Andrographolide Attenuates Short-Term Spatial and Recognition Memory Impairment and Neuroinflammation Induced by a Streptozotocin Rat Model of Alzheimer’s Disease

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Received: 14 June 2022 / Revised: 12 August 2022 / Accepted: 22 August 2022 / Published online: 27 August 2022
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Abstract
Alzheimer’s disease (AD) is a neurodegenerative disorder clinically manifested by a gradual cognitive decline. Intracerebroventricular injection (ICV) of streptozotocin (STZ), a model of sporadic AD (sAD), shows many aspects of sAD abnormalities (i.e., neuroinflammation, oxidative stress, protein aggregation), resulting in memory impairment. Andrographolide (ANDRO), a natural diterpene lactone, has numerous bioactivities including anti-inflammatory and antioxidant properties. Studies in rodents revealed that ANDRO has neuroprotective properties and restores cognitive impairment. In the present study, we investigated the effects of ANDRO in the ICV-STZ model relative to short-term spatial memory (object location test (OLT) and Y maze test), short-term recognition memory (object recognition test (ORT)), locomotor activity (open field test (OFT)), expression of amyloid precursor protein (APP), and activation of astrocytes (glial fibrillary acidic protein (GFAP) expression) and microglia (ionized calcium-binding adapter molecule-1 (Iba-1) immunohistochemistry) in the prefrontal cortex (PFC) and hippocampus (HIP). Wistar rats were injected ICV with STZ (3 mg/kg) or vehicle and treated with ANDRO (2 mg/kg, i.p.; three times per week). After four weeks, ANDRO attenuated the impairments of the Y maze and ORT performances, and the increase of astrocyte activation in the PFC induced by the ICV-STZ model. In addition, ANDRO decreased the number of activated microglia cells in the HIP of STZ-injected rats. The APP expression was not altered, neither by the STZ nor ANDRO. ANDRO showed a beneficial effect on memory impairment and neuroinflammation in the STZ model of AD.

Keywords Alzheimer · Andrographolide · Cognition · Microglia · Astrocyte · Streptozotocin

Introduction
Alzheimer’s disease (AD) is the most common age-related neurodegenerative disorder, accounting for nearly 80% of dementia cases and affecting an estimated 35 million individuals worldwide (Anand et al. 2014; Prince et al. 2013). AD is characterized by progressive and slow memory loss, especially memory related to recent events and to spatial orientation, leading to incapacitation (Blennow et al. 2006; Cummings 2004; Moura et al. 2020; Salardini 2019). Sporadic AD (sAD) is the preponderant form of the disease, and, despite its unclear etiology, many molecular disturbances have been detected in the sAD aging brain (Correia et al. 2011). The classical positive lesions consist of extracellular amyloid β (Aβ) and intracellular hyperphosphorylated tau (p-tau) accumulation. Protein aggregation is accompanied by oxidative and inflammatory damage, generating energy failure, synaptic and neuronal loss, and ultimately cognitive impairment (Querfurth and LaFerla 2010; Serrano-Pozo et al. 2011).

Preclinical animal models are useful for finding pathological mechanisms responsible for AD development and checking the effectiveness of neuroprotective agents (Dhami et al. 2021). An intracerebroventricular (ICV) injection of low sub-diabetogenic doses of streptozotocin (STZ) in rodents can model sAD (Bassani et al. 2017a; Grieb 2016; Salkovic-Petrisic and Hoyer 2007). STZ inhibits insulin receptors, promoting an
insulin-resistant brain state and brain glucose hypometabolism, abnormalities that appear early in sAD (Salkovic-Petrisic et al. 2013). In addition, the model causes cognitive impairment and neurodegeneration which are intimately related with the STZ-induced neuroinflammation, increase in free radical formation, increase in Aβ and p-tau levels, disturbances in mitochondrial calcium homeostasis, and cholinergic deficits. Features also present in brains of sAD patients (Chen et al. 2018; el Sayed and Ghomeou 2020; More et al. 2016; Sharma and Garabadu 2020; Zafer et al. 2019). All of which are observed in the brain regions responsible for cognition, as the hippocampus (HIP) and the prefrontal cortex (PFC) (Cisternas et al. 2019b; Gerzson et al. 2020; Kumar et al. 2015). The ICV-STZ model is a suitable strategy to mimic human sAD conditions, and it is widely used to evaluate the neuroprotective properties of various compounds (Kraska et al. 2012).

Current AD’s therapeutic approaches are limited, and most agents (e.g., acetylcholinesterase inhibitors) provide only cognitive deficit symptomatic relief (Stella et al. 2015; Lane et al. 2018). Researchers are making a significant effort toward the discovery of disease-modifying therapies which can block the progression of the disease and drugs targeting various molecular pathways (Kumar et al. 2015). Several bioactive compounds isolated from natural products can play an important role in the prevention and management of neurodegenerative disorders including AD (Tayanloo-Beik et al. 2022; Zhu et al. 2019). Several reports demonstrate positive effects of natural compounds in the ICV-STZ model. Xanthotoxin and umbelliferone, umuhengerin, fucoxanthin, paenol, and tannic acid, for example, enhanced cognitive impairment, neuroinflammation, oxidative stress, and various other features induced by STZ (Dhami et al. 2021; Gerzson et al. 2020; Hindlam et al. 2020; Sirwi et al. 2021; Tayanloo-Beik et al. 2022).

Andrographolide (ANDRO), the main bioactive component of the medicinal plant Andrographis paniculata, is a labdane diterpenoid lactone with multiple therapeutic uses and is largely known for its anti-inflammatory and antioxidative activities (Guan et al. 2013; Li et al. 2015; Zhang et al. 2021). ANDRO has shown beneficial effects in the central nervous system (CNS). It reduced inflammatory cytokines levels and astrocyte/microglia activation in rat models of cerebral ischemia and traumatic brain injury, improving neurological deficits and neurodegeneration in both models (Chan et al. 2010; Tao et al. 2018; Wang et al. 2019). Furthermore, ANDRO attenuated Parkinson’s disease-like phenotypes and preserved mitochondrial morphology in a mouse model of Parkinson’s disease (Geng et al. 2019). In various AD models, the treatment with ANDRO ameliorated crucial molecular mechanisms and reduced deficits in learning and memory (Hossain et al. 2022). For instance, in APPswe/PS1ΔE9 and J20 transgenic mice, which express a mutant form of amyloid precursor protein (APP) exhibiting high accumulation of amyloid aggregates, ANDRO decreased Aβ and p-tau levels, improved cellular energy metabolism markers and neurogenesis, and preserved spatial and recognition memory (Serrano et al. 2014; Cisternas et al. 2019a, b; Arredondo et al. 2021). Similarly, ANDRO protected recognition memory in aged Octodon degus, used as a natural model of AD, in addition to reducing Aβ and p-tau, and synaptic transmission deficits (Rivera et al. 2016).

