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Dose-dependent effects of vildagliptin (DPP-4 inhibitor) in a scopolamine-induced memory impairment model in rats

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A R T I C L E I N F O

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A B S T R A C T

Aims: Cognitive decline and dementia are closely linked with metabolic disorders such as type 2 diabetes mellitus, sharing common pathophysiological mechanisms including insulin resistance, inflammation, and oxidative stress. This study investigated the neuroprotective potential of vildagliptin, an antidiabetic medication, in a rat model of scopolamine-induced acute memory impairment, focusing on its effects on learning and memory as well as its association with cholinergic activity, inflammatory responses, and lipid peroxidation as an indicator of oxidative stress.

Materials and methods: Male Wistar Albino rats were orally administered vildagliptin (0.5, 5, or 50 mg/kg/day) or physiological saline for 4 weeks. Spatial learning and memory were assessed using the Morris water maze (MWM) test. Memory impairment was induced by intraperitoneal injection of scopolamine (1 mg/kg, *i.p.*) before the probe trial of the MWM. Following behavioral testing, rats were sacrificed, and hippocampal tissues were isolated for biochemical analysis.

Key findings: Vildagliptin significantly enhanced spatial learning performance in a dose-dependent manner during the acquisition phase of the MWM. Scopolamine administration markedly impaired memory performance in rats. Pretreatment with vildagliptin at all tested doses prevented these memory deficits during the MWM probe trial. In addition, vildagliptin robustly prevented scopolamine-induced increases in hippocampal acetylcholinesterase (AChE) activity and elevated levels of interleukin-1 beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and malondialdehyde (MDA).

Significance: These findings suggest that vildagliptin may exert protective effects against cognitive impairment by modulating cholinergic activity, inflammatory responses, and lipid peroxidation.

1. Introduction

Dementia has become a significant public health concern as the global population ages. The World Health Organization (WHO) estimates that over 55 million people worldwide currently have dementia. Around 10 million new cases are discovered each year [1]. Dementia is a clinical syndrome primarily characterized by progressive memory impairment, amnesia, and decline in other cognitive domains, ultimately leading to loss of functional independence. Alzheimer's disease accounts for approximately 60–80% of all dementia cases and is primarily defined by deficits in learning and memory [2].

Accumulating evidence indicates that dementia, including both Alzheimer's disease and vascular dementia, is closely associated with metabolic disorders such as type 2 diabetes mellitus. Epidemiological studies have demonstrated that individuals with diabetes have a 1.5–3-fold increased risk of developing dementia, particularly Alzheimer's disease and vascular dementia [3]. Furthermore, studies suggest that diabetes and Alzheimer's disease share common pathophysiological mechanisms, including insulin resistance, oxidative stress, and chronic inflammation, which has led some researchers to describe Alzheimer's disease as "type 3 diabetes" [4,5].

In recent years, growing interest has focused on investigating the potential effects of antidiabetic agents on dementia and cognitive function. Clinical studies indicate that antidiabetic treatments may improve cognitive performance in diabetic individuals, and that DPP-4 inhibitors are associated with greater improvements in cognitive test scores compared with metformin and sulfonylureas [6]. Vildagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, has been reported to preserve

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Mini-Mental State Examination (MMSE) scores in elderly diabetic patients with mild cognitive impairment [7]. Preclinical studies indicate that DPP-4 inhibitors reduce amyloid-β accumulation and tau phosphorylation, key features of the pathogenesis of memory impairment associated with Alzheimer's disease, and improve learning and memory performance [6].

Memory and learning processes rely heavily on cholinergic neurotransmission, with acetylcholine (ACh) acting as a key neurotransmitter. Degeneration or dysfunction of the basal forebrain cholinergic system (BFCS) leads to impaired ACh signaling, which is closely associated with memory impairment and amnesia, core features of dementia [8]. In this context, disruption of cholinergic signaling represents a widely used experimental approach to model cognitive dysfunction. Accordingly, scopolamine, a muscarinic acetylcholine receptor antagonist, is frequently employed in preclinical studies to induce memory impairment by transiently blocking cholinergic neurotransmission [9]. Furthermore, ACh signals through α7 nicotinic acetylcholine receptors that regulate the cholinergic anti-inflammatory pathway. Disruption of this signaling may facilitate the release of pro-inflammatory cytokines, thereby promoting oxidative stress and neuroinflammatory processes [10].

Clinical studies have indicated that the neuroprotective effects of vildagliptin reported to date have primarily been examined in the context of diabetes-related secondary cognitive impairment driven by chronic hyperglycemia, vascular dysfunction, and oxidative stress, rather than through direct assessment of its central nervous system actions [6]. In parallel, experimental studies in diabetic animal models have predominantly focused on diabetes-associated neurodegenerative changes [11,12]. Moreover, in intracerebroventricular Alzheimer's disease-like experimental models, the therapeutic effects of vildagliptin have mainly been evaluated with respect to abnormal amyloid-β accumulation and tau pathology [13]. However, despite these findings correlate diabetic and Alzheimer's disease-related conditions, the protective effects of vildagliptin on cognitive function in non-diabetic settings, particularly in models of cholinergic dysfunction, remain insufficiently explored. Given the critical role of the cholinergic system in learning and memory, the present study was designed to (1) evaluate the neuroprotective potential of vildagliptin in a scopolamine-induced memory impairment model, (2) to assess its effects on learning and memory performance, and (3) to investigate whether these effects are associated with modulation of cholinergic activity, inflammatory responses, and lipid peroxidation-related mechanisms.

