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RESEARCH ARTICLE

Acotiamide Can Be Used as an Anti-Cognitive Disorder Agent in Diseases Associated with Cognitive Dysfunction

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ABSTRACT

The steady increase in average lifespan of humans and animals has made the problem of aging-related cognitive impairment a significant issue. Alzheimer's disease (AD) in humans is a degenerative brain disease characterized by the loss of basic body functions due to neuronal damage, and canine cognitive dysfunction observed in dogs is stated to resemble human AD. Acetylcholine esterase (AChE) plays a role in the pathogenesis of the disease, and its inhibition prevents AChE-induced Aβ aggregation. Other pathological factors, including oxidative stress, inflammation and the Wnt pathway, also contribute to AD pathogenesis. The use of AChE inhibitor (AChEI) is the most effective way among the several available options for improving cholinergic symptoms. Although acotiamide is an AChEI, there is currently no information regarding its use in AD; therefore, this study aimed to examine the effects of acotiamide in an AD model for the first time. A total of 26 mice were divided into 4 groups: control, sham, Alzheimer and Alzheimer+Acotiamide. To create an experimental AD model, streptozotocin was administered intracerebroventricularly. In addition, acotiamide was administered intraperitoneally to the animals in the Alzheimer+Acotiamide group. The study evaluated AChE, choline acetyltransferase, β-secretase (BACE1), tumor necrosis factor-alpha, interleukin-10, α7nicotinic acetylcholine receptor, glycogen synthase kinase3beta, 8-hydroxy-2-deoxyguanosine and glutathione parameters; along with the number of neurons in the CA1 and CA3 regions, and the thickness in these areas. It can be concluded from results that acotiamide could prevent neuroinflammation, reduce AChE and BACE1 protein levels and may be effective in the treatment/prevention of AD.

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INTRODUCTION

Alzheimer's disease (AD) is the most common cause of progressive mental deterioration in older people, and there is increasing evidence that similar disorders such as Alzheimer-like diseases (ALD) are also occurring in animals. Given that most pet owners view their animals as family members, and since dogs exhibit cognitive impairment patterns comparable to human AD, examining ALD within the context of animal dementia research is essential (Gołaszewska *et al*., 2019). AD is a neurodegenerative disease of the central nervous system (CNS). The pathophysiology of AD is reported to be complex, and pathological events such as cholinergic dysfunction, oxidative stress, inflammation, immune dysregulation, vascular damage, Wnt pathway disruption, and calcium-mediated excitotoxicity are reported to play a role in its pathogenesis (Salkovic-Petrisic *et al*., 2013; Inestrosa and Varela-Nallar, 2014; Tarawneh, 2020).

An imbalance in the production or removal of abnormal, misfolded Aβ peptide and its accumulation in the CNS is reported to be the initiating factor leading to neurodegeneration (Panek *et al*., 2017). Amyloid precursor protein (APP) is abundant in the brains of healthy individuals and is cleaved by α-secretase to produce non-pathogenic soluble APPα. In individuals with AD, toxic Aβ is generated from APP in the brain by β-secretase (BACE1) and γ-secretase. The accumulation

of Aβ causes pathological conditions, including synaptic loss, altered neuronal activity, oxidative stress, neuroinflammation, neuronal damage, and ultimately neuronal death and dementia (Panek *et al*., 2017; Huat *et al*., 2019).

The enzyme choline acetyltransferase (ChAT) is responsible for synthesizing the neurotransmitter acetylcholine (ACh). A decrease in ChAT levels in the hippocampus and frontal cortex, coupled with the loss of cholinergic neurons in the nucleus basalis of AD brains, strongly suggests that ACh may play a key role in AD's development (Salkovic-Petrisic *et al*., 2013). According to the cholinergic hypothesis of AD, loss of cholinergic neurotransmission is proposed to significantly contribute to the cognitive impairment observed in patients (Francis *et al*. 1999). Furthermore, studies show that acetylcholinesterase (AChE) can accelerate the aggregation of Aβ peptides into amyloid fibrils, a process inhibited by AChE blockers (Inestrosa *et al.,* 1996; Mishra *et al*., 2015).

Normally, oxygen radical damage is managed by effective antioxidant systems, including enzymes and scavengers like vitamin E and ascorbate. However, in neurodegenerative conditions, this balance is disrupted, leading to the accumulation of free radicals and subsequent neuronal damage and AD is closely linked to oxidative stress. Biomarkers such as malondialdehyde and 8-hydroxy-2-deoxyguanosine can be measured in this condition, and antioxidant-based therapies are vital in combating the oxidative stress observed in AD (Moreira *et al*., 2005; Hafez and Gad, 2018).

Neuroinflammation is also a key element in the pathogenesis and development of AD. Increased levels of tumor necrosis factor (TNF) were found in the postmortem brains of individuals with AD, and co-localization with Aβ plaques revealed that its level correlated with disease severity. TNFalpha is reported to be important in neuroinflammation, tau hyperphosphorylation, and amyloid plaque accumulation (Montgomery and Bowers, 2012). Neuroinflammation is reported to trigger a decrease in α7nicotinic acetylcholine receptors (α7nAChRs), Aβ accumulation, and memory impairments in mouse brains (Lykhmus *et al*., 2015). The identification of these receptors as being associated with cognitive functions and the inhibition of these receptors by Aβ peptides demonstrate their importance in the treatment of symptomatic AD (Wallace and Porter, 2011). Furthermore, activation of the α7nAChR has been shown to reduce Aβ accumulation and prevent neurodegeneration (Liu *et al*., 2007).