Patel et al. (2021) have recently demonstrated that ANDRO can suppress the impairment of spatial memory caused by the ICV-STZ model in rats. ANDRO protected the hippocampus against STZ-induced increase in oxidative stress, neuroinflammatory markers (interleukin-1β (IL-1β), IL-16, and tumor necrosis factor-alpha (TNF-α)), Aβ1-42 and p-tau levels. However, the effects of prolonged ANDRO treatment on astrocyte and microglia activation and recognition memory were not yet studied in the ICV-STZ model. Therefore, in the present study, we investigated the potential of ANDRO for preserving spatial and recognition memory and reducing amyloid precursor protein (APP) expression and astrocytes and microglia activation in the prefrontal cortex and hippocampus of ICV-STZ injected rats.

### Material and Methods

#### Animals

Male Wistar rats (60–90 days old, weighing 300-350 g), provided by the animal facility of the Federal University of Paraná (UFPR), were housed in groups of 3–4 in polypropylene cages with wood shavings as bedding. The rats were maintained at 22 ± 2 °C on a 12 h/12 h light/dark cycle (lights on at 7:00 AM); water and standard chow were available ad libitum. Before the experiment, the rats were allowed to acclimatize for 1 week to reduce environmental stress. The experiments were performed following the Brazilian Law for Animal Experimental Ethics and Care (11.794/October 8, 2008) and the guidelines of the UFPR Committee on the Care and Use of Laboratory Animals. The experimental procedures were approved by the University Ethics Board (CEUA/BIO-protocol #1315).

#### Experimental Design and Treatments

The rats were randomly divided into four experimental groups: SHAM + VEH, SHAM + ANDRO, STZ + VEH, STZ + ANDRO (10–11 per group). All the animals were submitted to stereotaxic surgery, the STZ groups received a single bilateral injection of STZ (N-[methylnitrosocarbamoyl]-α-D-glucosamine, Santa Cruz Biotechnology, USA) in a total dose of 3 mg/kg, dissolved in sterile saline. The sham groups received a single bilateral injection of sterile saline. The animals were treated three times per week (Monday, Wednesday, Friday).
intraperitoneally (i.p.) with 2 mg/kg ANDRO (Sigma-Aldrich, USA) or its vehicle (0.9% saline and 2% dimethyl sulfoxide) for 4 weeks, starting 1 h after the surgery (Varela-Nallar et al. 2015; Kanazawa et al. 2021).

After surgery, the rats were allowed to recover for three weeks, and the behavioral tests were conducted on the fourth week. The spontaneous locomotor activity was assessed in the open field test (OFT) on day 21 following surgery, and cognitive performance was evaluated in the object location test (OLT) on day 28, in the object recognition test (ORT) on day 29, and the spatial version of the Y maze on day 30 (Bassani et al. 2017b). The experimental design is presented in Fig. 1.

Posterior to the behavioral tests, a subset of animals (n = 4/group) was decapitated under chloral hydrate anesthesia (400 mg/kg, i.p.). The brains were extracted, and the whole HIP and PFC were dissected for the quantification of APP and glial fibrillary acidic protein (GFAP) by Western blot. Another subset of animals (n = 4/group) was deeply anesthetized with chloral hydrate (400 mg/kg, i.p.) and intracardially perfused for immunohistochemical evaluation of the microglia-specific marker for ionized calcium-binding adaptor molecule (Iba-1).

**Stereotaxic Surgery**

Stereotaxic surgery was carried out as previously described (Bassani et al. 2017a, b; Moura et al. 2020). The animals were anesthetized with sodium thiopental (30 mg/kg, i.p.) and chloral hydrate (150 mg/kg, i.p.) and placed in a stereotaxic apparatus (David Kopf, USA). A 28-gauge stainless steel needle was lowered into each lateral ventricle (LV). The stereotaxic coordinates for ICV infusion, according to Paxinos and Watson (Paxinos and Watson 2007), were measured: anterior/posterior, −0.8 mm from bregma; medial/lateral, ±1.5 mm from the midline; dorsal/ventral, −3.8 mm from the skull. An electronic pump (Insight, Ribeirão Preto, SP, Brazil) was used to control the flow of the injections at a rate of 1.0 μl/min over 4.5 min. The lesioned group received bilateral ICV injections of STZ (3 mg/kg total dose) dissolved in sterile 0.9% saline (4.5 μl per injection site). Sham surgery followed the same procedure, but sterile saline was injected instead of STZ. After surgery, all the rats were allowed to recover from anesthesia for 2–4 h in a heated and well-ventilated room. Food and water were placed inside the cage for 10–15 days so that the animals could easily access it without physical trauma caused by head surgery.

**Open Field Test**

The OFT was performed 21 days after surgery. The open field apparatus was placed in a moderately lit room and consisted of a circular arena (97 cm diameter, 42 cm height) which was divided into three concentric circles and subdivided into 19 quadrants. A video camera was placed right above the arena to record the animal’s behavior for posterior analysis. Individually, the animals were placed in the center of the apparatus and allowed to freely explore it for 5 min. The total number of crossings from one quadrant to another was measured. A crossing was considered only when the animal entered another quadrant with its four paws. The apparatus was cleaned with a 20% ethanol–water solution between tests to eliminate possible odors left by other rats.

**Object Location Test (OLT) and Object Recognition Test (ORT)**

The OLT and ORT were performed between days 27 and 29 following surgery to evaluate short-term spatial and short-term recognition memory, respectively. Both tests took place
in a squared arena, 100 cm × 100 cm × 40 cm, made of wood and painted black. It was placed in a moderately lit room. A video camera was positioned over the arena, and the animals' behavior was recorded for later evaluation.

The first procedure consisted of the habituation of the animals in the box. Each animal was placed in the empty apparatus for 5 min for free exploration. Twenty-four hours later, a novel habituation session of 5 min was performed. After a 1-h delay, during the training session, two identical objects were placed in the apparatus in a symmetrical position about 10 cm away from the wall. The animals were allowed to explore them freely for 5 min and were then returned to their home cages. In the OLT (which was performed on day 28), after a 1-h delay, during the test session, each rat was put back into the box with one of the objects displaced 15 cm away from the original position (novel position); animals were allowed to freely explore the objects for 3 min. In the ORT (which was performed on day 29), a new training session was done with the two identical objects placed in a symmetrical position about 10 cm away from the wall, and the animals were allowed to explore them freely for 5 min and were then returned to their home cages. After a 1-h interval, the rats were put back into the arena for the test session; but now with two dissimilar objects, a familiar one (the sample) and a new one, the animals were allowed to freely explore the objects for 3 min.