2. Results

2.1. The effect of vildagliptin on the Morris water maze test (MWM)

In the MWM acquisition phase, rats treated with vildagliptin exhibited a dose-dependent improvement in learning performance, with the highest dose (50 mg/kg) producing a significant reduction in escape latency from day 3 onward (Day 3 $F(3,76) = 3.334$; Day 4 $F(3,76) = 4.364$; $p < 0.05$; Fig. 2A). Consistently, analysis of the area under the curve (AUC) revealed significant improvements at both 5 mg/kg and 50 mg/kg doses compared with the control group ($F(3,76) = 7.193$; $p <$ 0.01–0.001; Fig. 2B), that confirming the efficacy of vildagliptin in enhancing spatial learning.

In the subsequent probe trial, animals were divided into two subgroups: one received scopolamine (1 mg/kg, *i.p.*) to induce memory impairment. On the probe trial, scopolamine administration resulted in a marked reduction in the time spent in the target quadrant compared with the control group ($F(7,72) = 4.389$; $p < 0.01$). Remarkably, vildagliptin pretreatment effectively prevented the scopolamine-induced memory deficit, as evidenced by a significant increase in the time spent in the target quadrant ($p <$ 0.05–0.01; Fig. 2C). These behavioral findings strongly support the neuroprotective potential of vildagliptin against scopolamine-induced memory dysfunction.

2.2. Effects of vildagliptin on hippocampal acetylcholinesterase (AChE) activity

Scopolamine administration (1 mg/kg, *i.p.*) markedly increased hippocampal acetylcholinesterase (AChE) activity compared with the control group ($F(7,48) = 9.565$; $p < 0.001$; Fig. 3). Pretreatment with vildagliptin for four weeks significantly prevented this increase at all tested doses (0.5, 5, and 50 mg/kg/day, $p < 0.01$; Fig. 3). These findings indicate that vildagliptin pretreatment effectively counteracted the scopolamine-induced elevation in AChE activity, suggesting a potential protective role of vildagliptin on hippocampal cholinergic function.

2.3. The effect of vildagliptin on hippocampal inflammatory cytokine levels (TNF-α, IL-1β, and IL-6)

Scopolamine administration (1 mg/kg, *i.p.*) significantly increased hippocampal levels of the pro-inflammatory cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) compared with the control group ($F(7,48) = 6.934$; $F(7,48) = 28.53$ and $F(7,48) = 28.28$ respectively $p <$ 0.05–0.001 for all) (Fig. 4A–C). Pretreatment with vildagliptin at 0.5, 5, and 50 mg/kg/day (*p.o.*) for 4 weeks effectively prevented the scopolamine-induced increases in hippocampal IL-1β, IL-6, and TNF-α levels ($p <$ 0.01–0.001; Fig. 4A–C), maintaining cytokine levels close to those of the control group. These findings indicate that prophylactic administration of vildagliptin confers a protective effect against the development of scopolamine-induced neuroinflammatory responses.

Specifically, vildagliptin treatment at all relevant doses for four weeks significantly reduced IL-6 levels compared with the control group ($p < 0.01$), suggesting an intrinsic anti-inflammatory property of the medication even in the absence of any pathological condition (Fig. 4B).

Interestingly, vildagliptin treatment alone at 5 mg/kg/day caused a dose-independent paradoxical increase in TNF-α levels ($p < 0.001$), as neither the lower (0.5 mg/kg/day) nor the higher (50 mg/kg/day) doses showed a significant change compared with control (Fig. 4C).

2.4. The effect of vildagliptin on hippocampal MDA levels

Scopolamine administration significantly increased hippocampal malondialdehyde (MDA) levels compared with the control group ($F(7,48) = 7.957$; $p < 0.001$), indicating enhanced lipid peroxidation (Fig. 5). Pretreatment with vildagliptin at all relevant doses significantly prevented the scopolamine-induced increase in MDA levels ($p < 0.001$). These findings suggest a protective effect of vildagliptin against scopolamine-induced lipid peroxidation in the hippocampus.

3. Discussion

Cognitive decline and memory impairment are closely associated with alterations in cholinergic function, neuroinflammation, and lipid peroxidation. Impaired cognitive performance has been associated with reduced ACh availability and increased AChE activity, which breaks down ACh, leading to disruption of cholinergic neurotransmission [14]. In parallel, elevated levels of pro-inflammatory cytokines have been linked to learning and memory deficits, indicating a contributory role of neuroinflammatory mechanisms in cognitive decline [15]. In addition, increased malondialdehyde levels, reflecting enhanced lipid peroxidation, have been reported in association with memory impairment [16]. Together, these observations support a mechanistic relationship between cholinergic dysfunction, neuroinflammation, and lipid peroxidation in the development of cognitive decline and memory impairment.

In this context, the present study demonstrates that vildagliptin facilitates spatial learning and prevents scopolamine-induced memory impairment in rats. Scopolamine administration markedly increased hippocampal AChE activity, whereas vildagliptin pretreatment maintained AChE activity at levels comparable to controls. Moreover, vildagliptin pretreatment prevented increases in scopolamine-induced hippocampal IL-1β, IL-6, TNF-α, and MDA levels, indicating a protective effect against the development of neuroinflammatory responses and lipid peroxidation.