Due to its critical roles in various cellular processes, Wnt signalling has been linked to diseases such as cancer, cardiovascular disease, and AD. The brains of AD patients show a decrease in β-catenin levels and an increase in tau hyperphosphorylation, resulting from the activation of glycogen synthase kinase3beta (GSK3beta). Conversely, the use of GSK3beta inhibitors has been shown to alleviate spatial memory impairment by activating Wnt signalling in the hippocampus, which increases β-catenin levels and reduces Aβ aggregation and astrogliosis. Therefore, impaired Wnt signalling pathways likely contribute to AD neurodegeneration, suggesting that their activation could be a promising therapeutic strategy (Inestrosa and Varela-Nallar, 2014).

AD is the most common human dementia (Anonymous, 2022a). In veterinary medicine, canine cognitive dysfunction (CCD) is reported as a neurodegenerative disease in dogs with age-related cognitive and behavioural impairments that closely parallel human AD. Both diseases share similar neuropathological features and exhibit cholinergic hypofunction (Park *et al*., 2016; Žnidaršicˇ *et al*., 2023). Acetylcholinesterase inhibitors (AChEIs), such as donepezil and rivastigmine, are the primary and most effective symptomatic treatment for AD because the inhibition of the AChE enzyme increases ACh levels at cholinergic synapses, thereby improving cognitive function (Salkovic-Petrisic *et al*., 2013; Panek *et al*., 2017). This mechanism is also applicable to CCD, where AChEIs can be used for symptomatic treatment. AChE remains a valuable target for new anti-AD agents based on positive *in vitro* and *in vivo* results. Consequently, interest in new AChEIs for CCD has increased as researchers seek to develop therapeutic methods for this neurodegenerative disorder (Panek *et al*., 2017; Žnidaršicˇ *et al*., 2023). For example, acotiamide, an AChEI approved in Japan for functional dyspepsia, is among these agents (Shibli *et al*., 2020).

One of the key target regions associated with the development of AD or ALD is neurons in the CA1 and CA3 areas of the hippocampus, which is a crucial brain region for memory activity and functions. Given the importance of using a drug effective on multiple targets in multifactorial diseases like AD or ALD, the current study aimed to evaluate the effects of acotiamide on inflammation, oxidative stress, the Wnt pathway, Aβ aggregation, and the cholinergic system in the treatment/prevention of these diseases.

MATERIALS AND METHODS

Animals: Twenty-six female Swiss albino mice (11 weeks old) were used in the study. Food and water requirements were offered *ad libitum.* Animals were weighed at the beginning and end of the study to determine weight gain.

Experimental Design and Applications: Animals were divided into 4 groups: control (n:6); sham (n:6); Alzheimer (ALZ) (n:7) and Alzheimer+Acotiamide (ALZ+ACO) (n:7). Following the Morris Water Maze (MWM) test, all animals (except control) underwent intracerebroventricular (ICV) injections under ketamine/xylazine anesthesia using a stereotaxic device (Coordinates: 0.3mm posterior to Bregma; 1mm lateral to the sagittal suture; 2.5mm ventral). The sham group received 3µL of physiological saline. To establish the AD model, streptozotocin (STZ) (3mg/kg) was administered ICV bilaterally on days 1 and 3. Animals administered STZ were divided into two equal groups: ALZ and ALZ+ACO. One animal in the ALZ+ACO group died 1 day after the administration. Five days after the second ICV-STZ administration, animals in the ALZ+ACO group received intraperitoneal acotiamide at a dose of 0.2mg/mice twice a week (Mondays and Thursdays) for 8 weeks.

MWM Test: This test was performed to evaluate spatial learning and memory abilities. It was performed twice: before STZ application and final week of the study. A circular water tank (diameter = 120cm; height = 60cm) was divided into four quadrants. Visual cues were provided for each quadrant, and an escape platform was placed 1cm below the water level in the target quadrant. When the animal was released into each quadrant, it was given a 60-second swim to find the platform, which was repeated for four days. On the fifth day, the platform was removed, and the animals were allowed to swim for 60 seconds. All these processes were recorded using a video monitoring system overlooking the pool. The recordings were then analysed using the Animal Tracker plugin for ImageJ software 1.53, and the time and distance spent in the target quadrant were measured.

Sample Collection: Following the second MWM test, animals were euthanized by decapitation under anaesthesia. The brains were removed and the right cerebral hemisphere and cerebellum were fixed in 10% formalin for one day and then trimmed to 0.5cm thickness. After routine tissue processing, they were embedded in paraffin. 4-6 micrometer thick sections were cut for routine histopathology and stained with Hematoxylin-Eosin (HE), Congo Red (CR) for amyloid, and Bielschowsky's Silver Stain (BSS). Neurons in the CA1 and CA3 areas of the hippocampus were counted using Nissl staining, and the thickness of these areas was measured. Amyloid deposition and tau proteins were assessed in the CR and BSS-stained preparations. The left-brain hemisphere was stored under the necessary preservation conditions for ELISA [8-OHdG, glutathione (GSH)] and the hippocampus for Western blot [AChE, ChAT, BACE1, TNFalpha, interleukin (IL)-10, α7nAChR, GSK3beta].

