Research report

Thiazolidin-4-one prevents against memory deficits, increase in phosphorylated tau protein, oxidative damage and cholinergic dysfunction in Alzheimer disease model: Comparison with donepezil drug

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ABSTRACT

Alzheimer’s disease (AD) is characterized mostly by memory decline. The current therapeutic arsenal for treating AD is limited, and the available drugs only produce symptomatic benefits, but do not stop disease progression. The search for effective therapeutic alternatives with multitarget actions is therefore imperative. One such a potential alternative is thiazolidin-4-one, a compound that exhibits anti-amnesic, anticholinesterase, and antioxidant activities. The aim of this study was evaluated the effects of 2-(4-(methylthio)phenyl)-3-(3-(piperidin-1-yl)propyl) thiazolidin-4-one (DS12) on memory and neurochemical parameters in a model of AD induced by an intracerebroventricular injection of streptozotocin (STZ). Adult male rats were divided into five groups: I, control (saline); II, DS12 (10 mg/kg); III, STZ; IV, STZ + DS12 (10 mg/kg); V, STZ + donepezil (5 mg/kg). The rats were orally treated with DS12 and donepezil for a period of 20 days. Memory, acetylcholinesterase (AChE) activity, phosphorylated tau protein levels and oxidative stress were analyzed in the cerebral cortex, hippocampus, and cerebellum. Biochemical and hematological parameters were evaluated in the blood and serum. Memory impairment and the increase in AChE activity and phosphorylated tau protein level induced by STZ were prevented by DS12 and donepezil treatment. Streptozotocin induces an increase in reactive oxygen species levels and a decrease in catalase activity in the hippocampus, cerebral cortex, and cerebellum. DS12 treatment conferred protection from oxidative alterations in all brain structures. No changes were observed in serum biochemical parameters (glucose, triglycerides, cholesterol, uric acid, and urea) or hematological parameters, such as platelets, lymphocytes, hemoglobin, hematocrit, and total plasma protein. DS12 improved memory and neurochemical changes in an AD model and did not show toxic effects, suggesting the promising therapeutic potential of this compound.

1. Introduction

Alzheimer’s disease (AD) is the most common form of dementia and has become an ever-growing public health concern, with significant individual morbidity, mortality, and economic impact on the healthcare system (Dubois et al., 2016). AD is a highly debilitating neurodegenerative disease characterized by gradual decline in memory and other cognitive functions (Sereniki and Vital, 2008; Santos et al., 2020). Patients who suffer from AD progressively lose neurons and undergo several structural region-specific changes, these regions can include the...
The main neuropathological findings that characterize AD are the accumulation of senile plaques and neurofibrillary tangles consisting of hyperphosphorylated tau, as well as oxidative stress and cholinergic neuron dysfunction (Trevisan et al., 2019). Studies have indicated a synergistic relationship between these factors that affects the onset and progression of AD (Bitencourt et al., 2019; Dubois et al., 2016; Gemelli et al., 2013; López et al., 2012).

The cholinergic hypothesis was the first theory proposed to explain the pathogenesis of AD. The cholinergic system includes the neurotransmitter acetylcholine, enzymes that synthetize and degrade this neurotransmitter (choline acetyltransferase (ChAT) and acetylcholinesterase (AChE)) and muscarinic and nicotinic receptors (Ferreira-Vieira et al., 2016). This system plays a significant role in learning and memory and the loss of cholinergic neurons appear to be an important factor associated with memory deficits in AD (Ferreira-Vieira et al., 2016; Hampel et al., 2018). In addition, many alterations in cholinergic system also has been observed in AD. Thus, drugs that act on the cholinergic system is a target to treatment of patients with this neurodegenerative disease (Ferreira-Vieira et al., 2016; Hampel et al., 2018).