Spatial learning and memory were evaluated using the Morris Water Maze, a well-established paradigm for assessing hippocampal-dependent cognitive performance. During the acquisition phase of MWM, animals progressively reduce escape latencies as they learn the platform location, whereas the probe trial of the MWM assesses memory retention [17,18]. In the present study, Vildagliptin administration markedly enhanced spatial learning performance in a dose-dependent manner during the acquisition phase of the MWM in healthy rats. The group receiving 50 mg/kg exhibited a significant reduction in escape latency from the third training day onward, while AUC analysis confirmed improvement at both 5 and 50 mg/kg doses. These findings indicate that vildagliptin facilitates learning and cognitive adaptation.

Vildagliptin is an antidiabetic agent that increases endogenous GLP-1 levels by inhibiting DPP-4 [19]. Due to its limited blood-brain barrier permeability, the central effects of vildagliptin are generally thought to be mediated indirectly via enhanced GLP-1 signaling rather than direct brain penetration [12,20]. Notably, GLP-1 has been reported to exert multiple neuroprotective actions within the central nervous system by activating cyclic adenosine monophosphate/protein kinase A/cAMP response element-binding protein (cAMP/PKA/CREB), phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), and mitogen-activated protein kinase (MAPK) signaling pathways, thereby limiting neuronal apoptosis, suppressing neuroinflammation and lipid peroxidation, stabilizing the blood-brain barrier, and promoting neurogenesis and angiogenesis [21]. However, this raises the question of whether more efficient delivery of vildagliptin to the brain could further potentiate these protective effects. In support of this hypothesis, recent work has demonstrated that vildagliptin formulated as gold nanoparticles (VLD-AuNPs) exhibits enhanced brain bioavailability and reverses cognitive impairment at approximately half the conventional dose, underscoring the importance of central drug delivery for cognitive outcomes [12]. In this context, the more facilitation of spatial learning observed at the 50 mg/kg dose in the present study may reflect greater central engagement at higher doses. However, vildagliptin brain penetration was not directly measured in this study; therefore, this interpretation remains speculative.

Consistent with our findings, similar improvements in acquisition-phase performance have been reported in models of insulin resistance and high-fat diet-induced metabolic dysfunction, where vildagliptin administration shortened escape latency and normalized learning curves [22–24]. Likewise, in β-amyloid- and aluminum chloride-induced cognitive impairment models, vildagliptin treatment produced comparable enhancements in spatial learning performance [25–27]. Importantly, previous studies show that vildagliptin improves spatial learning under metabolic or neurotoxic challenges. Extending these findings, the present study demonstrates that vildagliptin enhances spatial learning and memory in healthy animals, potentially preventing the development of memory impairment.

After completion of the Morris Water Maze acquisition phase, the probe trial was conducted following scopolamine administration to assess the prophylactic potential of vildagliptin against acute cholinergic disruption. Scopolamine, a muscarinic acetylcholine receptor antagonist, is widely used to induce transient impairments in spatial learning and memory [28,29]. In the present study, scopolamine significantly impaired MWM probe trial performance, whereas vildagliptin pretreatment prevented these deficits across all doses. Comparable findings have been reported in other experimental models of memory impairment, including β-amyloid- and aluminum chloride ($AlCl_3$)-induced neurotoxicity, in which vildagliptin ameliorated spatial learning and memory deficits [25–27]. Similarly, in streptozotocin-induced diabetes [30], high-fat diet-induced insulin resistance [23], and cisplatin-induced neurotoxicity [31], vildagliptin improved Morris Water Maze performance by increasing target-quadrant time during the probe trial. Notably, in these studies, cognitive impairment was first established through metabolic or neurotoxic insults, and vildagliptin was subsequently administered as a therapeutic intervention to reverse or ameliorate existing deficits. In contrast, the present study was designed to evaluate the prophylactic potential of vildagliptin in preventing scopolamine-induced cholinergic disruption.

Overall, these behavioral findings suggest that vildagliptin provides dual benefits by facilitating spatial learning and memory under normal conditions and preserving memory performance against cholinergic dysfunction, as evidenced in the acquisition and probe phases, respectively.

Experimental evidence indicates that intraperitoneal administration of scopolamine increases AChE activity and concentration in the hippocampus and cerebral cortex [29,32]. Consistent with these findings, the present study showed a significant increase in hippocampal AChE activity ($p < 0.001$) following scopolamine administration. This increase was effectively prevented by vildagliptin pretreatment across all dose groups. In addition to this prophylactic approach, previous studies have reported normalization of AChE activity following vildagliptin treatment in pathological conditions, including streptozotocin-induced diabetic cognitive impairment [12] and intracerebroventricular amyloid-β_{1-40}-induced Alzheimer's disease models [33]. Similarly, in a cisplatin-induced neurotoxicity model, vildagliptin treatment significantly reduced elevated hippocampal AChE concentrations [31]. These findings indicate that vildagliptin preserves central cholinergic balance under both prophylactic and therapeutic conditions, supporting cholinergic integrity in the brain.

