Research paper

PMC-12, a traditional herbal medicine, enhances learning memory and hippocampal neurogenesis in mice

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\textbf{HIGHLIGHTS}

- Administration of PMC-12 increases hippocampal neurogenesis.
- PMC-12 improves hippocampus-dependent learning and memory.
- Neurogenic effects of PMC-12 are mediated by elevated BDNF-CREB signaling.

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\textbf{ABSTRACT}

The beneficial effects of traditional Korean medicine are recognized during the treatment of neurodegenerative conditions, such as, Alzheimer’s disease and neurocognitive dysfunction, and recently, hippocampal neurogenesis has been reported to be associated with memory function. In this study, the authors investigated the beneficial effects of \textit{polygonum multiflorum} Thunberg complex composition-12 (PMC-12), which is a mixture of four medicinal herbs, that is, \textit{Polygonum multiflorum}, \textit{Polystoma tenuifolia}, \textit{Rehmannia glutinosa}, and \textit{Acorus gramineus}, on hippocampal neurogenesis, learning, and memory in mice. PMC-12 was orally administered to male C57BL/6 mice (5 weeks old) at 100 or 500 mg/kg daily for 2 weeks. PMC-12 administration significantly was found to increase the proliferation of neural progenitor cells and the survival of newly-generated cells in the dentate gyrus. In the Morris water maze test, the latency times of PMC-12 treated mice (100 or 500 mg/kg) were shorter than those of vehicle-control mice. In addition, PMC-12 increased the levels of BDNF, p-CREB, and synaptophysin, which are known to be associated with neural plasticity and hippocampal neurogenesis. These findings suggest PMC-12 enhances hippocampal neurogenesis and neurocognitive function and imply that PMC-12 ameliorates memory impairment and cognitive deficits.

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1. Introduction

Adult hippocampal neurogenesis involves the generation of new cells in the hippocampus and their subsequent integration into neural networks in adults [32]. In the adult brain, neurogenesis continuously occurs in the subventricular zone of the lateral ventricles and in the subgranular zone of the dentate gyrus [5]. The integration of newly produced neurons into the hippocampal circuitry suggests that adult hippocampal neurogenesis plays a significant role in learning and memory [7]. Neurogenesis in the adult hippocampus can be impacted by neuronal network activities mediated by neurotrophic factors, neurotransmitters, growth factors, trophic factors, and environmental stimuli [17,21]. In addition, several affective disorders, cognitive aging and neuropsychiatric diseases are known to be closely associated with levels of hippocampal neurogenesis in the adult brain [32].

PMC-12 (\textit{polygonum multiflorum} Thunberg complex composition-12) consists of a mix of four medicinal herbs, \textit{P.
multiflorum, Polygonula tenuifolia, Rehmannia glutinosa, and Acorus gramineus, which are used in herbal medicine to treat age-related brain disease and cognitive disorders. Moreover, these herbal medicines and their extracts have been shown to lower blood cholesterol levels and improve learning and memory [13,27,30]. P. multiflorum is a popular traditional herbal medicine in East Asian, and its extracts have been found to protect from oxidative stress-associated neurodegenerative diseases [25], and to have potential therapeutic effects against aging, hyperlipidaemia, Alzheimer’s disease, Parkinson’s disease, inflammation, and cancer [19]. In addition, P. tenuifolia, R. glutinosa, A. gramineus, and their major active compounds have been reported to improve memory and cognitive function [1,13,16].

Recently, it was reported that PMC-12 effectively protected neurons in a mouse model of focal cerebral ischemia, and prevented Aβ-induced cognitive deficits by ameliorating neuroinflammatory responses [2,22], which suggest that PMC-12 might have potential application for the treatment of memory impairments associated with brain disorders and neurodegeneration. However, the effects of PMC-12 on brain under normal conditions have not been investigated. Therefore, in the present study, we sought to determine whether PMC-12 influences hippocampal neurogenesis and cognitive memory in young C57BL/6 mice in absence of any brain disorder.