Protein isolation and Western Blot Analysis: Whole protein lysate was isolated from the tissue samples using T-PER™ Tissue Protein Extraction Reagent (Thermo, 78510). Halt™ Protease Inhibitor Cocktail (Thermo, 87785) was added to the lysis buffer at a ratio of 1/1000. The tissue samples were mechanically disrupted with the help of a homogenizer (Heidolph homogenizers, SilentCrusher, Germany) along with the lysis solution. Then, centrifugation was carried out at 4000xg for 15 minutes at 4°C, and the supernatant was transferred into a new Eppendorf and stored at -20°C until analysis. Equal amounts of samples (30µg of protein) were separated by electrophoresis on 5–10% SDS-PAGE gels (Bio-Rad, Mini-PROTEAN®Tetra Cell). SDS-PAGE gels were transferred to Immun-Blot® PVDF membrane (Bio-Rad, 1620177) using the Trans-Blot^{R} Turbo^{TM} transfer system (Bio-Rad, USA). PVDF membranes were blocked in 5% skim milk (Biobasic, NB0669) solution for 1h and incubated overnight at 4°C with antibodies: AChE (Proteintech, 17975-1-AP, 1:500), ChAT (Proteintech, 20747-1-AP, 1:500), BACE1 (Proteintech, 12807-1-AP, 1:500), GSK3beta (Proteintech, 22104-1-AP, 1:1000), α7nAChR (Elabscience, E-AB-12583, 1:1000), TNFalpha (Proteintech, 26405-1-AP, 1:500), IL-10 (Elabscience, E-AB-93269, 1:500). Primary antibody solutions were removed and incubated with HRP-conjugated secondary antibody (Elabscience, E-AB-1003, 1:5000). Blots were visualized with the ChemiDoc XRS+ Gel (Bio-Rad) imaging system using Clarity™ Western ECL Substrate (Bio-Rad, 1705060). Quantitative analysis was performed with Image Lab software, and results were expressed by normalizing to GAPDH (Elabscience, E-AB-40337, 1:3000). At least one control sample was loaded on each blot as a calibrator for quantitative analysis.

Measurement of oxidative stress: Levels of GSH (Catalogue no: EA0104Mo, Bioassay Technology Laboratory, China) and 8-OHdG (Catalogue no: EA0007Mo, Bioassay Technology Laboratory, China) in the left cerebral hemisphere were determined using the commercially available ELISA kits using an ELISA reader (Bio-Tek Instruments Inc., MWGt Lambda Scan 200).

Statistical Analysis: Since one animal from the ALZ+ACO group died, statistical analysis was performed with 6 animals in each group. The data obtained in the study were statistically analysed using ANOVA and Duncan post-hoc tests (SPSS version 22.0). A p value of <0.05 was considered statistically significant.

RESULTS

Weight Gain: At the end of the study, the animals' weight gains were determined to be 4.45, 4.48, 3.62, and 3.67g in the control, sham, ALZ, and ALZ+ACO groups, respectively (Fig. 1).

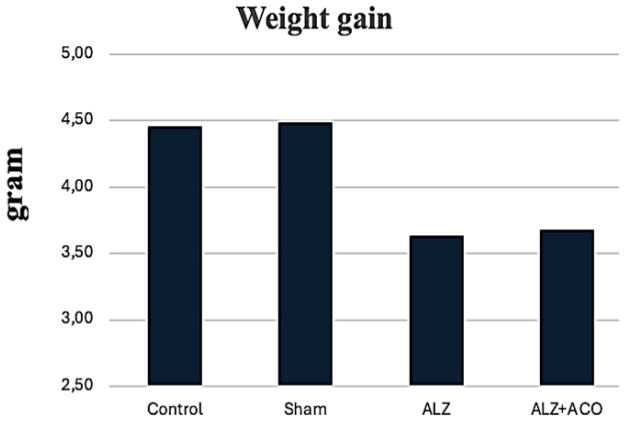

Fig. 1: Graphical representation of the weight gain of each group after weighing at the beginning and end of the experiment. ALZ; Alzheimer, ACO; acotiamide.

MWM test: The image of the MWM test performed on day 5 to investigate the animals' memory ability is presented in Fig. 2, and the values are presented in Fig. 3. According to the MWM test results, the ALZ group spent less time (P>0.05) and covered a shorter distance (P<0.05) compared to the other groups in the target quadrant. Acotiamide administration caused an increase in time (P>0.05) and distance (P<0.05) compared to the ALZ group in target quadrant.

Protein isolation and Western Blot analysis: In the current study, hippocampal AChE protein level in ALZ group was higher than in the control group, although there