Neuroinflammation driven by activated microglia and pro-inflammatory cytokines contributes substantially to cognitive decline and memory impairment [34,35]. Experimental models of scopolamine-induced cognitive dysfunction produce not only deficits in spatial learning and memory but also marked elevations in pro-inflammatory cytokines [36–38]. In the present study, scopolamine administration significantly increased hippocampal IL-1β, IL-6, and TNF-α levels, whereas vildagliptin pretreatment at all doses prevented this increase. This observation suggests that the protective effect of vildagliptin on neuroinflammatory parameters may be linked to preserved acetylcholine signaling, which exerts anti-inflammatory actions through nicotinic α7 (nAChRs) by supporting cholinergic integrity [10].

In agreement with our findings, previous studies have shown that vildagliptin reduces neuroinflammatory responses in different experimental models of cognitive impairment. In streptozotocin-based neurodegeneration models, elevations in hippocampal IL-1β and TNF-α were significantly reversed following vildagliptin treatment [13,39]. Similarly, vildagliptin reduced TNF-α levels in streptozotocin-induced diabetic cognitive impairment and aluminum chloride-induced Alzheimer's disease models [11,27]. Data on the effects of vildagliptin on hippocampal IL-6 are limited; however, in diabetic rats, vildagliptin reversed hyperglycemia-induced increases in IL-6 and TNF-α levels in brain tissue [12]. Consistent with these findings, liraglutide, a GLP-1 analog, reduced hippocampal IL-6 and TNF-α expression in an intracerebroventricular palmitate-induced neuroinflammation model [40]. Collectively, these observations suggest that the increase in GLP-1 induced by vildagliptin may protect the brain against neuroinflammation and oxidative damage by suppressing or modulating pro-inflammatory pathways such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3), and Toll-like receptor/mitogen-activated protein kinase (TLR/MAPK) that are activated under pathological conditions [21].

Evidence regarding the effects of vildagliptin in healthy animals remains limited. In the present study, one-month vildagliptin treatment significantly reduced hippocampal IL-6 levels at both 5 and 50 mg/kg doses. Comparable findings reported with sitagliptin suggest that DPP-4 inhibition may suppress basal pro-inflammatory cytokine expression.

Table 1
The main groups, subgroups, and treatments.

Groups	Subgroups	n	Treatment for four weeks (*p.o.*)	Treatment before the probe trial (*i.p.*)
Group I	C	10	1 mL/kg/day PS	1 mL/kg PS
	S	10	1 mL/kg PS	1 mg/kg scopolamine
Group II	0.5 V	10	0.5 mg/kg/day vildagliptin	1 mL/kg PS
	0.5 V + S	10	0.5 mg/kg/day vildagliptin	1 mg/kg scopolamine
Group III	5 V	10	5 mg/kg/day vildagliptin	1 mL/kg PS
	5 V + S	10	5 mg/kg/day vildagliptin	1 mg/kg scopolamine
Group IV	50 V	10	50 mg/kg/day vildagliptin	1 mL/kg PS
	50 V + S	10	50 mg/kg/day vildagliptin	1 mg/kg scopolamine

C: control; V: vildagliptin; S: scopolamine; PS: physiological saline; *i.p.*: intraperitoneal; *p.o.*: peroral.

This effect may reflect an intrinsic immunoregulatory action mediated by suppression of CD26 (DPP-4)-dependent costimulatory signaling in T helper Th1 and Th17 cells [41]. Vildagliptin also tended to reduce IL-1β levels; however, this effect did not reach statistical significance, which may be attributable to the tight inflammasome-dependent regulation of IL-1β that typically requires pathological stimuli for activation [42]. In contrast, a mild but statistically significant increase in TNF-α was observed at the 5 mg/kg dose. TNF-α is a pleiotropic cytokine in the central nervous system, capable of exerting both neuroprotective and neurotoxic effects depending on its concentration and receptor subtype engagement [43]. Importantly, previous studies have demonstrated that neither TNF-α nor IL-1β alone induces neuronal toxicity, whereas combined elevation of both results in marked neuronal injury [44]. In this context, the absence of concomitant IL-1β activation despite the significant increase in TNF-α observed in the present study suggests that this isolated TNF-α elevation is more likely to reflect a regulatory immune adjustment rather than an active neuroinflammatory process.

Overall, vildagliptin pretreatment appears to exert an intrinsic anti-inflammatory effect in healthy rats primarily through the suppression of IL-6, whereas modulation of IL-1β, IL-6, and TNF-α becomes evident under pathological conditions.

Malondialdehyde (MDA) is a compound generated as a result of oxidative damage to polyunsaturated fatty acids and is one of the most widely used biomarkers of lipid peroxidation and oxidative stress in medical research. The brain's high oxygen consumption promotes lipid peroxidation and depletes antioxidant defenses, leading to the formation of reactive metabolites such as MDA [45]. Studies have shown that MDA concentrations are significantly higher in individuals with cognitive impairment than in healthy controls [16]. In line with previous reports, scopolamine administration in our study significantly elevated hippocampal MDA levels [46]. Pretreatment with vildagliptin at all three doses effectively prevented this increase, consistent with a prophylactic effect against lipid peroxidation. The prophylactic effect observed in the present study appears to be similar to the previously reported therapeutic effects of vildagliptin in diabetic, obese, and neurotoxic models, which were associated with reduced brain MDA levels and improved mitochondrial function [11,12,23,31,33].