2. Materials and methods

2.1. PMC-12 extract

The dried roots of P. multiflorum, P. tenuifolia, R. glutinosa, and A. gramineus were obtained from Hwalim Natural Drugs (Busan, Korea). A voucher specimen (accession number PMC-12) was deposited at the Department of Korean Medical Science, Pusan National University (Yangsan, Korea). Dried powdered P. multiflorum (25.5 kg), P. tenuifolia (7.5 kg), R. glutinosa (9.5 kg), and A. gramineus roots (7.5 kg) were immersed in 450 L of distilled water and boiled at 115 ± 5 °C for 2 h. The resulting extract was centrifuged (2 000 g for 20 min at 4 °C) and filtered through a 0.2-μm filter. The filtrate was then concentrated in vacuo at 70 ± 5 °C under reduced pressure and converted into a fine spray-dried extract at a yield rate of 4.6% (2.3 kg) in a vacuum drying apparatus. The solid form of this spray-dried extract was dissolved in dimethyl sulfoxide (DMSO) for the experiments.

2.2. Reagents

5'-Bromo-2'-deoxyuridine (BrdU) was purchased from ACROS Organics (Fair Lawn, NJ, USA), Western blot detection reagent (ECL solution) was from Advansta (Menlo Park, CA, USA), and Alexa Flour 488 and 568 were from Invitrogen (Eugene, OR, USA).

2.3. In vivo drug administration

Male C57BL/6 mice (5 weeks, weight 18–20 g) were obtained from Daehan Biolink Co. Ltd. (Chungbuk, South Korea). Animals were housed under temperature- and light-controlled conditions (20–23 °C under a 12 h light/dark cycle). Animals were randomly assigned to three groups (100 mg/kg, 500 mg/kg, and vehicle-control groups) containing 5–6 animals per group. Mice were acclimatized for 1 week before orally administering PMC–12 at 100 or 500 mg/kg daily for 2 weeks. Vehicle-controls were administered same volumes of PBS at the same times. To evaluate newly survived cells (survival study), mice in each group were administered 6 i.p. injections of BrdU (100 mg/kg of body weight, twice daily for 3 days) before PMC–12 administration. To evaluate newly generated cells (proliferation study), mice in each group were injected with BrdU (100 mg/kg of body weight, twice daily for 3 days) on the last 3 days of PMC–12 administration. The animal protocol used in the present study was reviewed and approved by the Pusan National University-Institutional Animal Care and Use Committee (PNU-IACUC) with respect to ethicality (PNU-2014-0559).

2.4. Tissue preparation

For histological analysis, mice were anesthetized with diethyl ether and perfused with 0.1 M PBS (pH 7.4) containing 0.9% NaCl and then fixed with 0.1 M PBS containing 4% paraformaldehyde (PFA). Brains were removed, placed in the same fixation solution for 24 h at 4 °C and transferred to a 30% sucrose solution. Brains were rapidly frozen in liquid nitrogen cooled 2-methylbutane, and sectioned serially at 40 μm in the coronal plane using a freezing microtome (MICROM, Walldorf, Germany). The sections obtained were collected in Dulbecco’s phosphate buffered saline (DPBS) solution containing 0.1% sodium azide and stored at 4 °C. All sections containing the hippocampal formation were retained.

2.5. DAB immunostaining

To stain newly generated cells, brain sections were treated initially with 0.6% H2O2 in Tris-buffered saline (TBS; pH 7.5) followed by 50% sodium citrate buffer containing 50% formamide for 2 h at 65 °C and 2 N HCl for 30 min at 37 °C to denature DNA. Sections were neutralized with base (0.1 M borate buffer) for 15 min, blocked in TBS-TS buffer (TBS/0.1% Triton X-100/3% goat serum) for 30 min, and incubated with primary antibodies: anti-BrdU (rat, Abcam, Cambridge, MA, USA) or anti-p–CREB (rabbit, Cell Signaling Technology, MA, USA) in TBS-TS overnight at 4 °C. They were then treated with biotinylated secondary goat anti-rat IgG antibody (Vector Laboratories, Burlingame, CA, USA) or biotinylated secondary goat anti-rabbit IgG antibody (Vector Laboratories) at room temperature for 3 h, and incubated in ABC solution (Vector) for 30 min, placed on slides, dried, mounted with permanent mounting medium (Thermo Fisher Scientific, Fair Lawn, NJ, USA), and cover slipped. Images were obtained using a Nikon ECLIPSE TE 2000-U microscope (Nikon, Tokyo, Japan). BrdU-positive cells were counted in every 6th section throughout the entire hippocampal rostro-caudal region. All cell counts were performed by an investigator unaware of experimental details.

