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Inhibitory effect of sesquiterpene lactones and the sesquiterpene alcohol aromadendrane-4β,10α-diol on memory impairment in a mouse model of Alzheimer

Solomon K.S. Amoah [c], Maria Tereza Dalla Vecchia [a], Beatriz Pedrini [a], Gabriela Lazzarotto Carnhelutti [a], Ana Elisa Gonçalves [b], Diogo Adolfo dos Santos [b], Maique W. Biavatti [c,*], Márcia Maria de Souza [b]

[a] Núcleo de Investigações Químico Farmacêuticas (NIQFAR), Universidade do Vale do Itajaí, UNIVALI, Itajaí, Santa Catarina, Brazil
[b] Programa de Pós Graduação em Ciências Farmacêuticas, Universidade do Vale do Itajaí , UNIVALI, Itajaí, SC, Brazil
[c] Programa de Pós Graduação em Farmácia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

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ABSTRACT

Alzheimer's disease (AD), a progressive neurodegenerative disorder of the aged brain with no known cause or cures, has become a major medical and social problem for industrialized countries. Cerebral deposition of amyloid-β peptide (Aβ) is a critical feature of AD. The use of medicinal plants as an alternative form of prevention, or even as a possible treatment of AD, is therefore interesting areas of research. Sesquiterpene lactones and a sesquiterpene alcohol are compounds found in *H. brasiliense* that have several anti-oxidative and anti-inflammatory effects. In the present study, we investigated whether these compounds have neuroprotective effects in an amyloid-β peptide-induced Alzheimer's disease mouse model. Mice were injected with $A\beta_{1-42}$ peptide intracerebroventricularly and were subsequently injected (i.c.v.) with 1 μg/site of IGM-A (15-acetoxy-isogermafurenolide), IGM-H (15-hydroxy-isogermafurenolide), PDA (Podoandin), EHP (1,2-epoxy-10α-hydroxy-podoandin), HDS (13-hydroxy-8,9-dehydroshizukanolide), and ARD (aromadendrane-4β,10α-diol). Seven days after treatments the animals had their memory tested in the inhibitory avoidance. After the behavioral testing of animals the brains were removed and subjected to biochemical tests for oxidative stress. The results showed that ARD, HDS and PDA significantly ameliorated the $A\beta_{1-42}$ peptide-induced memory impairment in the passive avoidance task ($P < 0.05$). In addition, GSH activity was increased while the TBARS levels were decreased by treatment with these compounds. These results suggest that these compounds inhibit the cognitive deficit of animals induced peptide amyloid and may be potential candidates for Alzheimer's disease therapy.

1. Introduction

Alzheimer's disease (AD) is a progressive age-related neurodegenerative disorder and is the most common form of dementia among the elderly, generally diagnosed in individuals over the age of 65 years. The main pathological hallmarks of AD are the accumulation of amyloid plaques, or senile plaques, containing extracellular deposits of amyloid-β peptide (Aβ) and the presence of intraneuronal neurofibrillary tangles (NFTs), which result from hyperphosphorylated τ-protein (Konrath et al., 2013). The oxidation of lipids, proteins, and nucleic acids in neurons is also a common pathological feature of AD (Chen and Zhong, 2014).

The etiology of AD is still unknown, but several factors have been suggested that appear to reduce the incidence of the disease. Three main approaches have been taken: the first involves the re-establishment of neurotransmitters levels, with the inhibition of cholinesterases, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and also monoamine oxidase (MAO) enzymes. The second one concerns neuroprotection where oxidative stress is considered to be an early event in the pathological cascade for the disease, suggesting the potential use of antioxidants to limit the effects of free radicals on nerve cells. The third approach deals with specific aspects related to AD, including the decrease in the production or aggregation of Aβ peptide, and inhibition of γ and β-secretase enzymes which play a critical role in the amyloidogenic and t-protein pathways, among others (Konrath et al., 2013).

The current drug therapy used is palliative and drugs exhibit

* Correspondence to: Laboratório de Farmacognosia, CIF/CCS/UFSC, Campus Universitário, Trindade, 88040-900 Florianópolis, SC, Brazil.
E-mail address: maique.biavatti@ufsc.br (M.W. Biavatti).

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modest and transient effects in improving disease manifestation and can hardly prevent, halt, or reverse the disease. Exploration of natural active ingredients from medicinal herbs for treatment of AD has attracted substantial attention worldwide. Thus far, drugs including galantamine and huperzine which were originated from traditional Chinese herbs have been developed and used in clinics to treat mild to moderate AD (Gao et al., 2013).

