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Original Article

Ameliorative effects of *Matricaria chamomilla* L. hydroalcoholic extract on scopolamine-induced memory impairment in rats: A behavioral and molecular study

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ABSTRACT

Background: *Matricaria chamomilla* L. is a medicinal herb traditionally used as the anti-inflammatory, antimicrobial, antiviral, anxiolytic and antidepressant agent. Nevertheless, supporting evidence demonstrated its memory enhancing activity and antioxidant properties.
Purpose: To investigate the effects of the hydroalcoholic extract of *M. chamomilla* L. on memory processes in a scopolamine-induced a rat model of amnesia and to reveal its underlying mechanism of action.
Methods: The hydroalcoholic extract (25 and 75 mg/kg) was intraperitoneally administered to rats once daily for 7 days, and scopolamine (0.7 mg/kg) was injected 30 min before the behavioral testing to induce memory impairment. The phytochemical composition of the extract was quantified by HPLC/DAD analysis. Y-maze and radial arm-maze tests were employed for memory assessing. Acetylcholinesterase activity was measured in the rat hippocampus. Superoxide dismutase, glutathione peroxidase, and catalase specific activities along with the total content of reduced glutathione and protein carbonyl and malondialdehyde levels were also measured in the rat hippocampus. qRT-PCR was used to quantify BDNF mRNA and IL1β mRNA expression in the rat hippocampus.
Results: We first identified the chlorogenic acid, apigenin-7-glucoside, rutin, cynaroside, luteolin, apigenin and derivatives of apigenin-7-glucoside as the extract major components. Furthermore, we showed that the extract reversed the scopolamine-induced decreasing of the spontaneous alternation in the Y-maze test and the scopolamine-induced increasing of the working and reference memory errors in the radial arm maze test. Also, the scopolamine-induced alteration of the acetylcholinesterase activity and the oxidant-antioxidant balance in the rat hippocampus was recovered by the treatment with the extract. Finally, we demonstrated that the extract restored the scopolamine-decreased BDNF expression and increased IL1β expression in the rat hippocampus.
Conclusion: These findings suggest that the extract could be a potent neuropharmacological agent against amnesia via modulating cholinergic activity, neuroinflammation and promoting antioxidant action in the rat hippocampus.

Introduction

Neurochemical analyses of the brain samples from Alzheimer's disease (AD) patients indicated a significant loss of the cortical cholinergic innervation, and also cholinergic deficits in the cortex and hippocampus (Savonenko et al., 2012). Acetylcholinesterase (AChE) is a target for AD therapy and as inhibiting of its activity helps to maintain the acetylcholine (ACh) levels in the neuronal synapses with positive effects in AD patients. Evidence suggests that AChE inhibitors decrease extrasynaptic metabolism of ACh, being available high levels of ACh at the synaptic cleft and enhances postsynaptic stimulation. Recently, Haider et al. (2016) proposed that cholinergic dysfunction-induced

Abbreviations: AChE, acetylcholinesterase; ACh, acetylcholine; AD, Alzheimer's disease; ATC, acetylthiocholine; BCA, bicinchoninic acid; BDNF, brain-derived neurotrophic factor; CAT, catalase; DAD, multidiode array detector; DZP, diazepam; GPX, glutathione peroxidase; GSH, glutathione; LDH, lactatedehydrogenase; MDA, malondialdehyde; TRM, tramadol; SOD, superoxide dismutase; qRT-PCR, real-time quantitative PCR

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memory impairment is correlated with increased oxidative stress following administration of scopolamine. However, our group previously showed a strong correlation between memory dysfunctions and the oxidative stress in the rat hippocampus and scopolamine using a rat model of cognitive impairment (Aydin et al., 2016). Among different hallmarks of the AD, oxidative stress was reported (Xu et al., 2017). Moreover, decreasing of the antioxidant enzymes activity, such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) have been reported in the early stages of the AD (Boonruamkaew et al., 2017). The hippocampal neurogenesis is regulated by the normal cholinergic system activity through modulating neurogenic mechanisms such as those involving the brain-derived neurotrophic factor (BDNF) and cAMP response element-binding protein (CREB) (Bruel-Jungerman et al., 2011). Evidence suggested that a reduction in the BDNF levels in the entorhinal cortex and hippocampus of patients with the AD (Wu et al., 2016), resulting in decreasing of the patient's score on the mini-mental state examination. In addition, the alterations in BDNF, and phosphorylated CREB occurred following scopolamine treatment were evidenced (Park et al., 2016). Supporting information suggested that the involving of the neuroinflammation in AD pathogenesis which contributes to disease progression and severity (Heneka et al., 2015). Data suggested that IL-1β stimulate the progression of neurodegenerative diseases by inducing nitric oxide production and cholinergic function decline via increased AChE activity (Xian et al., 2015).

M. chamomilla L. is a well-known medicinal plant species from the Asteraceae family native to southern and eastern Europe (Singh et al., 2011), including Romania. Previous examinations have reported that *M. chamomilla* possess various biological activities such as neuroprotective activity against global cerebral ischemia/reperfusion injury-induced oxidative stress in rats, potent antidiarrheal and antioxidant properties in rats, analgesic and anti-inflammatory effects on mice, antioxidant effects against scopolamine-induced the rat brain oxidative stress, attenuation of motor deficits induced by scopolamine and anti-hyperglycemic potential in diabetic streptozotocin-induced rats supporting its use in folk medicine. However, the effects of *M. chamomilla* on cognitive function and hippocampal oxidative status, neurogenesis, and neuroinflammation have not been studied. Therefore, in the present study, we investigated the potential anti-amnesic effects of *M. chamomilla* on memory formation and hippocampal oxidative status, neurogenesis, and neuroinflammation in the scopolamine-induced model. Additionally, we investigated the hippocampal BDNF mRNA and IL1β mRNA expression in the scopolamine-induced amnesic rats.