2.6. Double-label immunostaining

For double-label fluorescence immunostaining, brain sections were blocked with TBS-TS buffer (TBS/0.1% Triton X-100/3% goat serum) for 30 min at room temperature, and then treated with the following primary antibodies, that is, anti-BrdU antibody (rat, Abcam), neuronal nuclear marker anti-NeuN antibody (mouse, Milipore Corporation, Bedford, MO, USA), immature neuronal marker anti-doublecortin (DCX) antibody (rabbit, Cell Signaling Technology), astrocyte marker anti-GFAP antibody (mouse, Cell signaling) and microglia marker anti-lba-1 antibody (rabbit, Wako, Tokyo) in TBS-TS at 4 °C overnight. Sections were washed three times with PBS, then incubated with Alexa Fluor 488 labeled anti-rat IgG, Alexa Fluor 568 labeled anti-mouse IgG or Alexa Fluor 568 labeled anti-rabbit IgG for 3 h at room temperature. Images were obtained using FV10i FLOUVIEW Confocal Microscope (Olympus, Tokyo). Z-stacked images of 20 consecutive brain sections (~2 μm taken from 40 μm brain sections) were acquired using a 60 × objective.
Fig. 1. PMC-12 increased the proliferation and survival of newly generated cells in the dentate gyrus. (A) Male C57BL/6 mice (5 weeks) treated with PMC-12 at 100 or 500 mg/kg or vehicle for 2 weeks were injected with BrdU (100 mg/kg of body weight, twice daily for 3 days) on the last 3 days of PMC-12 administration. Quantitative analysis showed that PMC-12 (500 mg/kg) significantly increased the proliferation of newly generated cells in dentate gyri. (B) Mice in the survival groups were administered six i.p. injections of BrdU before PMC-12 administration, and treated with PMC-12 at 100 or 500 mg/kg or vehicle for 2 weeks. Quantitative analysis showed that PMC-12 (100 or 500 mg/kg) increased numbers of BrdU-positive cells in dentate gyrus. Scale bar = 100 μm. Results are presented as means ±SEs (n = 5 mice/group). *p < 0.05 versus vehicle-controls (ANOVA with Fisher’s PLSD procedure).
2.7. Morris water maze behavior test

The Morris water maze test is used to examine spatial learning and memory, and requires mice to find a 10 cm diameter hidden platform just below the surface (1 cm) of a circular pool of water (120 cm in diameter, 35 cm high, maintained at 23–25 °C). The water tank was placed in a room with dimmed lights, and visible cues were placed on walls nearby to allow mice to determine the location of the platform. After vehicle or PMC-12 treatment for 2 weeks, mice were given 5 cumulative days of training (6 trials per day). At the beginning of each trial, mice were immersed in the pool, at one of three randomly assigned starting positions (located in the center of each quadrant not containing the platform). Mice were given a maximum of 60 s to find the platform. If a mouse failed to find the platform in 60 s, it was guided to the platform by the investigator. Once a mouse reached the platform, it was allowed to remain there for 5 s. Mice were videotaped during trials and swim paths were recorded using image tracking software. Times taken (latencies) to find the platform (sec), path lengths (cm), and swimming speeds (cm/sec) were recorded (Noldus EthoVision® XT, Nolus Information Technology, Wageningen, Netherlands).

2.8. BDNF assay

BDNF protein levels in the hippocampus were measured using an ELISA kit (Millipore Corporation). Initially, hippocampal homogenate samples (100 μl) and BDNF standards were added to wells pre-coated with rabbit anti-human BDNF polyclonal antibody. Plates were sealed with a plate sealer, incubated at 4 °C overnight, and washed with a washing buffer (at least 4 times). Diluted biotinylated mouse anti-BDNF monoclonal antibody (100 μl) was then added to each well and plates were incubated at room temperature for 2–3 h, then washed four times with washing buffer. Diluted streptavidin–HRP conjugate solution (100 μl) was added to each well, and plates were incubated at room temperature for 1 h on a shaker. TMB/E substrate solution (100 μl) was then added to each well and plates were incubated at room temperature for 15 min. The reaction was stopped by adding 100 μl of stop buffer to each well, which caused the blue

![Image](image1.png)