Concerning current treatment methods, the drugs used for AD treatment are the cholinesterase inhibitors galantamine, rivastigmine, and donepezil, and nementine, a (NMDA receptor antagonist). The
In this context considering the impact of AD on the lives of patients and public health, the lack of effectiveness of the currently available treatments in preventing disease progression, and the promising results obtained in brain studies with thiazolidin-4-ones; the aim of this study was therefore to evaluate the effects of 2-(4-(methylthio)phenoxy)– 3-(3-(piperidin-1-yl)propyl)thiazolidin-4-one (DS12) in an experimental model of AD. The effects of DS12 on memory, phosphorylated tau protein levels, oxidative stress parameters, and acetylcholinesterase activity in the brain structures were analyzed. Additionally, considering that there are few studies evaluating the therapeutic potential of the compound DS12 in vivo, here we also investigated the possible toxic effects of this compound on animals through the evaluation of hematological and biochemical parameters.

2. Material and methods

2.1. Chemicals

Streptozotocin (STZ), acetylhcyholine iodide (AcSCh), Coomassie Brilliant Blue G, dichlorodihydrofluorescein diacetate (DCFH-DA), and 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used in the detailed experiments were of analytical grade and had the highest purities. Compound 2-(4-(methylthio)phenoxy)– 3-(3-(piperidin-1-yl)propyl)thiazolidin-4-one (DS12) was synthesized in the Laboratory of Applied Chemistry to Bioactive at the Federal University of Pelotas according to the method described by Da Silva et al. (2016) and was accurately identified by gas chromatography-mass spectrometry (GC-MS).

2.2. Animals

Two-month-old adult male Wistar rats, weighing 300–350 g, were provided by the Central Animal House of the Federal University of Pelotas. The rats were kept in cages under a standard temperature (23 ± 1 °C), relative humidity (45–55 %), and lighting (12 h light/dark cycle) conditions. The rats had ad libitum access to standard rodent pelleted diet and water. All animal procedures were approved by the Ethics Committee of the Federal University of Pelotas (14002-2020).

2.3. Intracerebroventricular (icv) injection of streptozotocin (STZ)

The rats were anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg). After which, the head was positioned in the stereotaxic apparatus and a midline sagittal incision was made on the scalp. The stereotaxic coordinates for the lateral ventricle were measured accurately as anterior-posterior – 0.8 mm, lateral 1.5 mm and dorso-ventral – 4.0 mm relative to the bregma and ventral from the dura with the tooth bar set at 0 mm. Through the skull hole, a 28-gauge Hamilton® syringe of 10 μL attached to a stereotaxic apparatus, and the syringe piston was lowered manually into each lateral ventricle. STZ (3 mg/kg) was dissolved in citrate buffer (pH 4.5) and administered via bilateral icv injection (5 μL). STZ is a glucosamine nitrosourea compound that induces alterations in cerebral glucose metabolism. The dose of STZ used to induced sporadic dementia Alzheimer type was based in previous studies from literature (El Sayed and Ghoneum, 2020; Hindam et al., 2020). The group with the control rats received an icv injection with the same volume of citrate buffer. After stereotoxic surgery, the animals received painkiller (paracetamol solution 200 mg/ml) during three consecutive days. In addition, alterations such as motor activity, appearance (stopped posture, piloerection, ocular or nasal secretions), aggressive behavior, food and water consumption and stools were daily monitored.
2.4. Treatment with compound DS12 and donepezil

The rats were divided into five experimental groups (n = 10 animals each): I, control (C); II, DS12 (10 mg/kg); III, STZ; IV, STZ + DS12 (10 mg/kg); and V, STZ + donepezil (5 mg/kg). The rats in groups III, IV, and V received bilateral icv injections of STZ, whereas those in groups I and II received only citrate buffer. Seven days after the surgical procedure, the rats in groups II and IV were treated with DS12 (10 mg/kg), and the rats of group V were orally administered donepezil (5 mg/kg). DS12 was dissolved in canola oil and administered for 20 days (Fig. 1). Rats in groups I received only canola oil. DS12 and donepezil doses were chosen based on previous studies from literature (Saxena et al., 2008; Da Silva et al., 2021). Donepezil, an acetylcholinesterase inhibitor, was used in this study as a positive control because this drug is commonly used for the treatment of AD patients.