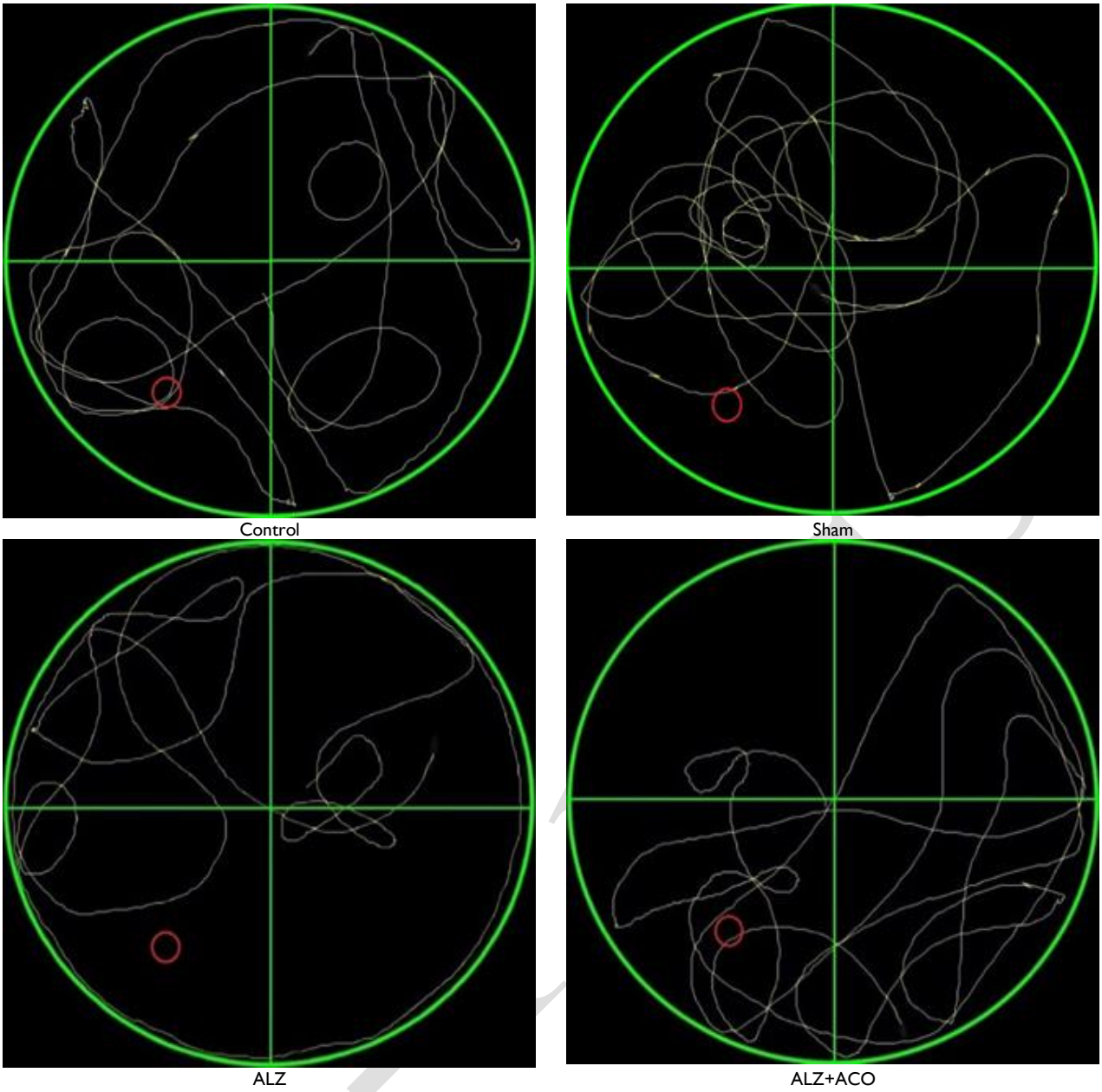

Fig. 2: At the end of the experiment, Morris Water Maze was made by removing the platform. The red circle represents the location of the removed platform in the target quadrant. ALZ; Alzheimer, ACO; acotiamide.

was no difference. Acotiamide administration prevented this increase ($P<0.05$, Figure 4B). No significant difference was found between the groups for ChAT and GSK3beta protein levels ($P>0.05$, Fig. 4C and 4E). BACE1 protein level was significantly higher in the sham and ALZ groups compared to the control and ALZ+ACO groups ($P<0.05$, Fig. 4D). ALZ caused a slight increase in α7nAChR protein level, while acotiamide administration caused a greater increase (Fig. 4F). TNFalpha protein level was the highest in the ALZ group, and acotiamide administration prevented this increase ($P<0.05$, Fig. 4G) while the IL-10 protein level was found to be the lowest in the ALZ group, it was determined to be the highest in the ALZ+ACO group ($P<0.05$, Fig. 4H).

Histopathological parameters: In the current study, no significant differences were observed in terms of degenerative and inflammatory changes in the brain. Furthermore, amyloid staining results were negative. While the number of neurons in the CA1 region decreased in the ALZ group ($P<0.05$), the ALZ+ACO group was found to be similar to the control group due to acotiamide administration reduced neuronal loss. In the CA3 region, all groups were statistically similar; with the fewest neurons in the ALZ group. Because acotiamide prevented neuronal loss in the CA1 and CA3 regions after ICV-STZ administration, the CA1/CA3 ratio was the highest in the ALZ+ACO group (Table 1). Furthermore, while the thickness in the CA1 region increased in the ALZ group, this increase was prevented in the ALZ+ACO group (Table 2, $P<0.05$). In the CA3 region, no statistical difference was found between the groups. The CA1/CA3 ratio showed a statistical difference; with the largest difference in the ALZ group (Table 2, $P<0.05$, Fig. 5).

Table 1: Effects of ICV-STZ and intraperitoneal acotiamide administration on neuron numbers in the CA1 and CA3 regions of the hippocampus (mean±SE).

	CA1	CA3	CA1/CA3
Control	346±16[a]	278±6	1.24±0.03[ab]
Sham	291±12[b]	286±11	1.02±0.05[b]
ALZ	299±12[b]	247±13	1.23±0.09[ab]
ALZ+ACO	327±14[ab]	255±23	1.35±0.16[a]

Table 2: Effects of ICV-STZ and intraperitoneal acotiamide administration on the thickness of hippocampus CA1 and CA3 regions (mean±SE).