Materials and methods

Plant material and extraction procedure

Dry flowers of the *M. chamomilla* were purchased from the Romanian pharmaceutical market in 2016 and identified in the Department of Pharmacognosy, University of Medicine and Pharmacy "Gr T. Popa", Iasi, Romania where a voucher specimen (No. C1-072016) was deposited. 2.5 g of the dry inflorescence was extracted with 100 ml of 50% ethanol, reflux 30 min in a water bath. The extract was filtered and concentrated by drying oven with a thermostat set to 40 °C, weighed (yield: 1.3 g) and stored at 4 °C, and used to treat the animals as needed (Gacea, 2010). The extract was resuspended in sterile saline for further work.

HPLC/DAD analysis

A Thermo UltiMate3000 HPLC system equipped with quaternary pumps controlled by Chromeleon interface, an autosampler and multidiode array detector (DAD) was used for the HPLC analyses. Solvents were filtered using a Millipore system and analysis was performed on an Accucore XL C18 column (150 × 4.6 mm, 4 µm). The mobile phase was acetonitrile (A) and water containing 0.1% acetic acid (B) and the composition gradient was:10%–23% (A) in 5 min; 23% (A) isocratic for 10 min and then 23% − 35% (A) in 12 min; 35% − 70% (A) for 5 min. The injection volume was 20 µL scanning absorbance wavelengths from 240 nm to 520 nm, typical for phenols. Each solution was injected in triplicate and the calibration curves were constructed with the averages.

Animals and groups

Male Wistar/25 rats (4-month-old) were purchased from Cantacuzino Institute (Bucharest, Romania) and were housed under temperature- and light-controlled conditions (22 °C, a 12-h cycle starting at 08:00 h) with food and water *ad libitum*. The rats were divided into five groups (n = 5 per group): (1) the Control group received 0.9% saline treatment; (2) the scopolamine (Sco, 0.7 mg/kg)-alone-treated group, as negative control; (3) the scopolamine-treated group received 25 mg/kg of the hydroalcoholic extract from *M. chamomilla* dry flowers (Sco + MC (25 mg/kg)), (4) the scopolamine-treated group received 75 mg/kg of the hydroalcoholic extract from *M. chamomilla* dry flowers (Sco + MC (75 mg/kg)) and (5) donepezil group (5 mg/kg), as positive control. The hydroalcoholic extract was injected for 7 continuous days before scopolamine injection. Except for control group, scopolamine (Sigma-Aldrich, Germany) was injected once intraperitoneally, 30 min before the behavioral experiments in specific tests.

Efforts were made to minimize the number of animals used per test and their potential suffering. This study was approved by the Committee on the Ethics of Animal Experiments of the Alexandru Ioan Cuza University of Iasi (permit number: 2195). All procedures were in compliance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Acute toxicity study

To establish the safety profile of the hydroalcoholic extract of *M. chamomilla* L., toxicity evaluation of the extract was carried out separately as previously described but with modification (Song et al., 2017). Rats were divided into three groups (5 animals/group). The extract was intraperitoneally delivered once daily in a single dose of 0 (control), 25 and 75 mg/kg. The control group received 0.9% saline treatment. The animals were monitored for 24 h after dosing and the number of deaths was recorded.

Y-maze task

The Y-maze task is a specific and sensitive test of spatial recognition memory in rodents. Spontaneous alternation behavior was analyzed on the day 8 after the hydroalcoholic extract administration by the Y-maze task as previously reported (Hritcu et al., 2017). Each animal was tested during 8 min session and the percent spontaneous alternation and the locomotor activity (the number of arm entries) was explored.

Radial arm maze task

The radial arm-maze is one of the standard apparatuses used in behavioral-based research to assess spatial memory. Working memory and reference memory was explored starting with 11th days after the hydroalcoholic extract administration using radial arm-maze task as previously indicated (Hritcu et al., 2017). Animals were individually tested during 7 days period and the number of working memory errors and the number of reference memory errors were recorded.

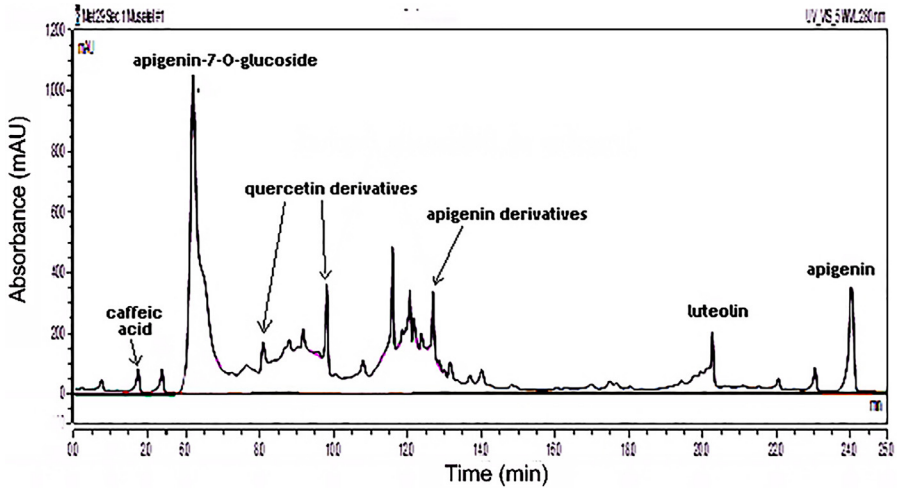

Fig. 1. Representative HPLC/DAD chromatography profile at 280 nm for the flavonoids and polyphenol carboxylic acids of the hydroalcoholic extract from *Matricaria chamomilla* aerial parts (dry flowers).

Table 1
Compounds identified in the hydroalcoholic extract from *Matricaria chamomilla* flowers.