2.5. Behavioral evaluation

2.5.1. Open-field test

Locomotor behavioral tests were performed using an open-field apparatus, as described previously by Pacheco et al. (2018). The open-field test was performed in an apparatus consisting of a box with the floor of the arena divided into 16 equal squares (18 cm × 18 cm) and placed in a sound-free room. The rats were placed in the rear left square and allowed to explore freely for 5 min. The number of squares crossed with all the paws (crossing) was manually counted. The apparatus was cleaned with 40% alcohol solution and dried after each rat session. This test was performed to identify motor disabilities that might influence the other behavioral tests performed.

2.5.2. Object recognition

Twenty-four hours after the open-field test, which was also used for habituation to the apparatus, the rats underwent an object recognition test to evaluate memory. The task was performed on the 23rd day after STZ injection. The rats were individually placed in a box with two identical objects (objects A and B) for 5 min for free exploration (training). After 24 h, the rats were returned to the box for 5 min, and one of the previous objects (B) was replaced with a novel object (object C). The time spent exploring the new and familiar objects was recorded manually by blinded experimenters. The results were calculated according to the recognition index = TC / (TA + TC) (Pacheco et al., 2018).

2.5.3. Y-maze test

Spatial recognition memory was evaluated in a three-armed apparatus: the start arm, in which rats were placed to start to explore (always open); the novel arm, which was blocked during the training session, but open during the test session; and another arm (always open). During the training session, the rat was placed in the apparatus on the start arm and was free to explore only the start arm and the other arm for five minutes. The novel arm was blocked during the training session. After three hours, the test session was performed with the novel arm open, and the rat could freely explore all three arms over a five-minute period. After each session, the apparatus was cleaned using 40% ethanol. Blinded experimenters manually determined the time spent in each arm, and the results were expressed as the number of entries and time spent in each arm (Teixeira et al., 2020). Thereafter, the rats were anesthetized with isofluorane and euthanized. Blood was obtained from the animals through cardiac puncture and brain structures (cerebral cortex, hippocampus, and cerebellum) were collected for biochemical analysis. Brain tissues were prepared and protein determination was performed according to each specific technique.

2.6. Acetylcholinesterase (AChE) activity

The following brain structures were homogenized on ice in a glass potter with 10 mM Tris-HCl solution (pH 7.4): cerebral cortex, hippocampus, and cerebellum. Protein content was determined using the
Coomassie blue method, with bovine serum albumin as the standard. AChE enzymatic assay was performed as previously described by Ellman et al. (1961). The reaction system, comprising 10 mM DTNB, 100 mM phosphate buffer (pH 7.5), and the enzyme (40–50 µg of protein), was pre-incubated for 2 min. The reaction was then initiated by adding 0.8 mM AcSCh, and the absorbance was read on a spectrophotometer at 412 nm. All samples were tested in duplicate and enzyme activity was expressed in µmol AcSCh/h/mg of protein.

2.7. Oxidative stress parameters

The hippocampus, cerebral cortex, cerebellum, liver, and kidneys were homogenized in 10 volumes (1:10 w/v) of sodium phosphate buffer (pH 7.4) containing KCl. Homogenates were centrifuged at 3500 rpm for 10 min at 4 °C. The pellet was discarded, and the supernatant was used for measurements. Protein content was determined using the method described by Lowry et al. (1951), with bovine serum albumin as the standard solution.

2.7.1. Reactive oxygen species (ROS) assay

ROS formation was determined as previously described by Ali et al. (1992). In this assay, the oxidation of DCFH-DA to DCF (fluorescent 2', 7'-dichlorofluorescin) was measured to detect intracellular reactive species. First, 5 µL of homogenate, 190 µL of Tris-HCl buffer and 10 µL of 1 mM DCF was incubated in the dark at 37 °C. Posteriorly, DCF fluorescence intensity emission was recorded at 525 and 488 nm excitation 30 min after addition of DCFH-DA to the medium. The results are expressed as µmol DCF per mg of protein.