	CA1	CA3	CA1/CA3
Control	88±0.34[b]	127±1.91	0.69±0.009[b]
Sham	90±0.29[a]	128±1.48	0.71±0.009[b]
ALZ	90±0.49[a]	124±0.77	0.73±0.007[a]
ALZ+ACO	89±0.37[b]	127±0.36	0.70±0.003[b]

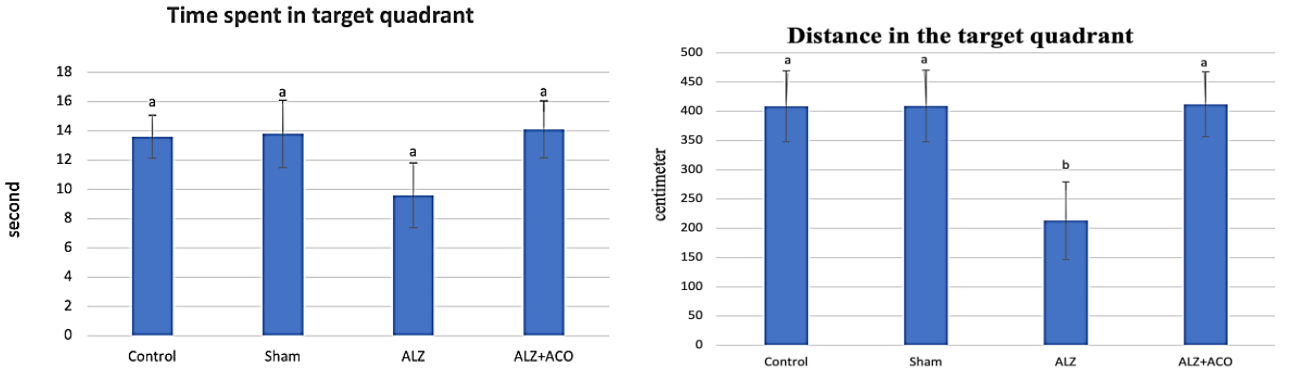

Fig. 3: Graphical representation of the time and distance spent in the target quadrant after the platform was removed. ALZ; Alzheimer, ACO; acotiamide.

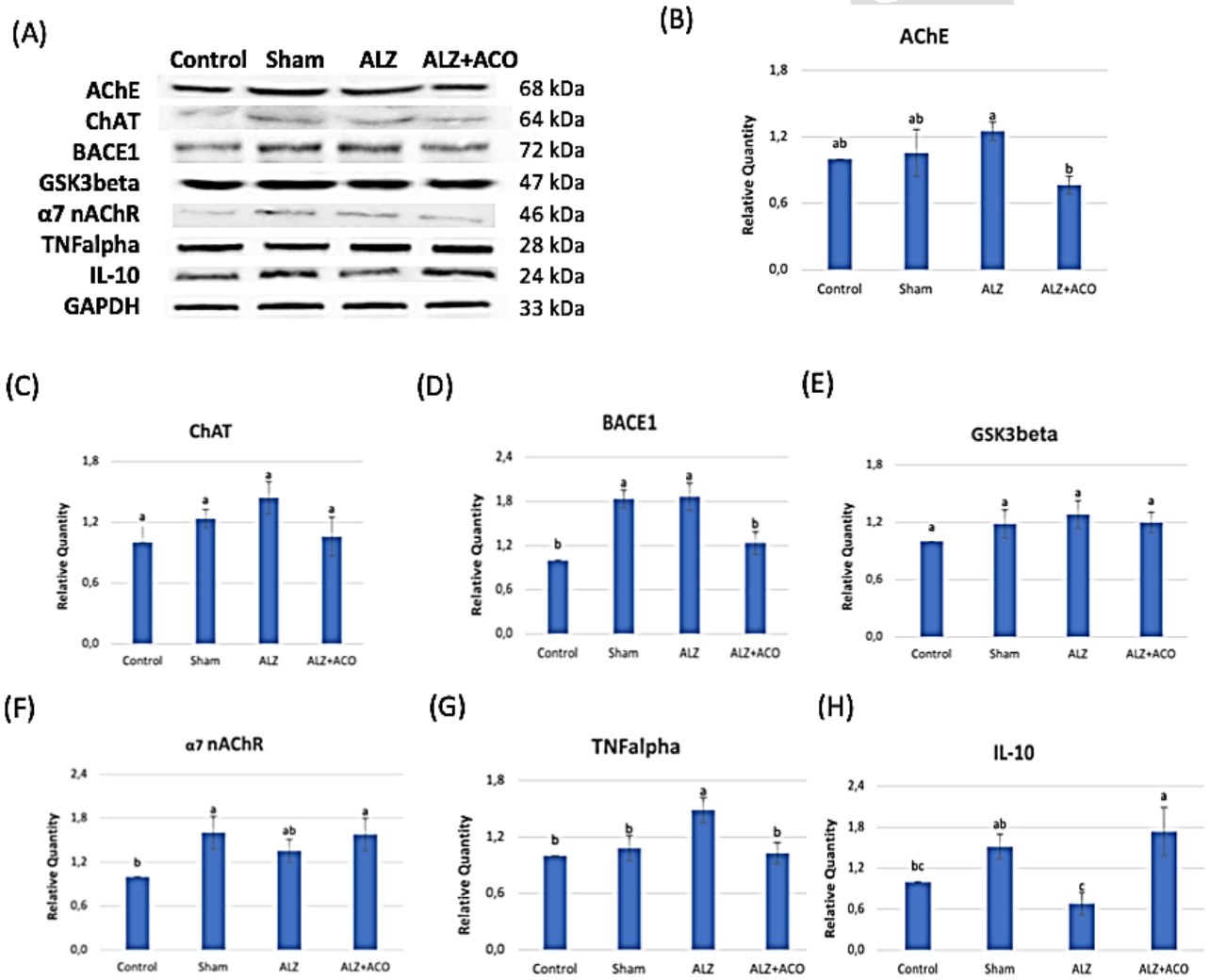

Fig. 4: Quantification of Western blot analyses. GAPDH was used as a loading control for normalization. (B–H) Densitometric quantification of normalized protein expression levels. (A) Representative Western blot images of hippocampal tissue samples showing the expression levels of (B) Acetylcholinesterase (AChE), (C) Choline acetyltransferase (ChAT), (D) β-secretase (BACE)1, (E) Glycogen synthase kinase (GSK3) beta, (F) α7nicotinic acetylcholine receptor (α7nAChR), (G) Tumor necrosis factor (TNF)alpha, and (H) Interleukin (IL)-10 protein levels across experimental groups. Data are normalized to GAPDH and presented relative to the Control group. Results are expressed as mean±SEM (n=6 per group). Statistical significance was determined using one-way ANOVA followed by Duncan post hoc test. P<0.05 was considered statistically significant.