Compound	Concentration (mg/100 g dry flowers)
Chlorogenic acid	222.54
Caffeic acid	57.04
Catechin	35.22
Apigenin-7-glucoside	927.62
Rutin	163.54
Cynaroside	72.32
Luteolin	139.08
Apigenin	377.64

Table 2
Results of acute toxicity of the hydroalcoholic extract from *Matricaria chamomilla* flowers in rats.

Group (n = 5 animals per group)	Dose (mg/kg body weight)
Control	0
All alive	
Matricaria chamomilla extract	25 mg
All alive	
Matricaria chamomilla extract	75 mg
All alive	

Biochemical parameter assay

After behavioral tests, all rats were deeply anesthetized (using sodium pentobarbital, 150 mg/kg b.w., i.p., Sigma-Aldrich, Germany), decapitated and whole brains were removed. The hippocampi were carefully excised. Each of the hippocampal samples was weighed and homogenized (1:10) with Potter Homogenizer coupled with Cole-Parmer Servodyne Mixer in ice-cold 0.1 M potassium phosphate buffer (pH 7.4), 1.15% KCl. The homogenate was centrifuged (15 min at 960 x *g*) and the supernatant was used for assays of acetylcholinesterase (AChE), superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), the total content of reduced glutathione (GSH), protein carbonyl and malondialdehyde (MDA) levels as previously described (Hritcu et al., 2015). The AChE, SOD, GPX and CAT activities, the GSH, protein carbonyl and MDA contents were normalized to protein content.

Real-time quantitative PCR (qRT-PCR)

The mRNA expression of BDNF and IL1β was performed by qRT-PCR analysis as previously described (Postu et al., 2017). Total RNA was isolated and purified using SV Total RNA Isolation System kit (Promega, Wi, USA) according to the manufacturer instructions. The reverse transcription and real-time amplification were performed in single-step amplification reactions using GoTaq® 1-Step RT-qPCR System (Promega, Wi, USA) on a 5-plex HRM Rotor-Gene 6000 (Corbett, AU) rotary real-time PCR. The reaction was carried out in a 20 µL total volume containing GoTaq® Probe qPCR Master Mix 2X (Promega, Wi, USA), GoScript™ RT Mix for 1-Step RT-qPCR 50X, forward and reverse primers, 100 ng of tRNA template and Nuclease-Free Water up to volume. Pre-designed specific primers for *Rattus norvegicus* were used as following: BDNF exon 5 - 300 nM forward and reverse primer (F: 5′-ATT ACC TGG ATG CCG CAA AC-3′; R: 5′-TGA CCC ACT CGC TAA TAC TGT-3′, 101 bp product size); IL1β - 300 nM forward and reverse primer (F: 5′-AGC ACC TTC TTT TCC TTC ATC TT-3′, R: 5′-CAG ACA GCA GGC ATT TT-3′, 144 bp product size). Levels of BDNF and IL1β were determined by absolute quantification using Rotor-Gene Q-Pure Detection Software v. 2.2.3. (Qiagen).

Statistical analysis

All results are expressed as a mean ± S.E.Ms. Statistical differences between groups were identified using two-way ANOVA with Tukey's *post hoc* test, taking $p < 0.05$ to indicate statistical significance.

Results

Chemical composition of the hydroalcoholic extract from M. chamomilla

The HPLC/DAD results indicated the presence of several flavonoids and polyphenol carboxylic acids such as chlorogenic acid, apigenin-7-glucoside, rutin, cynaroside, luteolin, apigenin and derivatives of apigenin-7-glucoside (Fig. 1). The amounts detected were: chlorogenic acid- 222.54 mg/100 g of dry flowers, cafeic acid - 57.04 mg/100 g of dry flowers, catechin - 35.22 mg/100 g of dry flowers, apigenin-7-glucoside - 927.62 mg/100 g of dry flowers, rutin - 163.54 mg/100 g of dry flowers, cynaroside - 72.32 mg/100 g of dry flowers, luteolin - 139.08 mg/100 g of dry flowers and apigenin - 377.04 mg/100 g of dry flowers (Table 1).

M. chamomilla extract exhibited safety profile

In the acute toxicity study, all rats survived and no sign of toxicity was observed at 25 mg/kg and 75 mg/kg (Table 2). There were no behavioral or body changes and no abnormal signs were observed.

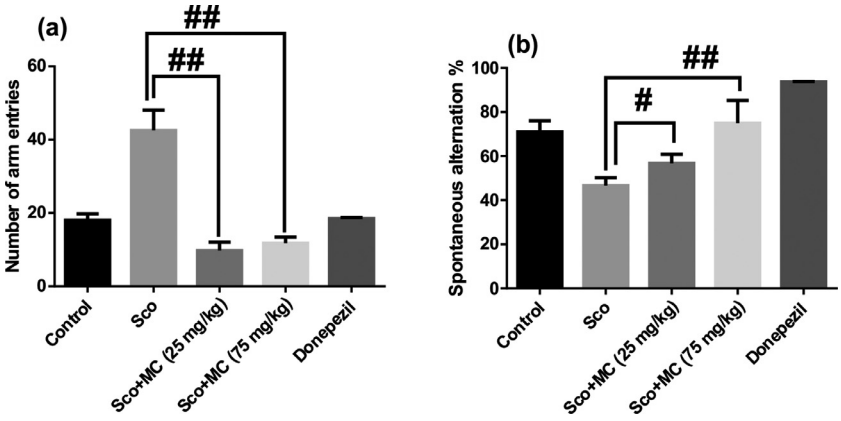

Fig. 2. Effects of the hydroalcoholic extract from *Matricaria chamomilla* aerial parts (25 and 75 mg/kg) administration in the Y-maze on the number of arm entries (a) and the spontaneous alternation % (b) in the scopolamine-treated rats. Values are means ± S.E.M. (n = 5 animals per group). For Tukey's *post hoc* analyses - $^{\#}$Sco vs. Sco + MC (25 mg/kg): $p < 0.01$ and $^{\#\#}$Sco vs. Sco + MC (75 mg/kg): $p < 0.001$ (b).