2.7.2. Total sulfhydryl content assay

Total sulfhydryl content was determined according to the method described by Aksenov and Markesbery (2011). Briefly, 10 µL of sample was mixed with 145 µL of PBS–EDTA (1 mM), pH 7.5. For the reaction, 10 µL of DTNB (10 mM) in PBS was added to the mixture. After 1 h of incubation at room temperature in the dark, the absorbance was read spectrophotometrically at 412 nm, and the results were expressed as nmol TNB/mg of protein.

2.7.3. Superoxide dismutase (SOD) activity

The SOD activity was measured using the method described by Misra and Fridovich (1972). This assay is based on the inhibition of superoxide-dependent adrenaline auto-oxidation of adenochrome. For this assay, the medium contained catalase (10 µM), 10 µL of sample, glycine buffer (50 mM, pH 10.2) and adrenaline (60 mM). The intermediate in this reaction is superoxide, which is scavenged by SOD and measured using a spectrophotometer adjusted to 480 nm. One unit of SOD was defined as the amount of enzyme necessary to inhibit 50% of adrenaline autoxidation. The specific activity of SOD was reported in units per mg of protein.
comparisons test was used. Values are expressed as mean (±SEM) of each group).

Table 1

|                 | Control | DS12  | STZ   | STZ + DON |
|-----------------|---------|-------|-------|-----------|
| Glucose (mg/dL) | 179.42  | 237.27| 206.79| 206.94    |
| ± 43.98         | ± 43.34 | ± 40.22| ± 26.09| ± 25.21  |
| Total (mg/dL)   | 97.84   | 125.54| 101.95| 113.96   |
| ± 30.16         | ± 24.52 | ± 24.91| ± 17.16| ± 15.22  |
| Cholesterol (mg/dL) | ± 54.80 | ± 50.05| ± 31.23| ± 73.66  |
| ± 5.62          | ± 6.62  | ± 6.62 | ± 6.62 | ± 6.62   |
| Triglycerides   | ± 155.22| ± 54.80| ± 44.34| ± 35.15  |
| ± 104.58        | ± 66.41 | ± 66.41| ± 66.41| ± 66.41  |
| Urea (mg/dL)    | ± 43.98 | ± 43.34| ± 40.22| ± 26.09  |
| ± 24.52         | ± 24.91 | ± 17.16| ± 15.22| ± 15.22  |
| ± 156.41        | ± 104.58| ± 113.96| ± 131.92| ± 131.92 |

2.7.4. Catalase (CAT) activity

The CAT activity was evaluated as described by Aebi (1984). For this analysis, 10 µL of sample was mixed with Triton X-100 (1:10 w/v) and potassium phosphate buffer (pH 7.0). The reaction was started with the addition of 30 mM of hydrogen peroxide (H₂O₂) in 50 mM potassium phosphate buffer (pH 7.0) in the reaction medium. This method is based on the ability of the CAT to decompose H₂O₂ and is measured by the decrease in absorbance at 240 nm. One unit of CAT was defined as one µmol of H₂O₂ consumed per minute, and the specific activity was expressed as units/mg of protein.

2.8. Phosphorylated tau protein

For in vitro study, the level of Phosphorylated tau protein was measured in brain tissue using ELISA Kit employing specific monoclonal antibodies for rat pMAPT/pTAU (Elabscience catalog No: E-EL-R1090,

USA), according to the manufacturer’s protocols. Calibrated curves were prepared in each plate employing a reference standard, performed in triplicate. The absorbance of the reactions was measured at 450 nm using SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA) and calibration curves fitted a linear regression with a correlation higher than 0.95 and a p value < 0.05 %. Samples were calculated by comparing the OD of the samples to the standard curve and results were expressed as phosphorylated tau protein (pg/ml).

2.9. Biochemical parameters

Serum glucose, cholesterol, triglyceride, and urea levels were determined using commercially available diagnostic kits supplied by Bioclin® (Bioclin MG, Brazil). Glucose (Bioclin catalog No: K082–2), cholesterol (Bioclin catalog No: K083–2), triglycerides (Bioclin, catalog No: K117–1) and urea kit (Bioclin catalog No: K047–1).

