Journal of Basic and Applied Pharmacology
Vol. 5 No.1 January - June 2025

Page: [O36-46]

Original research article

Aquilaria crassna leaf extract improves memory deficits in ovariectomized rats

Khachanan Inthiwong[1], Eakkaluk Wongwad[2], Kornkanok Ingkaninan[2,3], Onrawee Khongsombat[1], Pornnarin Taepavarapruk[1*]

[1]*Department of Physiology, Faculty of Medical Science, Naresuan University, Phitsanulok, 65000, Thailand*
[2]*Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand*
[3]*Center of Excellence for Innovation in Chemistry, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, 65000, Thailand*

Received 30 October 2024; Received in revised form 2 February 2025
Accepted 8 February 2025; Available online 31 March 2025

ABSTRACT

Aquilaria crassna is one Aquilaria species which can produce agarwood. Most agarwood is processed into oil which is used in perfumes and in the production of traditional medicines for treating asthma, stress, and other diseases. This study aimed to investigate the effect of *A. crassna* leaf extract on learning and memory of ovariectomized rats, an animal model of Alzheimer's disease (AD). Sprague Dawley rats were subjected to bilateral ovariectomy (OVX). Following a full recovery, *A. crassna* leaf extracts (10, 100, 1000 mg/kg B.W.) were orally administered for 60 days. The negative control group received RO water and the positive control group received donepezil. Novel object recognition (NOR) and Morris water maze (MWM) tests were employed to evaluate their cognitive functions. The activities of acetylcholinesterase (AChE), superoxide dismutase (SOD), and catalase (CAT), as well as the levels of malondialdehyde (MDA) in the hippocampus, were assessed. OVX rats receiving *A. crassna* leaf extract showed a significant improvement in both object recognition and spatial memories when compared to the OVX control group. Additionally, treatment of *A. crassna* leaf extract resulted in significant elevation in the activities of SOD and CAT, suppression of AChE activity, and reduction in MDA levels compared to the OVX group. *A. crassna* leaf extract could improve cognitive and memory impairment in OVX rats by increasing antioxidant activity and inhibiting acetylcholinesterase. Therefore, *A. crassna* leaf extract may be useful in reducing the risk of AD in postmenopausal women.

Keywords: *Aquilaria crassna*, Morris water maze, novel object recognition, acetylcholinesterase inhibitor, antioxidant

***Corresponding author:** pornnarint@nu.ac.th

1. Introduction

Alzheimer's disease (AD) is the most common type of senile dementia, characterized by the accumulation of senile plaques and neurofibrillary tangles in the brain.[1] This accumulation results in cognitive impairment associated with AD. However, synaptic loss is the principal correlate of disease progression in AD. In the late stages of the disease, a significant reduction in acetylcholine levels occurs, which is largely attributed to the increased activity of acetylcholinesterase (AChE). As AD progresses, AChE activity becomes more pronounced, accelerating acetylcholine degradation and exacerbating cognitive decline.[2] The increased activity of AChE in OVX rats can be attributed to the effect of estrogen depletion on the cholinergic neurotransmission system. Estrogen is also involved in the regulation of AChE activity and cognitive function. It has been reported to increase cholinergic function by enhancing the release of Acetylcholine (ACh) and regulating AChE activity.[3] On removing the ovaries, the regulatory role of estrogen is lost resulting in an increase in AChE activity, faster degradation of ACh and cognitive impairment.[4] Furthermore, estrogen deficiency is characterized by enhanced oxidative stress and neuroinflammation that can affect AChE activity. Oxidative stress has been found to increase the expression and activity of AChE, which may be a mechanism of response to increased levels of reactive oxygen species (ROS) in the brain.[5] Neuroinflammation, which is also a consequence of estrogen depletion, has also been found relate an increased AChE activity and, therefore, to worsen cognitive performance.[6] Previous studies on OVX rats have reported that AChE activity is increased in the absence of estrogen.[7]

It is common knowledge that cognitive impairment is usually accompanied by reduced cholinergic activity, however, the rise in AChE activity indicates that the brain may have a maladaptive response, an attempt to regulate cholinergic signaling in response to estrogen loss. These findings also show that estrogen, oxidative stress, and cholinergic function are interlinked and that AChE inhibition is a viable therapeutic target for postmenopausal cognitive impairment.

