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ORIGINAL PAPER

Sericin Improves Memory Impairment Via Activation of the PKA-CREB-BDNF Signaling Pathway and Suppression of Oxidative Stress in Ovariectomized Mice

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Abstract

Menopause results in estrogen hormone deficiency which causes changes in brain morphology and cognitive impairments. The risk of breast and ovarian cancer increases with estrogen therapy. Thus, finding a substitute treatment option for women in menopause is necessary. In the current study, the impact of chronic sericin treatment (200 mg/kg/day for 6 weeks, gavage) on memory process, oxidative stress markers, synaptic neurotransmission, and acetylcholinesterase (AChE) activity in the hippocampus (HIP) of ovariectomized (OVX) mice was examined and compared to the effects of 17β-estradiol (Es; 20 µg/kg, s.c.). The results demonstrated that sericin and Es administration improved spatial and recognition memory of the OVX animals in the both Lashley III maze and novel object recognition tests. Moreover, sericin-treated OVX mice showed decreased ROS levels, increased endogenous antioxidant defense capacity, and decreased AChE activity in the HIP. Additionally, sericin and Es therapy up-regulated pre-and-post-synaptic protein markers and increased BDNF, CREB, and protein kinase A (PKA) protein expressions in the HIP of OVX mice. Overall, the activation of the PKA-CREB-BDNF signaling pathway by sericin can provide protection against OVX-induced cognitive dysfunction, making it a potential alternative for managing cognitive deficits in postmenopausal women.

Keywords Ovariectomy · Cognitive dysfunction · Sericin · Acetylcholinesterase · Synaptic proteins · Oxidative stress

Introduction

Natural menopause refers to the permanent cessation of ovulatory function, which is characterized by 12 consecutive months of no menstruation [1, 2]. In addition to natural menopause, premature menopause may also happen through medical intervention such as bilateral ovariectomy or ovarian failure due to ovarian cancer, all of which result in the depletion of estrogens in circulation [3].

Besides the traditionally accepted roles of ovarian hormones in regulating reproduction, estrogens, namely 17β-estradiol (Es), are implicated in the regulation of brain functions such as neural activity, cognitive function, and behavior. Estrogens promote the growth and survival of memory-related cholinergic neurons, improve synaptic plasticity and cognitive function, and protect against oxidative stress, neuroinflammation, apoptosis, amyloid-beta (Aβ) aggregation, and hyperphosphorylation of tau protein [4–7]. Therefore, estrogen depletion, during natural menopause or ovariectomy, is closely associated with neurodegeneration and deteriorated cognitive performance [8, 9].

Moreover, menopause is linked to decreased activity of antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), and increased production of free radical or pro-oxidants, resulting in neural loss in the brain regions, particularly in the hippocampus (HIP) [10, 11]. The evidence suggests that lower cholinergic neurotransmission and acetylcholine levels in the HIP are connected to memory loss after menopause or ovariectomy [12, 13], due

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to an increase in the activity of the brain enzyme acetylcholinesterase (AChE), which breaks down acetylcholine [14, 15].

The transcriptional factor cAMP-response element binding protein (CREB) is one of the cornerstones of synaptic plasticity and subsequent potentiation in long-term memory [16, 17]. Activating CREB via Ser133 phosphorylation and subsequent cAMP- and Ca^{2+}/calmodulin-dependent kinase activation leads to the transcription of crucial proteins involved in neural plasticity, notably brain-derived neurotrophic factor (BDNF). BDNF is a potent modulator of neuronal functions of the HIP affecting learning and memory, synaptic plasticity, and neurogenesis [18]. Based on clinical and preclinical studies, the suppression of the CREB/BDNF pathway is associated with decreased memory and learning ability following surgical or natural menopause [19, 20]. Although treatment with Es reverses the negative impact of estrogen deficiency on memory function by boosting BDNF levels [21, 22], in chronic use it increases the risk of endometriosis and breast cancer in postmenopausal women [23]. Given this, scientists are currently searching for alternative treatments for estrogen in postmenopausal women that can negate its negative effects while retaining its benefits.

Silk is a kind of natural proteinous fiber that is woven into a cocoon by the silkworm (Bombyx mori) [24]. The major silk protein constituents of silk cocoons are fibroin (80%) and sericin (20%), which attach to each other by disulfide bonds at the center [25, 26]. Numerous biological activities of sericin, including antimicrobial, anti-inflammatory, antioxidant, anti-apoptotic, anti-cancer, anti-aging, and wound healing effects, have extended its application in regenerative medicine and neuroscience studies [24, 26–28]. Besides, several studies showed that sericin administration enhanced learning and memory in the experimental models by suppressing oxidative stress and inflammatory responses, and inhibiting AChE activity in the brain [26, 29, 30].

The aim of this study was to evaluate the effect of chronic sericin administration on spatial and recognition memories, oxidative stress status, PKA-CREB-BDNF signaling pathway, synaptic proteins, and AChE activity in the HIP of an OVX model in adult mice. We used estradiol as a positive control because of its established protective effects against menopause-related cognitive impairment. The objective was to compare the effects of sericin with those of estradiol, separately, to determine if sericin exhibits similar protective properties.

Materials and Methods

Animals

Forty adult female C57BL mice (8–10-weeks old and weighing 30–32 g) were obtained from the animal house of Tabriz University of Medical Sciences (Tabriz, Iran). Standard cages were used to socially house the animals (5 mice/cage) under controlled conditions with a constant temperature of 25 ± 1 °C, 50 ± 5% humidity, and a 12:12 h light–dark cycle. The animals had ad libitum access to food pellets and tap water. The guidelines of the National Institutes of Health (NIH; Publication No. 85-23, revised 1985) were strictly followed during all experimental procedures, and the Ethics Committee of Tabriz University of Medical Sciences approved the procedures (IR.TBZMED.VCR.REC.1398.217).