The objects were made of plastic or ceramic. To avoid an olfactory bias, before each trial, the objects were cleaned with a 20% ethanol solution. All objects and locations were balanced to reduce potential biases due to preferences for particular locations or objects. A rat could not displace the objects and the subjects were always placed into the box facing the same wall. Exploration was defined as sniffing at no more than 2 cm or touching the objects with the nose and/or forepaws. Sitting on or turning around the objects was not considered exploratory behavior. The animals that spent less than 10 s exploring the objects were excluded from the test.

The measures for both the OLT and ORT were the time spent by the rats exploring each object during the test session. The time spent exploring the familiar and the novel objects/locations, and a relative measure of discrimination that corrects for the latency to move from the start arm to another arm and the time spent in the center of the maze). An arm entry was considered when both hind paws were placed completely inside an arm. The maze was cleaned between sessions with a 10% ethanol solution to reduce olfactory bias (Kraeutler et al. 2019).

### Quantification by Western Blot

To investigate the effects of ANDRO on the astrogliosis in the HIP and PFC of ICV-STZ rats, we measured the level of GFAP, the commonly used marker for astrocytes, via western blot. The samples were homogenized and sonicated in lysis buffer (150 mM NaCl, 1.0% NP-40, 50 mM Tris–HCl, pH 8.0, and protease inhibitors). After centrifugation at 20,000 rpm and 4 °C for 20 min, the supernatant was collected, and the protein concentration was determined by the Bradford method (Bio-Rad, Germany). The supernatants were boiled with Laemmli buffer (4% SDS, 10% 2-mercaptoethanol, 20% glycerol, 0.004% bromophenol blue, 0.125 M Tris–HC with adjust final pH of buffer to 6.3) for 10 min at 95 °C. After reduction, the samples were subjected to electrophoresis and transferred to nitrocellulose membranes (Bio-rad, 0.45 μm). The membranes were blocked with 5% nonfat milk in TBS-Tween 20 solution and then incubated overnight at 4 °C with mouse monoclonal anti-β-amyloid antibody (1:750; sc-28365, Santa Cruz Biotechnology, Santa Cruz, USA), rabbit polyclonal anti-GFAP antibody (1:1000; ab7260, Abcam), rabbit polyclonal anti-β-actin (1:500; sc-130656, Santa Cruz Biotechnology, Santa Cruz, USA), or mouse monoclonal anti-GAPDH antibody (1:5000; sc-32233, Santa Cruz Biotechnology, Santa Cruz, USA). After incubation with the primary antibody, the membranes were extensively washed with TBS-T and incubated with anti-rabbit horseradish peroxidase-conjugated secondary

### Spatial Version of Y maze

A symmetrical Y maze was constructed of wood and painted black. It consisted of three arms. The arms (50 cm in length, 27 cm in height, 12 cm in width) were arranged at a 120° angle relative to each other. The test consisted of a training session and test session that were performed at 1-h intervals. In the training session, one arm was made inaccessible by a removable blockade that was placed in front of it. A rat was placed in one of the other arms (i.e., ‘start arm’). The start arm was randomized between groups. The animals were then allowed to explore these two arms for 5 min. The rat was then removed from the arena and returned to its home cage. After a 1-h interval, in the test session, the rat was returned to its corresponding start arm, but the blockade that prevented access to the third arm (i.e., ‘novel arm’) was removed, thus providing access to all three arms. The rat was allowed to explore the three arms for 3 min. The test session was video recorded for subsequent analysis. Short-term spatial memory was assessed as the percentage of time spent on the novel arm, which had to be significantly greater than 33.3% of the total time on the maze (corrected for the latency to move from the start arm to another arm and the time spent in the center of the maze). An arm entry was considered when both hind paws were placed completely inside an arm. The maze was cleaned between sessions with a 10% ethanol solution to reduce olfactory bias (Kraeutler et al. 2019).
antibody (Sigma, USA) in a blocking solution for 1 h at room temperature. The membranes were then washed, and the visualization was performed with Pierce ECL kit (Thermo scientific) chemiluminescence substrate on an Amersham™ Imager 600 detection system (GE Healthcare, São Paulo, SP, Brazil). Protein levels were quantified by densitometry using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

**Immunohistochemistry**

The effects of ANDRO on the microglia in the HIP and PFC of ICV-STZ rats were explored, and immunostaining to identify microglia was carried out using Iba-1 primary antibody. Brain sample processing and immunohistochemistry reactions were developed as described by Moura et al. (2020). The animals were deeply anesthetized and then euthanized with intracardiac perfusion of saline phosphate-buffered solution (pH 7.4; 1,000 mL/kg; 4 °C), followed by 4% paraformaldehyde in phosphate-buffered solution (pH 7.4; 1,000 mL/kg; 4 °C). Then, the brains were removed and placed in 30% sucrose solution for 4 days for tissue cryoprotection. After that, the tissue was protected with plastic, quickly frozen in liquid nitrogen, and stored in a freezer (–80 °C) for subsequent sectioning. The frozen tissue was cut into 30 µm semi-serial coronal sections along the dorsal hippocampus at a temperature of −25 °C using a cryostat (Leica Biosystems, Germany). The dorsal hippocampus was collected, and the sections were placed serially in ten wells-plates using −2.56 to −4.52 mm stereotaxic coordinates to the bregma, totaling six to eight 30 µm cuts per well-plate. Brain sections were stored at −20 °C in a cryoprotectant solution containing 30% ethylene glycol and 15% sucrose in a 0.05 M phosphate-buffered solution for subsequent processing.

The free-floating method was used for immunohistochemical reactions. The brain sections were initially washed with buffer A (0.1 M PBS, pH 7.4 with 0.5% Triton X-100) and incubated in 0.1 M citrate buffer, pH 6.0 in a water bath at 50 °C for 30 min. The steps described above characterize an antigen retrieval step for this marker. After that, the sections were cooled to room temperature, washed with buffer A, incubated with 0.5% H2O2 solution in 0.1 M PBS for 30 min at room temperature and protected from light, washed again with buffer A and incubated with 2% bovine serum albumin (BSA) in buffer A (blocking buffer) at room temperature for 1 h. Subsequently, the plates containing the sections were incubated overnight at 4 °C with the primary antibody diluted in anti-Iba1 goat polyclonal blocking buffer (1:500, Abcam, Cambridge, USA).