One therapeutic approach to managing AD involves inhibiting AChE to maintain higher acetylcholine levels in the brain, thereby temporarily improving cognitive symptoms.[8] Estrogen plays a vital role in neuroprotection, particularly in maintaining cognitive function. It has been shown to enhance synaptic plasticity, reduce neuro-inflammation, and regulate neurotransmitter systems. Additionally, estrogen has been implicated in protecting against beta-amyloid accumulation, a hallmark feature of AD. Studies suggest that estrogen replacement therapy can improve cognitive function and slow neurodegeneration in both animal models and human subjects.[9] Conversely, estrogen deficiency, as seen in postmenopausal women and modeled in OVX rats, has been associated with increased oxidative stress, neuroinflammation, and cholinergic dysfunction, all of which contribute to cognitive decline.[10,11]

Oxidative stress is a major factor in neurodegeneration, particularly in AD. It is defined as an imbalance between antioxidant systems and ROS, leading to lipid peroxidation and cellular damage. Key antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), play a crucial role in counteracting oxidative damage.[9] However, OVX models exhibit elevated oxidative stress due to increased ROS production and diminished antioxidant enzyme activity, contributing to neuronal apoptosis and cognitive impairments. This suggests that OVX-induced cognitive decline is strongly linked to oxidative stress, making it an important target for therapeutic intervention.

Aquilaria crassna (AC), locally known in Thailand as "Mai Kritsana," belongs to the Thymelaeceae family and has been traditionally used in medicine. Its leaves contain bioactive compounds with potential

AChE inhibitory and antioxidant effects, which may provide neuroprotective benefits.[12] Studies indicate that extracts from *A. crassna* leaves may help mitigate cognitive decline through their ability to inhibit AChE and enhance antioxidant defenses.[13]

Given the established link between estrogen deficiency, oxidative stress, and cognitive impairment, this study aimed to investigate the effects of *A. crassna* leaf extract on cognitive function in OVX rats. Note that in the MWM test, the study did not reveal any distinct variation in the spatial memory of the sham and OVX groups. There are several possible reasons for this outcome. It is possible that the duration of estrogen depletion in OVX rats was not enough to manifest clear-cut cognitive decline. According to some investigations, more prolonged estrogen deficiency results in more pronounced deficits. Furthermore, the OVX rats might have had some compensatory mechanisms including increased cholinergic activity or increased synaptic plasticity to counteract the estrogen depletion effects on cognition. Another possibility is that the behavioral tests employed in the study may not have been sensitive enough to pick up modest differences in cognitive performance between the two groups, particularly if there was variability in the sham group. These results are in opposition to previous research which indicates that estrogen depletion induced by OVX adversely affects cognitive performance. However, factors such as oxidative stress, alterations in neurotransmitters or neuroplastic adaptations of the brain may have contributed to the observed results. Specifically, this study assessed the impact on acetylcholinesterase activity, oxidative stress markers, and antioxidant enzyme levels to determine whether it could serve as a potential neuroprotective agent against AD-related cognitive decline.

2. Materials and Methods

2.1 Animal

This study utilized a total of 42 female Sprague Dawley rats. Rats were kept at 3 per cage with controlled temperature (22.0 ± 1.0 °C) and 12h/12h reverse dark-light cycle (lights on 23:00 – 11:00). All animals had free access to drinking water and food in their home cage. All protocols were approved by the committees for ethics in animal experimentation, Naresuan University Animal Care and Use Committee (NUACUC, Naresuan University, Phitsanulok, Thailand, ethics approval number: NU-AE590611).

2.2 Preparation of *A. crassna* leaf extracts

The process of extract preparation was facilitated by Dr. Eakkaluk Wongwad and Professor Dr. Kornkanok Ingkaninan, Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences and Center of Excellence for Innovation in Chemistry, Naresuan University. Young leaves of *A. crassna* were collected from Phitsanulok province, Thailand. *A. crassna* leaves were air-oven-dried at a temperature of 60°C for 48 h. Then, the leaves were transformed into a dry powder using a hot air oven set at 100°C for 3 h, and the dried specimens ground into powder. The extraction was performed using a ratio of 100 g of dried *A. crassna* leaves per 1 liter of hot water at 95-100°C for 30 min. The filtrate was then subjected to centrifugation until completely dried. The obtained aqueous extract was stored in a freezer at -20°C until further use.[14]

2.3 Ovariectomy procedure

Rats were induced and maintained under anesthesia with 3–4% isoflurane (Piramal Healthcare, USA) in an induction chamber until the time of the surgery. The lower abdomen was also shaved and alcohol was used to disinfect the area before making a small midline incision through the abdominal wall. For the OVX group, both ovaries were then carefully removed, and the incision closed in layers. For the sham group, the same surgery was done, but the ovaries were not removed, to mimic surgical stress and handling. All rats were placed on post-operative monitoring and supportive care to guarantee a good recovery.