Study Design

Animals were randomly allocated in 4 groups (n = 10 in each), including control, ovariectomized + normal saline (OVX + NS), OVX + Estradiol (OVX + Es), and OVX + sericin (OVX + Ser) groups. Ovariectomy surgery was performed on all groups except the control group, which underwent sham surgery. Animals in the control and OVX groups were treated with 10 ml/kg normal saline (NS) for 6 weeks. The OVX + Es group received Es (20 µg/kg/day, s.c.) [31] and the OVX + Ser group received sericin (200 mg/kg/day, p.o; Xi'an Liphar Biotech Co, Ltd) for six weeks. All treatments specified for each group were started a day after the surgery and continued for 6 weeks. Subcutaneous administration of ES allows for a gradual and sustained absorption of the drug into the bloodstream. Besides, the subcutaneous route allows for higher drug bioavailability by avoiding liver metabolism [32, 33]. In addition, it is important to note that evidence supports a higher risk of venous thromboembolism with oral estrogen therapy than transdermal estrogen therapy. This is because oral estrogen is processed in the liver, resulting in harmful hemostatic effects [34]. The oral administration of sericin was chosen to simulate its natural consumption and assess its oral delivery potential. The dose and route of administration were chosen based on our prior studies, indicating that gavage of sericin at 200 mg/kg dose enhanced memory in experimental models [35, 36].

Ovariectomy Surgery

The animals were anesthetized with isoflurane (5% for induction and 2% for maintenance) in oxygen. Two incisions were made on both side flanks, and the ovaries, oviduct, and upper part of the fallopian tubes were excised. Finally, the skin incisions were sutured. The control group (sham

surgery) received an identical surgical procedure, but the ovaries and their adjuncts were not extracted.

Behavioral Tests

Lashley III Maze

To assess spatial learning and memory, the Lashley III maze was used. The apparatus consists of a start box, maze arms, and a target box, all made of white Plexiglas®. The top part of the maze is made of transparent Plexiglas to observe animal movements and prevent escape. The mice were deprived of food for 12 h before the test. At the beginning of the test, the animal was placed in the starting box for 10 s, then the door was removed and the animal was allowed to enter the maze arms for 6 min. As the animal moves inside the maze arms, the number of errors until the animal found the correct path and the time to reach the target box were recorded. When the animal reached the target box at the end of the maze, the experiment was terminated. Mice were trained daily for 5 consecutive days, and all experimental procedures were recorded and analyzed using the Noldus EthoVision™ video monitoring software (Noldus, The Netherlands). The number of errors and latency to reach the goal box were calculated.

Novel Object Recognition (NOR) Test

In order to evaluate HIP-dependent episodic-like memory, the NOR test was performed. The test has three phases, including habituation, training, and retention. In the habituation phase, each animal was placed inside the chamber ($33 \times 33 \times 33$ cm) for 10 min and allowed to move freely. The next day, in the training phase, two similar objects were placed in the chamber, and the animals were separately placed in the arena to explore both objects for 5 min. One hour following the exposure to the familiar objects, the retention phase was performed. This step was similar to the previous stage, except that in the testing chamber there was a familiar object and one novel object. All sessions were video recorded, and the total time spent sniffing or exploring each object was measured. The discrimination index (DI), a criterion for evaluation of episodic-like memory, was calculated for each group as follows: $DI = (N\text{-}F)/(N + F)$, where N is the exploration time of the novel object and F is the exploration time of the familiar object.

Sampling

The mice were anesthetized with high-dose ketamine (100 mg/kg) and xylazine (10 mg/kg) a day after the behavioral tests, then sacrificed by decapitation, and their brains were removed. The HIP was then separated on the cold plate and stored at −70 °C for.

Biochemical Analysis

ROS Level Assessment

The first step to assess mitochondrial ROS production in the HIP tissues was to homogenize them in lysis buffer and then centrifuge at 12,000 g for 10 min at 4 °C. A bicinchoninic acid (BCA) protein assay kit (Sigma-Aldrich, Germany) was utilized to calculate protein concentration in the acquired supernatant. The supernatant was mixed with a dichlorodihydro-fluorescein diacetate (DCFDA) probe and kept in a dark place for 30 min at 37 °C. The fluorescence intensity of the solution was measured using a fluorescence microplate reader (BioTek Instruments, USA) with an excitation wavelength of 485 nm and an emission wavelength of 530 nm. The ROS level was normalized to the sample proteins and reported as fluorescence intensity per mg protein.

Measurement of Oxidative Stress Markers

After homogenizing the hippocampal samples in 1.15% KCl solution, the homogenates were centrifuged at 1000 rpm for 10 min at 4 °C, and enzyme activity of SOD and GPx, as well as total antioxidant capacity (TAC) were measured in the supernatant.

The activity of GPx and SOD were assessed spectrophotometrically using a RANSEL kit (Randox Crumlin, UK) at 340 nm and a RANSOD kit (Randox Laboratories Ltd,

Crumlin, United Kingdom) at 505 nm, respectively. The obtained results were expressed as U/mg of protein. TAC was assessed by measuring their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). A blue color is produced by Fe2 + -2,4,6-Tri(2-pyridyl)-s-triazine with absorbance at 593 nm. The findings were expressed as nmol/l.

Measurement of AChE Activity

The hippocampal AChE activity was detected by a colorimetric kit (Elabscience Biotechnology; China) according to

the instructions of the manufacturer, and the absorbance was read at 412 nm and reported as U/mg protein.

Protein Quantification

Protein levels of CREB, BDNF, PKA, growth-associated protein-43 (GAP-43), synaptophysin (SYP), and post-synaptic density-95 (PSD-95) were assessed using Western blotting technique, as previously described [36]. The membranes were incubated overnight with rabbit primary antibodies ((all purchased from Santa Cruz Biotechnology, USA, except Anti-BDNF antibody (Abcam, UK)) in 1:500 concentrations against BDNF (ab108319), CREB (sc-377154) PKA (sc-136231), GAP43 (sc-17790), PSD-95 (sc-32290), and SYP (sc-17750). Afterward, the membrane was washed three times with PBS and incubated with horseradish peroxidase-conjugated (HRP) anti-rabbit IgG secondary antibody (sc-2004, 1:500) for 2 h at room temperature. Finally, the membranes were soaked for 1 min in enhanced chemiluminescence (ECL) prime Western blotting detection solution (Amersham, United Kingdom) and exposed to autoradiography film (Kodak, USA) in order to visualize protein bands. The intensity of the captured signals was computed by Image J 1.62 software (NIH, USA) and normalized to the β-actin protein as the internal control.