Measurement of oxidative stress: Brain 8-OHdG and GSH levels are presented in Table 3. Compared with the control group, brain GSH levels increased in all groups, but this increase became statistical significance only in ALZ+ACO group. 8-OHdG level was similar in sham and ALZ groups when compared with the control group. Acotiamide administration resulted in a non-significant increase as compared to the ALZ group.

Table 3: Effects of ICV-STZ and intraperitoneal acotiamide administration on 8-OHdG and GSH levels (mean±SE).

	8-OHdG (ng/L)	GSH (mg/L)
Control	8.45±0.29^{b}	138±15.6^{b}
Sham	9.25±0.20^{ab}	166±10.9^{ab}
ALZ	8.85± 0.24^{ab}	170±9.80^{ab}
ALZ+ACO	9.41± 0.33^{a}	202±24.0^{a}

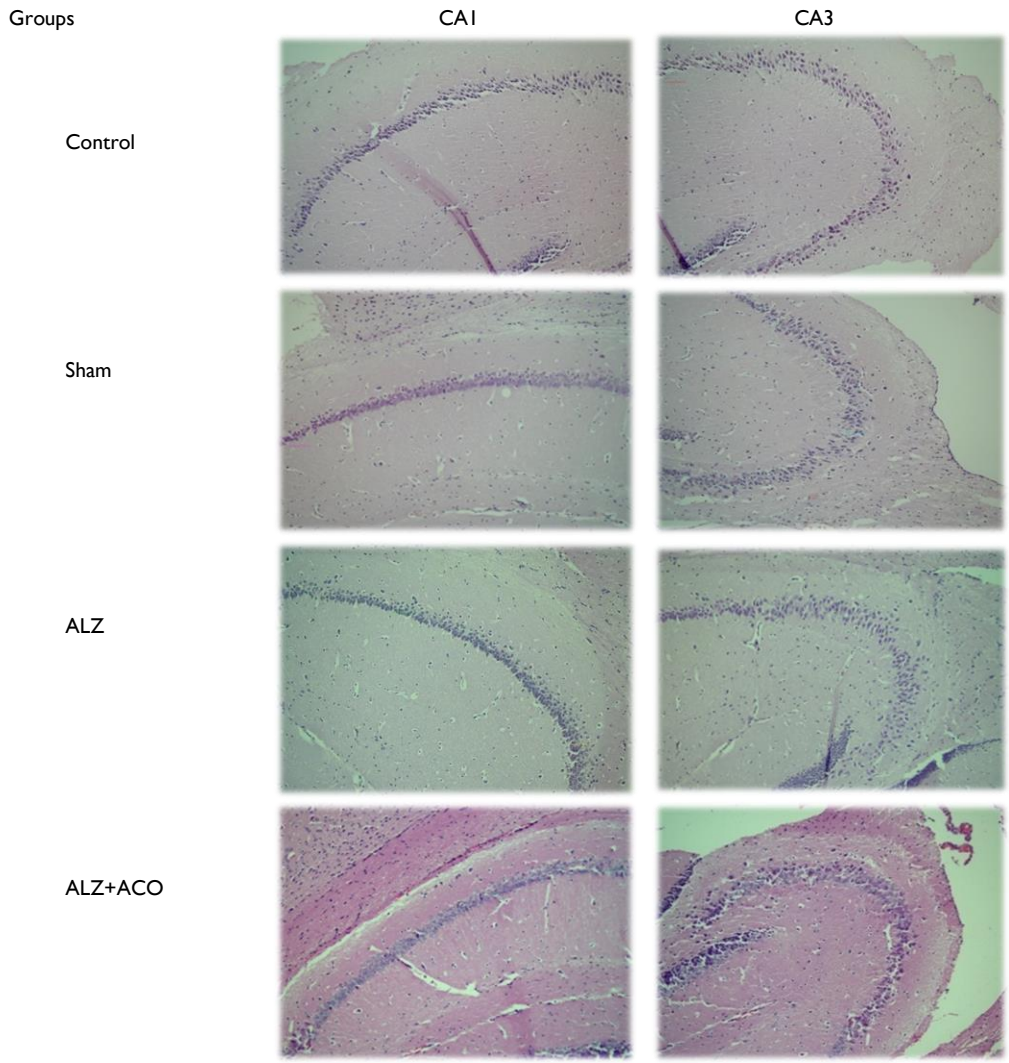

Fig. 5: Cell counts and layer thickness were measured in CA1 and CA3 regions. HE. X200 magnification.

DISCUSSION

AD is the most common form of dementia (Anonymous, 2022a). The pathophysiology of AD, a neurodegenerative disease of the CNS, is complex. Pathological events such as cholinergic dysfunction, oxidative stress, mitochondrial dysfunction, inflammation, immune dysregulation, vascular damage, Wnt pathway disruption, and calcium-mediated excitotoxicity are reported to play a role in the pathogenesis of AD. Neurodegeneration and synaptic dysfunction associated with these various pathologies cause behavioural, cognitive and functional decline in individuals (Salkovic-Petrisic *et al*., 2013; Inestrosa and Varela-Nallar, 2014; Tarawneh, 2020). Given the complexity of the disease, mice are frequently utilized in brain-related research to study these various mechanisms (Al-Gheffari *et al*., 2024; Wang *et al*., 2025).