2.4 Experimental groups

Forty-two female Sprague Dawley rats were used and they were allocated into six groups with seven rats in each group. Sham group: The surgical procedure was performed without performing OVX and the subjects received distilled water as a control. OVX control group: This group was subjected to bilateral OVX and also received distilled water. Three treatment groups were exposed to different concentrations of *A. crassna* leaf extract administered in the form of water extract; the OVX + AC10 group was given 10 mg/kg orally for 60 days, the OVX + AC100 group was given 100 mg/kg for 60 days and the OVX + AC1000 group was given 1000 mg/kg for the same duration. Donepezil at 5 mg/kg was used orally for 60 days in the OVX + DON group as a positive control.

2.5 Behavioral assessment

2.5.1 Morris water maze test

MWM test was used to assess spatial memory. The MWMT was carried out in a large circular pool. The tank was filled up so that the platform was one inch below the surface of the water (25 ± 2 °C). Powder was used to make the water opaque. The pool was divided into 4 quadrants corresponding to the 4 visual cues. The quadrant circular was the target zone where the hidden platform used to be located. The subjects were monitored by a video tracking system directly above the water tank as they swam and parameters were measured using Anymaze software (Stoelting Co.). During the training phase, each rat was allowed to swim in the circular pool to locate the hidden platform within 90 s. The rat was trained 3 times a day for 7 days. On the probe phase, the hidden platform was removed, and the rat could swim in the circular pool for 90 s. The spatial memory index was calculated according to the following formula:

$$\frac{\text{Time spent in the target quadrant}}{\text{Time spent in non} - \text{target quadrant}} X100$$

2.5.2 Novel object recognition test

For assessing recognition memory, the NOR Test was employed. In the training phase, each rat was introduced to an open-field box which had two identical objects and the rat was allowed to explore freely for 5 min. This was then followed by the rat being returned to its home cage for a 5-min retention interval. In the testing phase, one of the familiar objects was replaced with a new object, and the rat was again given 5 min to explore. The recognition index was used to evaluate recognition memory: [Time of novel object exploration during testing phase / (time of familiar object exploration during the testing phase + time of novel object exploration during the testing phase) x 100]. A high recognition index means that the rat has better memory because it prefers the novel object to the familiar object.

2.6 Biochemical assays

2.6.1 Determination of AChE activity

The assay for measuring AChE activity was modified from the assay described by Ellman GL, et al.[15] Briefly 50 µl of sample, 125 µl of 1mM DTNB, 25 µl of 1 mM ACTI and 25 µl of 0.1 M phosphate buffer were added into the wells. The microplate was read at 405 nm every 10 s for 3 min. Enzyme activity was calculated by an IEMS reader MF and Labsystem ascent software 2.4 (Labsystem, Finland).

2.6.2 Determination of superoxide dismutase activity

The hippocampus SOD activity was determined with a SOD assay kit (Merck, USA) according to the manufacturer's instructions. Briefly, 200 µl of radical detector, 10 µl of sample and 20 µl of xanthine oxidase were added into the wells. The solution was incubated for 20 min at room temperature. The absorbance was read with a microplate reader (Labsystem, Finland) at 450 nm.

2.6.3 Determination of catalase activity

CAT activity was determined by assessing the decomposition reaction of

H_2O_2. Briefly, adding 1 ml of 11 nM H_2O_2 solution in buffer, 2 ml catalase buffer and 0.1 ml sample were placed in the cuvette. Catalase activity was calculated using the molar extinction coefficient 40 cm-1 and absorbance at 240 nm was measured using a spectrophotometer (Bara Scientific Co., Ltd.).