Statistical Analysis

All data were reported as mean ± S.E.M, and statistical comparisons between different groups were carried out by one-way or two-way ANOVA followed by post-hoc Tukey's test using Graph Pad Prism 6.01 software (Graph Pad Software Inc., La Jolla, CA, USA). Moreover, an unpaired two-tailed Student's t-test was performed to compare the exploration time of each object in each group in the NOR test. A p-value < 0.05 was considered statistically significant.

Results

Sericin Amends Object Recognition Memory in OVX Mice

No significant differences in locomotor activity were observed among the groups during the habituation session (Data are not shown; $p > 0.05$). During the training phase, there was no significant difference in the exploration time of object A1 and A2, indicating no object preference (Fig. 1A, $p > 0.05$). During the retention session (Fig. 1B, $p < 0.001$), all groups with the exception of the OVX group, spent more time exploring the novel object than the familiar object. Moreover, the results of one-way ANOVA of DI showed a significant difference among the experimental groups ($F_{(3, 36)} = 6.344$, $p = 0.0014$). The intergroup analysis showed a significant reduction in DI in the OVX animals treated with NS compared to the control mice ($p < 0.01$). Of note, treatments with Es ($p < 0.05$) and Ser ($p < 0.01$) led to a significant increase in DI in the OVX animals (Fig. 1C).

Sericin Ameliorates Spatial Memory Deficits in OVX Mice

According to the results of two-way ANOVA repeated measures using treatments and days as factors, we found a significant main effect of treatments ($F_{(3, 170)} = 63.65$, $p < 0.0001$) and a significant main effect of days ($F_{(4, 170)} = 14.84$, $p < 0.0001$), but no significant main effect of treatments × days interaction ($F_{(12, 170)} = 0.3092$, $p = 0.9871$) for exploration time, and significant main effects of treatments ($F_{(3, 180)} = 36.52$, $p < 0.0001$) and days ($F_{(4, 180)} = 21.47$, $p < 0.0001$), but no main effect of treatments × days interaction ($F_{(12, 180)} = 0.6087$, $p = 0.8331$) for number of errors in the Lashley III maze. Post-hoc analysis showed that ovariectomy markedly increased latency time (Fig. 2A) and the number of errors (Fig. 2B) to find the target box compared to the control mice during training days. However, Es therapy significantly decreased latency time during 5 days of training, and diminished the number of errors on days 3–5 compared to the NS-treated OVX animals. The OVX animals treated with sericin found the goal box faster and made fewer errors than the NS group on days 1–5.

Sericin Attenuates ROS Generation and Enhanced Antioxidant Capacity in OVX Mice

The results of one-way ANOVA indicated significant effects of the treatments on ROS levels ($F_{(3, 20)} = 45.08$, $p < 0.001$), activities of SOD ($F_{(3, 20)} = 22.83$, $p < 0.001$) and GPx ($F_{(3, 20)} = 22.18$, $p < 0.001$), and TAC levels ($F_{(3, 20)} = 33.44$, $p < 0.001$). Figure 3 shows that induction of OVX considerably increased hippocampal ROS levels ($p < 0.001$, Fig. 3A), while it decreased the enzymatic activities of SOD ($p < 0.001$, Fig. 3B) and GPx ($p < 0.001$, Fig. 3C), and TAC levels ($p < 0.001$, Fig. 3D) compared to the control group. Nevertheless, Es treatment significantly diminished ROS levels ($p < 0.001$) and increased enzyme activities of SOD ($p < 0.01$) and GPx ($p < 0.01$), and TAC levels ($p < 0.05$). In comparison to the OVX group, administration of sericin resulted in decreased ROS levels and increased SOD and GPx activities and TAC levels ($p < 0.001$ for all).

Sericin Attenuated AChE Activity in the HIP of OVX Mice

Based on the One-way ANOVA results ($F_{(3, 20)} = 8.585$, $p = 0.0007$), there were significant differences in AChE

