A., Bartkowiak-Wieczorek, J., Szulc, M., Kaminska, E., Kujawska, M., Jodynis-Liebert, J., Gryszczynska, A., Opala, B., Lowicki, Z., Seremak-Mrozikiewicz, A., Czerny, B., 2016. Influence of the *Melissa officinalis* leaf extract on long-term memory in scopolamine animal model with assessment of mechanism of action. Evid. Based Complement. Alternat. Med. 2016, 9729818. https://doi.org/10.1155/2016/9729818.

Özbeyli, D., Sarı, G., Özkan, N., Karademir, B., Yüksel, M., Çilingir-Kaya, Ö.T., Çakır, Ö.K., 2017. Protective effects of different exercise modalities in an Alzheimer's disease-like model. Behav. Brain Res. 328, 159–177. https://doi.org/10.1016/j.bbr.2017.03.044.

Ozkol, H., Tuluce, Y., Dilsiz, N., Koyuncu, I., 2013. Therapeutic potential of some plant extracts used in Turkish traditional medicine on streptozocin-induced type 1 diabetes mellitus in rats. J. Membr. Biol. 246, 47–55. https://doi.org/10.1007/s00232-012-9503-x.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, 2nd ed. Academic Press, London.

Rahimzadegan, M., Soodi, M., 2018. Comparison of memory impairment and oxidative stress following single or repeated doses administration of scopolamine in rat hippocampus. Basic. Clin. Neurosci. 9, 5–14. https://doi.org/10.29252/NIRP.BCN.9.1.5.

Rossi, A., Di Paola, R., Mazzon, E., Genovese, T., Caminiti, R., Bramanti, P., Pergola, C., Koeberle, A., Werz, O., Sautebin, L., Cuzzocrea, S., 2009. Myrtucommulone from *Myrtus communis* exhibits potent anti-inflammatory effectiveness in vivo. J. Pharmacol. Exp. Ther. 329, 76–86. https://doi.org/10.1124/jpet.108.143214.

Schliebs, R., Arendt, T., 2011. The cholinergic system in aging and neuronal degeneration. Behav. Brain Res. 221, 555–563. https://doi.org/10.1016/j.bbr.2010.11.058.

Sen, A., Ozkan, S., Recebova, K., Cevik, O., Ercan, F., Kervancıoglu-Demirci, E., Bitis, L., Sener, G., 2016. Effects of *Myrtus communis* extract treatment in bile duct ligated rats. J. Surg. Res. 205, 359–367. https://doi.org/10.1016/j.jss.2016.06.094.

Singh, M., Dang, T.N., Arseneault, M., Ramasamy, C., 2010. Role of by-products of lipid oxidation in Alzheimer's disease brain: a focus on acrolein. J. Alzheimers Dis. 21, 741–756. https://doi.org/10.3233/JAD-2010-100405.

Tumen, I., Senol, F.S., Orhan, I.E., 2012. Inhibitory potential of the leaves and berries of *Myrtus communis* L. (myrtle) against enzymes linked to neurodegenerative diseases and their antioxidant actions. Int. J. Food Sci. Nutr. 63, 387–392. https://doi.org/10.3109/09637486.2011.629178.

Utkan, T., Gocmez, S.S., Ozer, C., Gacar, N., Aricioglu, F., 2012. Selective and non-selective neuronal NOS inhibitors impair cognitive function in the three panel runway and passive avoidance tasks in rats. Pharmacol. Biochem. Behav. 101, 515–519. https://doi.org/10.1016/j.pbb.2012.02.020.

Verma, S., Kumar, A., Tripathi, T., Kumar, A., 2018. Muscarinic and nicotinic acetylcholine receptor agonists: current scenario in Alzheimer's disease therapy. J. Pharm. Pharmacol. 70, 985–993. https://doi.org/10.1111/jphp.12919.

Verri, M., Pastoris, O., Dossena, M., Aquilani, R., Guerriero, F., Cuzzoni, G., Venturini, L., Ricevuti, G., Bongiorno, A.I., 2012. Mitochondrial alterations, oxidative stress and neuroinflammation in Alzheimer's disease. Int. J. Immunopathol. Pharmacol. 25, 345–353. https://doi.org/10.1177/039463201202500204.

Wang, J., Wang, X., Lv, B., Yuan, W., Feng, Z., Mi, W., Zhang, Ho., 2014. Effects of Fructus Akebiae on learning and memory impairment in a scopolamine-induced animal model of dementia. Exp. Ther. Med. 8, 671–675. https://doi.org/10.3892/etm.2014.1775.

Woodruff-Pak, D.S., Vogel, R.W., Wenk, G.L., 2001. Galantamine: effect on nicotinic receptor binding, acetylcholinesterase inhibition, and learning. Proc. Natl. Acad. Sci. 98, 2089–2094.

Yoo, D.Y., Choi, J.H., Kim, W., Nam, S.M., Jung, H.Y., Kim, J.H., Won, M.H., Yoon, Y.S., Hwang, I.K., 2013. Effects of luteolin on spatial memory, cell proliferation, and neuroblast differentiation in the hippocampal dentate gyrus in a scopolamine-induced amnesia model. Neurol. Res. 35, 813–820. https://doi.org/10.1179/1743132813Y.0000000217.