2.6.4 Determination of tissue lipid peroxidation

The level of homogenized tissue malondialdehyde (MDA), as an index of lipid peroxidation, was determined by the thiobarbituric acid reaction according to Tsikas D.[16] Briefly, 100 µl sample, 200 µl of 8.1% SDS, 1.5 ml of 20% acetic acid and 1.5 ml TBA were added into the tube. The solution was incubated for 60 min at 95 °C. After incubation, the reaction product was added into the microplate wells. The absorbance was read with a microplate reader (Labsystem, Finland) at 532 nm.

2.6.5 Determination of protein content

Protein concentration was determined using the bicinchoninic acid (BCA) assay kit, with bovine serum albumin (BSA) as the standard. Briefly, 25 µL of each sample was mixed with 200 µL of the working reagent in a 96-well plate. The mixture was incubated at 37°C for 30 min, allowing color development. Following incubation, absorbance was measured at 562 nm using a microplate reader (Labsystem, Finland).

2.7 Statistical analysis

All data are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism software. Oneway ANOVA with Dunnett's post hoc comparison was used to compare groups to the control. A *p* value of less than 0.05 was considered statistically significant.

3. Results

3.1 Effect of OVX on cognitive function and biochemical parameters

OVX resulted in significant cognitive impairment, as indicated by decreased performance in the MWMT and NORT. In the MWMT, the spatial memory index was significantly lower in the OVX group (22.8 ± 1.2) compared to the Sham group (28.2 ± 2.1, $p < 0.05$), indicating impaired spatial learning and memory. Similarly, in the NORT, the recognition memory index was substantially lower in the OVX group (42.2 ± 3.3) compared to the Sham group, (78.5 ± 2.3) suggesting deficits in object recognition memory (Fig.1).

At the biochemical level, OVX led to a significant increase in hippocampal AChE activity, which was markedly higher in the OVX group (129.5 ± 4.3 nmol/min/mg protein) than in the Sham group (97.3 ± 6.5 nmol/min/mg protein, $p < 0.05$). Additionally, OVX-induced oxidative stress was evident, as demonstrated by a significant decrease in SOD activity (0.9 ± 0.1 U/mg protein in OVX vs. 1.7 ± 0.1 U/mg protein in Sham, $p < 0.05$) and CAT activity (5.1 ± 0.2 U/mg protein in OVX vs. 7.5 ± 0.4 U/mg protein in Sham, $p < 0.05$). Concurrently, MDA levels, a marker of lipid peroxidation, were significantly elevated in the OVX group (1.5 ± 0.1 nmol/mg protein) compared to the Sham group (1.0 ± 0.1 nmol/mg protein, $p < 0.05$), further confirming increased oxidative stress.

3.2 The effect of AC on the Novel object recognition test

NORT was used to evaluate the effect of the AC extract on recognition memory in ovariectomized rat. The recognition memory indices of the OVX+AC10, OVX+AC100 and OVX+AC1000 groups were 72.0 ± 4.4, 74.6 ± 1.6 and 78.8 ± 2.6, which were significantly higher compared to the OVX group (42.2 ± 3.3) ($p < 0.05$). The values of these test groups were close to that of the OVX+DON group in which the recognition index was 71.5 ± 0.8 (Fig.2).

3.3 The effect of AC on the hippocampus AChE activity

There was a significant increase in AChE activity in OVX group (129.5 ± 4.3 nmol/min/mg protein) as compared to the sham group (97.3 ± 6.5 nmol/min/mg protein).the

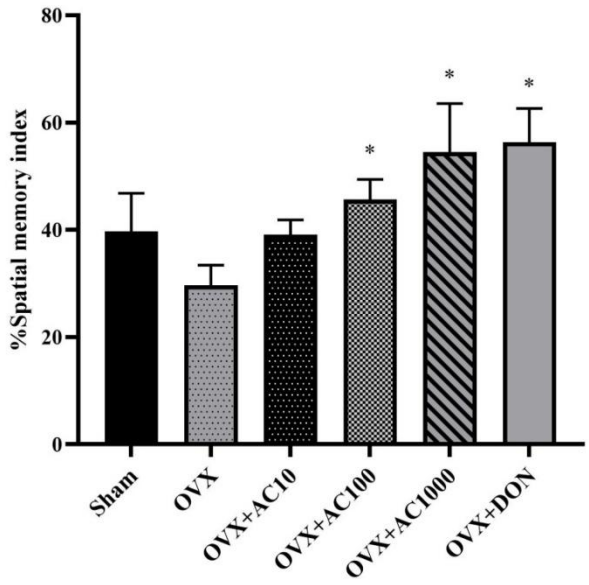

Fig. 1. Effect of AC on the spatial memory in an ovariectomized rat model of AD. Values are expressed as mean ± SEM. *, $p < 0.05$ versus OVX group.