The following day the sections were washed in buffer A and incubated with biotinylated secondary antibody at 4 °C for 2 h, washed again in buffer A and incubated with ABC reagent (Vectastain Elite ABC Kit, Vector Laboratories, USA) in 0.1 M PBS in room temperature for 2 h. After being washed with 0.1 MPBS, the reaction was revealed by incubating the sections with 3,3'-diaminobenzidine (DAB, Vector Laboratories, USA) in 0.1 MPBS at room temperature for 7 min. The sections were washed in 0.1 M PBS, mounted on gelatinized slides, and air-dried. The brain slices were mounted on microscope slides, dehydrated in solutions of ascending ethanol concentrations, cleared in xylene, and coverslipped.

The areas of the hippocampus (CA1, CA3, DG) were photographed using a BX50 optical microscope (Olympus Optical, USA) at 200× magnification. Microglial cells were identified as Iba-1-positive (Iba1+) cells and manually counted using the Cell Counter of the ImageJ software. Iba1+ cells were morphologically classified as Types I, II, III, IV, and V as previously described by as (Diz-Chaves et al. 2012). Types I, II, and III were categorized as non-reactive glia, whereas Types IV and V were taken as reactive glia (Zappa Villar et al. 2018). Results were expressed as a percentage of the number of sham Iba-1+ cells and sham reactive Iba-1+ cells.

**Statistical Analysis**

The data are presented as mean ± standard error of the mean (SEM) and analyzed using a two-way analysis of variance (ANOVA) (factor lesion: STZ or vehicle injection; factor treatment: ANDRO or vehicle treatment). Pearson’s correlation coefficient was used to evaluate the degree of association between variables. The level of significance was p < 0.05. The analysis was performed with the software GraphPad Prism 8.0 (GraphPad Software, Inc) and STATISTICA (data analysis software system) version 12 (StatSoft, Inc).

**Results**

**ANDRO Mitigates Short-Term Spatial Memory Impairment Caused by ICV-STZ**

It is extensively described in the literature that the ICV infusion of STZ causes impairments in cognitive tasks that rely on the HIP (Prickaerts et al. 1999; Rodrigues et al. 2010; Li et al. 2020). STZ promotes hippocampal damage, severely disturbing the ability of rodents to remember a location in space (Martin and Clark 2007; Eichenbaum 2017). We hypothesized that ANDRO would preserve rats spatial memory. Therefore, short-term spatial memory was assessed in the spatial version of the Y maze on day 30 after surgery, with an 1 h interval between the training and test sessions. Results indicate that STZ infusion impaired short-term spatial memory, STZ + VEH group showed a significantly lower percentage of time spent in the novel arm (p < 0.05) (Fig. 2) compared with the SHAM + VEH group (treatment x lesion interaction [F(1, 38) = 5.881; p = 0.020]).
The STZ+ANDRO group performed similarly to the SHAM+VEH group, suggesting that ANDRO prevented the spatial memory disruption induced by STZ; however, the percentage of time spent in the novel arm was not significantly higher compared with the STZ+VEH group. There were no differences between the groups regarding the total entries in the arms of the maze (Fig. S1a) and the total distance travelled (Fig. S1b).

**ANDRO Worsens OLT Performance and Mitigates Short-Term Recognition Memory Impairment Induced by ICV-STZ**

Short-term spatial memory was also assessed in the OLT on day 28 after surgery, with a 1-h interval between the training and test sessions. In the OLT (Fig. 3), the STZ-infused animals exhibited a significantly lower discrimination index compared with SHAM animals, as indicated by the lesion factor [F (1, 30) = 10.12; p = 0.003] and treatment x lesion interaction ([F (1, 30) = 6.939; p = 0.0132]. The post hoc test revealed that the STZ+ANDRO group had a decrease in the index in comparison with SHAM+VEH group (p < 0.05), but the STZ+VEH did not, which suggests that ANDRO impacted negatively short-term spatial memory in this test, contradicting the Y maze results.

Likewise, the ICV-STZ disturbs recognition memory and ANDRO showed potential to protect it in previous studies. Thus, short-term recognition memory was assessed in the ORT on day 29 after surgery. In the ORT (Fig. 4), STZ+VEH animals presented deficits in short-term memory, as indicated by treatment x lesion interaction [F (1, 30) = 7.601; p = 0.009]; there was a significant decrease in the discrimination index (p < 0.05) in comparison with the SHAM+VEH group. The STZ+ANDRO group performed similarly to the SHAM+VEH group, indicating a protective effect of ANDRO; however, the discrimination index was not higher in the STZ+ANDRO group than in the STZ+VEH group. There were no significant differences between the groups regarding the total time exploring the objects (e) both in the OLT (Fig. S2a) and in the ORT (Fig. S2b).
ANDRO Reduces Locomotion Impaired by ICV-STZ

Regarding the performance in the OFT (Fig. 5), assessed 21 days after surgery, the post hoc test revealed only one significant difference in locomotion frequency between groups, the STZ+ANDRO group exhibited a decrease in relation to the SHAM+VEH group. This result could suggest an alteration due to ANDRO treatment; however, the two-way ANOVA showed that the STZ infusion had greater influence in locomotion frequency (lesion factor $F_{(1, 38)} = 9.126; p = 0.004$).

ANDRO Mitigates Astrogliosis Induced by ICV-STZ in the Prefrontal Cortex

Neuroinflammation, as reflected by astrogliosis and microglial activation, is a pathological hallmark of AD and an important feature of ICV-STZ model (Guo et al. 2017). We hypothesized that ANDRO would reduce neuroinflammation, protecting the HIP and the PFC against astrocyte reactivity. Reactive astrocytes are characterized by increased expression of GFAP (Heneka et al. 2015). We observed a significantly increase in GFAP expression in the PFC (Fig. 6a) of the STZ+VEH group compared with the SHAM+VEH group 30 days after the surgery ($p < 0.05$), as showed by the post hoc test, but not by the two-way ANOVA (treatment x lesion interaction $F_{(1, 12)} = 4.180; p = 0.063$, treatment $F_{(1, 12)} = 0.234; p = 0.637$, and lesion $F_{(1, 12)} = 3.965; p = 0.069$ factors). In the STZ+ANDRO group, GFAP expression in the PFC was comparable with SHAM+VEH animals; however, there was also no significant difference in relation to the STZ+VEH group. In the hippocampus (Fig. 6b), there were no differences between the groups (treatment x lesion interaction $F_{(1, 12)} = 1.521; p = 0.241$, treatment $F_{(1, 12)} = 3.350; p = 0.092$, and lesion $F_{(1, 12)} = 0.824; p = 0.381$). These results suggest that the ICV-STZ model increases the GFAP expression in the PFC after 30 days, and that ANDRO attenuated the astrogliosis in this cerebral region.
Correlation Between Short-Term Memory and GFAP Expression in the HIP and PFC

The Pearson’s correlation coefficients revealed negative correlations between the percentage of time spent in the novel arm in the Y maze and GFAP expression of in the PFC ($r = -0.711$, $p = 0.014$; Fig. 7a), and between the ORT discrimination index and GFAP expression in the HIP ($r = -0.653$, $p = 0.021$; Fig. 7b). No correlation was found between the percentage of time spent in the novel arm in the Y maze and GFAP expression in the HIP ($r = -0.137$, $p = 0.688$; data not shown), and between the ORT discrimination index and GFAP expression in the PFC ($r = -0.162$, $p = 0.615$; data not shown).