Hedyosmum brasiliense Miq. is identified in folk medicine as "Cidrão", "Âmbar vegetal", "Canela-cânfora" "Chá-de-bugre", "Hortelã-do-brejo" among others. Although it is widely used as a sedative, hypnotic, antidepressant, stomachic and for aphrodisiac purposes (Di Stasi et al., 1988; Reitz, 1965), studies which seek to validate its phytochemical composition and also to demonstrate its pharmacological effects are few. The neurochemical properties of the ethanol leaf extract and its fractions showed sedative, hypnotic, anxiolytic and antidepressant effects, (Tolardo et al., 2010) and the antidepressant effect of the isolated sesquiterpene lactones 13-hydroxy-8,9-dehydroshizukanolide (or onoseriolide) and podoandin have recently been studied by this group (Goncalves et al., 2012). The plant itself is not used in folk medicine in cognitive deficits, but the presence of lactones as a component in their chemical constitution sparked interest for research in this area. Has been reported that these compounds inhibit oxidative stress (Gach et al., 2015) In addition, triterpene lactones are acetylcholinesterase inhibitors (Hajimehdipoor et al., 2014). Since anticholinesterase agents are used in the treatment of the Alzheimer's disease, and that oxidative stress may be involved in neurodegenerative processes, the present work describes the effects of sesquiterpene lactones and of a sesquiterpene alcohol from the leaves of *H. brasiliense*. These compounds were tested against cognitive deficit induced by the amyloid peptide $A\beta_{1\text{-}42}$, with the purpose of identifying their anti-Alzheimer potential.

2. Materials and methods

2.1. Plant material

Aerial parts of *H. brasiliense* Miq (Chloranthaceae) were collected in the municipal area of Antonio Carlos, Santa Catarina, Brazil in October 2009. It was identified by the botanist Dr. Ademir Reis and a voucher specimen (no. 2031) was deposited at the Lyman Bradford Smith Herbarium (UNIVALI, Itajaí – Santa Catarina).

2.2. Isolation of compounds

Fresh leaves of *H. brasiliense* (5 kg) were extracted, at room temperature, with ethanol (95%) for 15 days. The solvent was removed under reduced pressure and used to re-extract the plant material. The resulting crude extracts were combined (190 g) and stored in a desiccator in vacuo, to remove any solvent residue. The extract was dissolved in water and partitioned with solvents of increasing polarity, yielding *n*-hexane (16 g), dichloromethane (CH_2Cl_2, 4 g), and ethyl acetate (EtOAc, 13 g) fractions, as well as a residual aqueous fraction. All fractions were stored at −18 °C.

The *n*-hexane fraction was subjected to flash silica gel column chromatography (CC, 240–400 mesh), eluting with a gradient of 0–70% CH_2Cl_2 in *n*-hexane (200 ml) followed by 0–70% EtOAc in CH_2Cl_2 (200 ml), yielding eight sub-fractions (A–H).

Sub-fraction B was re-crystallized to produce **PDA** (300 mg), which was identified as podoandin by comparison with previously reported NMR spectroscopic data (Blay et al., 2000; Kubo et al., 1992). **HDS** (40 mg), crystallized spontaneously from sub-fraction C and was identified as onoseriolide by comparing its NMR spectroscopic data with those reported in the literature (Trentin et al., 1999). Sub-fraction F was submitted to silica gel CC with eluting with *n*-hexane–CH_2Cl_2–acetone (4:5:1), yielding **IGM-A** which was elucidated as 15-acetoxy-isogermafurenolide (35 mg) (Amoah et al., 2013). 3 g of the dichloromethane fraction was subjected to flash silica gel (240–400 *mesh*) column chromatography (CC) and eluted in a gradient system consisting of increasing concentrations of CH_2Cl_2 in *n*-hexane (0–70%, 200 ml) followed by ethyl acetate in dichloromethane (0–70%, 200 ml) which produced sub-fractions (I-P). Sub-fraction M was purified on silica gel CC, eluting with 9:1 to 3:7 acetone in *n*-hexane, which yielded 11 new sub-fractions (M1–M11). Sub-fraction M8 (111 mg) was subjected to further silica gel CC, eluting with 9:1 to 3:7 EtOAc in dichloromethane, which gave **IGM-H**, elucidated as 15-hydroxy-isogermafurenolide (15 mg) (Amoah et al., 2013). Subfraction P was also subjected to CC on silica which led to the isolation of **EHP** and **ARD** and were identified as sesquiterpenoids 1,2-epoxy-10α-hydroxy-podoandin (Amoah et al., 2013) and aromadendrane-4β,10α-diol (Moreira et al., 2003) respectively. The elucidation of these substances was achieved by 1D, 2D NMR and in comparison with published data, as cited above.

2.3. Chemicals and reagents

All drugs were freshly prepared before use. 5,5′-Dithiobis (2-nitro benzoic acid) DTNB, bovine serum albumin (BSA), and reduced glutathione (GSH) standard were purchased from SIGMA (EUA). Thiobarbituric acid and $A\beta_{1\text{-}42}$ were obtained from (Demoleris/SA, BRAZIL)

2.4. Animals

Experiments were conducted using 3-month-old male Swiss albino mice weighing 25–35 g, obtained from central bioterium of UNIVALI. They were kept in groups of 10 animals per cage ($41 \times 34 \times 16$ cm) and maintained in a room under controlled temperature (23 ± 1 °C). They were subjected to a 12 h light cycle (lights on 7:00 a.m.) with free access to food and water. All procedures used in the present study complied with the guidelines on animal care of the local Ethics Committee on the Use of Animals which follows the NIH publication "Principles of laboratory animal care". The experimental protocols were submitted to the ethical committee for animal use CEUA / UNIVALI being approved with the number CEUA/21/12.