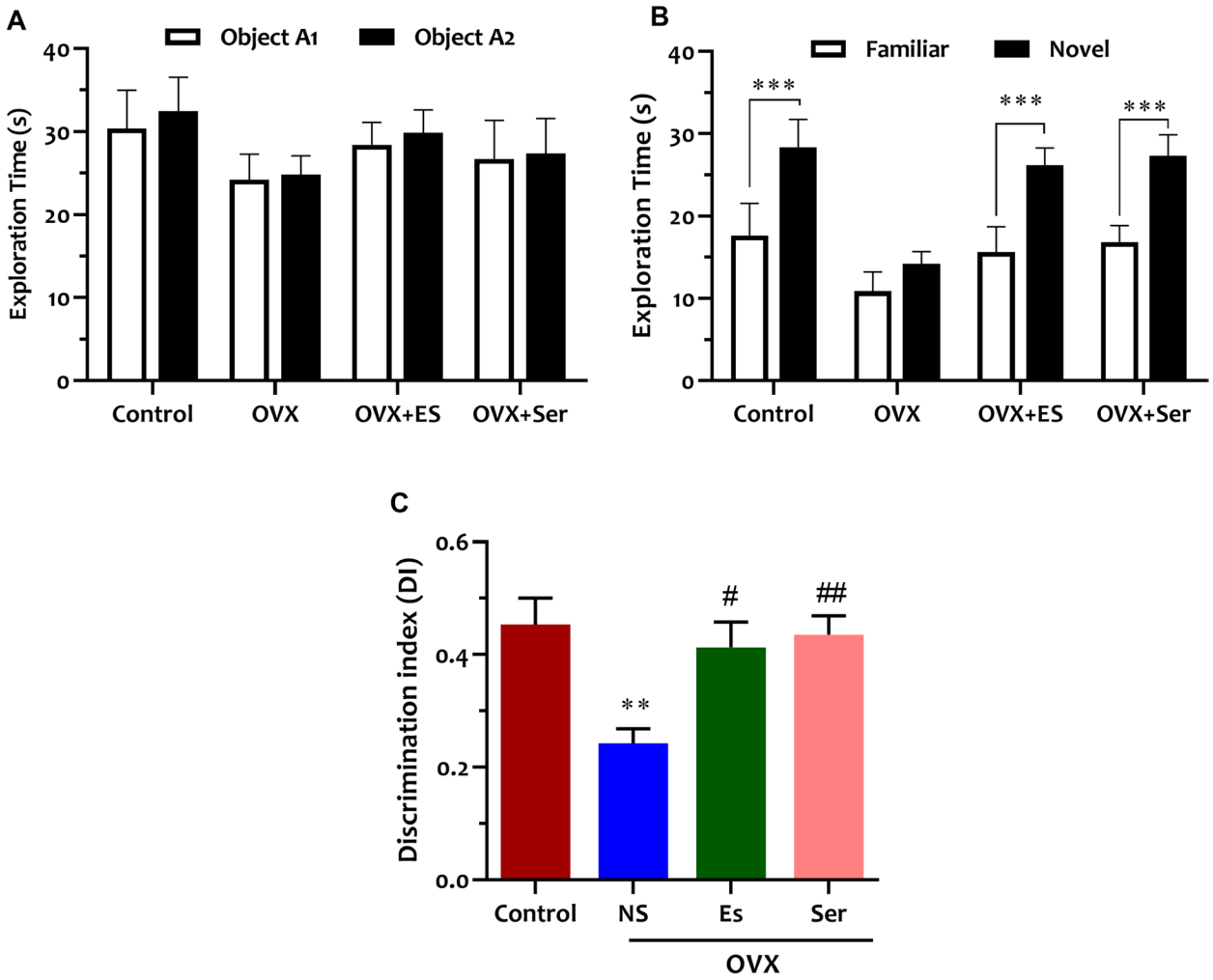

Fig. 1 The effect of Es and sericin on recognition memory in the OVX mice. (**A**) Exploration time of two familiar objects and (**B**) Exploration time of familiar or novel object in the retention phase of the NOR test. Unpaired Student's t-test, $^{***}p<0.001$. (**C**) Discrimination index calculated as follows: (novel–familiar)/(novel + familiar), in the NOR test. One-way ANOVA, $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ vs. control group. $^{\#}p<0.05$, $^{\#\#}p<0.01$ and vs. OVX + NS group. Values are presented as the means ± SEM (n = 10). OVX, ovariectomized; NS, normal saline; Ser, sericin; Es, estradiol; NOR, novel object recognition

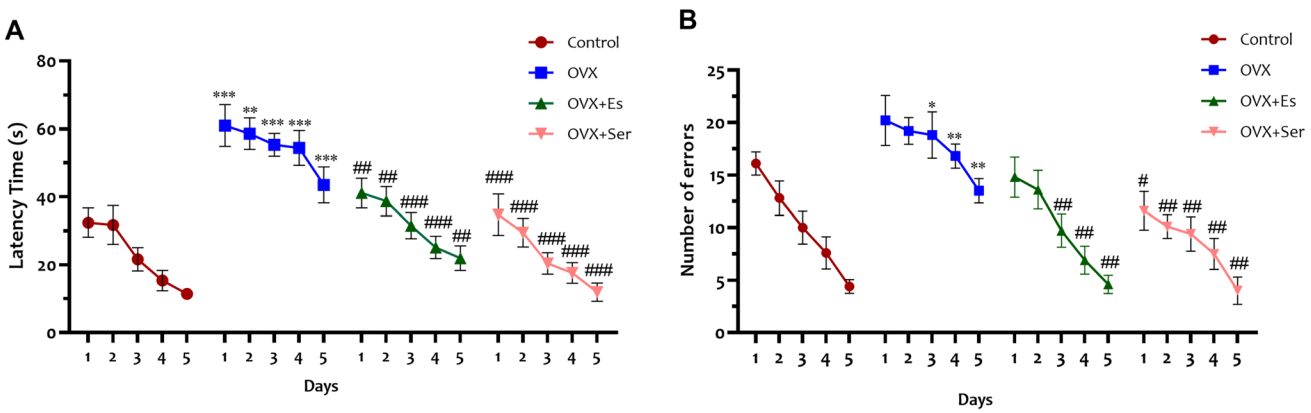

Fig. 2 The effect of Es and sericin treatments on spatial memory in the OVX mice. (**A**) latency time and (**B**) the number of errors to reach the target box in the Lashley III maze task. Values are presented as the means ± SEM (n = 10). Two-way ANOVA repeated measure, $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ vs. control group. $^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ vs. OVX + NS group. OVX, ovariectomized; NS, normal saline; Ser, sericin; ES, estradiol