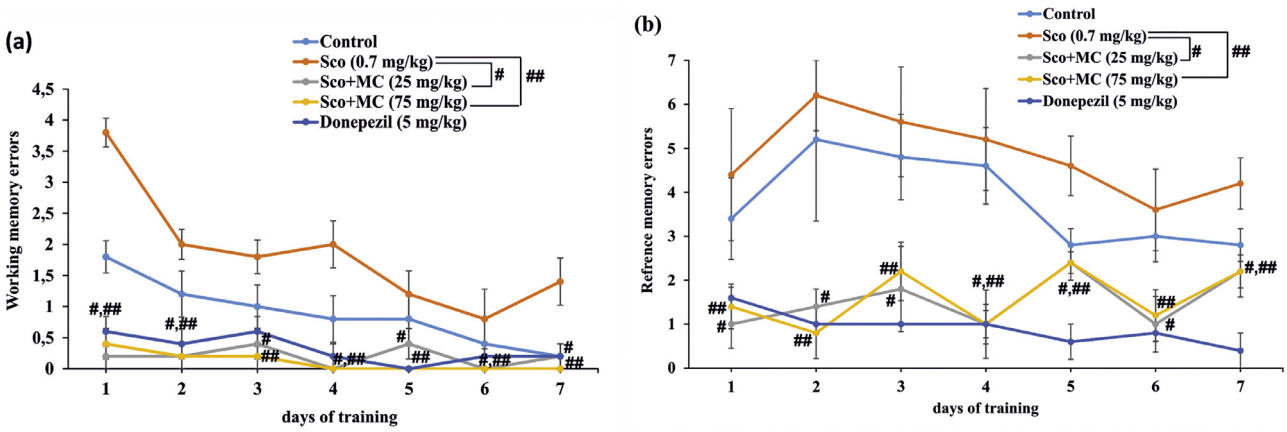

Fig. 3. Effects of the hydroalcoholic extract from *Matricaria chamomilla* aerial parts (25 and 75 mg/kg) administration on the working memory errors (a) and the reference memory errors (b) during 7 days training in the radial arm maze in the scopolamine-treated rats. Values are means ± S.E.M. (n = 5 animals per group). For Tukey's *post hoc* analyses – $^{\#}$Sco vs. Sco + MC (25 mg/kg): $p < 0.001$ and $^{\#\#}$Sco vs. Sco + MC (75 mg/kg): $p < 0.001$ (a) and $^{\#}$Sco vs. Sco + MC (25 mg/kg): $p < 0.001$ and $^{\#\#}$Sco vs. Sco + MC (75 mg/kg): $p < 0.001$ (b).

M. chamomilla extract stimulated spatial memory in Y-maze and radial arm-maze tasks

Scopolamine treatment significantly decreased ($p < 0.01$) the spontaneous alternation percentage and increased locomotor activity (the number of arm entries, $p < 0.0001$) in the Y-maze task as compared to control group. Administration of the extract significantly ameliorate the decreasing of the spontaneous alternation percentage ($p < 0.01$ for 25 mg/kg and $p < 0.001$ for 75 mg/kg) induced by scopolamine (Fig. 2b) and also decreased the locomotor activity ($p < 0.0001$ for 25 mg/kg and $p < 0.0001$ for 75 mg/kg) to the values closed related to normal level (Fig. 2b). In contrast, donepezil had different effects than the extract treatment.

In the radial arm maze task, the scopolamine-treated rats exhibited a significant increase of both the working memory errors ($p < 0.01$, Fig. 3a) and the reference memory errors ($p < 0.01$, Fig. 3b) as compared to control group, but this increase was significantly reversed by the administration of the extract. Additionally, repeated-measures ANOVA revealed a significant time difference ($F(6,112) = 2.39$, $p < 0.01$) and a significant group difference ($F(3,56) = 10.89$, $p < 0.001$) and a significant time-group interaction ($F(3,112) = 32.23$, $p < 0.001$) for the working memory errors evaluation (Fig. 3a). Moreover, repeated-measures ANOVA revealed a significant group difference ($F(3,112) = 32.02$, $p < 0.001$) for the reference memory errors evaluation. Donepezil has effects similar to those of the extract treatment.

M. chamomilla extract exhibited cholinesterase inhibitory activity

Significant overall differences between groups ($F(3, 16) = 20.90$, $p < 0.001$) regarding the AChE specific activity in the rat hippocampal homogenates were indicated (Fig. 4a). AChE activity in the hippocampal tissue was increased significantly by scopolamine injection compared with the control group ($p < 0.01$), while the treatment with the extract significantly attenuated ($p < 0.0001$) the increase of the AChE activity.

M. chamomilla extract exhibited antioxidant profile in the rat hippocampus

Estimated significant overall differences between groups for SOD (Fig. 4b) with ($F(3, 16) = 21.61$, $p < 0.0001$), GPX (Fig. 4c) with ($F(3, 8) = 11.81$, $p < 0.001$) and CAT (Fig. 4d) specific activities with ($F(3, 16) = 48.20$, $p < 0.0001$), the total content of reduced GSH (Fig. 4e) with ($F(3, 16) = 50.49$, $p < 0.0001$), the levels of protein carbonyl (Fig. 4f) with ($F(3, 16) = 37.64$, $p < 0.0001$) and MDA (Fig. 4g) with ($F(3, 16) = 31.87$, $p < 0.0001$) in the rat hippocampal homogenates were demonstrated. Scopolamine injection significantly depleted the antioxidant activities in hippocampal tissue, including SOD activity ($p < 0.0001$), GPX activity ($p < 0.001$) and CAT activity ($p < 0.0001$), GSH content ($p < 0.0001$), and increased the levels of protein carbonyl and MDA ($p < 0.0001$) compared to the control group. These changes were reversed by the extract treatment and for all biomarkers.

