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The protective and therapeutic effects of vinpocetine, a PDE1 inhibitor, on oxidative stress and learning and memory impairment induced by an intracerebroventricular (ICV) injection of amyloid beta (aβ) peptide

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A R T I C L E I N F O

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A B S T R A C T

Alzheimer's disease (AD) is a neurodegenerative disease leading to cognitive and memory impairment. This study aimed at investigating the therapeutic and preserving effects of vinpocetine on amyloid beta (Aβ)-induced rat model of AD. Sixty male adult Wistar rats were randomly divided into 6 groups (n = 10 per group) as follows: 1; control, 2; sham, 3; Aβ, 4; pre-treatment (vinpocetine + Aβ): oral gavage administration of vinpocetine at 4 mg/kg for 30 days followed by intracerebroventricular (ICV) injection of Aβ, 5; treatment (Aβ + vinpocetine): Aβ ICV injection followed by vinpocetine administration for 30 days, 6; pre-treatment + treatment (vinpocetine + Aβ + vinpocetine): vinpocetine administration for 30 days before and 30 days after AD induction. Following treatments, the animals' learning and memory were investigated using passive avoidance learning (PAL) task, Morris water maze (MWM), and novel object recognition (NOR) tests. The results demonstrated that Aβ significantly enhanced escape latency and the distance traveled in the MWM, decreased step-through latency, and increased time spent in the dark compartment in PAL. Vinpocetine ameliorated the Aβ-infused memory deficits in both MWM and PAL tests. Administration of vinpocetine in the Aβ rats increased the discrimination index of the NOR test. It also significantly diminished the nitric oxide and malondialdehyde levels and restored the reduced glutathione (GSH) levels. Vinpocetine can improve memory and learning impairment following Aβ infusion due to its different properties, including antioxidant effects, which indicates that vinpocetine administration can lead to the amelioration of cognitive dysfunction in AD.

1. Introduction

Alzheimer's disease (AD), as a degenerative brain disease, is regarded as one of the most common types of dementia [1]. Brain alterations resulting from AD can appear before its symptoms, such as the emergence of memory impairment and a reduction in thinking capabilities [2]. The accumulation of the amyloid beta (Aβ) protein (named Aβ plaques) outside of the neurons and also agglomeration of an unusual form of the protein tau (named tau tangles) inside the neurons are two common types of brain alterations, which result in the deterioration of neurons leading to memory loss and other symptoms of AD [3].

In AD, some changes can be found in the expression of phosphodiesterases (PDEs), such as PDE1, PDE4, PDE9 and PDE10 [4]. Eleven families of PDE isoforms have been identified [5,6]. 1A, 1B, and 1C are three subtypes of PDE1, which have been demonstrated to be distributed in certain areas of the nervous system. These subtypes can be observed in the cerebral cortex, hippocampus, striatum, and thalamus, and also a great deal of PDE1B is available in the nucleus accumbens and caudate nucleus [7]. In the AD brain tissue, PDE10 and PDE1, as the cGMP and cAMP special enzymes, are extremely expressed [8]. Accordingly, PDE inhibitors, due to their effect on synaptic function, can presumably be helpful and accessible to treat AD [4,9].

Cyclic nucleotides (cAMP/cGMP) are omnipresent second messengers, which are upregulated upon neuronal activity. PDEs are enzymes, which hydrolyze and inactivate these second messengers, and as a result, can terminate signaling of cAMP and cGMP [5,6]. These second messengers have been shown to be greatly associated with motor and cognitive mechanisms and also controlling signal conduction and

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synaptic transfer of diverse neurotransmitters in the brain as a cellular function [4,10]. It has been shown that cAMP and cGMP facilitate attachments to the protein kinase A (PKA) and protein kinase G (PKG), as their target enzymes [11]. In addition, various expression processes, such as cyclic nucleotide-mediated transactivation of brain-derived neurotrophic factor (BDNF) [12] and cAMP response element-binding protein (CREB) [13] have been known to play an important role in cognitive function [14].

Phosphodiesterase inhibitors (PDE-Is) have recently been considered as possible candidates to treat dementia as well as AD [15]. Vinpocetine is an alkaloid and one of the PDE-Is, which is regarded as a classic inhibitor of PDE1 function [16,17], especially the PDE1B subtype [18]. It also facilitates long-term potentiation (LTP) [19,20] and has strong anti-inflammatory [21] and antioxidant [22,23] activities. Vinpocetine can increase cerebral blood flow [24,25] and selectively inhibit voltage-sensitive sodium (Na) channels [26], which are responsible for its neuroprotective and anticonvulsant activities [27].

Effectiveness of Vinpocetine on cognitive impairment, dementia, and especially AD has been reported in several studies. In this regard, the present study aimed at investigating the effects of long-time administration of vinpocetine on oxidative stress biomarkers and learning and memory in rat models of AD using three common accepted learning and memory tests, including passive avoidance learning (PAL) task, novel object recognition (NOR) and Morris water maze (MWM) tests.

2. Materials and methods

2.1. Animals

Sixty adult male Wistar rats (230 ± 15 g) were prepared from the Animal House of Hamadan University of Medical Sciences, Hamadan, Iran to perform the study. They were placed in cages (two per cage) in an animal room at a temperature of 22–25 °C with 60 ± 5 % comparative humidity and with a 12-h light-dark cycle (lights on at 7:00, off at 19:00). The rats had free access to water and dry pellets. The treatment procedures and the protocols of animal health surveillance were in accordance with the Veterinary Ethics Committee of the Hamadan University of Medical Science, based on the National Institutes of Health Guidelines for studies involving animals (NIH Publication 80–23, 1996).

2.2. Experimental design

The rats were randomly divided into the six following groups (n = 10): 1) The control group (without any intervention), 2) sham group, which received phosphate-buffered saline (PBS) via intracerebroventricular (ICV) injection as a solvent of Aβ1– 42, 3) The Aβ model group, which received a single injection of Aβ into the lateral ventricle, 4) The pre-treatment group (vinpocetine + Aβ), which received oral administration of vinpocetine (4 mg/kg) for 30 days prior to AD induction, 5) The treatment group (Aβ + vinpocetine), which received oral administration of vinpocetine (4 mg/kg) following AD induction for 30 days, and 6) The pre-treatment + treatment group (vinpocetine + Aβ + vinpocetine), which received vinpocetine (4 mg/kg) for 30 days, before and 30 days after AD induction. The experimental timeline is shown in Fig. 1.