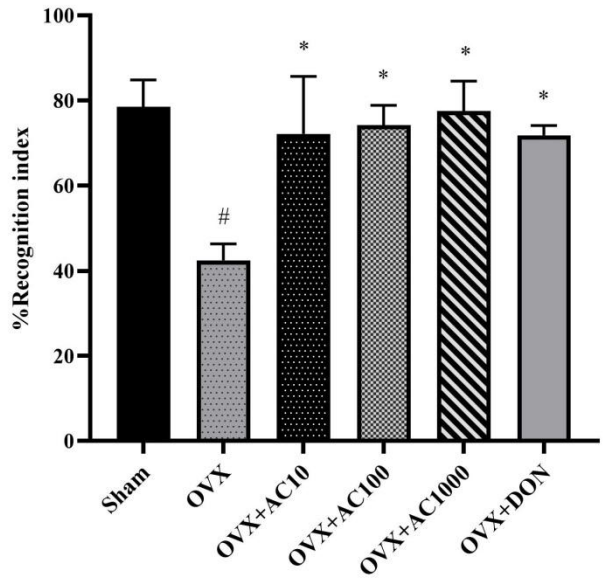

Fig. 2. Effect of AC on the recognition memory in an ovariectomized rat model of AD. Values are expressed as mean ± SEM. #, $p < 0.05$ versus the Sham group. *, $p < 0.05$ versus the OVX group.

OVX+AC10, AC100, and AC1000 groups (100.9 ± 2.1, 96.3 ± 7.1 and 97.9 ± 6.1 nmol/min/mg protein respectively) and the OVX+DON group (58.2 ± 7.6 nmol/min/mg protein) showed decreased activity in the hippocampus in OVX rats ($p < 0.05$) (Fig. 3).

3.4 The effect of AC on the hippocampus SOD activity

There were no significant changes in SOD activity among the OVX (0.9 ± 0.1 U/mg protein), OVX+AC10 (1.0 ± 0.1 U/mg protein) and OVX + DON (1.4 ± 0.1 U/mg protein) groups. In the OVX+AC100 (2.5 ± 0.3 U/mg protein) and OVX+AC1000 (2.4 ± 0.3 U/mg protein) groups, SOD activity was significantly higher than in the OVX group. In the Sham group, SOD activity was found to be 1.7 ± 0.1 U/mg protein. In the OVX group, SOD activity was significantly less than that in the Sham group ($p < 0.05$) (Fig. 4).

3.5 The effect of AC on the hippocampus CAT activity

CAT activity was decreased in the OVX group (5.1 ± 0.2 U/mg protein) compared with value in the sham group (7.5 ± 0.4 U/mg protein). CAT activity in the OVX+AC10, AC100, AC1000 and OVX+DON groups (7.1 ± 0.4, 6.8 ± 0.2, 7.5 ± 0.9 and 7.1 ± 0.3 U/mg protein respectively) was significantly higher than that in the OVX group ($p < 0.05$) (Fig. 5).

3.6 The effect of AC on the hippocampus MDA level

In the OVX group (1.5 ± 0.1 nmol/mg protein), the MDA level was significantly higher than in the sham group (1.0 ± 0.1 nmol/mg protein). The MDA level decreased in the OVX+AC10, AC100, AC1000 and OVX+DON groups (0.8 ± 0.1, 0.9 ± 0.1, 0.9 ± 0.1 and 0.8 ± 0.1 U/mg protein respectively) compared to the OVX group ($p < 0.05$) (Fig. 6).