M. chamomilla extract decreased LDH activity in the hippocampus

For the LDH specific activity estimated in the rat hippocampal homogenates, significant overall differences between groups ($F(3,16) = 9.46$, $p < 0.001$) were noticed (Fig. 4h). LDH activity in the hippocampal tissue was enhanced significantly by scopolamine injection ($p < 0.001$) compared to the control group. Administration of the extract significantly attenuated increasing of LDH activity.

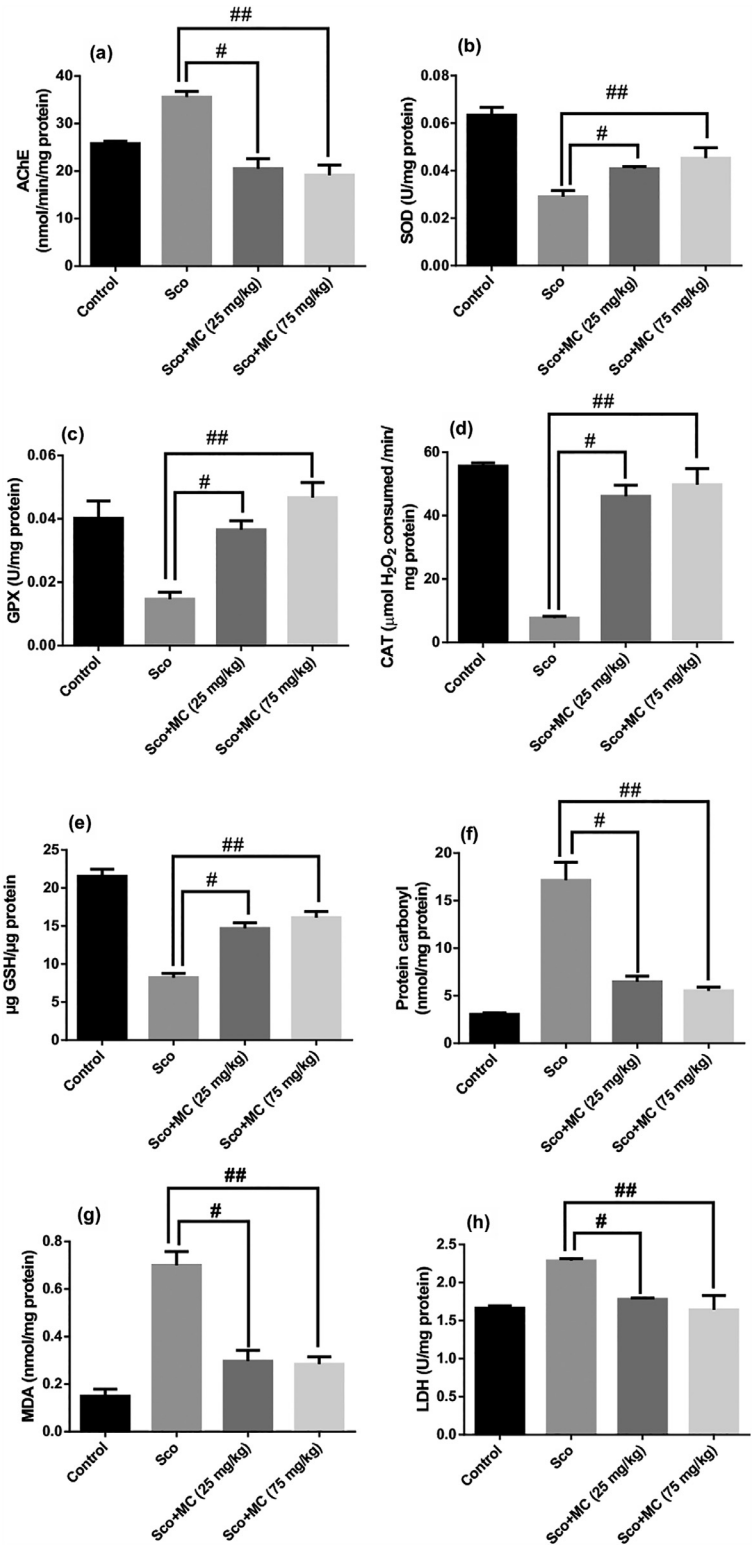

Fig. 4. Effects of the hydroalcoholic extract from *Matricaria chamomilla* aerial parts (25 and 75 mg/kg) administration on the AChE (a), SOD (b), GPX (c) and CAT (d) specific activities, the total content of reduced GSH (e), protein carbonyl (f) and MDA (g) levels and on the LDH (h) estimated in the rat hippocampal homogenates of the scopolamine-treated rats. Values are means ± S.E.M. (n = 5 animals per group). For Tukey's *post hoc* analyses – $^{\#}$Sco vs. Sco + MC (25 mg/kg): $p < 0.0001$ and $^{\#\#}$Sco vs. Sco + MC (75 mg/kg): $p < 0.0001$ (a), $^{\#}$Sco vs. Sco + MC (25 mg/kg): $p < 0.01$ and $^{\#\#}$Sco vs. Sco + MC (75 mg/kg): $p < 0.001$ (b), $^{\#}$Sco vs. Sco + MC (25 mg/kg): $p < 0.01$ and $^{\#\#}$Sco vs. Sco + MC (75 mg/kg): $p < 0.001$ (c), $^{\#}$Sco vs. Sco + MC (25 mg/kg): $p < 0.01$ and $^{\#\#}$Sco vs. Sco + MC (75 mg/kg): $p < 0.001$ (d), $^{\#}$Sco vs. Sco + MC (25 mg/kg): $p < 0.001$ and $^{\#\#}$Sco vs. Sco + MC (75 mg/kg): $p < 0.0001$ (e), $^{\#}$Sco vs. Sco + MC (25 mg/kg): $p < 0.0001$ and $^{\#\#}$Sco vs. Sco + MC (75 mg/kg): $p < 0.0001$ (f), $^{\#}$Sco vs. Sco + MC (25 mg/kg): $p < 0.0001$ and $^{\#\#}$Sco vs. Sco + MC (75 mg/kg): $p < 0.0001$ (g) and $^{\#}$Sco vs. Sco + MC (25 mg/kg): $p < 0.01$ and $^{\#\#}$Sco vs. Sco + MC (75 mg/kg): $p < 0.001$ (h).