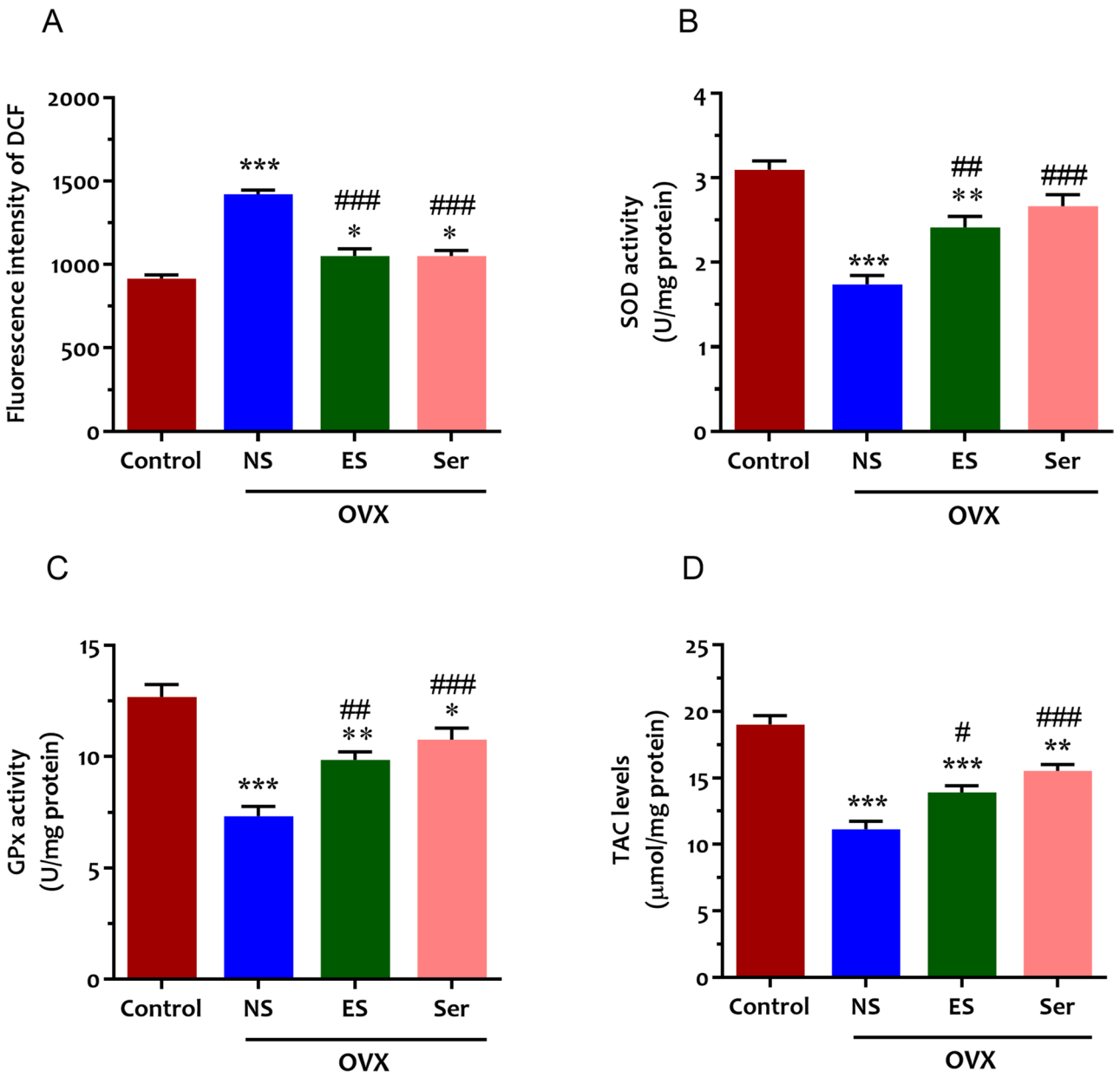

Fig. 3 The effect of Es and sericin on hippocampal (**A**) intracellular ROS levels, (**B**) superoxide dismutase activity (SOD), (**C**) glutathione peroxidase activity (GPx), and (**D**) total antioxidant capacity (TAC) in the OVX animals. One-way ANOVA, followed by Tukey's post-hoc test. Values are presented as the means ± SEM (n = 10). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ vs. OVX + NS group. OVX, ovariectomized; NS, normal saline; Ser, sericin; Es, estradiol

activity among the study groups. A marked increase in AChE activity was observed in OVX + NS group ($p < 0.001$) compared to the control animals. On the contrary, both Es ($p < 0.05$) and sericin ($p < 0.01$) treatments led to a significant decline in AChE activity as compared to the OVX group treated with NS (Fig. 4).

Sericin Improves Synaptic Protein Markers in the HIP of OVX Mice

Statistical examination using one-way ANOVA underlined significant differences in protein expressions of SYP ($F_{(3, 8)} = 10.33$, $p = 0.0040$), GAP-43 ($F_{(3, 8)} = 14.68$,

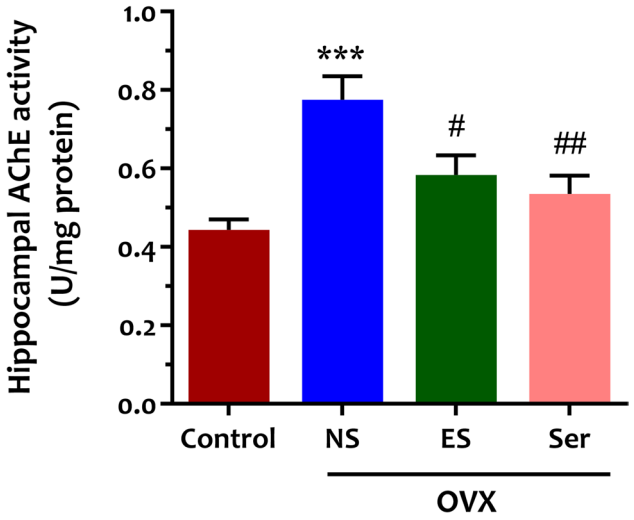

Fig. 4 The effect of Es and Sericin on hippocampal activity of AChE in the OVX mice. Values are presented as the means ± SEM (n = 6). $^{***}p < 0.001$ vs. control group. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. OVX + NS group. OVX, ovariectomized; NS, normal saline; Ser, sericin; ES, estradiol; AChE, Acetylcholinesterase

p = 0.0013), and PSD-95($F_{(3, 8)} = 15.75$, p = 0.0010) in the HIP among the groups. As Fig. 5 shows, a marked decline in protein levels of SYP (p < 0.01, Fig. 5A), GAP-43 (p < 0.01, Fig. 5B), and PSD-95 (p < 0.001, Fig. 5C) was observed in the HIP of the NS group compared to the control group. However, Es significantly up-regulated hippocampal SYP (p < 0.05), GAP-43 (p < 0.01), and PSD-95 (p < 0.05) proteins in the OVX mice. Additionally, Sericin treatment increased protein levels of SYP (p < 0.05), GAP-43 (p < 0.01), and PSD-95 (p < 0.01) compared to the NS-treated OVX mice.