2.5. Amyloid- β peptide administration

$A\beta_{1\text{-}42}$ (Tocris, MO, USA) (Bachem, CA, USA) was prepared as stock solution at a concentration 0.6 μg/μl in sterile 0.1 M phosphate-buffered saline (PBS) (pH 7.4), and aliquots were stored at −20 °C. $A\beta_{1\text{-}42}$ was aggregated by incubation in sterile distilled water at 37 °C for 4 days before use as described previously (Prediger et al., 2007) $A\beta_{1\text{-}42}$ (400 pmol/mouse), or control solution (PBS) were administered by intracerebroventricular (i.c.v.) route using a microsyringe with a 28-gauge stainless-steel needle 3.0 mm long (Hamilton®) according to the procedure previously described by Piermartiri et al. (2010). After counted anesthesia, each animal was immobilized carefully and in brief, the needle was inserted unilaterally 1 mm to the right of the midline point equidistant from each eye, at an equal distance between the eyes and the ears and perpendicular to the plane of the skull. The injection volume (3 μl) of $A\beta_{1\text{-}42}$, was delivered gradually. After 5 min after application of the peptide, microsyringes were changed and the compounds were applied in the same manner as the peptide. Mice exhibited normal behavior recovery from anesthesia. The injection placement or needle track was visible and was verified at the time of dissection. The present $A\beta_{1\text{-}42}$ dose is comparable to that of previous literature on the use of $A\beta_{1\text{-}42}$

(Prediger et al., 2007).

2.6. Experimental design

The behavioral tasks studies were performed 7 days after i.c.v. administration of $A\beta_{1-42}$ (400 pmol/mouse), compounds, vehicle (PBS/0.5 μl) or galantamine (0.1 mg/kg, s.c.) as follows illustrative scheme:

2.6.1. Open field test

To verify the effects of i.c.v. treatment with compounds on locomotor activity, the animals were placed for 5 min in the open field arena. The apparatus, made of wood covered with impermeable Formica®, had a black floor of 30 cm × 30 cm (divided by white lines into nine squares of 10 cm × 10 cm) and transparent walls, 15 cm high. The experiments were conducted in a sound-attenuated room under low-intensity light (12 lx). Each mouse was placed in the centre of the open field and the numbers of squares crossed (crossings) and rearings were registered (Tolardo et al., 2010).

2.6.2. Elevated plus-maze test

In order to determine if compounds might have affected mobility, locomotion or pro- or anti-conflict behaviours, animals were also submitted to an elevated plus-maze (EPM) (Pellow et al., 1985). The EPM is made of wood, in the form of a Greek cross with two open arms (30 × 5 × 0.2 cm) and two closed (30 × 15 × 5 cm), the arms are connected by a central open area (5 × 5 cm). The EPM is raised to a height of 45 cm above the floor. Animals were allowed to explore the apparatus, which is used to measure pro- or anti-conflict behaviours as animal models of anxiety (Pellow et al., 1985; Tolardo et al., 2010). After 5 min of i.c.v. administration of the compounds (1 μg/0.5 μl/side,) or vehicle, each animal was placed in the centre of the apparatus, facing one of the enclosed arms. The total number of entries into the four arms, the number of entries and the time spent into the open arms were recorded over a 5 min session. After each trial, the open field apparatus was wiped clean with ethanol (10%) solution.

2.6.3. Step-Down inhibitory avoidance test.

The animals were submitted to one trial step-down inhibitory avoidance and this test was performed according to Izquierdo et al. (2004). The animals were gently placed on a 2.5-cm high, 7.0-cm wide, 25.0-cm long Formica® platform at the left side of a 50 × 25 × 25-cm apparatus, floor of which was a series of parallel 0.1-cm caliber stainless-steel bars spaced 1.0 cm apart. A 15 W lamp at the top center of the device illuminates the apparatus. In the training session, latency to step down placing the four paws on the grid was measured, immediately followed by a 3.0-s, 0.4-mA foot-shock. Retention test sessions, procedurally identical to the training session, were carried out 24 h after training, without foot-shock and with step-down latency limited to 180 s, i.e., test session values higher than 180 s were counted as 180 s. Retention test performances at 24 h were taken as measures of retention of long-term memory.

2.7. Biochemical determinations

Animals were killed by cervical dislocation, brains were removed and homogenized in phosphate buffer (pH=7.4). The homogenates were than centrifuged at 3000 g for 15 min. The supernatant of homogenates were used for biochemical estimations using the methods described below.