Canine cognitive dysfunction (CCD), a neurodegenerative disease characterized by age-related cognitive impairments that parallel the behavioural symptoms of AD in dogs, has been reported in the veterinary field. This progressive neurodegenerative disease in animals exhibits behavioural changes as well as changes in cognitive abilities. In addition to behavioural symptoms, the neuropathology of both diseases is reported to be similar in several ways. Furthermore, cholinergic hypofunction is present in dogs, similar to AD in humans (Žnidaršicˇ *et al*., 2023) as in AD, AChE inhibitors can also be used for symptomatic treatment in CCD. In recent years, interest in the use of new AChE inhibitors in CCD has increased. While their use is limited, the potential for therapeutic use of these drugs in CCD is increasing as researchers develop new treatment methods to alleviate the symptoms of the neurodegenerative disorder in CCD. Acotiamide is an AChE inhibitor approved for the

treatment of functional dyspepsia in Japan (Shibli *et al*., 2020; Žnidaršicˇ *et al*., 2023).

The weight gains at the end of the study were determined to be 4.45, 4.48, 3.62, and 3.67g for the control, sham, ALZ, and ALZ+ACO groups, respectively. The weight loss observed in the experimental AD model animals in this study is consistent with the findings reported by Silva *et al*. (2023). The lack of weight gain in the ICV-STZ administration treated groups may be attributable to malnutrition resulting from brain inflammation.

Based on the MWM test results, the ALZ group spent less time ($P>0.05$) and covered a shorter distance ($P<0.05$) in the target quadrant compared to the other groups. Acotiamide administration caused an increase in both time ($P>0.05$) and distance ($P<0.05$) spent in the target quadrant compared to the ALZ group. These findings align with results reported in other studies (Go *et al*., 2022; Saleh *et al*., 2024; Chik *et al*., 2025). Spatial memory impairment is characterized by a delay in reaching the target quadrant and spending less time there (Rao and Bairy, 2015), so the shorter time recorded in the ALZ group likely indicates this impairment. Consequently, the observed increase in time and distance spent in the target quadrant following acotiamide treatment suggests an improvement in spatial memory.

In the current study, ICV-STZ administration caused a non-significant increase in hippocampal AChE protein levels ($P>0.05$), while acotiamide administration in the treatment group significantly attenuated this increase ($P<0.05$). In an experimental AD model in mice, increased AChE protein levels were reported to be reduced by Korean red pine (*Pinus densiflora*) bark extract (Go *et al*., 2022). Acotiamide, an AChEI, is approved for functional dyspepsia (Shibli *et al*., 2020). The drug is reported to have minimal CNS penetration. Therefore, although the likelihood of inhibiting AChE in the brain has been reported to be low (Anonymous 2022b), the amount of acotiamide that reaches the CNS is expected to inhibit central AChE. The decrease in AChE levels in the hippocampus following acotiamide administration in the current study also supports this expectation. This study is the first to demonstrate this effect of acotiamide in the hippocampus. As a result of the current study, it is predicted that acotiamide, used in dyspepsia due to its AChE inhibitor effect, may also have a therapeutic/protective effect for AD.

In the current study, ICV-STZ administration was determined to cause an increase in ChAT protein levels, similar to AChE ($P>0.05$) while acotiamide administration during treatment caused a slight decrease when compared with the ALZ group and this result was determined to be non-significant ($P>0.05$). In an experimental AD model study by Knezovic and Salkovic-Petrisic (2025), it was suggested that increased ChAT protein expression was a compensatory response to STZ-induced decreased activity. A similar result in this study could be due to a compensatory response, while the lack of an increase in ChAT protein levels, despite the desired increase in treatment, could be due to a balance in ACh levels.

In the current study, ICV-STZ administration caused a significant increase in BACE1 protein levels, while acotiamide administration significantly prevented this increase ($P<0.05$). In animals undergoing an experimental AD model, increased BACE1 protein levels have been reported to be reduced using mesenchymal stem cell-derived extracellular vesicles (Sha *et al*., 2021). Although histopathological features of AD were not observed in this study, the increased BACE1 levels in AD suggest that a long-term study is necessary to evaluate plaque formation. Furthermore, since acotiamide has been shown to reduce BACE1 protein level for the first time, it can be emphasized that it may be an important agent in the treatment/prevention of AD.

In the current study, ICV-STZ administration was found to cause an insignificant increase in GSK3beta protein levels, similar to ChAT ($P>0.05$) while acotiamide administration during treatment caused a slight decrease as compared to the ALZ group, no statistical change was observed ($P>0.05$). It has been reported that GSK3beta protein levels increased in an experimental AD model and decreased with treatment (Saleh *et al*., 2024). GSK3beta is a key member of the Wnt pathway. Disruption of Wnt canonical signalling plays a role in the pathogenesis of AD. Increased GSK3beta activity in AD increases the inactivation of the Wnt canonical pathway and the formation of neurofibrillary tangles. Conversely, activation of the Wnt canonical pathway suppresses GSK3beta and reduces AD pathology by protecting cells against Aβ and tau toxicity, resulting in neuroprotection (Hadi *et al.*, 2020). Although GSK3beta did not increase significantly in this study, the slight decrease caused by acotiamide suggests that the drug may be effective by preventing the inactivation of the Wnt canonical pathway.