Despite these findings, several limitations should be acknowledged. First, the assessment of oxidative stress was restricted to MDA levels as an index of lipid peroxidation; inclusion of additional antioxidant and redox-related parameters would have allowed a more comprehensive characterization of oxidative balance. Second, the molecular mechanisms underlying the observed behavioral and biochemical effects were not fully explored, particularly regarding intracellular signaling pathways and glial activation. Moreover, DPP-4 inhibitors have limited penetration into the central nervous system. In this study, the absence of direct measurements of dose-dependent central bioavailability of vildagliptin limits our ability to determine whether the observed effects are mediated by direct central actions, indirect peripheral mechanisms, or both. Therefore, further studies are needed to clarify dose-dependent central exposure. In addition, the exclusive use of male rats may limit the generalizability of the findings across sexes. Further studies addressing these limitations will help clarify the mechanisms underlying the present findings.

The present findings demonstrate that vildagliptin improves learning and memory performance in a scopolamine-induced model of acute cognitive impairment. In parallel with its behavioral effects, vildagliptin

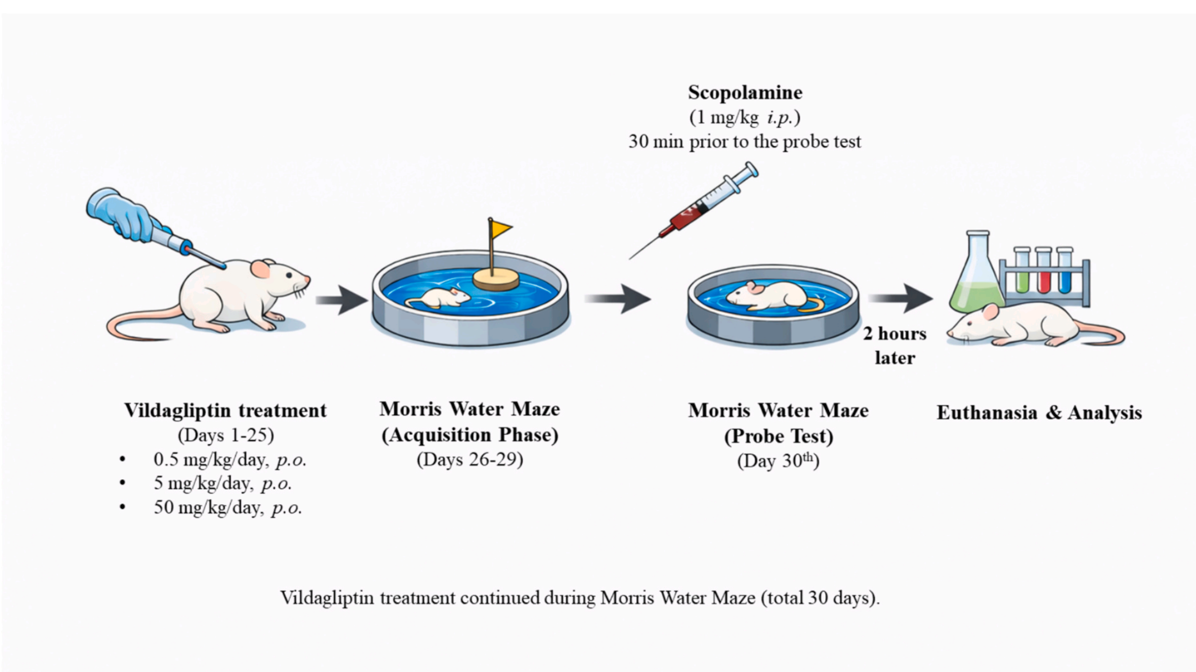

Fig. 1. Study design.