2.7.1. Estimation of brain thiobarbituric acid reactive species (TBARS) level

Lipid peroxidation is measured by the production of malondialdehyde (MDA), achieved spectrophotometrically by the method of Ohkawa et al. (1979) using 1,1,3,3-tetraethoxypropane as standard and expressed as nmol per mg protein. The reagents acetic acid 1.5 ml (20%) pH 3.5, 1.5 ml thiobarbituric acid (0.8%) and 0.2 ml sodium dodecyl sulfate (8.1%) were added to 0.1 ml of the processed sample. This mixture was then heated at 100 °C for 60 min, cooled under tap water and 5 ml of *n*-butanol:-pyridine (15:1% v/v) plus 1 ml of distilled water was added. The mixture was shaken vigorously on vortex. After centrifugation at 4000 g for 10 min, the organic layer was withdrawn and absorbance measured at 532 nm.

2.7.2. Estimation of brain reduced glutathione (GSH) level

The whole brain GSH level was measured by the method of (Beutler et al., 1963) with slight modifications. The absorbance was measured at 412 nm (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA). Different concentration of the GSH standard was processed similarly to prepare a standard curve (5–50 μg). Results were expressed as nmol of GSH/mg of protein.

2.8. Statistical analysis

For tests as open field and plus maze, the values are expressed as mean ± S.E.M. Statistical significance was determined by ANOVA followed by Newman-Keuls post-hoc test. Values with $P < 0.05$ were considered significant. For inhibitory avoidance task, because of the ceiling of 180 s imposed on test session measures, non-parametric statistics were used. Data are expressed as median (interquartile range). The two-tailed Mann-Whitney *U*-test evaluated test session differences from controls. The Wilcoxon test evaluated differences between training and test session performances in each group.

3. Results

3.1. Effects of compounds on the behavioral parameters in the open field test

The tested compounds are presented in the Fig. 1. The Table 1 shows that none of these compounds and galantamine when administered in animals produced changes in behavioral parameters like crossings and rearing's when compared to the control group ($P > 0.05$). These results indicate that there were no sedative effects in mice, as assessed by the open field apparatus (Table 1).

3.2. Effects of compounds on the behavioral parameters in the plus maze test

Table 2 shows that the number of entries and the time spent by the animals in the open arms were not altered by treatment with the compounds tested and galantamine when compared with the control group ($P > 0.05$). Behavioral changes were also not observed in the frequency of entries and time spent in the closed arms ($P > 0.05$). These results also demonstrated that treatment of animals with the compounds and galantamine did not alter the behavioral parameters related to anxiety.

3.3. Effects of compounds on aversive memory of Swiss mice in the inhibitory avoidance test

Results for the inhibitory avoidance test are displayed in Fig. 2. In the test session, groups that received the amyloid peptide $A\beta_{1-42}$ (400 pmol/mouse) and were treated with ARD, HDS and PDA