2.3. The main reagents and drugs

Lyophilized powder Aβ1– 42 (Tocris Bioscience, Bristol, UK; 100 μg) was dissolved in 100 μL of PBS as a solvent and incubated at 37 °C for 7 days before utilization, which is essential to provide amyloid fibrils as a neurotoxic factor [28].

2.4. Clinical dose of vinpocetine conversion

Vinpocetine (ethyl apovincaminate; Eburnamenine-14-carboxylic acid) was used once a day at a dosage of 4 mg/kg [29,30] for 30 consecutive days by oral administration (gavage).

2.5. Aβ injections and surgery

To establish the animal model of AD, the rats were anesthetized using anesthetics (10 mg/kg of xylazine plus 100 mg/kg of ketamine), and they then were placed in a stereotaxic device (Stoelting Co., Wood Dale, IL, USA). The rats' body temperature was kept at 37 °C using an electrically protected heating pad through the Aβ injection procedure [31]. Through a tiny hole 5 μl of Aβ1–42 was unilaterally injected into the right lateral ventricle (medial/lateral: 1.4 mm, dorsal/ventral: 4.0 mm, and anteroposterior: -0.8 mm from Bregma) via a 5 μl Hamilton microsyringe and its stainless steel cannula (Hamilton Laboratory Products, Reno, NV, USA). Injections lasted 5 min and the needle of microsyringe was kept in the hole for 2 min to ensure the proper injection of Aβ1–42. The rats in the sham group were injected by the same volume of PBS as the Aβ1- 42-injected rats [32]. After surgeries, the rats were separately placed in their cages and had free access to the water and food [33].

2.6. Behavioral study

2.6.1. Passive avoidance learning (PAL) test

2.6.1.1. Passive avoidance instrument. In this research, the passive avoidance apparatus (step-through method) was used to evaluate passive avoidance memory and learning [34,35]. The apparatus consists of a light compartment (22 × 22 × 32 cm) made by crystal plastic and a dark compartment made by the opaque and dark plastic (22 × 22 × 32 cm). The floor of the two compartments is made of 3 mm diameter stainless steel rods spaced 1 cm, separately. The floor of the dark compartment can be electrified by a shock generator (Burj Sanat Co. Tehran, Iran). A 6 cm × 8 cm rectangular gateway is placed between both compartments, which can be closed by a turbid guillotine door [34].

2.6.1.2. Training procedure. For adaptation, the rats were first subjected to two trials. They were located in the light compartment of the apparatus facing away from the door and 30 s later, the guillotine door was opened, manually. The rats moved to the dark compartment, based on their natural tendency. In the next step, the door was closed and

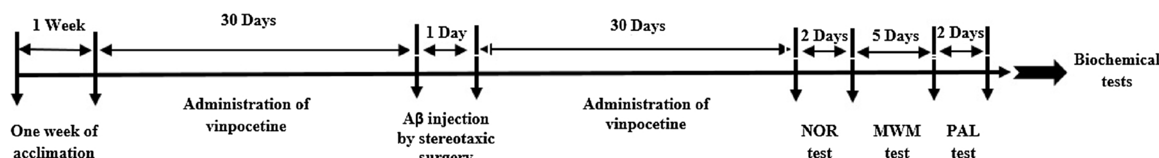

Fig. 1. Experimental timeline. Following 30 days of vinpocetine administration (4 mg/kg) in experimental groups, to generate a rat model of Alzheimer's disease, the rats were anesthetized with xylazine (10 mg/kg) and ketamine (100 mg/kg) and transferred to a stereotaxic device. The amyloid beta (Aβ) solution (2 μL) was injected intraventricularly at a rate of 1 μL/2 min. After recovery, vinpocetine was re-administered by daily oral gavage for 30 days. The animals' memory and learning were then investigated using passive avoidance learning (PAL) task, and Morris water maze (MWM) and novel object recognition (NOR) tests. At the end of the experiments, the levels of the biomarkers of oxidative stress were determined after serum analysis.

after spending 30 s in the dark compartment, the rats were returned into their home cages. This habituation trial was repeated after 30 min and pursued after the same pause by the first acquisition trial. When the rats were located with their entire four claws in the dark compartment, the entrance latency to the dark compartment (step-through latency (STL)) was recorded. The guillotine door was closed after the rats instinctively entered the dark compartment and an electrical foot shock was applied (50 Hz, 1.2 mA) for 1.5 s. The animals were kept in the dark compartment for 30 s and were then returned to their home cages. The experiment was repeated 2 min and 120 s later. Whenever the rats reentered and placed all their four claws in the compartment, they were received a foot-shock. By remaining the animal in the light compartment for two continuous min, training was terminated and the number of trials was recorded. The number of trials (entries into the dark chamber) (NTa) and also the step-through latency in the acquisition trial (STLa) were recorded.

2.6.1.3. Retention test. Long-term memory was appraised 24 h after the PAL acquisition trial. Similar to the PAL training session, the animals were kept in the light compartment for 10 s, then the door was opened. Then, the step-through latency in the retention trial (STLr) and the time spent in the dark section (TDC) were recorded for 10 min. Through the retention test, the electric shocks were not applied to the floor. If animals did not enter the dark compartment within 300 s, the test was stopped and the extra time of 300 s was considered [36].

2.6.2. Evaluation of spatial memory

The Morris water maze (MWM) test is used to assess spatial learning and memory in animal models [37,38]. The MWM is composed of a circular pool (65 cm height, 185 cm in diameter) filled with water to a depth of 45 cm maintained at 25 ± 1 °C. The pool (painted black) is separated into four equal quadrants with four start points denominated as the East (E), West (W), North (N), and South (S). An escape route (an invisible platform that is 10 cm in diameter) is situated 1.5 cm below the water surface in the center of the northern quadrant. This distinguishing quality remained consistent for all rats across the training trials. This stage of training was performed between 10:30 AM and 12:30 PM for 4 days, including two sections with four trials. Each rat in all groups was allowed to swim for 60 s, from start points (E, W, N, and S) in the pool to reach the hidden platform. The rats were remained in the platform for 30 s after detecting the hidden platform. In this procedure, an inter-trial interval of 5 min was also regarded. A video camera is fixed above the tank connected to an exploring system to record the requested parameters, including escape latency to reach the hidden platform and the distance traveled by swimming. The platform was removed from the pool on day 5, and the animals were subjected to swimming for 1 min, and the probe trial was then performed. In this phase, the time spent in the target quadrant was recorded [32].