M. chamomilla extract increased BDNF mRNA and decreased IL1β mRNA levels

Important overall differences between groups for BDNF mRNA copy number (Fig. 5a) with ($F(3,16) = 14.92$, $p < 0.0001$) and for IL1β mRNA copy number (Fig. 5b) with ($F(3,16) = 21.55$, $p < 0.0001$) were evidenced. Scopolamine injection significantly down-regulated BDNF mRNA copy number ($p < 0.0001$) and up-regulated IL1β mRNA copy number ($p < 0.0001$) in the hippocampal tissue as compared to the control group, while treatment with the extract significantly reversed these changes in a dose-dependent manner.

Pearson's correlation coefficient and regression analysis were used in order to evaluate the connection between behavioral measures, the antioxidant defense and lipid peroxidation. A strong correlation between the spontaneous alternation percentage vs. MDA ($r = -0.634$, $p < 0.01$) (Fig. 6a), working memory errors vs. AChE ($r = 0.782$, $p < 0.001$) (Fig. 6b), working memory errors vs. MDA ($r = 0.546$, $p < 0.01$) (Fig. 6c) and reference memory errors vs. AChE ($r = 0.820$, $p < 0.001$) (Fig. 6d), was evidenced by linear regression. Moreover,

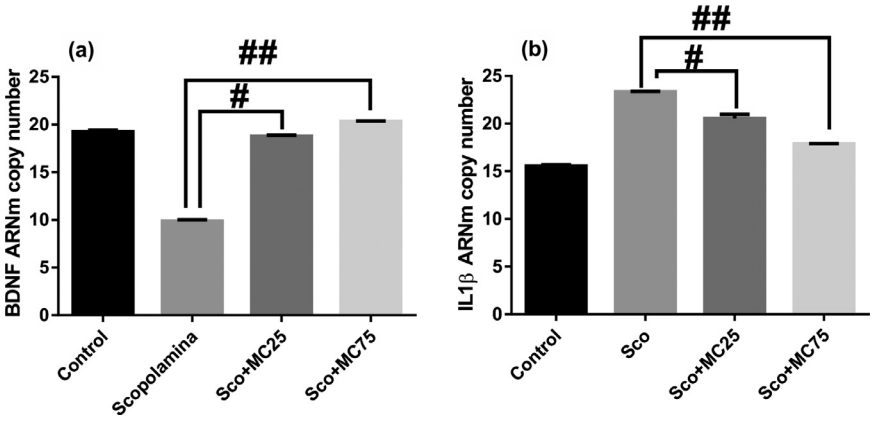

Fig. 5. BDNF mRNA copy number (a) and IL1β mRNA copy number (b) in the scopolamine groups pretreated with the hydroalcoholic extract from *Matricaria chamomilla* aerial parts (25 and 75 mg/kg). Values are means ± S.E.M. (n = 5 animals per group). For Tukey's *post hoc* analyses – $^{\#}$Sco vs. Sco + MC (25 mg/kg): $p < 0.0001$ and $^{\#\#}$Sco vs. Sco + MC (75 mg/kg): $p < 0.0001$ (a) and $^{\#}$Sco vs. Sco + MC (25 mg/kg): $p < 0.001$ and $^{\#\#}$Sco vs. Sco + MC (75 mg/kg): $p < 0.0001$ (b).