Sericin Stimulates the PKA-CREB-BDNF Signaling Pathway in the HIP of OVX Mice

We also found significant differences in hippocampal protein expression of PKA ($F_{(3, 8)} = 55.99$, p < 0.0001), CREB ($F_{(3, 8)} = 12.43$, p = 0.0022), and BDNF ($F_{(4, 10)} = 14.80$, p = 0.0013) among the experimental groups. Post-hoc analysis showed that OVX induced significant decrease in PKA (p < 0.001, Fig. 6A), CREB (p < 0.01, Fig. 6B), and BDNF (p < 0.01, Fig. 6C) levels compared to the control animals. Nevertheless, Es treatment up-regulated the expression of PKA (p < 0.001), CREB (p < 0.05), and BDNF (p < 0.01) in the HIP compared to the NS-treated OVX group. Additionally, sericin administration resulted in a notable rise in protein expressions of PKA (p < 0.001), CREB (p < 0.01), and BDNF (p < 0.05) in the HIP of the OVX animals.

Discussion

The study aimed to determine the effect of long-term sericin treatment in ameliorating learning and memory impairments caused by OVX and to identify the associated intracellular signaling pathway. Our results substantiated that sericin administration effectively: (1) improved spatial and recognition memories; (2) reduced ROS levels and restored endogenous antioxidant levels; (3) enhanced AChE activity; (4) activated PKA-CREB-BDNF signaling pathway; and (5) increased synaptic protein markers in the HIP of OVX animals.

Surgical menopause through bilateral ovariectomy can replicate human ovarian hormone loss, causing an acute decline in blood estrogens and androgens [37]. The higher brain functions like mood and cognition are profoundly affected by ovarian hormones. This is accomplished through modulation of synapse structure and function, and protection against oxidative damage and neuroinflammation [38]. Studies suggest that Alzheimer's disease progression and cognitive decline are more likely to occur with menopause or ovariectomy-induced estrogen depletion [39–41], and hormone replacement therapy may help manage these conditions [42]. In accordance with our findings, it has been reported that bilateral ovariectomy severely affects cognitive abilities, whereas Es therapy can recover spatial and recognition memories [43, 44]. Likewise, mice in the OVX + Ser group exhibited better memory performance in the Lashley III maze and the NOR tests. The ability of sericin for improvement of cognitive disabilities has been previously proven in different conditions, including sleep deprivation, aging as well as cerebral ischemia, indicating its pro-cognitive potential [35].

Besides, estrogen depletion is accompanied by excessive production of free radicals and deterioration of the endogenous antioxidant defense system, resulting in neuronal loss [44–46]. In line with previous reports, we showed that ovariectomy perturbed redox homeostasis, manifested by an increase in hippocampal ROS levels and reduced TAC levels and SOD and GPx activities. Conversely, treatment with sericin and Es restored redox balance in the OVX mice. In agreement with our results, free radical scavenging capacity of sericin and Es has been established in animal models [30, 35, 36, 47–50]. Emerging evidence shows that oxidative stress impairs synaptic transmission and plasticity by damaging strategic proteins involved in storing and release of neurotransmitter and synaptic signaling. Besides, oxidative stress can decrease neurotrophic factors, which are essential for neuronal survival and growth, ultimately leading to cognitive decline [51, 52].

Neurotrophins like BDNF are the major regulator of neurogenesis, synaptogenesis, neurotransmission, and synaptic