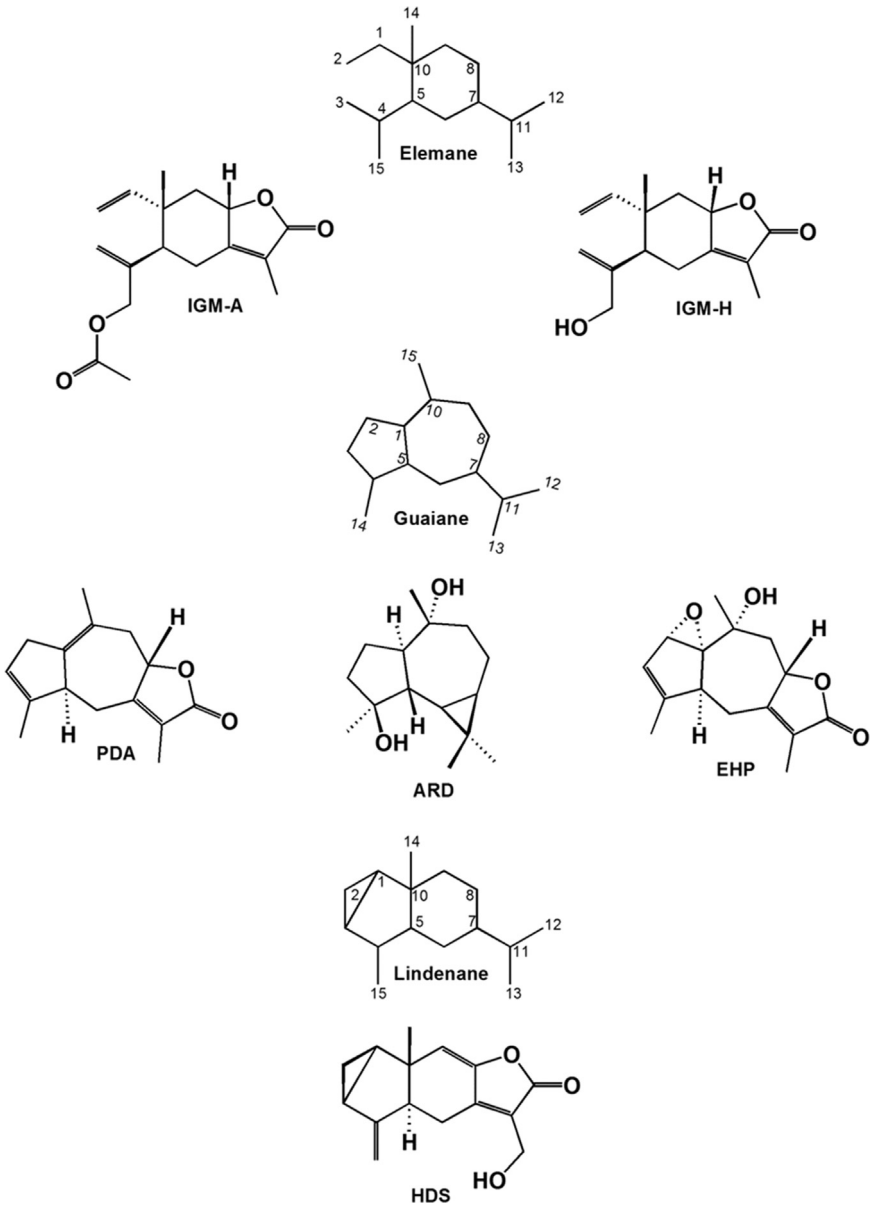

Fig. 1. Structures of substances evaluated in Alzheimer model and their respective carbonskeletons. **IGM-A**-15-acetoxy-isogermafurenolide, **IGM-H** – 15-hydroxy-isogermafurenolide, **PDA** – Podoandin, **EHP**-1,2-epoxy-10α-hydroxy-podoandin, **HDS** – onoseriolide, **ARD**-aromadendrane-4β,10α-diol.

Table 1
Effect of the administration of compounds (1 μg/mouse, i.c.v.) and galantamine (0.1 mg/kg, s.c.) on animal behavior in the Open Field test. Data are presented as mean ± S.E.M. $N=8–10$ per group. Does not differ from vehicle.

Treatments	Crossing	Rearing
Vehicle	112.2 ± 3.18	48.10 ± 2.20
ARD	109.6 ± 5.44	53.60 ± 1.62
HDS	120.1 ± 3.86	55.10 ± 2.72
IGM-H	109.5 ± 5.79	51.10 ± 1.86
IGM-A	114.2 ± 4.72	61.20 ± 2.22
PDA	109.7 ± 4.38	57.90 ± 2.19
EHP	110.2 ± 4.33	57.70 ± 1.22
Galantamine	106.4 ± 3.31	50.20 ± 6.33

Table 2
Effect of the administration of compounds (1 μg/mouse, i.c.v.) and galantamine (1 μg/mouse, i.c.v.) on animal behavior in the plus maze test. Data are presented as mean ± S.E.M. $N=8–10$ per group. Does not differ from vehicle.

Treatments	% Frequency entry into the open arms	%Frequency entry into the closed arms	% Time of permanence in the open arms	% Time of permanence in the closed arms
Vehicle	34.51 ± 2.12	76.90 ± 2.10	42.50 ± 1.52	61.23 ± 2.15
ARD	36.35 ± 4.21	83.00 ± 3.70	48.50 ± 1.56	64.53 ± 3.55
HDS	44.11 ± 3.17	81.00 ± 2.17	43.50 ± 1.23	67.22 ± 4.52
IGM-H	35.52 ± 2.23	79.00 ± 1.27	42.50 ± 1.57	60.29 ± 1.25
IGM-A	45.12 ± 4.23	81.40 ± 3.70	47.50 ± 2.56	65.45 ± 1.65
PDA	38.56 ± 2.63	69.14 ± 2.6	47.30 ± 3.50	57.56 ± 4.15
EHP	37.52 ± 3.23	63.29 ± 4.65	42.34 ± 3.50	64.53 ± 1.45
Galantamine	37.15 ± 5.31	69.11 ± 1.32	42.34 ± 2.15	61.45 ± 3.51

(1 μg, i.c.v.) and galantamine (0.1 mg/kg, s.c.) showed higher memory retention in test session when compared with animals treated with vehicle (control), an effect which was statistically significant. These results show that these compounds reverse the cognitive deficits induced by the amyloid peptide $A\beta_{1-42}$. In addition, the Fig. 2 also shows that SHAM animals (who did not receive treatment with amyloid peptide) had higher memory retention in test session when compared with animals treated with