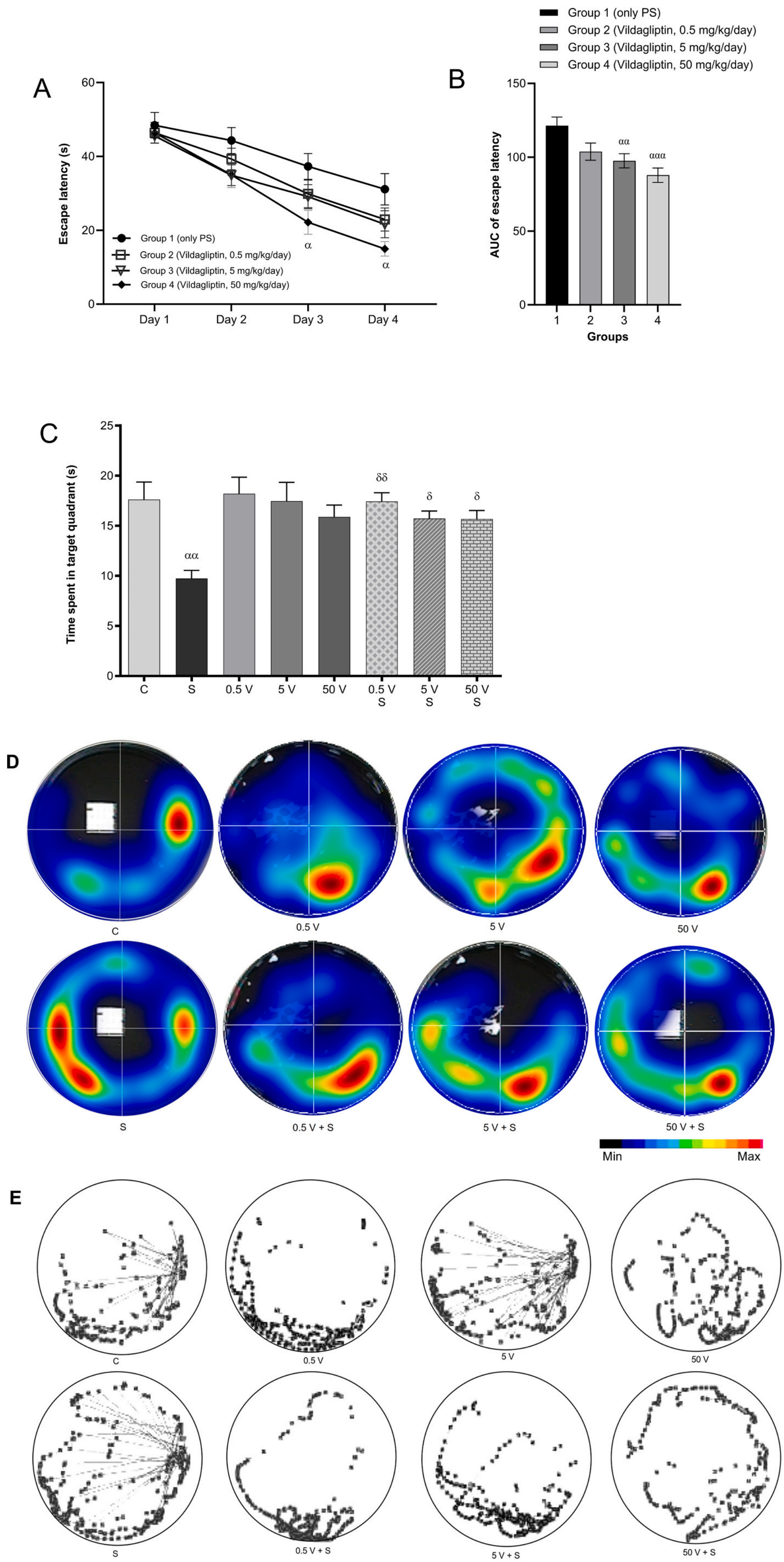

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Fig. 2. Effects of vildagliptin administration on spatial learning and memory performance in the Morris water maze (MWM) test. (A) Escape latency during the acquisition phase ($n = 20$), (B) Area under the curve (AUC) values for escape latency (n = 20), (C) Time spent in the target quadrant during the probe trial ($n = 10$). (D) Heat maps and (E) swimming trajectories of representative animals from each group during the probe trial.
Values are presented as mean ± SEM.
α: vs. control; δ: vs. scopolamine; $^{\alpha,\ \delta}p < 0.05$; $^{\alpha\alpha,\ \delta\delta}p < 0.01$; $^{\alpha\alpha\alpha}p < 0.001$; p values as indicated.
Statistical analysis was performed using one-way ANOVA.

attenuated neuroinflammatory responses and reduced hippocampal lipid peroxidation. These results suggest that vildagliptin may exert neuroprotective effects by preserving cholinergic signaling and limiting hippocampal lipid peroxidation and inflammation.

4. Materials and methods

4.1. Subjects

A total of 80 male Wistar Albino rats, aged three months and weighing 250–300 g, were used in this study. The animals were housed under a 12 h light/dark cycle at a controlled temperature of 22 ± 2 °C, with free access to standard chow and water. Before behavioral testing, all rats were acclimated to the laboratory environment for one week. Behavioral experiments were conducted between 09:00 and 12:00. The Local Ethics Committee approved all experimental procedures for Animal Experiments of Balıkesir University (Approval No: 2022/6-5; Date: 03.09.2022) and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

4.2. Drugs and experimental design

Vildagliptin (Novartis, Switzerland) and scopolamine hydrobromide (Sigma, USA) were freshly dissolved each day in sterile 0.9% physiological saline (PS), which served as the vehicle control. Vildagliptin solutions were prepared to ensure that each animal received a constant administration volume of 1 mL/kg per dose. Scopolamine was dissolved immediately before use to prevent degradation. All solutions were prepared under aseptic conditions.

A total of 80 male rats were randomly allocated to 4 experimental groups, with 20 rats per group. Animals received vildagliptin (0.5, 5, or 50 mg/kg/day) or saline (1 mL/kg/day) by oral gavage (*p.o.*) for four consecutive weeks.

The control group received only the vehicle (physiological saline, 0.9% NaCl) to match the volume and route of administration used in the treatment groups. All medications were administered at consistent times each day to minimize circadian effects. Each group was further divided into two subgroups ($n = 10$): one subgroup received intraperitoneal scopolamine (1 mg/kg, *i.p.*) to induce cognitive impairment, while the other received saline (*i.p.*) 30 min before the probe trial (Table 1). Two hours after completion of the behavioral tests, all rats were sacrificed, and hippocampal tissues were collected for biochemical analyses (Fig. 1).