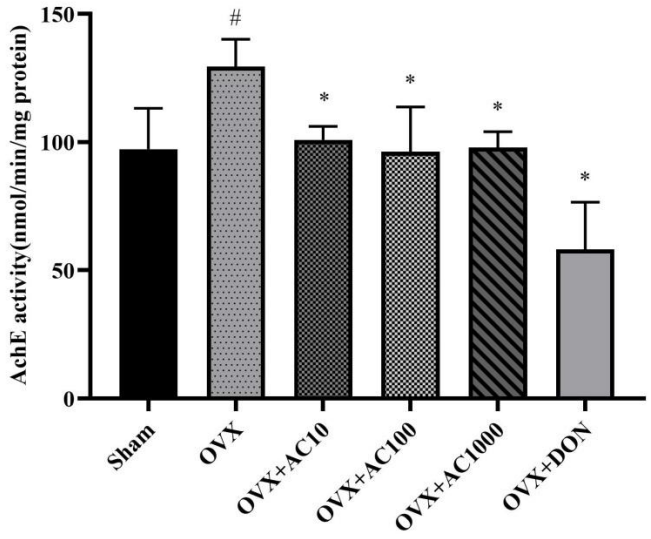

Fig. 3. Effect of AC on the hippocampus AChE activity in an ovariectomized rat model of AD. Values are expressed as mean ± SEM. #, $p < 0.05$ versus the Sham group. *, $p < 0.05$ versus the OVX group.

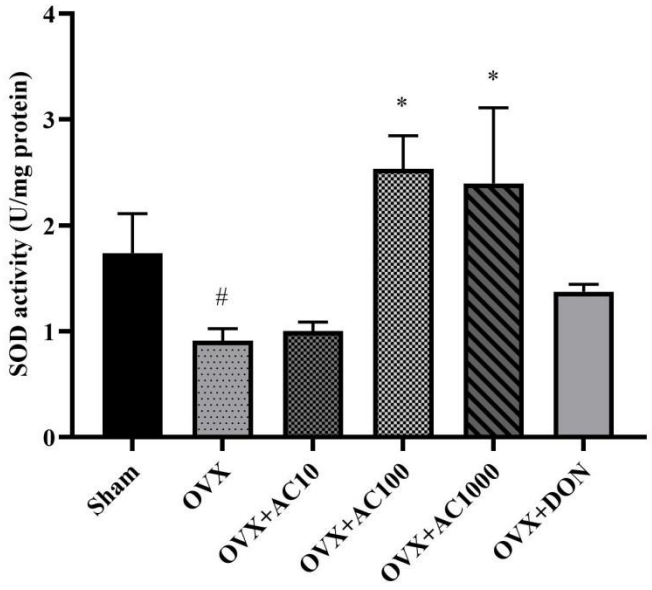

Fig. 4. Effect of AC on the hippocampus SOD activity in an ovariectomized rat model of AD. Values are expressed as mean ± SEM. #, $p < 0.05$ versus the Sham group. *, $p < 0.05$ versus the OVX group.

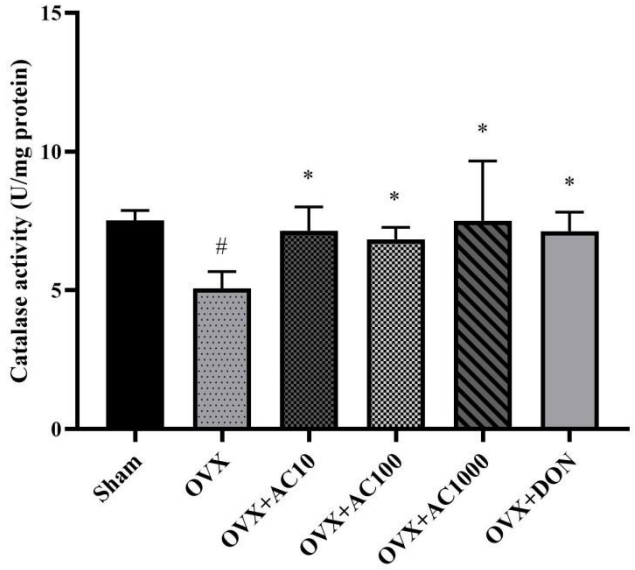

Fig. 5. Effect of AC on the hippocampus CAT activity in an ovariectomized rat model of AD. Values are expressed as mean ± SEM. #, $p < 0.05$ versus the Sham group. *, $p < 0.05$ versus the OVX group.