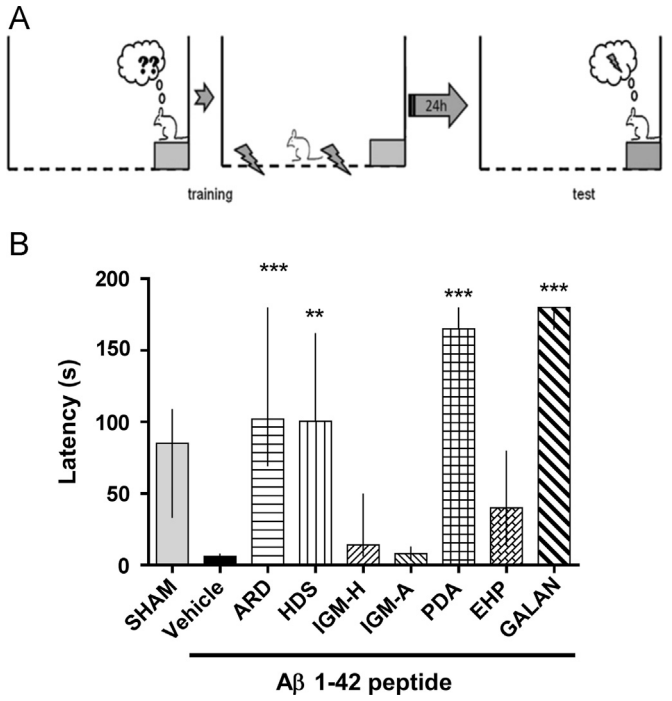

Fig. 2. (A) The representation scheme of the experiment of inhibitory avoidance. (B) Effect of intracerebroventricular infusion of vehicle (PBS/0.5 μl) and compounds: **IGM-A**, **IGM-H, PDA, EHP**, **HDS, ARD** (1 μg/mouse, i.c.v.) that received amyloid peptide $A\beta_{1-42}$ (400 pmol/mouse, i.c.v) on inhibitory avoidance memory test sessions. Bars represent median (interquartile range) of step-down latencies during a memory retention test session carried out 24 h after training. *N* 8–10 per group; * $P<0.05$ and ** $P<0.001$, comparing with vehicle in Dunn's comparison after Kruskal–Wallis test, #$P<0.05$ comparing the treatments in the test sessions. SHAM=group of animals that received no treatment with $A\beta_{1-42}$.

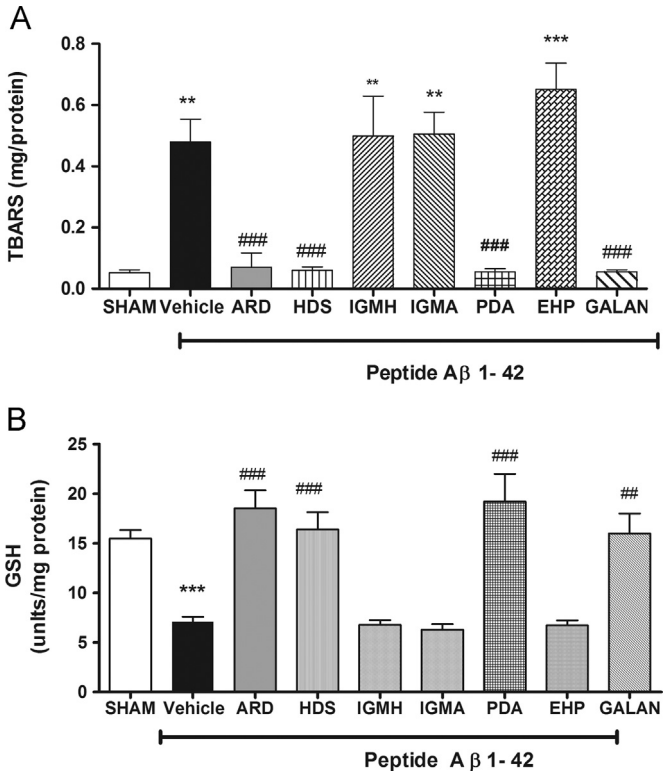

Fig. 3. Effect of intracerebroventricular infusion of vehicle (PBS/0.5 μl), compounds: **IGM-A**, **IGM-H, PDA, EHP**, **HDS, ARD** (1 μg/mouse, i.c.v.) and galantamine (0.1 mg/kg, s.c.) that received amyloid peptide $A\beta_{1-42}$ (400 pmol/mouse, i.c.v.) on TBARS content (A) and GSH activity (B). Bars represent means ± S.E.M. N 8-10 per group; **$P<0.001$, comparing with SHAM and, ##$P<0.001$ or ##$P<0.0001$ comparing with vehicle. $A\beta_{1-42}$ infusion leads to significant alterations in the activities of antioxidant enzymes (GHS), and TBARS in hippocampus. Pretreatment of ARD, HDS, PDA and galantamine significantly increased the activity of this enzyme and decreased of lipid peroxidation. SHAM=group of animals that received no treatment with $A\beta_{1-42}$.

vehicle (control). In groups of animals treated with compounds IGM-H, IGM-A and EHP, no significant results regarding the reversal of cognitive deficits induced by $A\beta_{1-42}$ were observed.

3.4. *Effect on brain oxidative stress levels*

I.c.v. $A\beta_{1-42}$ markedly enhanced oxidative stress due to the increase of the TBARS (Fig. 3 A) and the reduction of the GSH levels in the brain (Fig. 3 B) when compared to the control (SHAM) group. The compounds IGM-H, IGM-A and EHP (0.1 mg/kg/day, i.p.) produced significant increase on TBARS levels. However, significant reduction in oxidative stress by enhanced GSH level was observed with ARD, HDS, PDA and galantamine (0.1 mg/kg/day, s.c.) in $A\beta_{1-42}$ mice.