4.3. Morris water maze (MWM) test

The MWM test was conducted in a circular pool (150 cm in diameter, 50 cm deep) filled with opaque water maintained at 25 ± 1 °C. The pool was divided into four equal quadrants, and a hidden platform (12 cm in diameter) was placed 1 cm below the water surface in the southeast quadrant.

Days 1–4 (Acquisition phase): Rats underwent three trials per day, starting from different quadrants. Each rat was allowed a maximum of 60 s to locate the hidden platform; those failing to find it within this time were guided to it and allowed to remain there for 20 s. The escape latency (time to reach the platform) was recorded as a measure of learning performance.

Day 5 (Probe trial): The platform was removed, and rats received either scopolamine (1 mg/kg, *i.p.*) or saline (*i.p.*) 30 min before the probe trial. Each rat was released from the center of the pool and

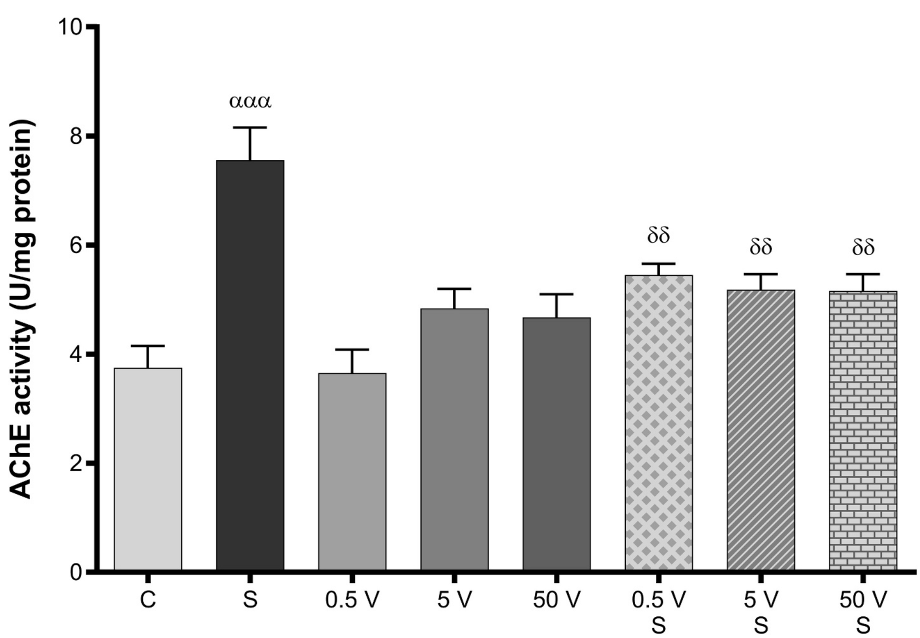

Fig. 3. Effects of vildagliptin administration on hippocampal acetylcholinesterase (AChE) activity (U/mg protein) in control and scopolamine-treated rats.
Values are presented as mean ± SEM ($n = 10$).
α: vs. control; δ: vs. scopolamine; $^{\alpha\alpha\alpha}p < 0.001$; $^{\delta\delta}p < 0.01$; p values as indicated.
Statistical analysis was performed using one-way ANOVA.