2.6.3. Novel object recognition test

The NOR is designated to assess the visuospatial memory of animals in a familiar environment [39,40]. Each animal, 24 h before testing was located in the NOR apparatus (60 × 60 × 45 cm) for 20 min for acclimatization, their exploratory behavior does not interfere with their interaction with objects. Twenty-four hours later, two similar squares or round targets were placed in the box and the rats were placed separately at the midpoint and beside the front wall of the box with their heads positioned opposite to the objects. In the familiarization phase, the rats were allowed to explore the objects for 10 min, and then returned to their cages. One hour later, one of the familiar objects was exchanged with a novel object used in the testing phase, and then the rats were located in the apparatus with a novel object and the familiar object for 5 min. This process was recorded by a video camera [41]. The time spent exploring the novel object to the total time spent with both objects was defined as the discrimination ratio. The testing timeline indicates the time spent exploring two objects. Object presentation was counterbalanced and randomized across the groups and rats. The field and the objects were cleaned through the intervals with 70 % ethanol to ensure the absence of olfactory cues [39].

2.7. Biochemical parameters

The animals were anesthetized with ether, and blood sampling was done from the inferior vena cava after behavioral studies. Plasma samples were separated from the whole blood for estimation and analysis of the oxidative stress biomarkers levels and the biochemical parameters.

2.7.1. Estimation of malondialdehyde (MDA)

According to Ohkawa et al., the level of lipid peroxidation was assessed as the concentration of thiobarbituric acid-reactive malondialdehyde (MDA) production [42]. Briefly, MDA was evaluated based on the following procedure: 1) mixing 1.0 ml of 1 % thiobarbituric acid and 1.0 ml of 20 % trichloroacetic acid reactive substances with 100 μL of the supernatant, 2) incubating the solution for 80 min at 100 °C, 3) cooling the solution on ice and centrifuging for 20 min at 3000 rpm, and 4) reading the absorbance of the supernatant at 532 nm [31]. The level of MDA was expressed as nmol/mg protein.

2.7.2. Evaluation of the reduced glutathione (GSH)

The reduced glutathione (GSH) was measured based on the technique developed by Ellman (1959) [43] as follows: 1) mixing 1 ml of 4 % sulfosalicylic acid with 1 ml supernatant and cold digesting for 60 min at 4 °C, 2) centrifugation of the samples for 15 min at 1200 × g, 3) adding 0.2 ml of 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) and 2.7 ml of phosphate buffer (0.1 M, pH 8) to 1 ml of the supernatant, and 4) measuring the yellow color produced directly using a spectrophotometer at 412 nm. Condensation of GSH in the supernatant was measured by a standard curve and represented as μmol per mg protein [44].

2.7.3. Estimation of nitrite as an indicator of nitric oxide (NO) production

Nitrite accumulation in the supernatant as an index of NO production representative of a free radical was estimated by colorimetric test, based on the method developed by Green et al. using Greiss reagent (2.5 % phosphoric acid, 1 % sulfanilamide, and 0.1 % N-(1-naphthyl) ethylenediamine dihydrochloride) [45]. The procedure is briefly as follows: 1) mixing the equal volumes of supernatant and Greiss reagent and incubating for 10 min at 25 °C in the dark, and 2) assaying the absorbance by spectrophotometer at 540 nm. Condensation of the nitrite in the supernatant was measured using a sodium nitrite standard curve and represented as μmol per mg protein [44].

2.8. Data analysis

Data were analyzed using one-way and two-way analysis of variance (ANOVA) confirmed by Tukey's Post Hoc test for multiple comparisons. Values are expressed as mean ± S.E.M. $P < 0.05$ was assumed as statistically significant.

3. Results

3.1. The effect of vinpocetine on memory performance in the PAL test in the Aβ-injected rats

3.1.1. PAL acquisition

In the acquisition test, no significant difference was observed among the groups in STLa ($F_{5, 54} = 1.84$, $P = 0.1224$; Fig. 2A). It showed that the exploratory and intrinsic behavior of the rats in the studied groups did not differ in the dark compartment. In addition, there was no significant difference among the groups in NTa ($F_{5, 54} = 1.396$, $P = 0.2416$; Fig. 2B).

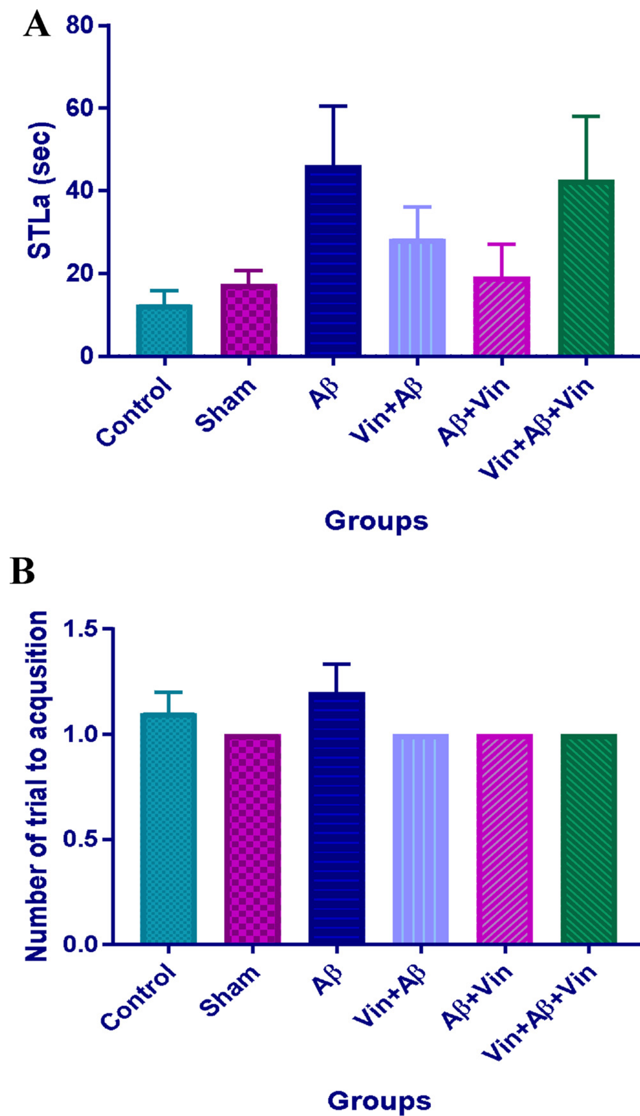

Fig. 2. Effect of vinpocetine administration in the ICV amyloid beta (Aβ)-injected rats on the step-through latency in the acquisition trial (STLa) **(A)** and the number of trials to acquisition (NTa) **(B)** of passive avoidance learning (PAL) task in all experimental groups.