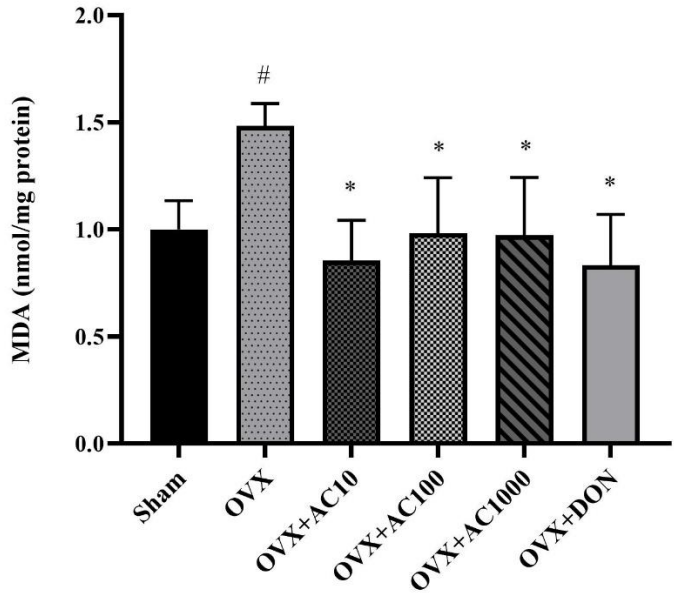

Fig. 6. Effect of AC on the hippocampus MDA levels in an ovariectomized rat model of AD. Values are expressed as mean ± SEM. #, $p < 0.05$ versus the Sham group. *, $p < 0.05$ versus the OVX group.

4. Discussion

In this study, the leaves of *A. crassna* were extracted and the extract was used to manage cognitive dysfunction in female ovariectomized rats. The effects were dose dependent. Surprisingly, the lowest dose of (10 mg/kg) exhibited maximum positive effect in some of the outcomes like recognition memory and AChE inhibition. However, it did not show a significant effect on spatial memory index in the MWM test or SOD activity. The lack of effect of AC10 on spatial memory index might be because of its low bioavailability in the brain at this dose, which results in inadequate modulation of hippocampal synaptic plasticity that is required for spatial learning. Previous research has shown that the effectiveness of plant-based cognitive enhancers is strongly determined by their ability to penetrate the blood-brain barrier and act at the synaptic level.[13] Whereas AC10 was sufficient to improve recognition memory in the NOR test which is dependent on the perirhinal cortex, it might not have been powerful enough to support the hippocampal- dependent spatial learning. Likewise, the absence of improvement in SOD activity at this dose suggests that AC10 did not induce a sufficient antioxidant response to counter the oxidative stress associated with OVX, although higher doses (100 and 1000 mg/kg) elevated SOD activity. This may mean that there is a certain dose that must be reached in order to switch on the endogenous antioxidant defense mechanisms.[14] With regard to the safety of the highest dose (1000 mg/kg), there were no apparent behavioral toxicity observed throughout the study. Nevertheless, previous research has indicated that high doses of the *A. crassna* extracts have CNS depression properties such as sedation and decreased spontaneous activity.[18] Although this study did not directly measure sedation, it is possible that the highest dose could have had slight sedative effects, which may help to explain why cognitive enhancement was not as dramatic as in the 100 mg/kg group. Also, high concentrations of bioactive compounds can sometimes result in receptor desensitization

or adaptive physiological responses that reduce the effectiveness of the stimulus.[19] In general, the results of the present work suggest that intermediate dose of *A. crassna* (100 mg/kg) could be the most appropriate dose for achieving the best balance between efficacy and safety, as well as for improving cognitive performance and antioxidant status. More studies are needed to elucidate the specific dose-related mechanisms that mediate these effects and to investigate the possible consequences of administration at high doses in the long term.

5. Conclusion

This study demonstrated that *A. crassna* leaf extract effectively improved cognitive deficits in OVX rats, suggesting its potential as a neuroprotective agent. The extract enhanced both spatial and recognition memories, likely through its antioxidant properties and AChE inhibition, which counteract oxidative stress and cholinergic dysfunction induced by estrogen deficiency. While the 10 mg/kg dose showed the most pronounced effects on recognition memory and AChE inhibition, it did not significantly improve spatial memory or SOD activity, possibly due to insufficient bioavailability in the hippocampus. Higher doses (100 and 1000 mg/kg) demonstrated broader neuroprotective effects, enhancing both cognitive function and oxidative stress markers. However, no overt toxicity was observed even at the highest dose. These findings suggest that *A. crassna* leaf extract, particularly at 100 mg/kg, may offer an optimal balance between efficacy and safety for mitigating cognitive decline associated with estrogen deficiency. Future research should explore its long-term effects and molecular mechanisms to further validate its therapeutic potential.

Conflicts of Interest

None to declare.