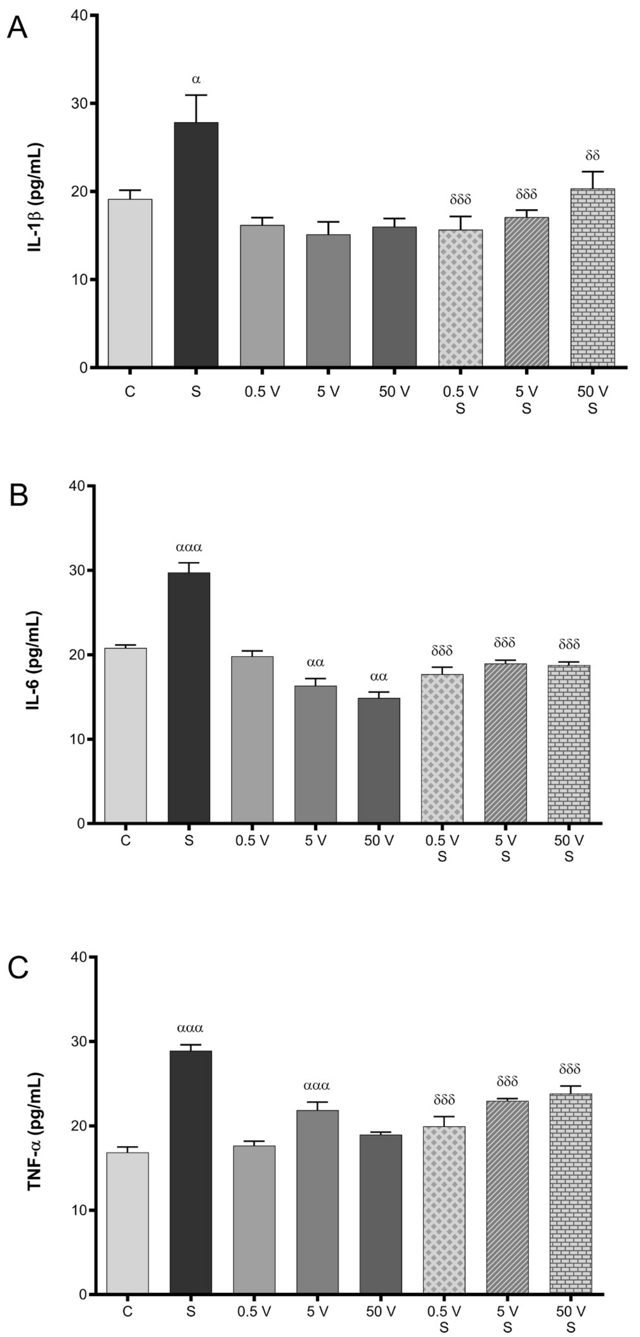

Fig. 4. Effects of vildagliptin administration on hippocampal inflammatory parameters in control and scopolamine-treated rats. (A) Tumor necrosis factor-α (TNF-α), (B) interleukin-1β (IL-1β), and (C) interleukin-6 (IL-6) levels (pg/mL). Values are presented as mean ± SEM ($n = 10$).
α: vs. control; δ: vs. scopolamine; $^{\alpha}p < 0.05$; $^{\alpha\alpha\alpha,\ \delta\delta\delta}p < 0.001$; $^{\delta\delta}p < 0.01$; p values as indicated. Statistical analysis was performed using one-way ANOVA.

allowed to swim freely for 60 s. The time spent in the target quadrant was recorded as an index of memory retention.

All trials were recorded by a video camera and analyzed using EthoVision XT v10 software (Noldus Information Technology, Wageningen, The Netherlands).

4.4. Sample preparation and biochemical analyses

Two hours after the completion of behavioral experiments, rats were sacrificed by decapitation. Brains were rapidly removed, and

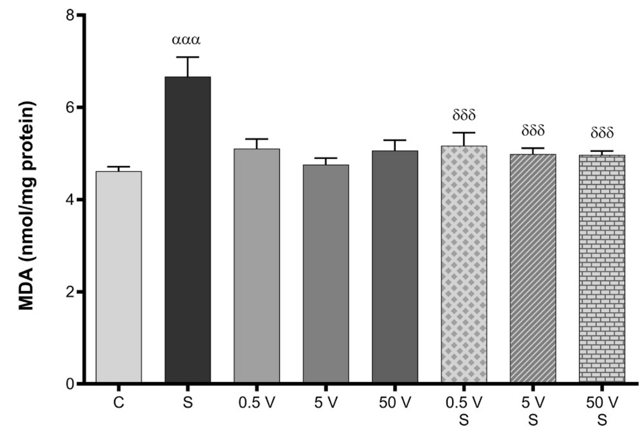

Fig. 5. Effects of vildagliptin administration on hippocampal malondialdehyde (MDA) levels (nmol/mg protein) in control and scopolamine-treated rats. Values are presented as mean ± SEM ($n = 10$).
α: vs. control; δ: vs. scopolamine; $^{\alpha\alpha\alpha}p < 0.001$; $^{\delta\delta\delta}p < 0.001$; p values as indicated.
Statistical analysis was performed using one-way ANOVA.

hippocampal tissues were dissected and stored at −80 °C until analysis. Biochemical parameters were analyzed using commercial assay kits, as follows:

Acetylcholinesterase (AChE) activity was measured using the AChE Activity Assay Kit (ELABSCIENCE, USA). The levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) were determined using Rat Interleukin-1β, Rat Interleukin-6, and Rat Tumor Necrosis Factor-α Enzyme-Linked Immunosorbent Assay (ELISA) Kits (FINETEST, China), respectively. Malondialdehyde (MDA) levels were measured with the MDA Colorimetric Assay Kit (ELABSCIENCE, USA). All biochemical analyses were performed by Atlas Biotechnology (Ankara, Türkiye) in accordance with the manufacturers' protocols.

4.5. Statistical analysis

Data were analyzed using Prism 10 software (GraphPad Software, Inc., San Diego, CA, USA), and results were expressed as the mean ± standard error of the mean (SEM). The acquisition phase, area under the curve (AUC) values, and probe trial parameters in the Morris water maze (MWM) were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc tests. The biochemical analyses were also evaluated using one-way ANOVA followed by Bonferroni post hoc tests. Differences were considered statistically significant at $p < 0.05$.

5. Conclusions

This study demonstrates that vildagliptin facilitates learning and spatial memory in healthy animals. In addition, vildagliptin pretreatment exerted neuroprotective effects against scopolamine-induced memory impairment. Vildagliptin prevented increases in scopolamine-induced hippocampal AChE activity as well as elevations in inflammatory cytokines and MDA levels. These findings suggest that the protective effects of vildagliptin on memory may be mediated through preservation of cholinergic signaling, limitation of lipid peroxidation, and attenuation of inflammatory responses. Taken together, vildagliptin may have potential as a prophylactic therapeutic approach in conditions characterized by memory deficits, although further studies are required to clarify its molecular mechanisms and long-term efficacy.

CRediT authorship contribution statement

Fazilet Şen Metin: Writing – original draft, Methodology, Investigation, Conceptualization. **Elif Aksöz:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

Institutional review board statement

The Local Ethics Committee approved the animal study protocol for Animal Experiments of Balıkesir University, Türkiye (Approval No: 2022/6-5; Date: 03 September 2022).

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Fazilet Sen Metin reports financial support was provided by Balıkesir University. Fazilet Sen Metin reports equipment, drugs, or supplies was provided by Novartis AG. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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