In the current study, STZ administration caused a non-significant increase in α7nAChR protein levels ($P>0.05$). Acotiamide administration during treatment resulted in an increase compared to the ALZ group ($P>0.05$). In a study, quercetin significantly ameliorated the STZ-induced decrease in α7nAChR expression in STZ-exposed animals, demonstrating the α7nAChR-dependent mechanism of quercetin (Singh and Garabadu, 2021). α7nAChR has been shown to be important for growth, development, and aging, regulating neural circuit plasticity, neuronal differentiation, proliferation, apoptosis, and the clearance of senescent neurons (Fabiani and Antollini, 2019). In this study, acotiamide, which causes an increase in nicotinic receptors, may be important in the therapeutic/protective effect of AD.

The current study investigated neuroinflammation in an experimental AD model, finding that ICV-STZ administration caused increase in TNFalpha protein level ($P<0.05$) and a decrease in the IL-10 protein level ($P>0.05$). Crucially, acotiamide administration significantly prevented the TNFalpha increase ($P<0.05$) while causing a significant increase in IL-10 levels ($P<0.05$). This anti-neuroinflammatory action of acotiamide, characterized by reducing TNFalpha and boosting IL-10, mirrors the mechanism reported for other compounds like astaxanthin (Chik *et al*., 2025). Despite the unknown full molecular basis AD, evidence suggests that slow, long-term neuroinflammation, potentially triggered by Aβ or associated with aging (Meraz-Ríos *et al*., 2013), is a crucial driver of neuronal destruction and clinical symptoms, potentially being more central to the disease than amyloid plaque accumulation alone. The current study supports this inflammatory hypothesis by showing that acotiamide, a drug currently used for dyspepsia, may possess therapeutic

utility in AD by inhibiting this neuroinflammation, specifically resulting in a beneficial decrease in the proinflammatory TNFalpha level and an increase in the anti-inflammatory IL-10 level.

In the current study, no significant differences were observed in terms of degenerative and inflammatory changes in the brain, and amyloid staining yielded negative results. It can be concluded that longer-term model trials would be appropriate in future studies. While the number of neurons in the CA1 region decreased in the sham and ALZ groups ($P<0.05$), the ALZ+ACO group was found to be similar to the control group because acotiamide administration reduced neuronal loss. While no statistical difference was found in the number of neurons in the CA3 region between the groups, the lowest was found in the ALZ group. The CA1/CA3 value was highest in the ALZ+ACO group, but similar to the control and ALZ groups. In the experimental AD model, although there was no statistical difference in the CA1 and CA3 regions of the hippocampus, it was noted that cells decreased, and that these decreases were prevented by treatment (Chik *et al*., 2025). Furthermore, the thickness of the CA1 region increased in the ALZ group, while this increase was prevented in the ALZ+ACO group ($P<0.05$). Furthermore, a significant decrease in cell numbers and an increase in layer thickness were associated with edema due to inflammation. In the CA3 region, although there was no statistical difference between the groups, the CA1/CA3 ratio was highest in the ALZ group ($P<0.05$). Although there was no statistical difference in the CA3 region, a decrease in layer thickness was found in association with the decrease in cell number. The hippocampus is a brain region important for memory activity and functions. Numerous structural, morphological, and electrophysiological changes are observed in many neurodegenerative disorders such as AD. CA1 is essential for mediating temporal component associations and can preserve short-term memories, while CA3 is involved in processes related to the rapid formation of spatial or contextual memories. The CA3 and CA1 hippocampal areas respond differently to ischemic/hypoxic conditions. In patients with cerebral ischemia/hypoxia, CA1 pyramidal neurons are among the most vulnerable. In fact, because hippocampal CA1 circuits are fundamental to memory formation processes, the deterioration of CA1 neurons contributes to memory deficits in patients with hippocampal damage (Lana *et al*., 2020). In the current study, acotiamide reduced neuronal loss in the CA1 region, which is more susceptible to damage, and the high CA1/CA3 value, that is, the increased neuronal survival rate, may indicate that acotiamide may have neuroprotective effects against damage in the hippocampus. Furthermore, activation of the Wnt pathway resulting from GSK3beta inhibition may also play a role in reducing this neuronal loss.

Brain 8-OHdG and GSH levels are presented in Table 3. Compared to the control group, brain GSH levels increased in all ICV-treated groups, but this increase reached statistical significance only in the treatment group, the ALZ+ACO group. Acotiamide administration caused a statistically insignificant increase in 8-OHdG levels compared to the ALZ group. A study by Go *et al*. (2022) reported a decrease in GSH levels in the disease model and a prevention of this decrease in the treatment groups. In this study, it can be concluded that acotiamide's increase in GSH levels may be beneficial by alleviating oxidative stress.

In conclusion, although the molecular basis of AD, whose pathophysiology is complex, remains unknown, inflammation is crucial in the neurodegenerative process. Slow but steady inflammation occurring in the brain over long periods can destroy neurons and contribute to clinical symptoms. The current study demonstrates that acotiamide may be a potential agent for the treatment/prevention of CCD in dogs or AD due to its neuroprotective effects, including preventing neuronal loss, improving spatial memory, and inhibiting the increase in AChE, BACE1, and TNFalpha protein levels, as well as the decrease in IL-10 and α7nAChR protein levels. However, further research is needed on this topic.

Ethical statement: The procedure was approved by the Selcuk University Chair of Experimental Medicine Research and Application Center's Ethics Committee (Decision Number: 2022/10, Decision Date: 22.04.2022).

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