4. Discussion

An immense health burden has been imposed by AD, for which there is no prevention and no disease-modifying agents which have been approved so far. AD is a progressive neurodegenerative disorder characterized, at least in part, by abnormal accumulation of amyloid-β peptide (Aβ) in the brain (Huang and Mucke, 2012). The accumulated Aβ is believed to play an important role in the pathogenesis of AD (Maltsev et al., 2011) hence, an important target for prevention and treatment of AD (Lemere and Masliah, 2010). In AD treatment, acetylcholinesterase inhibitors are among the most extensively studied drugs, which have shown to significantly improve cognitive function in AD. However, these drugs are known to have limitations for clinical use due to their short-half-lives and/or unfavorable adverse effects (Gongadze et al., 2008; Singh et al., 2012). Thus, the study of new compounds with anti-Alzheimer potential is fundamental.

Essa et al. (2012) and Howes and Houghton (2012) proved that herbal medicines containing phytochemicals can modify brain aging. Various factors which may contribute to the impairment of learning and memory change in the brain neurotransmitter levels include cholinergic and monoamines and free radicals in the brain region.

Galantamine, unlike the other anticholinesterases, is an alkaloid obtained from natural sources. It was first isolated from *Galanthus nivalis* L., which belongs to the Amaryllidaceae family. This product was first marketed in 2001 and is a long-acting, relatively selective AChE inhibitor, with less BChE inhibitory activity and some gastrointestinal adverse effects described. Galantamine also improves cholinergic transmission by allosteric modulation of nicotinic receptors and has been used to treat the symptoms of other forms of dementia. It is currently isolated from daffodil bulbs (*Narcissus* spp) by the pharmaceutical industry, which has engendered an exponential interest in the isolation and characterization of alkaloids from Amaryllidaceae bulbs (e.g. lycorine, homolycorine and hipeastrine), most of them having anticholinesterase activity. Besides, it also exhibits less toxicity than other AChEIs like tacrine (Fang et al., 2014; Konrath et al., 2013).

Sesquiterpenoids are natural compounds with a 15-carbon frame skeleton that are considered to be important for plants protection and for humans, presenting many biological activities, such as anti-inflammatory, antimicrobial, antitumor, and cytotoxic properties (Chadwick et al., 2013; Chen et al., 2011). About 100 different skeleton types of sesquiterpenoids have been isolated

(Kawabata and Mizutani, 1988; Schmidt, 2006), and in *H. brasiliense,* sesquiterpenoids with the elemane, guaiane and lindenane carbon skeletons (Fig. 1) have been found. In this present work, the results showed that only PDA and ARD (guaiane group), and HDS (lindenanolide group) significantly ameliorated the $A\beta_{1\text{-}42}$ peptide-induced memory impairment in the passive avoidance test. Step-down inhibitory avoidance task is a classic task of memory with a strong aversive component. This is a quick, easy to perform model without the need for multiple training sessions (Alonso et al., 2005). How quickly learned, the inhibitory avoidance allows for the precise time after learning in which treatments affect memory consolidation difficult to discern in tasks that require many procedures. In humans, this represent an inhibitory avoidance constantly used and a necessary form of memory (Furini et al., 2014) This model does not only depend on brain structures such as the hippocampus, but others such as entorhinal cortex and amygdala (Blank et al., 2014), all affected by the neurodegenerative process in the Alzheimer's disease (Singh et al., 2014).

Another important aspect evaluated in this study was that treatment of animals with these compounds produced no detectable adverse effects such as increased or decreased locomotor activity. These effects on the locomotor activity were investigated using the open field test to rule out the possibility that the effect of the compounds in the animals subjected to the inhibitory avoidance task could be a consequence of increased locomotor activity. However, it should be emphasized that drugs that induce hyperlocomotion may give a "false" positive effect in this test, whereas drugs that decrease locomotion may give a 'false' negative result (Tolardo et al., 2010). Similarly, treatment with the compounds causes no detectable emotional changes in the model of elevated plus-maze, which is frequently used to detect and evaluate the anxiolytic/anxiogenic properties of drugs (Pellow and SE, 1987).