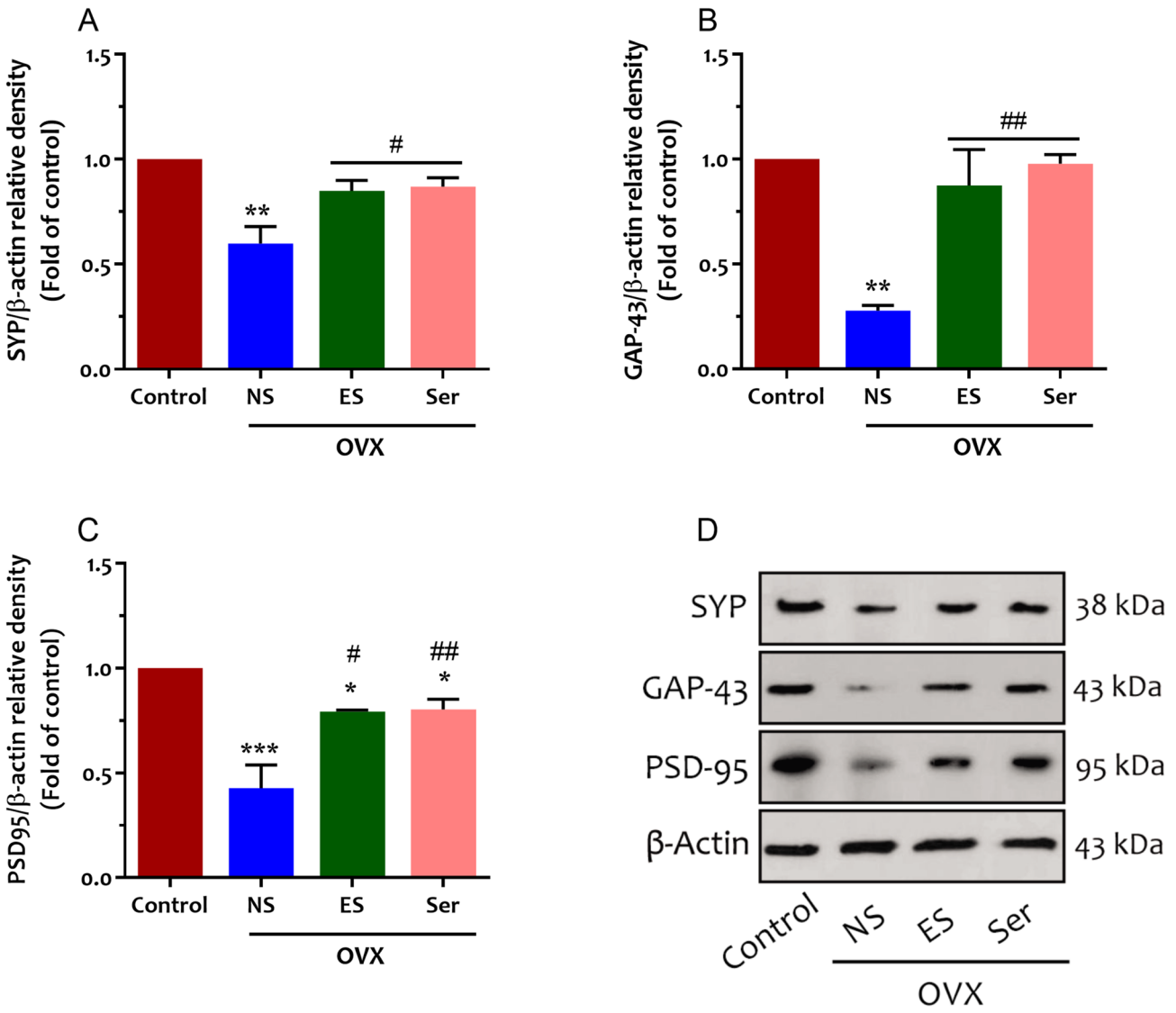

Fig. 5 The effect of ES and Sericin on the expression of (**A**) SYP, (**B**) GAP-43, (**C**), and PSD-95 proteins in the HIP of the OVX mice. (**D**) Representative blot images of synaptic proteins established by the immunoblotting. Values are presented as the means ± SEM (n = 3). One-way ANOVA, followed by Tukey's post-hoc test. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. control group. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. OVX + NS group. OVX, ovariectomized; NS, normal saline; Ser, sericin; Es, estradiol; SYP, synaptophysin; GAP-43, growth-associated protein-43; PSD-95, post-synaptic density-95

plasticity. The activation of PKA-CREB-BDNF signaling promotes synaptic efficiency and shapes synaptic plasticity by modulating levels of pre-and post-synaptic proteins [53, 54]. Accordingly, suppression of this signaling pathway impairs memory formation and synaptic plasticity in OVX animals [19, 20, 55–57], though Es treatment improves memory function by up-regulation of BDNF levels and potentiating synaptic plasticity [21, 22]. Similarly, we observed that OVX mice demonstrated prominent reductions of BDNF protein expression as a consequence of inhibition of the PKA-CREB pathway in the HIP. These results were coincident with the down-regulation of pre-synaptic proteins (SYP and GAP-43) and post-synaptic protein (PSD-95) in the HIP of OVX mice. Conversely, the application of Es or sericin prevented OVX-induced changes in synaptic proteins, leading to improvement of learning and memory processes through the activation of the PKA-CREB-BDNF signaling pathway. According to several studies, sericin can regulate synapse plasticity by increasing the hippocampal protein content of BDNF and up-regulating synaptic proteins, including PSD-95, SYP, and synapsin-1 [35, 58].

The brain may undergo structural changes such as neuron shrinkage, shorter dendrites, and lower spine density due to surgical menopause and estrogen depletion, leading