ANDRO Attenuates the Increase in Hippocampus Iba-1-Positive Cells Induced by ICV-STZ

In previous reports, ANDRO showed potential for decreasing microglial proliferation and reactivity, reducing the neuroinflammation burden (Chan et al. 2010; Tao et al. 2018). Thus, the dorsal HIP was evaluated in three regions, CA1, CA3 and dentate gyrus (DG), regarding the number of Iba-1+ cells and reactive Iba-1+ cells. The two-way ANOVAs showed significant differences between the groups variances in the CA1 (treatment x lesion interaction [$F (1, 12) = 5.218$; $p = 0.041$]; treatment [$F (1, 12) = 5.671$; $p = 0.035$]) and CA3 (treatment x lesion interaction [$F (1, 12) = 7.378$; $p = 0.019$]; treatment [$F (1, 12) = 4.987$; $p = 0.045$]), but not in the DG. In relation to the number of reactive Iba-1+ cells, significant differences were observed in the CA3 (treatment x lesion interaction [$F (1, 12) = 6.809$; $p = 0.023$]) and in the DG (treatment x lesion interaction [$F (1, 12) = 10.540$; $p = 0.007$]). However, different from what we expected, the multiple comparisons test did not reveal differences between the STZ+ VEH and SHAM+ VEH groups (Iba-1+ cells in CA1, $p = 0.176$, and CA3, $p = 0.069$; reactive Iba-1+ cells in CA3, $p = 0.059$, and DG, $p = 0.051$). Yet, in the comparison between the STZ-injected groups, the treatment with ANDRO reduced ($p < 0.05$) Iba-1+ cells in CA1 (Fig. 8a) and CA3 (Fig. 8c), and reactive Iba-1+ cells in CA3 (Fig. 8d) and DG (Fig. 8f). These results indicate that the treatment with ANDRO caused a mild improvement against the increase in microglia cells and microglia reactivity in the HIP (Fig. 9).

Correlation Between Short-Term Memory and the Number of Iba-1+ Cells in the HIP

The Pearson’s correlation coefficients revealed negative correlations between the percentage of time spent in the novel arm in the Y maze and the number of Iba-1+ cells in the CA3 area ($r = -0.530$, $p = 0.042$; Fig. 10a), and between the percentage of time spent in the novel arm in the Y maze and the number of reactive Iba-1+ cells in the CA3 ($r = -0.567$, $p = 0.027$; Fig. 10b) and the DG ($r = -0.529$, $p = 0.043$; Fig. 10c).

APP Expression in the PFC and HIP is Not Affected by ICV-STZ and ANDRO

ICV-STZ infusion can increase APP (Pierzynowska et al. 2019). Furthermore, pro-inflammatory cytokines when chronically activated affect the processing of APP through beta-secretase, accelerating the production of Aβ (Kaur et al. 2019). ANDRO is capable of reducing neuroinflammation and Aβ deposition (Zhang...
Fig. 8 Effect of andrographolide (2 mg/kg) prolonged administration and ICV-STZ (3 mg/kg) on the number of Iba-1-positive cells in the A CA1, C CA3 and E DG areas of the HIP, and on the number of reactive Iba-1-positive cells in the B CA1, D CA3 and F DG areas of the HIP 30 days after surgery. The data are expressed as mean ± SEM (n = 4/group); *p < 0.05, in comparison with the SHAM + VEH group; #p < 0.05, in comparison with the STZ + VEH group (two-way ANOVA followed by Tukey’s post hoc test)
et al. 2021). We sought to evaluate the APP expression in the ICV-STZ model. The two-way ANOVA did not show differences between the groups in the amyloid precursor protein expression (Fig. 11), both in the PFC (treatment x lesion interaction $[F (1, 11) = 0.008; p = 0.930]$; treatment $[F (1, 11) = 0.746; p = 0.406]$; lesion $[F (1, 11) = 0.725; p = 0.412]$) and the HIP (treatment x lesion interaction $[F (1, 12) = 0.122; p = 0.732]$; treatment $[F (1, 12) = 0.665; p = 0.430]$; lesion $[F (1, 12) = 0.006; p = 0.940]$).

**Discussion**

The current study investigated the neuroprotective potential of a prolonged andrographolide treatment (2 mg/kg, 30 days) in rats submitted to the ICV-STZ model of sAD. We assessed short-term spatial memory, short-term recognition memory, neuroinflammation, and APP expression, aspects altered in the sAD rat model similarly to AD patients (Grieb 2016).

The STZ-injected animals showed impairments in short-term spatial memory. STZ worsened the Y maze and OLT performances. Regarding the treatment with ANDRO, previous studies demonstrated a protection of spatial memory in different AD models (Serrano et al. 2014; Rivera et al. 2016). Here, ANDRO exhibited an ambiguous role. In the Y maze test, ANDRO had a positive and protective effect. However, in the object location test, results suggest a negative impact of ANDRO on short-term spatial memory. The conflict between these results is unexpected since the two tasks have fundamental similarities in assessing spatial memory, both are inherently not stressful and based on exploiting rodents’ innate preference for novelty (Dellu et al. 1997; Vogel-Ciernia and Wood 2014). Despite the poor performance of the STZ+ANDRO group, all the groups showed a low discrimination index in the OLT ($-0.1 < d < 0.2$), including the control group, which could have occurred due to performance confounds, like stress and anxiety (Vogel-Ciernia and Wood 2014), interfering on results interpretation. We also observed a disruption in rats short-term recognition memory induced by ICV-STZ.

In line with previous studies (Cisternas et al. 2019a, b; El Sayed and Ghoneum 2020), STZ impaired rats’ ORT performance. ANDRO was able to preserve it, having a protective effect on recognition memory. Our study is the first to report an improvement in short-term recognition memory caused by ANDRO in the ICV-STZ model of sAD. In a recent report, it was observed that ANDRO (15, 30, and 60 mg/kg, 14 days) protected rats against STZ-induced learning and memory impairment (Patel et al. 2021). However, cognition was assessed 21 days after STZ infusion in the Morris Water
Maze Test and in a modified version of the Elevated Plus Maze Test, which measures spatial long-term memory. In addition, the doses were substantially higher than the dose used in our study.