**Fig. 2**. PMC-12 enhanced neuronal differentiation and hippocampal neurogenesis. (A) To determine the effect of PMC-12 on hippocampal neurogenesis, double labeling immunohistochemistry was performed using primary antibodies against anti-BrdU (green) and anti-NeuN (mature neuronal marker, red). Most BrdU-labeled cells co-labeled with NeuN, and more double-labeled cells were observed in the dentate gyrus of PMC-12 treated mice. (B) To determine the effect of PMC-12 on neuronal differentiation, double labeling immunohistochemistry was performed using anti-BrdU (green) and DCX (immature neuronal marker, red) antibodies. PMC-12 administration significantly increased DCX-positive cell numbers in dentate gyri. Scale bar = 50 μm. (C) Quantitative analysis of DCX immunostaining in the dentate gyrus of hippocampus. The values are presented as means ± SEs (n = 4–5 mice/group). *p < 0.05, compared with vehicle-controls (ANOVA with Fisher's PLSD procedure). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
**Fig. 2.** (Continued)
reaction solution to turn yellow. Plates were immediately read at 450 nm. BDNF amounts were obtained using standard curves prepared using pure BDNF.

2.9. Western blotting

After treatments, tissue homogenates were solubilized in SDS-polyacrylamide gel electrophoresis sample buffer and protein concentrations were determined using a Bio-Rad protein assay kit (Hercules, CA) using bovine serum albumin standard. Samples (50 µg protein per lane) were separated in 12% SDS-polyacrylamide gels (SDS-PAGE) and transferred to Immobilon-P transfer membranes (Millipore Corporation), which were then placed in blocking solution (nonfat milk 5%) at room temperature for 30 min and then incubated with diluted primary antibodies of anti-synaptophysin (rabbit, Thermo Fisher Scientific Inc) or anti-β-actin (mouse, Sigma-Aldrich Co, St. Louis, MO, USA) in TBS-T buffer (Tris–HCl based buffer containing 0.2% Tween 20, pH 7.5) at 4°C overnight. Membranes were then washed (4 × 10 min) and incubated with secondary antibody in TBS-T buffer at room temperature for 2 h. Horseradish-conjugated secondary antibody labeling was detected using ECL solution. For quantitative analysis, blots were imaged by using a Cooled CCD Camera System ATTO Ez-Capture II (Atto-corporation, Tokyo).

2.10. Nissl staining

To perform Nissl staining, brain sections were mounted on slides and dried overnight, hydrated using an ethanol series, stained with cresyl violet. Then the sections were dehydrated in series of ethanol, cleared with xylene, mounted with permanent mounting medium (Thermo Fisher Scientific) and then coverslipped. Images were taken by Nikon ECLIPSE TE 2000-U microscope (Nikon).

2.10.1. Statistical analysis

The significances of differences between groups were determined by analysis of variance (ANOVA) with Fisher’s protected least significant difference (PLSD) test. The analysis was performed using Statview software (Version 5.0.1., SAS Institute Inc., Cary, NC, USA). Results are expressed as means ± SEs, and P values of <0.05 were considered statistically significant.

3. Results

3.1. Effects of PMC-12 on the proliferation and survival of newly generated cells in the dentate gyrus.

To evaluate the effects of PMC-12 on hippocampal neurogenesis, male C57BL/6 mice (5-week-old) were orally administered with either PMC-12 (100 or 500 mg/kg) or vehicle for 2 weeks. For the proliferation study, six doses of BrdU were administered i.p. for 3 days from PND 53–55 to label newly generated cells in the hippocampus. It was found that numbers of newly-generated cells in the dentate gyrus were significantly increased in the PMC-12 500 mg/kg-treated mice (Fig. 1A). For the survival study, BrdU was administered for 3 days before PMC-12 treatment from PND 39–42. Most surviving cells had migrated into the granule cell layer of the dentate gyrus. Numbers of BrdU-positive cells in the dentate gyrus were significantly greater in mice treated 100 or 500 mg/kg of PMC-12 than in vehicle-controls (Fig. 1B).