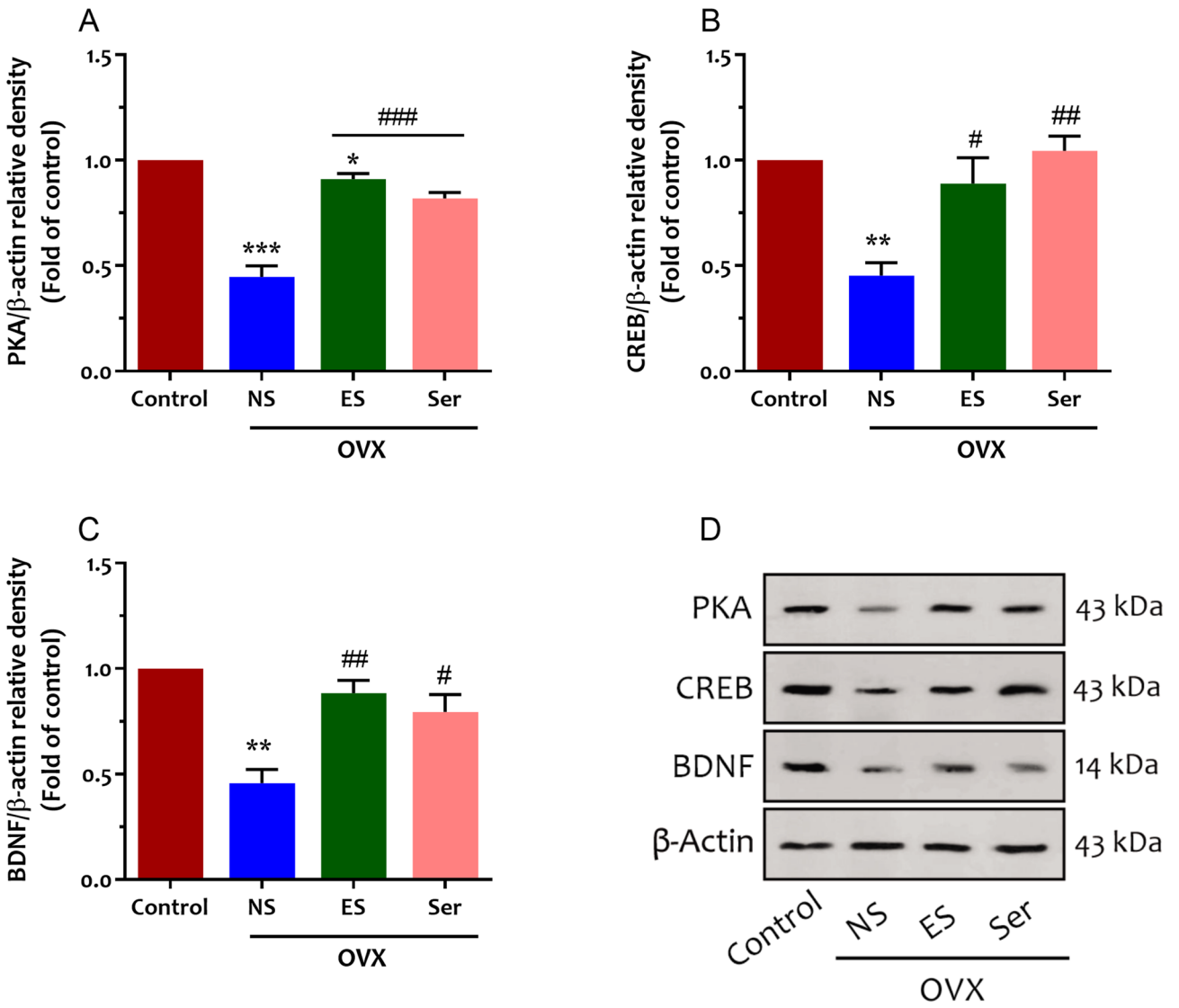

Fig. 6 The effect of Es and sericin on the hippocampal expression of (**A**) PKA, (**B**) CREB, and (**C**) BDNF proteins in OVX mice. (**D**) Representative blot images of the relevant proteins detected by immunoblotting method. Values are presented as the means ± SEM (n = 3). One-way ANOVA followed by Tukey's post-hoc test. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. OVX + NS group. OVX, ovariectomized; NS, normal saline; Ser, sericin; Es, estradiol; BDNF, brain-derived neurotrophic factor; CREB, cAMP response element-binding protein; PKA, protein kinase A

to neurotransmission dysregulation [59, 60]. Cholinergic deficiency, which is caused by a decrease in choline acetyltransferase activities and an abnormal increase in AChE activities in the brain, is one of the major causes of memory and cognitive deficits after menopause or ovariectomy [12–15]. Basically, compounds with AChE inhibitory effects improve cognitive dysfunctions via increasing synaptic transmission of acetylcholine and boosting of cerebral cholinergic network activity [61]. Our study validated earlier reports that ovariectomy leads to a significant increase in AChE activity in the HIP, which impairs memory performance. The exact mechanism of increased AChE activity under ovariectomy is complex and multifaceted, but evidence suggests a connection with estrogen deficiency. Cholinergic neurotransmission in the brain is enhanced by estrogen, leading to higher choline acetyltransferase activity, increased choline uptake, and enhanced acetylcholine release, which can reduce AChE activity [62, 63]. Moreover, the decline of estrogen during ovariectomy can contribute to neuroinflammatory processes and oxidative stress, which may affect cholinergic function and result in elevated AChE activity [64]. Thus, estrogen indirectly inhibits AChE activity in the brain by

modulating cholinergic neurotransmission. Investigating the connection between ovariectomy and AChE activity may offer treatment possibilities for addressing menopausal symptoms and enhancing cognitive function in postmenopausal women. In our study, treatment of OVX mice with Es or sericin restored hippocampal AChE activity to the sham levels and enhanced memory function. Studies showed that estrogen therapy improved memory deficit in the OVX animals by augmentation of the cholinergic neurotransmission [15, 65]. Likewise, Peera et al. showed that sericin improved cognitive impairments in rat model of Alzheimer's disease via inhibiting of AChE activity and increasing acetylcholine content [30]. Nevertheless, there has been no research on how sericin inhibits AChE activity. While mechanistic studies are lacking, a previous study found that sericin can counteract AChE activity in rat models of Alzheimer's disease, suggesting it may be a cholinesterase inhibitor [26]. Hence, further mechanistic studies are needed to fully comprehend the specific pharmacodynamic effects of sericin on AChE activity.

Limitations and Future Directions

The effectiveness of sericin as a therapeutic agent can be affected by its stability during oral administration. However, the stability of sericin during oral administration has not been studied. The presence of hydrogen bonds, hydrophobicity, and crystalline structure in silk sericin contribute to its stability compared to globular proteins [66]. Therefore, sericin may exhibit favorable stability characteristics during oral administration, contributing to its potential therapeutic benefits. However, conducting additional specific studies on sericin stability in the gastrointestinal environment and after oral administration would offer more concrete insights on this matter.

Moreover, we did not assess plasma estradiol levels in the treatment groups due to specific focus on the cognitive impacts of sericin in OVX animals, aiming to investigate alternative mechanisms beyond estrogen-like effects. Furthermore, the risk of endometriosis and breast cancer increases with prolonged estrogen usage in postmenopausal women, and current treatment approaches for estrogen depletion side effects are insufficient. Scientists are currently exploring alternative treatments for estrogen in postmenopausal women that can counteract its negative effects while preserving its advantages. In order to tackle this constraint, forthcoming research should incorporate assessments of plasma or serum estradiol levels in all experimental groups and investigate how sericin administration affects hormonal balance in OVX mice.

Conclusion

Sericin treatment has been found to protect against behavioral and molecular changes caused by ovariectomy. This is achieved by enhancing synaptic protein markers, activating the PKA-CREB-BDNF signaling pathway, improving cholinergic neurotransmission, and restoring redox homeostasis in the HIP. These results provide a foundation for further research into the potential of sericin as a therapeutic agent for cognitive disorders related to menopause.

Author Contributions JM and FF conceived and designed the study. FF, SMV, and JM performed the experiments and collected the data. SSE and PGS analyzed the data. The first draft of the manuscript was written by SM, HF, and LH. All authors commented on previous versions of the manuscript, and reviewed and approved the final version of the manuscript.

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Data Availability The data that support the findings of this study can be obtained from the corresponding author upon reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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