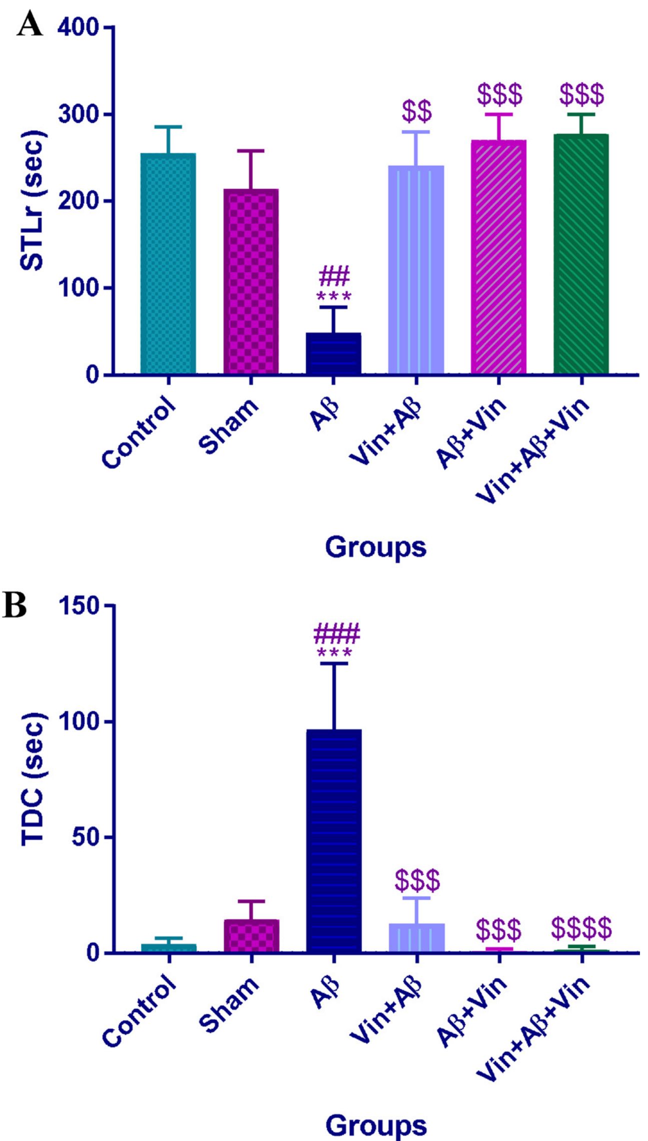

Fig. 3. Effect of vinpocetine administration in all experimental groups on the step-through latency in the retention trial (STLr) **(A)** and the time spent in the dark compartment (TDC) **(B)** in the retention test, which was performed 24 h after the passive avoidance learning (PAL) acquisition trial. Values are expressed as mean ± S.E.M. ***p < 0.001 compared with the control group. ## p < 0.01 and ### p < 0.001 compared with the sham group. $$ p < 0.01, $$$ p < 0.001, and $$$$ p < 0.0001 in comparison with the amyloid beta (Aβ) group.

3.1.2. PAL retention

Twenty-four hours after the PAL acquisition trial, a long-term memory retention test was done and a significant difference was found in STLr among the experimental groups ($F_{5, 54} = 6.856$, $P < 0.0001$; Fig. 3A). The STLr of the Aβ group was significantly reduced compared with the control and sham groups ($P < 0.001$; $P < 0.01$). STLr values in the pre-treatment, treatment, and pretreatment + treatment groups were significantly higher than the Aβ group ($P < 0.01$ and $P < 0.001$, respectively).

There was no significant difference in STLr values in the pretreatment, treatment, and pretreatment + treatment groups in comparison with the control group. Moreover, the TDC value differed significantly among the experimental groups ($F_{5, 54} = 7.838$, $P < 0.0001$; Fig. 3B). TDC in the pretreatment, treatment, and pretreatment + treatment groups was significantly lower than that of the Aβ group ($P < 0.001$, $P < 0.0001$), whereas TDC in the Aβ group was significantly more than the control and sham groups ($P < 0.001$). There was no significant difference in the TDC results in the pretreatment, treatment, and pretreatment + treatment groups than the control group.

3.2. Effects of vinpocetine administration on memory performance in MWM task in the Aβ-injected rats

The results obtained through the four training days demonstrated that all groups spent less time to find the hidden platform (escape latency). However, in the Aβ group, it was less than other experimental groups (Fig. 4A). Through this period, there were significant differences between the escape latency in the AD model and the control and sham groups ($P < 0.0001$, $P < 0.001$, and $P < 0.01$, respectively). According to the results, vinpocetine administration (pre-treatment, treatment, and pre-treatment + treatment groups) significantly reduced escape latency compared with the Aβ group through all four days ($P < 0.0001$, $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively).