3.2. PMC-12 increased neuronal differentiation and hippocampal neurogenesis.

After 2 weeks after BrdU injection, most BrdU-positive cells had migrated into the GCL and co-labeled with NeuN in the dentate gyrus, indicating that newly-generated cells had survived and differentiated into mature neurons. In order to observe the neuronal differentiation of newly generated cells in the dentate gyrus, double label confocal immunohistochemistry was performed using anti-NeuN antibody (a mature neuron marker) or anti-DCX antibody (an immature neuron marker) in combination with BrdU antibody. Mice in the PMC-12 treated groups had more NeuN/BrdU co-labeled cells in dentate gyrus than vehicle-controls (Fig. 2A), and more DCX-positive immunostaining was detected in the dentate gyrus of PMC-12 treated mice (Fig. 2B). Quantitative analysis showed that PMC-12 (500 mg/kg) increased DCX immunostaining in hippocampus (Fig. 2C). These results suggest that PMC-12 effectively enhanced hippocampal neurogenesis by increasing the survival and differentiation of new neurons.

3.3. PMC-12 enhanced spatial learning and memory.

Since hippocampal neurogenesis is associated with hippocampus-dependent learning and memory, the Morris water
Fig. 4. Hippocampal BDNF, p-CREB and synaptophysin protein levels were elevated in PMC-12 treated mice. (A) Levels of BDNF in hippocampi from vehicle or PMC-12 treated mice were measured using the BDNF ELISA assay kit. PMC-12 at 100 or 500 mg/kg significantly elevated BDNF protein levels in hippocampi. (B) The activation of CREB signaling was evaluated by immunohistochemistry using anti-p-CREB antibody, and it was observed that PMC12 significantly increased numbers of p-CREB-positive cells in dentate gyri. Scale bar = 100 μm. (C) Synaptophysin protein levels in hippocampi were assessed by Western blotting, and PMC-12 at 500 mg/kg increased synaptophysin levels. β-actin was used as a protein loading control. Results are presented as means ±SEs (n = 4–5 mice/group). *p<0.05 versus vehicle-controls (ANOVA with Fisher’s PLSD procedure).
Fig. 5. PMC-12 has no effect on neuroinflammation, neuronal damage and loss in the dentate gyrus of hippocampus. (A) Representative images showing GFAP and Iba-1 double immunostaining. There were no effects of PMC-12 on immunostaining intensities or patterns of astrocytes and microglia. Scale bar = 50 μm. (B) Nissl staining was performed with cresyl violet. Representative images showed that no specific brain pathological signs of shape change or neuronal density of dentate gyrus in hippocampus. Scale bar = 100 μm.

maze test was used to determine whether PMC-12 influences cognitive learning and memory. Repeated trainings and guidance decreased latency times in all experimental groups, indicating no significant learning impairment in any group. However, mice in the PMC-12 treated groups found the platform more quickly on days 3 and 4 of training than vehicle-controls (Fig. 3). Spatial reference memory test findings revealed no differences between the PMC-12 treated groups and the vehicle-controls in terms of swim speed or total distance (data not shown).
3.4. PMC-12 elevated levels of BDNF, p-CREB, and synaptophysin in hippocampi.

Hippocampal BDNF levels are related to hippocampal neurogenesis and synaptic plasticity, and BDNF expression is mediated by the transcription factor CREB (cAMP response element–binding protein) [4,26]. Therefore, we examined hippocampal BDNF and p-CREB levels to investigate the involvement of BDNF-CREB signaling in the enhancement of neurogenesis caused by PMC-12. It was found that PMC-12 significantly increased levels of BDNF (Fig. 4A) and p-CREB (Fig. 4B) in hippocampi, suggesting the neurogenic effect of PMC-12 on hippocampal neurogenesis is associated with the up-regulations of BDNF and p-CREB signaling. Synaptophysin expression was also examined because it is involved in synapse formation, synaptic plasticity, and learning and memory, and its expression was found to be significantly higher in the hippocampi of PMC-12 (500 mg/kg) treated mice than in vehicle-controls (Fig. 4C). These results suggest that high dose (500 mg/kg) PMC-12 enhanced neurogenesis and cognitive memory by elevating BDNF-CREB signaling and synaptic plasticity.

3.5. PMC-12 had no effects on glial activations and neuronal survival in the hippocampus.

In order to evaluate the effect of PMC-12 on neuroinflammation in the hippocampus, we performed double-immunostaining using anti-GFAP (astrocyte marker) and anti-Iba-1 (microglia marker) antibodies. There were no obvious differences in intensities of GFAP and Iba-1 immunofluorescence between vehicle-control and PMC-12 treated mice (Fig. 5A). In addition, Nissl staining was performed to evaluate the potential toxicity of PMC-12 in hippocampal neurons. PMC-12 did not change hippocampal shape, neuronal density, or cause neuronal loss (Fig. 5B). These suggest that PMC-12 has no potency to cause neuronal cell loss or neuroinflammation.