References

[1] Armstrong R. What causes Alzheimer's disease? Folia Neuropathol. 2013;51(3):169-188.
[2] Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM. Alzheimer's disease: targeting the cholinergic system. Curr Neuropharmacol. 2016;14(1):101-115.
[3] Matyi JM, Rattinger GB, Schwartz S, Buhusi M, Tschanz JT. Lifetime estrogen exposure and cognition in late life: the Cache County Study. Menopause. 2019;26(12):1366-1374.
[4] Yun J, Yeo IJ, Hwang CJ, Choi D-Y, Im H-S, Kim JY, et al. Estrogen deficiency exacerbates Aβ-induced memory impairment through enhancement of neuroinflammation, amyloidogenesis and NF-κB activation in ovariectomized mice. Brain Behav Immun. 2018;73:282-293.
[5] Murphy KE, Park JJ. Can co-activation of Nrf2 and neurotrophic signaling pathway slow Alzheimer's disease? Int J Mol Sci. 2017;18(6):1168.
[6] Takuma K, Matsuo A, Himeno Y, Hoshina Y, Ohno Y, Funatsu Y, et al. 17beta-estradiol attenuates hippocampal neuronal loss and cognitive dysfunction induced by chronic restraint stress in ovariectomized rats. Neuroscience. 2007;146(1):60-68.
[7] Meng Y, Wang R, Yang F, Ji ZJ, Fang L, Sheng SL. Amyloid precursor protein 17-mer peptide ameliorates hippocampal neurodegeneration in ovariectomized rats. Neurosci Lett. 2010;468(3):173-177.
[8] Sozio P, Cerasa LS, Marinelli L, Di Stefano A. Transdermal donepezil on the treatment of Alzheimer's disease. Neuropsychiatr Dis Treat. 2012;8:361-368.
[9] Colović MB, Krstić DZ, Lazarević-Pašti TD, Bondžić AM, Vasić VM. Acetylcholinesterase inhibitors: pharmacology and toxicology. Curr Neuropharmacol. 2013;11(3):315-335.
[10] Bahrani H, Mohamad J, Paydar MJ, Rothan HA. Isolation and characterisation of acetylcholinesterase inhibitors from Aquilaria subintegra for the treatment of Alzheimer's disease (AD). Curr Alzheimer Res. 2014;11(2):206-214.
[11] Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot. 2003;91 Spec No(2):179-194.
[12] Zhang X, Wang J, Xing Y, Gong L, Li H, Wu Z, et al. Effects of ginsenoside Rg1 or 17β-estradiol

on a cognitively impaired, ovariectomized rat model of Alzheimer's disease. Neuroscience. 2012;220:191-200.

[13] Khodamoradi M, Asadi-Shekaari M, Esmaeili-Mahani S, Esmaeilpour K, Sheibani V. Effects of genistein on cognitive dysfunction and hippocampal synaptic plasticity impairment in an ovariectomized rat kainic acid model of seizure. Eur J Pharmacol. 2016;786:1-9.

[14] Wongwad E, Pingyod C, Saesong T, Waranuch W, Wisuitiprot W, Sritularak B, et al. Assessment of the bioactive components, antioxidant, antiglycation and anti-inflammatory properties of Aquilaria crassna Pierre ex Lecomte leaves. Ind. Crops Prod. 2019;138: 111448.

[15] Ellman GL, Courtney KD, Andres V Jr, Feather-stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88-95.

[16] Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal Biochem. 2017;524:13-30.

[17] Bahrani H, Mohamad J, Paydar MJ, Rothan HA. Isolation and characterisation of acetylcholinesterase inhibitors from Aquilaria subintegra for the treatment of Alzheimer's disease (AD). Curr Alzheimer Res. 2014; 11(2):206-214.

[18] Okugawa H, Ueda R, Matsumoto K, Kawanishi K, Kato A. Effect of jinkoh-eremol and agarospirol from agarwood on the central nervous system in mice. Planta Med. 1996;62(1):2-6.

[19] Pattarachotanant N, Rangsinth P, Warayanon W, Leung GP-H, Chuchawankul S, Prasansuklab A, Tencomnao T. Protective Effect of Aquilaria crassna Leaf Extract against Benzo[a]pyrene-Induced Toxicity in Neuronal Cells and Caenorhabditis elegans: Possible Active Constituent Includes Clionasterol. Nutrients. 2023; 15(18):3985.