In the OFT, the STZ+ANDRO group reduced locomotion frequency suggests a negative effect of ANDRO on the STZ-injected rats spontaneous exploratory behavior and locomotor activity (Walsh and Cummins 1976; Choleris 2001). Yet only the lesion factor was significantly different. Therefore, the STZ infusion caused the reduction in locomotion frequency. It is known that changes in these parameters could worsen the animals’ performance in subsequent cognitive tests (Moura et al. 2020). However, the exploratory behavior and ambulation were affected only in the OFT. In the OLT and ORT, the groups did not show differences in the total time spent exploring the objects. In addition, in the Y maze, both the distance covered and the number of entries in the arms were similar between groups. An explanation is that the OFT was performed 8 to 9 days before the other behavioral tests, and the animals could have recovered the exploratory and locomotor capacity before the cognitive tests.

Several reports demonstrate that the GFAP immunoreactivity and expression increase in the PFC and the HIP in the ICV-STZ model (Pilipenko et al. 2019; Rai et al. 2014; Rajasekar et al. 2017; Ravelli et al. 2017). In the present study, STZ infusion increased GFAP expression only in the PFC. Treatment with ANDRO protected the rats’ PFC against astrocyte reactivity induced by STZ. In the HIP, there were no differences between groups. In relation to microglia, we evaluated different regions of the HIP, and STZ did not increase the number of cells and reactivity, contrary to previous reports (Pilipenko et al. 2020; Zappa Villar et al. 2018), although in the CA3 and the DG there were important tendencies. On the other hand, ANDRO reduced microglia cells in CA1 and CA3, and reactive microglial cells in CA3 and DG, agreeing with Zhang et al. (2021). We identified

Fig. 10 Correlations between the percentage of time spent in the novel arm in the Y maze and the number of A Iba-1-positive cells in the CA3 area of the HIP and reactive Iba-1-positive cells in the B CA3 and C DG areas. r: Pearson correlation coefficient; n = 15
different negative correlations between the performance in cognitive tests and neuroinflammation: correlation between the Y maze performance and astrogliosis in the PFC; correlation between the ORT performance and astrogliosis in the HIP; and correlations between the Y maze performance and microgliosis in CA3 and DG. Altogether, these data reinforce the association between cognitive impairment and neuroinflammation, as well as demonstrate ANDRO’s neuroprotective effect, inhibiting astrocyte reactivity in the PFC and microglia hypertrophy and proliferation in the HIP.

Regarding the expression of APP, no significant differences were observed between the groups both in the PFC and the HIP. There is evidence that the ICV-STZ infusion can increase APP levels. For example, Pierzynowska et al. (2019) observed in Wistar rats augmented APP expression in the PFC, HIP, and the rest of the brain after 30 days of the STZ infusion (3 mg/kg). Retinasamy et al. (2020) observed an increase of APP gene expression in the PFC and HIP 21 days post-surgery in Sprague Dawley (SD) rats (STZ 3 mg/kg). However, there are also contrasting data, as in the study of Zappa Villar et al. (2018), in which the infusion of 1 and 3 mg/kg of STZ did not increase APP expression in the HIP of SD rats after 25 days; and in the work of Gupta et al. (2018), the gene expression of APP was increased by STZ infusion only in the cortex, but not in the HIP of SD rats 30 days after surgery. As the levels of the pathogenic fragments (e.g., Aβ 1–40, Aβ 1–42) were not measured in the present report, it is difficult to state if the amyloidogenic pathway influenced the effects of STZ and ANDRO.

It is important to mention that the mechanisms of andrographolide neuroprotection involve its anti-inflammatory and antioxidative activities (Lu et al. 2019). These activities were not directly assessed in the current report. For example, ANDRO can reduce glia-mediated oxidative damage and production of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-18, nitric oxide) by down-regulating the nuclear factor kappa B (NF-κB) pathways and activating the NF-E2-related factor-2 (Nrf2) and the heme oxygenase-1 (HO-1) (Wong et al. 2016; Xu et al. 2019; Zhang et al. 2021). Thus, further studies addressing these and other mechanisms are necessary for better understanding the ANDRO role in the ICV-STZ model.

In summary, ANDRO attenuated the impairment of short-term spatial memory and short-term recognition memory induced by the ICV-STZ model of sAD in rats. Additionally, ANDRO decreased astrogliosis in the prefrontal cortex and microgliosis in the hippocampus of the animals, which could be related with the memory improvement. Further investigations of the effects of ANDRO on the ICV-STZ model are necessary to elucidate the adjacent mechanisms of neuroprotection and the treatment optimal dose.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s12640-022-00569-5.

**Acknowledgements** We thank Professor Marcelo M.S. Lima (Department of Physiology, Federal University of Parana) for allowing us to conduct part of the experiments in the facilities of the Laboratory of Neurophysiology and Professor Silvio M. Zanata (Department of Basic Pathology, Federal University of Parana) for the support.
Authors Contribution Leonardo C. Souza contributed to the conceptualization, methodology, validation, formal analysis, investigation, data curation, writing—original draft, writing—review and editing, and visualization. Marcos K. Andrade and Evellyn M. Azevedo were involved in the formal analysis, investigation, and data curation. Daniele C. Ramos contributed to the investigation and writing—review and editing. Ellen L. Bail contributed to the investigation, Maria A. B. F. Vital helped in the conceptualization, methodology, resources, writing—review and editing, visualization, supervision, project administration, and funding acquisition.

Funding This work was supported by grants from Coordination for the Improvement of Higher Education Personnel (CAPES) and Araucaria Foundation to Support Scientific and Technological Development of the State of Paraná (No. 88882.168580/2018-01), which had no further role in the study design, collection, analysis, and interpretation of the data, writing the report, and decision to submit the paper for publication. MARBV is a recipient of a National Council for Scientific and Technological Development (CNPq) fellowship (No. 309567/2019-0).

Declarations

Ethics Approval The experiments were performed following the Brazilian Law for Animal Experimental Ethics and Care (11.794/October 8, 2008) and the guidelines of the UFPR Committee on the Care and Use of Laboratory Animals.

Conflict of Interest The authors report no conflicts of interest.