A significant difference was observed in the distance traveled in the Aβ group on the day 1 and day 2 compared with the control and sham groups ($p < 0.0001$ and $p < 0.05$, respectively; Fig. 4B). Vinpocetine

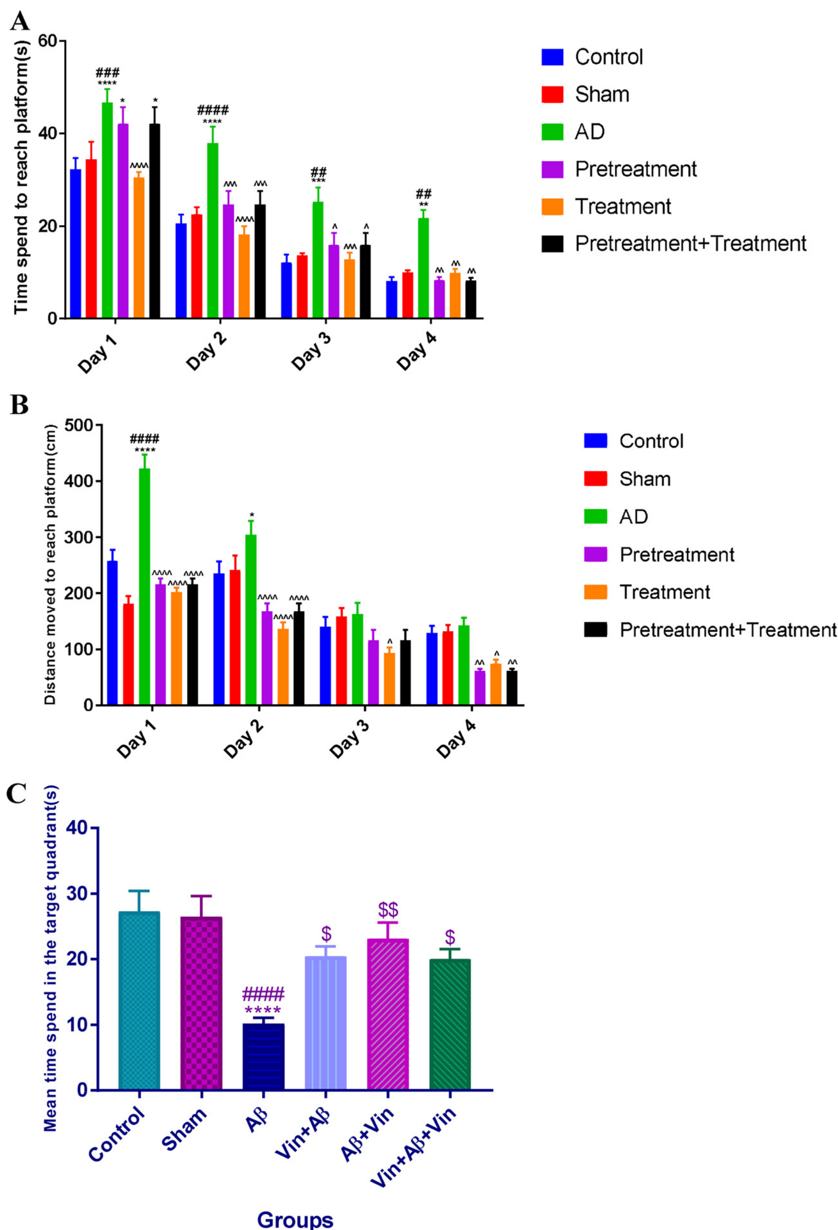

Fig. 4. The mean of latencies to discover the hidden platform in the Morris water maze (MWM) test. The bars indicate the average latency of four successive trial days **(A)**, and the mean of the distance traveled in the MWM **(B)**. The bars demonstrate the mean time spent in the target quadrant in MWM on the fifth day **(C)**. Values are expressed as mean ± S.E.M. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared with the control group. ## $p < 0.01$ and ### $p < 0.001$ in comparison with the sham group. ^ $p < 0.05$, ^^ $p < 0.01$, ^^^ $p < 0.001$, and ^^^^ $p < 0.0001$ compared with the amyloid beta (Aβ) group. \$ $p < 0.05$, \$\$ $p < 0.01$, \$\$\$ $p < 0.001$, and \$\$\$\$ $p < 0.0001$ in comparison with the Aβ group.

administration (pre-treatment, treatment, pre-treatment + treatment groups) caused a significant reduction in the distance traveled compared with the Aβ group through the experiments ($P < 0.0001$, $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively).

The mean time spent in the target quadrant was studied in the probe trial. The mean time spent in the target quadrant of the AD model group was diminished significantly in comparison with the control and sham groups ($P < 0.0001$). Based on the results, the pre-treatment, treatment, and pre-treatment + treatment groups spent more time in the target quadrant than the Aβ group (Fig. 4C).

3.3. The effect of vinpocetine administration on memory performance in NOR-related cognitive abilities in the Aβ-injected rats

The discrimination index is considered as an indicator of the NOR test. It is calculated as the time spent exploring the novel object divided by the total time exploring both familiar and novel objects on the second day of the test. The results of one-way ANOVA demonstrated a significant difference in the discrimination index of the NOR test among the groups ($F5, 54 = 8.189$, $P < 0.0001$; Fig. 5). Accordingly, a significant decrease was observed in Aβ group compared with the control and sham groups ($P < 0.01$), whereas there was a significant increase in the pre-treatment, treatment, and pre-treatment + treatment groups in comparison with the Aβ group ($P < 0.05$, $P < 0.001$, and $P < 0.0001$, respectively). This indicates that treatment and pre-treatment with vinpocetine could improve the visuospatial memory abilities in the Aβ group (Fig. 5).

3.4. Efficacy of vinpocetine in the ICV Aβ-injected rats on serum malondialdehyde (MDA) levels

MDA levels were significantly increased in the ICV Aβ-injected rats in comparison with the control and sham groups ($P < 0.0001$), whereas the pre-treatment, treatment, and pre-treatment + treatment of the ICV Aβ-injected rats with vinpocetine significantly reduced MDA levels compared with the Aβ rats group ($P < 0.001$, $P < 0.0001$, and $P < 0.0001$, respectively; Fig. 6).