2.10. Hematological parameters

Hematological parameters were measured at the Clinical Analysis Laboratory of HCV – UFPel. Erythrocyte counts and hemoglobin concentrations were determined using an automated counter (PocH-101iV). Hematocrit was determined using a micro-hematocrit centrifuge rotating at 19,720g for 5 min. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined by indirect calculations.

2.11. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test for comparison of means using GraphPad Prism version 5.0 Program (Intuitive Software for Science, São Diego, CA, USA). Differences with P < 0.05 were considered statistically significant in the analysis. All data are expressed as mean ± standard error (SEM).

3. Results

3.1. DS12 prevents memory deficits induced by STZ

The results of the behavioral tests are shown in Fig. 2. First, no significant changes were observed in locomotor activity in the open field test in any experimental group compared to the control group (P > 0.05), which excludes the possibility of interference of locomotor deficits in memory tasks. In the object recognition task, the percentage of exploratory preference for the new object in the STZ group was significantly lower than that in the control group (P < 0.05), indicating memory impairment. Treatment with DS12 (10 mg/kg), as well as treatment with donepezil (5 mg/kg), increased the percentage of exploratory preference of the new object when compared to the STZ group (P < 0.001 for DS12 and P < 0.01 for donepezil), demonstrating that DS12 is able to restore memory deficits induced by this experimental model (Fig. 2B). In the Y-maze test, STZ also decreased the time spent (P < 0.01, Fig. 2C) and the number of entries (P < 0.001; Fig. 2D) on the new arm. Treatment with DS12 (10 mg/kg) effectively attenuated the spatial memory deficits, showing results similar to those of standard donepezil (5 mg/kg).

3.2. DS12 prevented AchE activity alterations induced by STZ in cerebral cortex and hippocampus

As shown in Fig. 3, STZ induced a significant increase in AchE activity in the cerebral cortex and hippocampus (P < 0.05), and DS12 (10 mg/kg) and donepezil (5 mg/kg) treatment prevented this enzyme alteration (P < 0.001). Regarding AchE activity in the cerebellum, no significant differences were observed in rats in the STZ group compared to the control group.
to the control (Fig. 3). However, treatment with donepezil caused a decrease in enzyme activity compared to that in the STZ group (P < 0.001). DS12 alone or in combination with STZ treatment did not alter AChE activity in the cerebellum (P > 0.05, Fig. 3).

3.3. DS12 prevents oxidative damage induced by STZ administration

In relation to oxidative stress parameters, our results showed an increase in ROS levels in the hippocampus, cerebral cortex, and cerebellum of rats in the STZ group when compared to the control group (Fig. 4A, B, and C, P < 0.05). Treatment with DS12 (10 mg/kg) prevented the increase in ROS levels in all evaluated brain structures (P < 0.05). Our findings also showed a decrease in ROS levels in the brains of rats treated with donepezil (5 mg/kg) (Fig. 5A, B, and C, P < 0.05). DS12 per se did not alter ROS levels under these experimental conditions. Additionally, no significant changes were observed in SH levels in the hippocampus, cerebral cortex, and cerebellum in any of the groups evaluated (Fig. 4 D, E, and F, P > 0.05).

The results of antioxidant enzyme activities are shown in Figs. 5 and 6. SOD activity in the hippocampus was increased in the STZ group (P < 0.05) compared to that in the control group. Neither DS12 nor donepezil treatments were capable of preventing SOD enzyme alterations in the hippocampus (Fig. 6). In contrast, an increase in SOD activity was observed only in STZ animals treated with DS12 and donepezil in the cerebral cortex (Fig. 5). No significant changes were observed in the SOD activity in the cerebellum of any of the groups evaluated (Fig. 5, P > 0.05). A decrease in CAT activity was observed in the STZ group in the hippocampus and cerebral cortex of STZ group when compared to the control group (Fig. 6 P < 0.05). However, DS12 and donepezil treatments were effective in preventing alterations in brain antioxidant enzymes in the hippocampus and cerebral cortex when compared to the STZ group (Fig. 6). In addition, no significant changes were observed in catalase activity in the cerebellum of any of the groups evaluated (Fig. 6).