References

Anand R, Gill KD, Mahdi AA (2014) Therapeutics of Alzheimer’s disease: Past, present and future. Neuropharmacology 76(PART A):27–50. https://doi.org/10.1016/j.neuropharm.2013.07.004
Arredondo SB, Reyes DT, Herrera-Soto A, Mardones MD, Inestrosa NC, Varela-Nallar L (2021) Andrographolide promotes hippocampal neurogenesis and spatial memory in the APP/PS1ΔE9 mouse model of Alzheimer’s disease. Sci Rep 11(1):22904. https://doi.org/10.1038/s41598-021-01977-x
Bassani TB, Bonato JM, Machado MMF, Cöppola-Segovia V, Moura ELR, Zanata SM, Oliveira RMWM, Vital MABF (2017a) Decrease in adult neurogenesis and neuroinflammation are involved in spatial memory impairment in the streptozotocin-induced model of sporadic Alzheimer’s disease in rats. Mol Neurobiol. https://doi.org/10.1007/s12035-017-0645-9
Bassani TB, Turnes JM, Moura ELR, Bonato JM, Cöppola-Segovia V, Zanata SM, Oliveira RMWM, Vital MABF (2017b) Effects of curcumin on short-term spatial and recognition memory, adult neurogenesis and neuroinflammation in a streptozotocin-induced rat model of dementia of Alzheimer’s type. Behav Brain Res 335(75):41–54. https://doi.org/10.1016/j.bbr.2017.08.014
Blennow K, de Leon MJ, Zetterberg H (2006) Alzheimer’s disease. Neurobiol. https://doi.org/10.1016/j.brainres.2017.08.014
Blenno K, de Leon MJ, Zetterberg H (2006) Alzheimer’s disease. Neurobiol. https://doi.org/10.1016/j.brainres.2017.08.014
Blennow K, de Leon MJ, Zetterberg H (2006) Alzheimer’s disease. Neurobiol. https://doi.org/10.1016/j.brainres.2017.08.014
Blennow K, de Leon MJ, Zetterberg H (2006) Alzheimer’s disease. Neurobiol. https://doi.org/10.1016/j.brainres.2017.08.014
Blennow K, de Leon MJ, Zetterberg H (2006) Alzheimer’s disease. Neurobiol. https://doi.org/10.1016/j.brainres.2017.08.014
Choleris E (2001) A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci Biobehav Rev 25(3):235–260. https://doi.org/10.1016/S0149-7634(01)00011-2
Cisternas P, Oliva CA, Torres VI, Barrera DP, Inestrosa NC (2019a) Presymptomatic treatment with andrographolide improves brain metabolic markers and cognitive behavior in a model of early-onset Alzheimer’s disease. Front Cell Neurosci 13(July):1–18. https://doi.org/10.3389/fncel.2019.00295
Cisternas P, Zolezzi JM, Martínez M, Torres VI, Wong GW, Inestrosa NC (2019b) Wnt-induced activation of glucose metabolism mediates the in vivo neuroprotective roles of Wnt signaling in Alzheimer disease. J Neurochem 149(1):54–72. https://doi.org/10.1111/jnc.14608
Correia SC, Santos RX, Perry G, Zhu X, Moreira PL, Smith MA (2011) Insulin-resistant brain state: The culprit in sporadic Alzheimer’s disease? Ageing Res Rev 10(2):264–273. https://doi.org/10.1016/j.arr.2011.01.001
Cummings JL (2004) Alzheimer’s disease. N Engl J Med 351(1):56–67. https://doi.org/10.1056/NEJMra040223
de Bruin NMWJ, Prickaerts J, van Loevezijn A, Venhorst J, de Groote L, Houba P, Reeneerkens O, Akkerman S, Kruse CG (2011) Two novel 5-HT6 receptor antagonists ameliorate scopolamine-induced memory deficits in the object recognition and object location tasks in Wistar rats. Neurobiol Learn Mem 96(2):392–402. https://doi.org/10.1016/j.nlm.2011.06.015
Dellu F, Fauchey V, Moal ML, Simon H (1997) Extension of a new two-trial memory task in the rat: influence of environmental context on recognition processes. Neurobiol Learn Mem 67(2):112–120. https://doi.org/10.1006/nlme.1997.3746
Dhami M, Raj K, Singh S (2021) Neuroprotective effect of fucoxanthin against intracerebroventricular streptozotocin (ICV-STZ) induced cognitive impairment in experimental rats. Curr Alzheimer Res 18(8):623–637. https://doi.org/10.2174/1567205018666201111184460
Diz-Chaves Y, Pernía O, Carrero P, Garcia-Segura LM (2012) Prenatal stress causes alterations in the morphology of microglia and the inflammatory response of the hippocampus of adult female mice. J Neuroinflammation 9(1):580. https://doi.org/10.1186/1742-2094-9-71
Eichenbaum H (2017) On the integration of space, time, and memory. Neuron 95(5):1007–1018. https://doi.org/10.1016/j.neuron.2017.06.036
el Sayed NS, Ghoneum MH (2020) Antia, a natural antioxidant product, attenuates cognitive dysfunction in streptozotocin-induced mouse model of sporadic Alzheimer’s disease by targeting the amyloidogenic, inflammatory, autophagy, and oxidative stress pathways. Oxid Med Cell Longev. https://doi.org/10.1155/2020/4386562
Geng J, Liu W, Gao J, Jiang C, Fan T, Sun Y, Qin Z, Xu Q, Guo W, Gao J (2019) Andrographolide alleviates Parkinsonism in MPTP-PD mice via targeting mitochondrial fission mediated by dynamin-related protein 1. Br J Pharmacol 176(23):4574–4591. https://doi.org/10.1111/bph.14823
Gerzon MFB, Bona NP, Soares MSP, Teixeira FC, Rahmeier FL, Carvalho FB, da Cruz Fernandes M, Onzi G, Lenz G, Gonçalves RA, Spanevello RM, Stefanello FM (2020) Tannic acid ameliorates STZ-induced mouse model of sporadic Alzheimer’s disease by targeting the amyloidogenic, inflammatory, autophagy, and oxidative stress pathways. Oxid Med Cell Longev. https://doi.org/10.1155/2020/4386562
Griep P (2016) Intracerebroventricular streptozotocin injections as a model of Alzheimer’s disease: in search of a relevant mechanism. Mol Neurobiol 53(3):1741–1752. https://doi.org/10.1007/s12035-015-9132-3
Guan S, Tee W, Ng D, Chan T, Peh H, Ho W, Cheng C, Mak J, Wong W (2013) Andrographolide protects against cigarette smoke-induced...
oxidative lung injury via augmentation of Nrf2 activity. Br J Pharmacol 168(7):1707–1718. https://doi.org/10.1111/bph.12054
Guo Z, Chen Y, Mao Y-F, Zheng T, Jiang Y, Yan Y, Yin X, Zhang B (2017) Long-term treatment with intranasal insulin ameliorates cognitive impairment, tau hyperphosphorylation, and microglial activation in a streptozotocin-induced Alzheimer’s rat model. Sci Rep 7(1):45971. https://doi.org/10.1038/srep45971
Gupta S, Yadav K, Mantri SS, Singhal NK, Ganesh S, Sandhir R (2018) Evidence for compromised insulin signaling and neuronal vulnerability in experimental model of sporadic Alzheimer’s disease. Mol Neurobiol 55(12):8916–8935. https://doi.org/10.1007/s12052-018-0985-0
Heneka MT, Carson MJ, el Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM, Herrup K, Frautschy SA, Finski B, Brown GC, Verkhratsky A, Yamanaka K, Koistinaho J, Latz E, Halle A, Kummer MP (2015) Neuroinflammation in Alzheimer’s disease. Lancet Neurol 14(4):388–405. https://doi.org/10.1016/S1474-4422(15)70016-5
Hindam MO, Sayed RH, Skalicka-Woźniak K, Budzynska B, el Sayed NS (2020) Xanthotoxin and umbelliferone attenuate cognitive dysfunction in a streptozotocin-induced rat model of sporadic Alzheimer’s disease: The role of JAK2/STAT3 and Nrf2/HO-1 signalling pathway modulation. Phytother Res. https://doi.org/10.1002/ptr.6686
Hossain R, Quispe C, Herrera-Bravo J, Beltrán JF, Islam MT, Guo Z, Chen Y, Mao Y-F, Zheng T, Jiang Y, Yan Y, Yin X, Zhang B (2019) A review for the neuroprotective effects of andrographolide and its derivatives on various neurodegenerative disorders. Biomed Pharmacother 117:109078. https://doi.org/10.1016/j.biopha.2019.109078
Martin SJ, Clark RE (2007) The rodent hippocampus and spatial memory: from synapses to systems. Cell Mol Life Sci 64(4):401–431. https://doi.org/10.1007/s00018-007-6336-3
More SV, Kumar H, Cho DY, Yun YS, Choi DK (2016) Toxin-induced experimental models of learning and memory impairment. Int J Mol Sci. https://doi.org/10.3390/ijms1701447
Moura ELR, dos Santos H, Celes APM, Bassani TB, Souza LC, Vital MABF (2020) Effects of a nutritional formulation containing caprylic and capric acid, phosphatidylserine, and docosahexaenoic acid in Streptozotocin-lesioned rats. J Alzheimers Dis Rep 4(1):353–363. https://doi.org/10.3233/ADR-200175
Patel R, Kaur K, Singh S (2021) Protective effect of andrographolide against STZ-induced Alzheimer’s disease in experimental rats: possible neuromodulation and Aβ-lowering effects. Inflammopharmacology 29(4):1157–1168. https://doi.org/10.1007/s10787-021-00843-6
Paxinos G, Watson CR (2007) The rat brain in stereotaxic coordinates (6th ed.). Academic Press
Pierzyłowska K, Podlacha M, Gaffike L, Majkutewicz I, Mantejko A, Andreatini R (2021) Andrographolide blocks 50-kHz ultrasonic vocalizations, hyperlocomotion and oxidative stress in an experimental rat model of Alzheimer’s disease. Biomedicines 8(5):1–15. https://doi.org/10.3390/biomedicines8050104
Rivera DS, Lindsay C, Codocedo JF, Morel I, Pinto C, Cistermans P, Bozinovic F, Inestrosa NC (2016) Andrographolide recovers cognitive performance in a natural model of Alzheimer’s disease