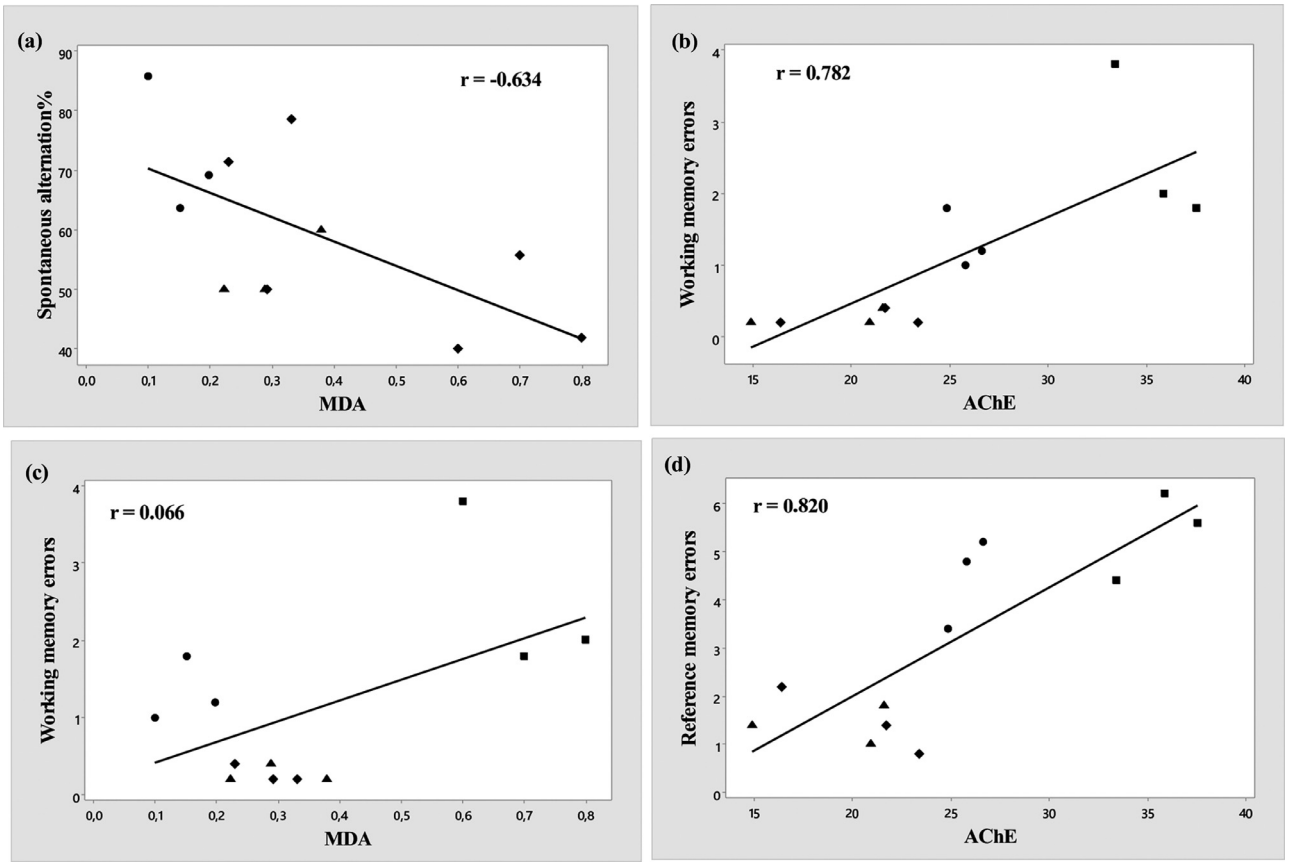

Fig. 6. Pearson's correlation between the spontaneous alternation percentage vs. MDA (a), the working memory errors vs. AChE (b), the working memory errors vs. MDA (c) and the reference memory errors vs. AChE (d) in control group (●), scopolamine (Sco) alone-treated group (■), Sco + MC (25 mg/kg) group (▲) and Sco + MC (75 mg/kg) group (♦).

several correlation between SOD vs. MDA ($r = -0.829$, $p < 0.01$) (Fig. 7a), CAT vs. MDA ($r = -0.829$, $p < 0.01$) (Fig. 7b), GPX vs. MDA ($r = -0.821$, $p < 0.001$) (Fig. 7c), GSH vs. MDA ($r = -0.830$, $p < 0.001$) (Fig. 7d), protein carbonyl vs. MDA ($r = 0.958$, $p < 0.0001$) (Fig. 7e) and AChE vs. MDA ($r = 0.857$, $p < 0.0001$) (Fig. 7f).

Discussion

Matricaria chamomilla is used in folk medicine because of its medicinal values and remarkable pharmacological properties and also because this plant has confirmed a safety profile (Keefe et al., 2016). Our findings showed no sign of toxicity. The present study evidenced that treatment with scopolamine impaired memory processes in laboratory rats. Various behavioral and molecular experiments were conducted and revealed decreased memory performance, a changed in oxidant/antioxidant balance along with an altered BDNF and IL-1β expression in the rat hippocampus.

Results from the Y-maze and radial arm maze tasks showed that scopolamine-treated rats displayed decreased scores during training sessions. However, co-treatment with the extract in both doses prevented scopolamine-induced memory deficits as evidenced by increased spontaneous behavior in Y-maze test along with decreased of working memory errors and reference memory errors by performing radial arm maze task. These findings suggest that the extract has anti-amnesic effects in the scopolamine-induced model. Furthermore, this evidence is supported by the decrease of the AChE activity in pretreated scopolamine rats with the hydroalcoholic extract, suggesting that the extract could prevent scopolamine-induced cholinergic dysfunction in the