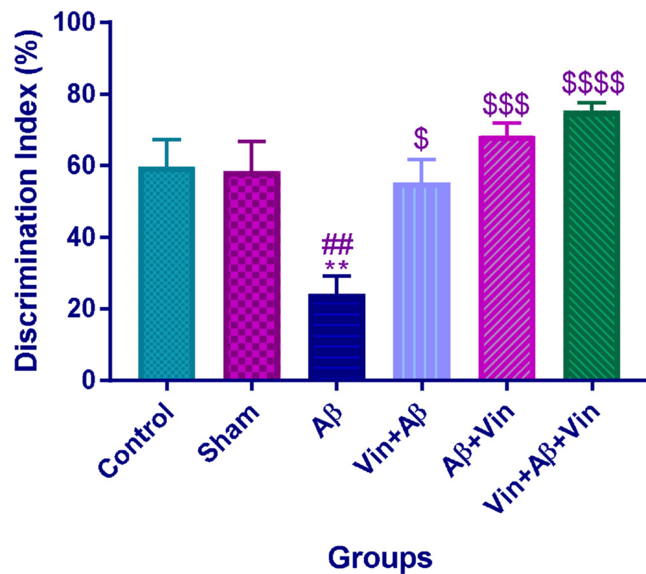

Fig. 5. Exploratory preference in the novel object recognition (NOR) test and the discrimination index on the second day. The blocks show the exploration preference in the NOR test 24 h after habituation. Values are expressed as mean ± S.E.M. **p < 0.01 compared with the control group. ## p < 0.01 in comparison with the sham group. $ p < 0.05, $$$ p < 0.001, and $$$$ p < 0.0001 compared with the amyloid beta (Aβ) group.

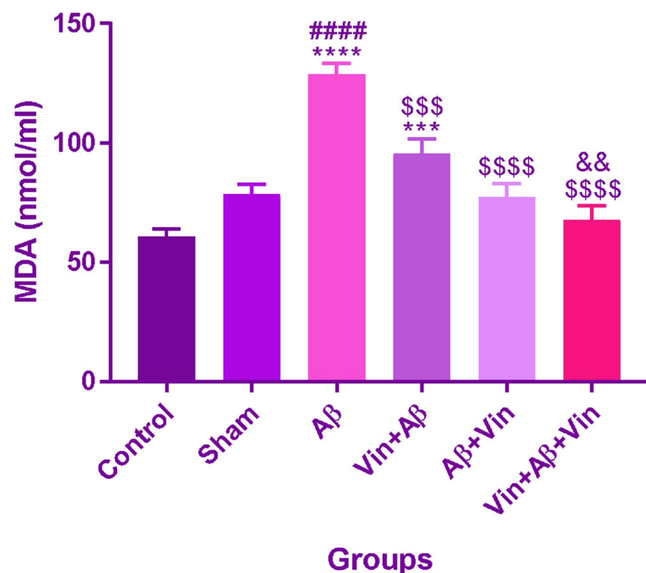

Fig. 6. Effects of vinpocetine administration in the ICV amyloid beta (Aβ)-injected rats on serum malondialdehyde (MDA) levels. Values are expressed as mean ± S.E.M (n = 10). MDA levels significantly increased in the Aβ group compared with the control and sham groups (**** P < 0.0001 and #### P < 0.0001, respectively). Vinpocetine significantly declined serum levels of MDA than the Aβ group ($$$ P < 0.001 and $$$$ P < 0.0001 compared with the Aβ group).

3.5. Efficacy of vinpocetine in the ICV Aβ-injected rats on serum glutathione (GSH) levels

GSH levels declined significantly in the ICV Aβ-infused rats than the control and sham groups (P < 0.0001 and P < 0.01, respectively); however, the pre-treatment, treatment, and pre-treatment + treatment of these rats with vinpocetine significantly increased GSH levels compared with the Aβ rats group (P < 0.05, P < 0.05, and P < 0.001, respectively; Fig. 7).

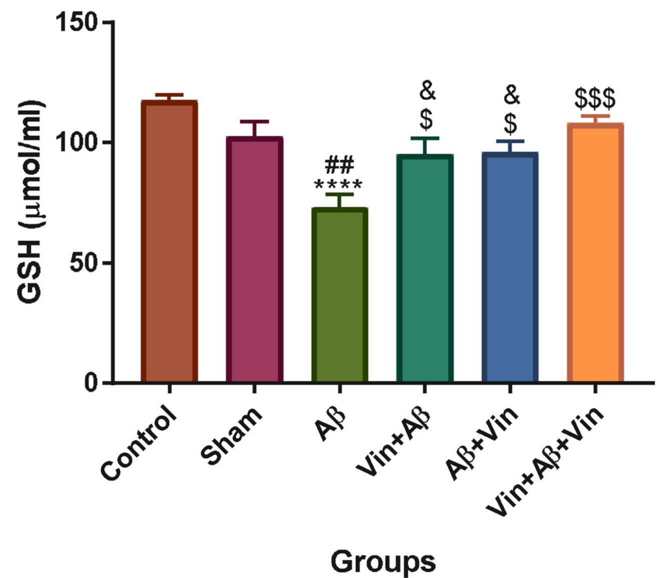

Fig. 7. Effects of vinpocetine administration in the ICV amyloid beta (Aβ)-injected rats on serum glutathione (GSH) levels. Values are expressed as mean ± S.E.M (n = 10). GSH levels significantly decreased in the Aβ group compared with the control and sham groups (**** P < 0.0001 and ## P < 0.01, respectively). Vinpocetine significantly increased GSH level than the Aβ group ($ P < 0.05 and $$$ P < 0.001 compared with the Aβ group, and P < 0.5 compared with control group).

3.6. Efficacy of vinpocetine in the ICV Aβ-injected rats on serum nitrite (NO) levels

Nitrite levels as an indicator of NO (a free radical) increased significantly in the ICV Aβ-injected rats compared with the control and sham groups (P < 0.0001). However, treatment and pre-treatment + treatment of these rats with vinpocetine significantly decreased nitrite levels in comparison with the Aβ rats (P < 0.01 and P < 0.0001, respectively; Fig. 8).

4. Discussion

The ICV Aβ [1–42] injection induced a significant memory and learning impairment in the NOR, MWM, and PAL tasks in rats. These three types of memory and learning tests in the current research were considered to differentiate the consequence of the pre-treatment and treatment effects on different types of memory and learning. In this regard, passive avoidance learning and memory were evaluated by the passive avoidance task, spatial memory was assessed using the MWM test, and visual recognition memory was investigated by the NOR test. In the current study, ICV Aβ [1–42] injection resulted in a decrease in the STLr, an increase in TDC in the PAL test, and a reduction in the discrimination index in the Aβ group compared with the control group in NOR test. In addition, it caused an increase in the time spent to reach the platform as well as the distance traveled to reach the platform in the MWM test as spatial memory. In AD, due to brain tissue damage, memory and learning processes are impaired. Many experimental studies have shown a decline in memory and learning in AD models, including the reduced memory and learning in behavioral tests, such as the MWM test and passive avoidance learning task [46–48]. In another study, ICV injections of Aβ [1–42] in mice caused memory impairment in the Y-maze as well as MWM tests [49].