In this present work, i.c.v. $A\beta_{1-42}$ markedly produced cognitive deficits and abnormal biochemical alterations in the mice brain. The oxidative stress is the state of imbalance between the level of antioxidant defense mechanism and production of the free radicals that favors potential brain damage. Brain tissue is particularly susceptible to oxidative damage due to its high oxygen content, low level of antioxidant protection, and high level of polyunsaturated fatty acids (Moreira et al., 2005). Therefore, it is believed that pharmacological modification of oxidative damage is one of the most promising strategies in the treatment of neurodegenerative diseases. Reactive oxygen species (ROS)-induced damage to biomolecules (lipids, proteins, DNA and RNA) plays an important role in the advancement of aging and age-related neurodegenerative disorders such as AD (Liu et al., 2003). In vivo studies have shown that rats showed AD-like behavioral and pathological changes induced by injection of Aβ into the lateral cerebral ventricle or hippocampus (Cheng et al., 2006; Nobakht et al., 2011). However, the mechanisms underlying Aβ-mediated neurotoxicity remain unclear. It is evidenced that Aβ can accumulate in mitochondria and lead to enhanced reactive oxygen species (ROS) generation and mitochondrial energy metabolism impairment (Alikhani et al., 2011; Manczak et al., 2004). Reduced GSH is the most abundant non-protein thiol, which buffers free radicals in brain tissue (Dringen et al., 2000). The redox system of glutathione consists of GSH and an array of functionally related enzymes, of which GR is responsible for the regeneration of GSH, whereas GPx works together with GSH in the decomposition of H_2O_2 and other organic hydroperoxides. Consistent with previous works, in the present study, $A\beta_{1-42}$ caused a decrease in GSH levels (Ding et al., 2013). A reduction in the levels of GSH may impair H_2O_2 clearance and promote formation of OH, the most toxic moiety to the brain, leading to more oxidant load and consequently oxidative damage. In this study, the treatments of animals with compounds ARD, HDS and PDA prevented this process which may be the mechanism responsible for facilitating effects of inhibitory avoidance memory. Damage to myelin sheath by Aβ oxidative stress may lead to cognitive dysfunction (Sims-Robinson et al., 2013). In line with these studies, Aβ treated mice performed poorly on inhibitory avoidance indicating impairment in their learning abilities and memory capabilities. Furthermore, there was a significant rise in brain activity and oxidative stress levels (indicated by an increase in TBARS and decrease in GSH levels) which is consistent with earlier reports (Yin et al., 2014).

The majority of sesquiterpene lactones exert their biological activities through a reaction called the Michael-type addition where they react with cellular nucleophiles. The α,β-unsaturated carbonyl, epoxy-, aldehyde-, and peroxy have been considered potential reactive moieties (Merfort, 2011; Schmidt, 2006). However, other factors, such as lipophilicity, molecular geometry, and chemical environment or the target sulfhydryl may also influence the activity of the sesquiterpene lactones (Chaturvedi, 2011).

With regards to this work, it has been observed that the lactone ring moiety may not be essential for the activity in the evaluated model. Only three substances PDA, ARD and HDS exerted good activities with PDA > ARD > HDS. It can further be observed that ARD which is a sesquiterpene alcohol showed a better activity than the sesquiterpene lactone HDS. Apparently the hydrophobic and rigid moiety C1-C5 ring present in guaiane skeletons of PDA and ARD (Fig. 1) are the most interactive part of the compounds, since the elemane compounds IGM-H and IGM-A possess an open chain in C1-C5 and exhibited weak activity, despite being sesquiterpene lactones. Furthermore, it can also be observed that the most active compounds were those with the guaiane carbon skeletons without the epoxy group, as present in EHP. Hence for this evaluation, the epoxy group may not be necessary for this activity as stated by Schmidt (2006), but instead could be exerting a steric or polar hindrance. Through virtual screening, sesquiterpenoids from *Chicorium intybus* exerted anti-acetylcholinesterase activities (Rollinger et al., 2006; Rollinger et al., 2005). The AChE inhibition of these compounds was confirmed through an activity-guided fractionation of the chicory root extract which led to the isolation of two sesquiterpene lactones, 8-deoxylactucin and lactucopicrin. These two guaiane-type sesquiterpenoids showed significant and dose-dependent inhibitory activity on AChE. Recently, guaianolides obtained from *Amberboa ramosa* showed good inhibitory activities against AChE and BChE. The authors indicated that the effect of substituents at C-10 (Fig. 1., guaiane skeleton) is more important for AChE inhibition (Ibrahim et al., 2013).

Previous studies have shown that PDA acts on the central nervous system by exerting antidepressant-like effect *in-vivo* (Goncalves et al., 2012) while ARD has previously been isolated from *Xylopia brasiliensis* which is used in folk medicine as a sedative and analgesic (Moreira et al., 2003). Meanwhile, germacrane-type sesquiterpenoids have also been found to be effective against AD. These compounds which were isolated from *Valeriana amurensis* were investigated for their protective effect on PC 12 neuronal cells and they seem to afford protection against Aβ-induced toxicity in PC 12 cells (Wang et al., 2012) Some sesquiterpenoids appear to show neuroprotective activities and can act as pharmacological targets for neurodegenerative diseases like Alzheimer and Parkinson (Chung et al., 2010).

5. Conclusions

Sesquiterpenoids obtained from *Hedyosmum brasiliense* exhibited important effects on the nervous system of mice. The compounds studied here, exert nootropic effect inhibiting the cognitive deficits induced by Aβ1-42 and that may be potential

candidates for Alzheimer's disease therapy. These compounds appear to have an effect on oxidative stress and thus enhance memory in mice that received $A\beta_{1-42}$ infusion. Unlike the recognized mechanism of biological action of the sesquiterpene lactones (Michael-type nucleophilic addition), the tested compounds produced neuroprotective effects more related to the presence of the five-membered guaiane ring than to the presence of the lactone ring.

Conflict of interest

The authors have declared that there is no conflict of interest.

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