4. Discussion

P. multiflorum is widely used as a traditional medicine in East Asia, and tetrahydroxyxilobene glucoside (a major component in the extract of P. multiflorum), has been reported to protect against cerebral ischemia/reperfusion injury through multifunctional pathways in vitro and in vivo [28]. Furthermore, tetrahydroxyxilobene has significant neuroprotective effects against MPP+-induced damage and apoptosis through a PI3 K/Akt pathway in PC12 cells [23], and P. multiflorum extract reduces brain pathological changes, and promotes learning and memory [3]. R. glutinosa, a component of PMC-12 has been previously reported to improve cognitive function and alleviate inflammatory responses [16]. In addition, catalpol from R. glutinosa was found to inhibit apoptosis induced by H2O2 in PC12 cells [14], and an extract of P. tenuifolia improved memory and cognitive function in vivo in a scopolamine-induced model of amnesia [20]. Polygalasaponins extracted from the P. tenuifolia were observed to have potential antipsychotic activity [6], and α-asarone (1-propenyl-2, 4, 5-methoxybenzol) from A. gramineus decreased memory impairment and Aβ25-35–neurotoxicity in rats [18].

Recently, the neuroprotective effects of PMC-12 were studied in an in vivo model of brain disease. PMC-12 was found to have anti-neuroinflammatory effects and to prominently improves cognitive deficits in an Aβ25-35–induced mouse model [22] and to enhance spatial memory in a mouse model of focal cerebral ischemia [2]. These findings suggest PMC-12 might ameliorate neurodegeneration and potential have therapeutic effects on memory and cognitive impairments. However, studies on the beneficial effects of PMC-12 have not been established its effects on the normal brain in vivo. The present study indicates that PMC-12 markedly enhances spatial learning memory and hippocampal neurogenesis in unstressed mice.

In the adult brain, new neurons are generated in two neurogenic areas: the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus [9]. Newly-generated cells in the hippocampus have the ability to proliferate, differentiate, migrate into subgranular zone, and subsequently integrated into neural network in adult brain [32]. Moreover, hippocampal neurogenesis is associated with learning and memory formation [10,12], and is known to be modulated by diverse factors, such as, stress, depression, drugs, environmental stimuli, aging, and physical exercise [8,29]. These studies suggest hippocampal neurogenesis can be affected by natural products and components in medical herbs, for example, curcumin and Ginkgo biloba promote adult hippocampal neurogenesis [15,31]. The present study shows that PMC-12 (at 100 or 500 mg/kg) increased the survival and proliferation of newly-generated cells in the dentate gyrus. In addition, PMC-12 at both doses increased the neuronal differentiation of newly-generated cells in dentate gyrus. These findings show PMC-12 has neurogenic effects, and that in particular it promotes hippocampal neurogenesis.

BDNF is an important regulator of hippocampal neurogenesis, and promotes neuronal plasticity and differentiation, and modulates synaptic function [4]. In particular, CREB phosphorylation regulates BDNF mRNA expression [26]. In present result, BDNF protein levels in hippocampus were significantly elevated in PMC-12 (100 or 500 mg/kg) treated mice, and PMC-12 treatment also increased p-CREB levels in the dentate gyrus. These findings imply PMC-12 increased BDNF expression by increasing CREB activation. In addition, levels of hippocampal synaptophysin were elevated by PMC-12 at 500 mg/kg. It has been reported that environmental enrichment, a positive neurogenic paradigm, accelerates spatial memory and increases synaptophysin levels [11], and that synaptophysin deficient mice exhibit retarded learning and impaired memory based on Morris water maze test findings [24]. The present study shows PMC-12 treatment improved hippocampus-dependent spatial learning and memory as determined by the Morris water maze test. Taken together, our findings indicate increased levels of BDNF, p-CREB, and synaptophysin are associated with PMC-12-mediated memory enhancement, possibly by boosting synaptic plasticity and hippocampal neurogenesis.

In conclusion, the present study suggests PMC-12 has possibly therapeutic value for in the contexts of hippocampal neurogenesis, learning memory enhancement, neurocognitive function.

Conflict of interest

The authors have no potential conflict of interest to declare.

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