This study revealed that learning and memory deterioration following ICV injection of Aβ could be improved by vinpocetine. The AD rats that received Aβ had deficiencies in passive avoidance learning and spatial learning as demonstrated by the impaired acquisition and retention in PAL, MWM, and NOR tasks. Nevertheless, pre-treatment and

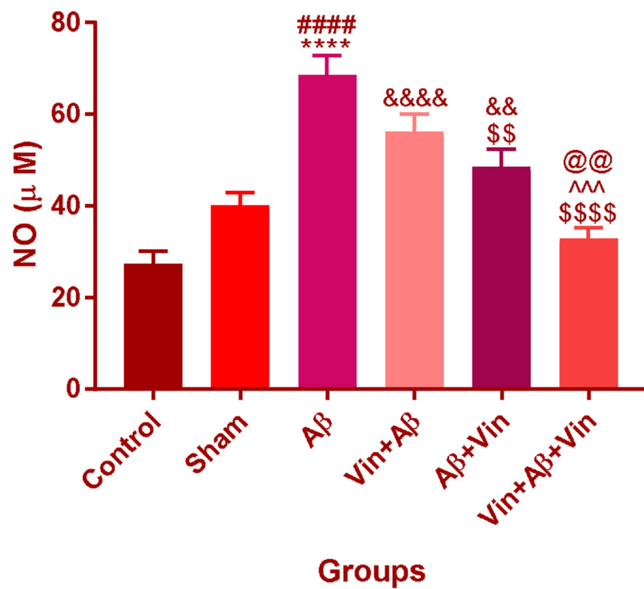

Fig. 8. Effects of vinpocetine administration in the ICV amyloid beta (Aβ)-injected rats on serum nitric oxide (NO) levels. Values are expressed as mean ± S.E.M (n = 10). The levels of NO significantly increased in the Aβ group compared with the control and sham groups (**** $P < 0.0001$ and #### $P < 0.0001$, respectively). Vinpocetine significantly decreased NO levels compared with the Aβ group ($$ $P < 0.01$ and $$$$ $P < 0.0001$ compared with the Aβ group). &&&& $P < 0.0001$ and && $P < 0.01$ in comparison with the control group. @@ $P < 0.01$ compared with the Aβ + vinpocetine group. ^^^ $P < 0.001$ compared with the vinpocetine + Aβ group.

treatment with vinpocetine could significantly ameliorate learning and memory impairment following the ICV injection of Aβ. This effect was more effective through both pretreatment and treatment before and after AD induction via Aβ injection. Aβ plays a key role in the pathophysiology of AD and also there is a close interaction between the role of Aβ and the neuroinflammatory process of AD [50].

Vinpocetine, as an antioxidant and a PDE1, has shown to be effective in improving memory and learning impairments. In ICV streptozocin-induced rats, which is a model that almost mimics cognitive impairments induced by AD, treatment with vinpocetine restored their performance in the passive avoidance and the MWM tests [44]. Indeed, vinpocetine has been reported to improve memory deficits in other experimental patterns, like hypoxia, scopolamine, and streptozocin-induced retrieval impairment in rats and it has also shown effective in clinical studies on patients suffering from depression, psychosyndromes, cognitive impairment, AD, and other dementia [18,44,51–54].

Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system, which buffers the system from oxidative damage [55,56]. Oxidative stress can cause disruptions in normal mechanisms of cellular signaling [57]. More severe oxidative stress can cause cell death, and even moderate oxidation can trigger apoptosis, whereas more intense stresses may lead to necrosis [58]. The role of oxidative stress has been implicated in the pathogenesis of several diseases [55,56,59]. The reactive species produced in oxidative stress can cause direct damage to the DNA [60]. Since the brain is highly vulnerable to oxidative stress [61], pathologically induced oxidative stress could induce brain dysfunction and result in impaired learning and memory [62,63]. Oxidative stress may be associated with learning and memory deficits following oxidative stress-induced brain damage in animal models [64]. Oxidative stress has widely shown to be increased in the AD brain, and also ROS has indicated to play an important role in the pathogenesis of neuronal degeneration and death in this disorder [65,66]. It has been indicated that several oxidative injuries can be found in the AD brain, depending on both indication pathologies (senile plaques and neurofibrillary tangles) as well as natural appearance of pyramidal neurons [44,67–69]. Although ROS have a significant effect on the neuronal failure and death more often in different neurodegenerative disorders, such as AD [70], the effect of vinpocetine on oxidative stress as a potential neuroprotective strategy has also been investigated.

Aβ is a major source of oxidative free radicals and/or a toxic agent in AD [70]. It has widely approved that the aggregation of Aβ is associated with its neurotoxicity and forms free radicals. Aβ has shown to cause H_2O_2 agglomeration in neuroblastoma cultures as well as in the cultured hippocampal neurons [65,66]. Electron paramagnetic resonance analysis of gerbil synaptosomes and in vitro analysis demonstrated that Aβ induced lipid peroxidation and generation of free radical peptides [70,71]. Aβ can cause the generation of ROS in cell cultures, and can also produce an overplus of superoxide radicals in endothelial structure leading to vascular abnormalities and neurodegeneration interfered by free radicals in AD [70,72]. In contrast, the marked antioxidant activity, due to the destruction of hydroxyl radicals is one of the mechanisms leading to neuroprotection presented by vinpocetine [22,73]. It has reported that the inhibitory effects of natural products on Aβ fibril formation are related to their antioxidative properties [74]. In this respect, investigations on the *in vitro* models of oxidative stress have reported the inhibitory effects of vinpocetine against ROS [75]. Accordingly, the potential neuroprotective effects of PDE1 inhibitors has been shown [76], which can especially be helpful in treating neurodegenerative disorders.