3.4. DS12 decreased the phosphorylated tau protein levels in cerebral cortex

Level of phosphorylated tau protein was found to be significantly elevated (P < 0.01) in STZ group as compared to the control group in both hippocampus and cerebral cortex. However, the treatment with DS12 and donepezil prevented these alterations only in cerebral cortex (Fig. 7).

3.5. DS12 treatment did not alter biochemical and hematological parameters

The results of the biochemical analyses are presented in Table 1. Regarding biochemical parameters, no significant changes were observed in glucose, triglycerides, cholesterol, and urea, and in any of the groups evaluated in this study (P > 0.05). With regard to hematological parameters, neither STZ nor STZ associated with DS12 or donepezil treatment induced significant changes in the following counts: platelets, lymphocytes, monocytes, basophils, eosinophils, red blood cells, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean cellular hemoglobin concentration (CHCM), total plasma protein, fibrinogen, and leukocytes (Table 2).

4. Discussion

AD, a progressive neurodegenerative disorder, can be divided in familial and sporadic cases based on age onset and genetic predisposition. Familial AD is characterized by early onset dementia (< 65 years) due to mutations in the amyloid precursor protein and presenilin genes (Dorszewska et al., 2016). Most patients with AD (>95 %) have the sporadic form, which is characterized by a late onset (after the age of 65 years). Although the cause of sporadic form of the disease is unknown, has been suggested that many genetic and environmental factors may contribute to development of this AD form (Knoepman et al., 2021).

Intracerebroventricular injection of STZ in rodents has been shown to produce dysfunctions in cholinergic signaling, neuroinflammation, oxidative stress, hyperphosphorylation of tau protein, insulin resistance, and changes in glucose metabolism, similar to those described in the brains of AD patients. In this regard, STZ-induced sporadic dementia Alzheimer type is considered a valid experimental model for the study of early pathophysiological changes in this neurodegenerative disease (Kamat, 2015).

Here, we showed that brain injection of STZ induced memory impairment in rats, which were observed in behavioral tasks, such as object recognition and the y-maze. Although desensitization of the cerebral insulin receptor and metabolic abnormalities in cerebral glucose are considered the main causes of STZ-induced memory impairment (Henneberg and Hoyer, 1995); studies from research group have shown that other mechanisms, such as oxidative stress, alterations in genes and activities of cholinergic enzymes, disturbances in ion pump activities, neurotrophic factors, and adenosinergic signaling also contribute to memory deficits induced by STZ (Gutierrez et al., 2014; Pacheco et al., 2018; Teixeira et al., 2020; 2022).

Our results showed that DS12 treatment improved memory similar to donepezil. In another study, we also demonstrated that previous administration of DS12 (5 and 10 mg/kg) for seven days was capable of
preventing the amnesic effects induced by scopolamine in inhibitory avoidance tasks, which can be associated with the anticholinesterase action of this compound (Da Silva et al., 2021).

The association between cholinergeric signaling dysfunction and memory decline in patients with AD has been described in the literature (Chen et al., 2022). Similar to other studies that used the STZ model for induced sporadic dementia of the Alzheimer type (Pacheco et al., 2018); here we also demonstrated an increase in AChE activity in the cerebral cortex and hippocampus. AChE is responsible for rapid degradation of the neurotransmitter acetylcholine into acetate and choline (Chen et al., 2022). Considering that acetylcholine is involved in mechanisms associated with memory and learning, an increase in AChE activity may lead to a decrease in neurotransmitter levels in the brain, contributing to memory deficits. Additionally, the presence of AChE has been associated with increased neurotoxicity of amyloid components (Talesa, 2021). Based on this, the most successful therapeutic strategies for AD treatment are drugs capable of inhibiting AChE activity, such as donepezil (Maruccia et al., 2021). Donepezil is considered the first-line treatment for patients with mild to moderate AD and is a highly selective reversible AChE inhibitor that exhibits a good pharmacological profile in relation to cognitive improvement (Jia et al., 2020; Maruccia et al., 2021). However, adverse effects have also been associated with its use (Dun et al., 2020).