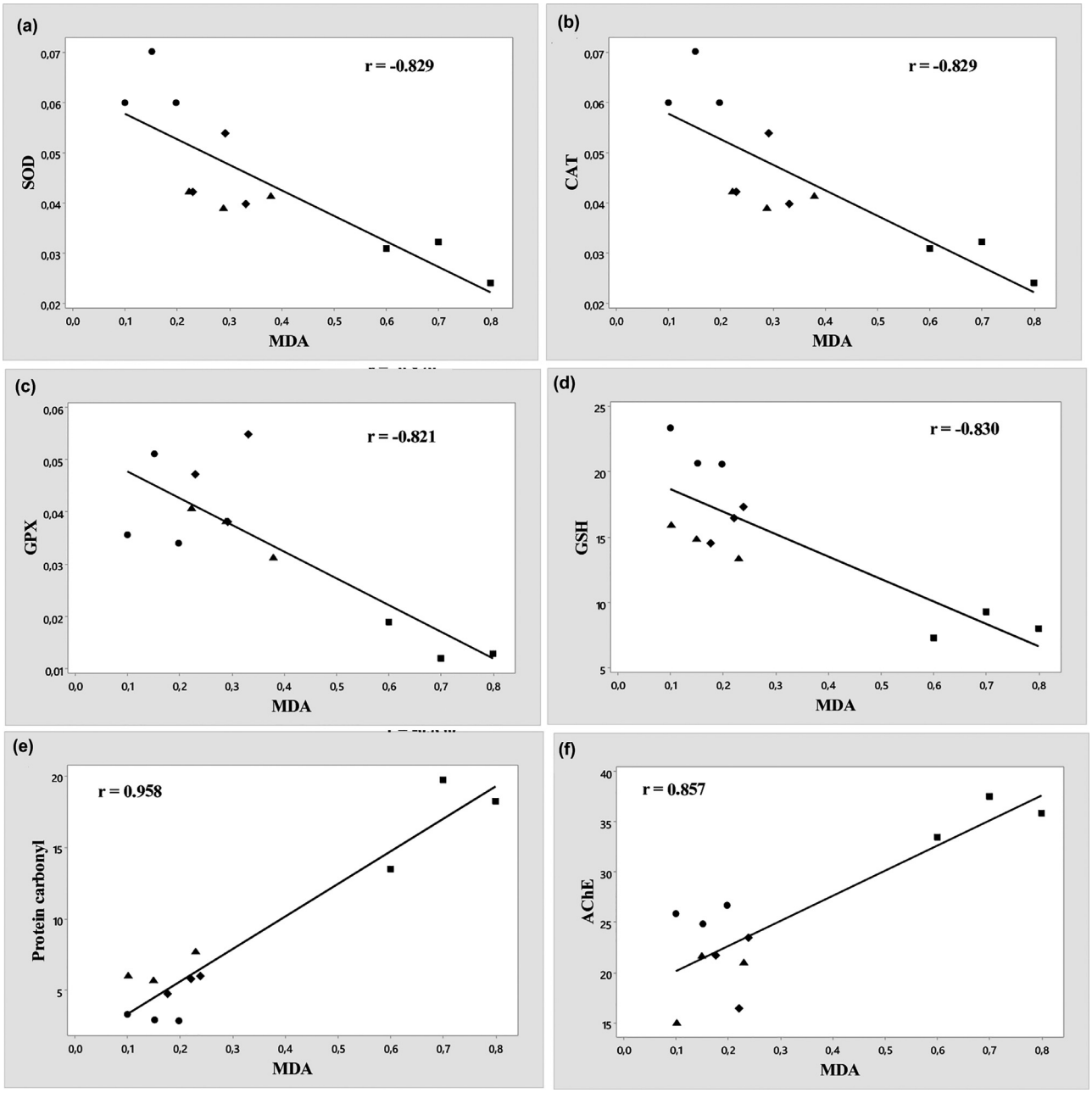

Fig. 7. Pearson's correlation between the SOD vs. MDA (a), the CAT vs. MDA (b), the GPX vs. MDA (c), the GSH vs. MDA (d), protein carbonyl vs. MDA (e) and AChE vs. MDA (f) in control group (●), scopolamine (Sco) alone-treated group (■), Sco + MC (25 mg/kg) group (▲) and Sco + MC (75 mg/kg) group (♦).

hippocampus by modulating the cholinergic activity. The previous study has demonstrated that decreased in hippocampal ACh level due to over-activity of AChE could disrupt the brain cholinergic system activity, resulting in cognitive impairment (Rogers and Kesner, 2004). HPLC analyses data of the extract identified the presence of eight active compounds such as chlorogenic acid, cafeic acid, catechin, apigenin-7-glucoside, rutin, cynaroside, luteolin and apigenin. We believe that the anti-AChE activity and cognitive-enhancing effects of the extract are induced by the synergic action of the active phenol compounds.

Accumulating evidence suggested that scopolamine-induced memory impairment by an attenuation of the cholinergic neurotransmission that parallels to decrease the anti-oxidative enzyme activity and increase the level of radical (Zhou et al., 2016). The elevation of brain oxidative status after scopolamine administration further substantiates the value of scopolamine-induced amnesia as an animal model to test for drugs with potential therapeutic benefits in dementia (El-Sherbiny et al., 2003). In the present study, scopolamine injection significantly depleted antioxidant capacity of SOD, GPX, CAT and the total content of reduced GSH in the rat hippocampus, whereas these abnormalities were restored significantly by the treatment with the extract. As expected, scopolamine injection induced oxidative stress in the hippocampus, as evidenced by increased levels of the protein carbonyl and MDA. These alterations were significantly attenuated by the extract pretreatment. Increased LDH activity-induced neuronal apoptosis (neurotoxicity) was evidenced in AD associated with aging (Ho et al., 2007). Scopolamine administration induced neurotoxicity in the hippocampus, as observed by enhancing the LDH activity, while treatment with the extract lowered the LDH activity close to normal values. Our results demonstrated that the extract exhibits an antioxidant and neuroprotective potential by contributing to improving the memory performance in the scopolamine rat model.

Supporting evidence suggested that dysfunction of BDNF is a

possible contributor to the pathology and symptoms of the AD (Qin et al., 2017). It has been suggested that scopolamine-induced suppression of the expression of the BDNF, nerve growth factor (NGF) and their receptors in the hippocampus (Lee et al., 2016), resulted in alteration of memory function in mice. In our study, scopolamine suppressed BDNF mRNA which is similar to the results of the previous studies. As expected, treatment of scopolamine rats with the extract reversed the BDNF mRNA copy number close to normal conditions. These results indicate that the cognitive-enhancing effects of the extract may be associated with the activation of BDNF gene. Inflammation, as well as cholinergic neuron degeneration, may play a critical role in the pathogenesis of the degenerative changes and cognitive impairments of the AD. Previous data have evidenced that scopolamine-induced a strong inflammatory response such as the hyperexpression of proinflammatory cytokines IL-1β and IL-6 and astrocyte activation in the mice hippocampus (Xu et al., 2016). In our study, hippocampal IL-1β mRNA copy number was increased by scopolamine injection, which was decreased by treatment with the extract, suggesting powerful antineuroinflammatory potential.

Results from Pearson correlation indicated that enhancing of memory in specific behavioral tasks are related to decreasing of oxidative stress damage and cholinesterase activity in the hippocampal tissue of treated scopolamine rats with the extract.

Conclusions

The present study supports that the extract improved the memory deficits induced by scopolamine through modulation of AChE activity, and increasing of BDNF along with decreasing of IL1β expression in the rat hippocampus. Therefore, our extract may be a promising natural therapeutic drug for the prevention of amnesia and aging-/neurodegenerative disease-related cognitive impairment.

Author contributions

RI, PAP, MM, DLG, MH, OC and LH performed the experimental studies and drafted the manuscript. LH, MM, MH and OC played roles in the writing and editing of the manuscript. LH and OC participated in the design and coordination of the study, supervised the study and revised the manuscript. All authors have read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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