In this experiment, Aβ increased the levels of MDA and NO, whereas it decreased the levels of GSH in the serum of AD model animals. Consistent with our results, Aβ could increase MDA levels and reduce GSH levels in AD and the experimental model of AD, as well [44,67–69]. For buffering free radicals in brain tissue, the antioxidant system utilizes the reduced GSH as the most abundant non-protein thiol source [77]. The reduced GSH is a component of the antioxidant system with a thiol group as a reducing agent in its structure [77]. GSH participates directly in the neutralization of free radicals and reactive oxygen compounds, and also maintaining exogenous antioxidants, such as vitamins C and E in their reduced (active) forms [77–79]. In addition, GSH plays an important role in the progression of the cell cycle, including cell death [80]. GSH levels regulate redox changes to nuclear proteins necessary for the initiation of cell differentiation. Differences in GSH levels can also determine the expressed mode of cell death, such as apoptosis or cell necrosis [81]. GSH removes H_2O_2 and organic peroxides by glutathione peroxidase (GPxs) [82]. A decrease in the levels of GSH leads to the defective H_2O_2 clearance and increases OH level as an oxidative stress inducer in the brain [77,83]. On the other hand, the increased levels of H_2O_2 result in the peroxidation of polyunsaturated fatty acids leading to the formation of end products of lipid peroxidation, like MDA [77,84]. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and forms protein covalent adducts as the advanced lipoxidation end products (ALEs), similar to the advanced glycation end products (AGEs) [85]. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism [86,87].

In this study, vinpocetine increased the levels of GSH and decreased the levels of MDA and NO in the serum of Aβ-induced AD in rats. It has been reported that the marked antioxidant activity related to the scavenging of hydroxyl radicals is a mechanism responsible for the neuroprotection represented by vinpocetine, which is consistent with our results [73]. Based on the previous studies, vinpocetine has several various mechanisms, which are effective in its antioxidant activity. Vinpocetine blocks the sodium channels, by which the accumulation of sodium in neurons ameliorates the excitotoxic neuronal injury that may be beneficial in lowering the toxic effects of oxidative stress caused by anoxia and other disorders in the brain [88–90]. In one study, vinpocetine lowered the increase of $[Na^+]i$ induced by veratridine, by inhibiting the voltage-dependent Na^+ channels [91]. In another study,

vinpocetine was reported as potent as phenytoin to block voltage-gated Na^+ channels in rat cortical neurons [92]. On the other hand, vinpocetine protects neuronal cells against glutamate toxicity and ROS produced by excitotoxic levels of extracellular glutamate [93–95]. Vinpocetine inhibits lipid peroxidation in synaptosomes and is an effective scavenger of hydroxyl radicals [73]. Evaluation of lipid peroxidation and ROS formation has shown that vinpocetine is highly effective against ascorbate/Fe^{2+}-induced peroxidation in synaptosomes by inhibiting ROS formation and lipid peroxidation [23].

Vinpocetine has been described as a specific inhibitor of PDE1 [17], which can lead to an increase in the cAMP and cGMP [96]. Intracellular increase in cAMP/cGMP levels can establish a cascade that leads to the activation of CREB, a transcription factor, and this activation eventually can result in the expression of plasticity-related genes [97–99]. CREB protein is an important molecule for learning and memory [100,101], LTP [102,103], the expression of many neuroprotective and anti-apoptotic molecules [104], protective neurotrophic factors, such as BDNF, fibroblast growth factor (FGF), and transforming growth factor (TGF) [105,106], the expression of BCL2 anti-apoptotic protein [107,108], and the expression of the peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α), which acts as a ROS sweeper [109]. In addition, PDE inhibitor can increase cAMP/cGMP levels leading to the phosphorylation of AMPA receptors, which can increase the conjunctions in the synapses and actually facilitate the transmission of glutamatergic [110].

In brain diseases as well as neurodegenerative disorders, such as AD neuronal plasticity is interrupted [111,112]. Therefore, as PDE1 inhibitors are appropriate plasticity boosters, they seem to be potential therapeutic options. In this regard, vinpocetine treatment has been indicated to facilitate LTP [19,20], boost the structural dynamics of dendritic spines [113], recover memory retrieval [51], and elevate the efficiency on cognitive tests in humans [114]. In addition, the vinpocetine effect in inhibiting Na channel is possibly associated with its neuroprotective and anticonvulsant function [27]. Vinpocetine, in a selective manner, inhibits Ca-calmodulin-affiliated cGMP-phosphodiesterase, therefore it can increase intracellular cGMP levels in the vascular smooth muscle leading to the decreased resistance in cerebral vessels and an increase in cerebral blood flow. This exclusivity is responsible for its neuroprotective activity, as well [24,115,116]. Vinpocetine can inhibit IκB kinase (IKK), prohibit IkB degradation, and the result in translocation of NF-κB to the nucleus. This properties of vinpocetine along with its potential to expand neuronal plasticity indicate that, it might be effective in pathologic conditions with inflammation and negligible neuronal plasticity, such as AD and Parkinson's disease [117].

The present research had several limitations, which should be considered in interpreting the results. For example, no valid natural laboratory rodent model of AD is available. Therefore, AD models have a number of limitations, including the fact that they do not recapitulate the morphological and behavioral patterns observed in clinical human AD. The AD model created in this study by ICV Aβ injection was done based on previous studies. However, it was associated with some limitations. The time spent to induce AD (30 days) in rats was one of the most important of these restrictions, which was not long enough for the development of neuronal loss or neural degeneration, a specific feature in AD. On the other hand, AD has been associated with changes and genetic disorders. In the model of AD induced by ICV injection of Aβ, genetic differences in AD are ignored, which is a significant limitation that should be considered in future studies.

5. Conclusion

In summary, in this research, we showed that the ICV injection of Aβ induced considerable memory and learning deficiencies in the PAL task, MWM, and NOR tests. Vinpocetine administration improved the Aβ-induced memory impairment in all behavioral experiments i.e. PAL task and MWM and NOR tests. Its neuroprotective, anti-inflammatory, antioxidant, and especially, phosphodiesterase 1 inhibitory effects are possibly associated with its effectiveness on memory impairment observed in this study. The most considerable finding of the current research was that vinpocetine pre-treatment and treatment had beneficial effects in a rat model of AD, which can be used to prevent AD.

CRediT authorship contribution statement

Meysam Shekarian: Conceptualization, Methodology, Investigation. **Alireza Komaki:** Supervision, Conceptualization, Writing - review & editing, Resources. **Siamak Shahidi:** Writing - review & editing, Data curation. **Abdolrahman Sarihi:** Writing - original draft, Resources. **Iraj Salehi:** Formal analysis, Software. **Safoura Raoufi:** Software, Validation.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bbr.2020.112512.

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