Here we showed that DS12 prevented alterations in AChE activity in a manner similar to donepezil. Data from literature using molecular docking analysis have suggested a good overlap of thiazolidinones with the active site of AChE, similar to the neurotransmitter acetylcholine and donepezil drug (Da Silva et al., 2020). In fact, we recently demonstrated that DS12 is a mixed inhibitor capable of inhibiting in vitro the activity of AChE total as well as the isoforms G1 and G4 of the cerebral cortex and hippocampus (Da Silva et al., 2020). Moreover, DS12 (5 and 10 mg/kg) administered orally for seven days also improved memory and prevented alterations in AChE activity induced by scopolamine in the cerebral cortex, hippocampus, and lymphocytes (Da Silva et al., 2021). Taken together, these findings suggest that the anticholinesterase action of DS12 is an important mechanism associated with the improved memory observed in other studies, as well as in the model of memory dysfunction used here.

Furthermore, DS12 was able to prevent alterations in oxidative stress in brain structures. It is well established that the administration of STZ causes oxidative damage in the brain (Huang et al., 2016; Kheradmand et al., 2018; Pacheco et al., 2018; Teixeira et al., 2020). Oxidative stress is considered an important mechanism in AD development and progression because it is associated with mitochondrial dysfunction, neuroinflammation, Aβ accumulation, and tau hyperphosphorylation, leading to subsequent loss of synapses and neurons and memory deficits (Dias-Santagata et al., 2007; Chen and Zhong, 2014). Thus, compounds with antioxidant activities may be useful for AD treatment.

The antioxidant potential of thiazolidin-4-one derivatives has been reported previously (El Nezhawy et al., 2009; Apostrosoaei et al., 2014). Lopez and colleagues showed that 3-(3-(dietilamino)propil)-2-(4-(metiltio)fenil)thiazolidin-4-ona prevented the increase in ROS, nitrite, and IL-6 levels, and alterations in antioxidant enzymes in astrocyte cultures exposed to lipopolysaccharide (Lopez et al., 2022). Most importantly, DS12 also prevented the increase in ROS levels and decrease in superoxide dismutase activity in the cerebral cortex and hippocampus in a model of scopolamine-induced amnesia (Da Silva et al., 2021). Although it is not possible to determine the exact mechanism involved in the antioxidant activity of DS12, we suggest that the increase in superoxide dismutase and catalase activities by this compound can contribute to the clearance of free radicals, preserving brain function and memory.

The antioxidant effects of DS12 are similar to those of donepezil. Previous studies demonstrated that donepezil has in vitro antioxidant effects with radical scavenging activity similar to that of vitamin C (Munishamappa et al., 2019) capable also to inhibiting quinonilic acid and Fe⁺ induced lipid peroxidation in the rat brain (Oboh et al., 2017). Acetylcholine has been shown to reduce ROS levels, attenuate cell apoptosis and mitochondrial dysfunction, and enhance the protein expression and activity of SOD1 and SOD2 induced by hypoxia/reoxygenation in cultured rat cardiomyoblasts (Zun et al., 2014). These findings suggest to role in modulating oxidative stress. Thus, a compound with dual action, such as DS12, capable of increasing acetylcholine levels and acting as an antioxidant, is an important aspect to consider in AD neuroprotection.