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Martin SJ, Clark RE (2007) The rodent hippocampus and spatial memory: from synapses to systems. Cell Mol Life Sci 64(4):401–431. https://doi.org/10.1007/s00018-007-6336-3
More SV, Kumar H, Cho DY, Yun YS, Choi DK (2016) Toxin-induced experimental models of learning and memory impairment. Int J Mol Sci. https://doi.org/10.3390/ijms1701447
Moura ELR, dos Santos H, Celes APM, Bassani TB, Souza LC, Vital MABF (2020) Effects of a nutritional formulation containing caprylic and capric acid, phosphatidylserine, and docosahexaenoic acid in Streptozotocin-lesioned rats. J Alzheimers Dis Rep 4(1):353–363. https://doi.org/10.3233/ADR-200175
Patel R, Kaur K, Singh S (2021) Protective effect of andrographolide against STZ-induced Alzheimer’s disease in experimental rats: possible neuromodulation and Aβ-lowering effects. Inflammopharmacology 29(4):1157–1168. https://doi.org/10.1007/s10787-021-00843-6
Paxinos G, Watson CR (2007) The rat brain in stereotaxic coordinates (6th ed.). Academic Press
Pierzyłowska K, Podlacha M, Gaffike L, Majkutewicz I, Mantejko A, Andreatini R (2021) Andrographolide blocks 50-kHz ultrasonic vocalizations, hyperlocomotion and oxidative stress in an experimental rat model of Alzheimer’s disease. Biomedicines 8(5):1–15. https://doi.org/10.3390/biomedicines8050104
Rivera DS, Lindsay C, Codocedo JF, Morel I, Pinto C, Cistermans P, Bozinovic F, Inestrosa NC (2016) Andrographolide recovers cognitive performance in a natural model of Alzheimer’s disease

Neurotoxicity Research (2022) 40:1440–1454

Martin SJ, Clark RE (2007) The rodent hippocampus and spatial memory: from synapses to systems. Cell Mol Life Sci 64(4):401–431. https://doi.org/10.1007/s00018-007-6336-3
More SV, Kumar H, Cho DY, Yun YS, Choi DK (2016) Toxin-induced experimental models of learning and memory impairment. Int J Mol Sci. https://doi.org/10.3390/ijms1701447
Moura ELR, dos Santos H, Celes APM, Bassani TB, Souza LC, Vital MABF (2020) Effects of a nutritional formulation containing caprylic and capric acid, phosphatidylserine, and docosahexaenoic acid in Streptozotocin-lesioned rats. J Alzheimers Dis Rep 4(1):353–363. https://doi.org/10.3233/ADR-200175
Patel R, Kaur K, Singh S (2021) Protective effect of andrographolide against STZ-induced Alzheimer’s disease in experimental rats: possible neuromodulation and Aβ-lowering effects. Inflammopharmacology 29(4):1157–1168. https://doi.org/10.1007/s10787-021-00843-6
Paxinos G, Watson CR (2007) The rat brain in stereotaxic coordinates (6th ed.). Academic Press
Pierzyłowska K, Podlacha M, Gaffike L, Majkutewicz I, Mantejko A, Andreatini R (2021) Andrographolide blocks 50-kHz ultrasonic vocalizations, hyperlocomotion and oxidative stress in an experimental rat model of Alzheimer’s disease. Biomedicines 8(5):1–15. https://doi.org/10.3390/biomedicines8050104
Rivera DS, Lindsay C, Codocedo JF, Morel I, Pinto C, Cistermans P, Bozinovic F, Inestrosa NC (2016) Andrographolide recovers cognitive performance in a natural model of Alzheimer’s disease