The hippocampus and cerebral cortex are brain regions that are involved in memory and other cognitive abilities (Preston and Eichenbaum, 2013). In fact, alterations in the structure and function of the hippocampus and cerebral cortex have been well documented in the literature in both AD patients and experimental models (Mullaart et al., 1990; Pacheco et al., 2018; Gaunitz et al., 2021; Teixeira et al., 2020, 2022). Although the cerebellum has been less studied in relation to AD, previous studies have shown that this structure has many pathological changes, such as microglial reactive, diffuse, and amyloid deposits, dystrophic neurites, and nodular formation, as well as contribute to DA progression (Larner, 1997; Jacobs et al., 2018; Singh-Bains et al., 2019). Thus, in our study, we also evaluated neurochemical changes in the cerebellum, but only an increase in ROS levels was observed in the AD model used here.

In addition, it is well established that one of the hallmark of AD is the presence the protein tau hyperphosphorylated. Tau is a protein responsible to stability of microtubules, however, when this protein is hyperphosphorylated disrupt microtubules leading to synapses dysfunctions, memory deficits and neuronal degeneration (Gao et al., 2018). Corroborating with previous studies (Akhtar et al., 2020) here we also showed an increase in hyperphosphorylated tau levels in structures. Insulin contribute to the regulation of tau phosphorylation through the activation of PI3K/AKT and inactivation of GSK-3β. In this line, previous studies have showed that dysregulation of insulin signaling induced by STZ administration leading the upregulated gene expression of GSK-3β, a kinase responsible for hyperphosphorylation of tau protein (Akhtar et al., 2020). Concerning DS12 treatment, our results showed that this compound was capable to reduce the protein tau hyperphosphorylated only in cerebral cortex. Data from literature have demonstrated that thiazolidin-4-one derivatives can ameliorate insulin resistance (Raza et al., 2013) in this line, is plausible suggest that DS12 can improve neural insulin signaling leading to a decrease in the activity of GSK-3β e tau phosphorylation. Unfortunately, it is not possible to explain the exact mechanism involved in the effects on DS12 in specific brain structure in regard tau hyperphosphorylation, however, is important to consider that tau protein can be phosphorylated at many sites by several protein kinases (Ferrer et al., 2005).

Finally, it is important to note that DS12 administrated orally for 20 days did not alter the glucose, triglyceride, total cholesterol, and urea levels in the serum of the animals, which is consistent with previously published results (Da Silva et al., 2021). In addition, DS12 did not alter erythrogram and leukogram parameters, suggesting that the time and dosage of DS12 were well tolerated by the animals and did not cause toxic effects. However, a limitation of this present study is that only young male rats were evaluated, and data from literature support sex and age differences in regard prevalence and development of AD (Zhu et al., 2021). Therefore, it is imperative to evaluate in future studies the efficacy of DS12 compound in mitigating the effects induced by AD experimental in young and old male rats, as well as in old male rats et al., 2022). In conclusion, treatment with DS12 prevented the alterations in memory, AChE, and oxidative damage, phosphorylated tau protein induced by STZ in a manner similar to that of donepezil. Our results provide evidence that DS12 may be a promising compound for the prevention of brain alterations associated with AD. In addition, DS12 could be also useful in DA resistant to conventional treatments, in patients that not responding to donepezil or in patients that need to discontinue the treatment due the adverse effects of the anticholinesterase
drugs. However, further studies need to be conducted to evaluate other mechanisms involved in the neuroprotective effects of this compound.

CRediT authorship contribution statement

Alessandra dos Santos: Conceptualization, Methodology, Data curation, Writing – original draft preparation. Fernanda Cardoso Teixeira: Conceptualization, Methodology, Data curation, Writing – original draft preparation. Daniel Schuch da Silva: Methodology (compound synthesis). Tayna Amaral Veleda: Methodology (compound synthesis). Julia Eisenhart de Mello: Methodology (animal’s experiments and biochemical analysis). Karina Pereira Ludovico: Methodology (animal’s experiments and biochemical analysis). Rejane Giacomelli Tavares: Methodology (tau protein analysis). Francesc Teixeira: Writing – reviewing and editing. Wilson Cunico: Methodology (compound synthesis). Roselia Maria Spavenello: Supervision, Experimental design, Funding, Writing – reviewing and editing.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Data availability

Data will be made available on request.

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