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Tadalafil modulates intracerebroventricular streptozotocin-induced cognition and memory impairment in the young and middle middle-aged rats

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Abstract

Background: Learning and memory may decline due to Alzheimer's disease (AD) in older adults. A reduction in cyclic guanosine monophosphate concentration and an increase in phosphodiesterase activity have been reported in the process of aging. Although phosphodiesterase (PDE) type 5 inhibitor, Tadalafil is used to treat erectile dysfunction; PDE inhibitors possibly prevent cognition impairment in aging. This study was designed to investigate the effects of tadalafil on memory in middle-aged and young healthy and AD rats.

Methods: Memory impairment was induced by intracerebroventricular (ICV) administration of streptozotocin (STZ; 3 mg/kg) in AD rats. Male Wistar rats (middle-aged and young) were distributed into six groups as follows: two control, two AD, and two AD+tadalafil (1 mg/kg) groups. Saline or tadalafil was administered once a day orally for 40 consecutive days. Animals were tested using novel object recognition (NOR), passive avoidance learning (PAL), and Morris water maze (MWM) tests.

Results: Aged AD rats exhibited a significant impairment in cognition in the NOR test and impaired learning and memory in PAL and MWM tests compared with the control aged rats. Tadalafil treatment in aged AD rats significantly improved the discrimination index in the NOR test, decreased the time spent in the dark compartment in the PAL test, and increased time spent in the target quadrant in MWM tests compared with aged AD rats. In young AD rats, treatment with tadalafil significantly enhanced cognition, learning, and memory in the NOR, PAL, and MWM tests compared with young AD rats treated with saline.

Conclusion: Tadalafil treatment in aged rats improves cognition and memory after STZ-induced (ICV) memory impairment. It can be concluded that chronic treatment with tadalafil is protective against cognitive, learning, and memory impairment in both young and aged subjects.

Keywords: Aging; Cognition; Memory; Rat; Streptozotocin; Tadalafil

Introduction

Memory is an ability to encode, store, retain, and recall information and past experiences in the brain[1]. Learning and memory are cognitive functions[2] that are dependent on physical and chemical changes in neurons of the brain[3]. Learning promotes information processing and storage in a variety of brain regions[3]. Aging is associated with dendritic spine loss, cellular decline, a decrease in spine densities, and changes in different neurotransmitter systems (such as acetylcholine) and intracellular signaling and enzymes (such as cyclic guanosine monophosphate and phosphodiesterase) in the brain[4]. Learning and memory may decline due to neurodegenerative diseases, such as Alzheimer's disease (AD) through aging[3,5]. AD is characterized by the accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles[6,7]. It is associated with progressive brain volume loss, brain inflammation, loss of a large number of neurons, cognitive disorders, and memory deficits[8,9].

The cyclic guanosine monophosphate (cGMP) is an important secondary messenger. It is essential in a variety of cellular functions, including cognition, learning, memory, and neuroplasticity[10-12]. Changes in various neurotransmitters, cyclic adenosine monophosphate (cAMP), and cGMP signaling have been reported in the aged brain[13,14]. A decline in the level of cyclic nucleotides is associated with cognitive dysfunction[13-15]. Phosphodiesterase type 5 (PDE5) enhances cGMP signaling by reducing the degradation of this cyclic nucleotide[16]. PDE5 is expressed in neurons of the hippocampus and cortex[17]. Tadalafil is a selective PDE5 inhibitor[18]. It is widely used for the management of erectile dysfunction [19]. The effectiveness of tadalafil on anxiety[20], morphine withdrawal syndrome[21], cerebral ischemia injury[22], and ischemic stroke[23] has been

reported. It is suggested that phosphodiesterase enzyme inhibitors may exert their therapeutic effects on some neurologic diseases through controlling intraneuronal cGMP and cAMP levels in the brain[17,24,25].

There are different animal models of dementia[26]. Sporadic AD was induced by intracerebroventricular (ICV) injection of streptozotocin (STZ)[7,27,28]. . ICV administration of STZ has been described as a useful approach for sporadic AD induction in rodents[6,29,30]. In addition, aging animals are suitable models that mimic the neurochemical and morphological alterations and cholinergic hypofunction similar to the pathophysiology of AD[26,31-33]. Tadalafil is a drug that is used for the treatment of nervous system disorders. It also improves memory in aged animals[31].

Aging is associated with a decline in learning and memory and AD is linked to the severity of cognitive impairment impairm[3,5] However, PDE inhibitors have been suggested as a target for cognition enhancement[24]. They enhance working memory and reduce hippocampal oxidative stress in aged mice[34] and a rat model of hyperhomocysteinemia[35]. However, in some studies, they have been reported with no effects on memory[36] they did not prevent the cannabis-induced memory impairment[37] and caused amnesia[38]. On the other hand, no study has yet been conducted on the effect of long-term tadalafil administration on cognitive function in both normal aging and AD. Therefore, this study was conducted to investigate the cognitive effect of long-term tadalafil administration in normal aging and AD.

Materials and methods

Animals

The experiments were carried out in young (2 months) and middle-aged (13-14 months old) male Wistar rats obtained from the animal house of Hamadan University of Medical Sciences. Rats were kept under controlled environmental conditions (temperature: 21 ± 2 °C, 12:12h light/dark cycle) with ad libitum food and water.

Surgery and administration

Briefly, the rats were anesthetized with ketamine (100 mg/kg, Behbod Darou, Tehran, Iran) and xylazine (10 mg/kg, Alfasan, Woerden, Netherlands). The anesthetized rats were placed in a stereotaxic apparatus (Stoelting Co., Chicago, IL). The heads of the rats were positioned according to the following coordinates: 0.9 mm posterior to bregma, 1.6 mm lateral to the sagittal suture, and 2 mm ventral to the surface of the brain (Paxinos). Then, the rats were recovered for ten days. STZ (3 mg/kg, Tocris Bioscience, UK) was dissolved in 0.9% saline (Shahid Ghazi Co, Tabriz, Iran) and the ICV microinjection was carried out on the first and the third days (Fig. 1)[30,39-41].

Both young and old rats were randomly divided into six groups (n= 10 per group): two control (young and aged groups), two AD models (young and aged groups), and two AD+ tadalafil (young and aged groups) groups. The Control group received 10 µL of saline via ICV injection during operation, followed by receiving saline; AD groups received STZ via ICV injection during operation followed by saline; AD+tadalafil group received STZ via ICV injection during operation followed by tadalafil (Fig. 1). Tadalafil was freshly prepared each day in physiological saline to reach a volume of 1 mg/kg. Tadalafil or saline was orally given by gavage for 40 consecutive days.

Novel object recognition (NOR) test

The apparatus and procedure were similar to those utilized in our previous studies[42,43]. The apparatus consists of a wooden open box (48 cm × 41.5 cm × 36 cm) and a video recording system. The habituation and acquisition phases were done on the first day. The rats were given two habituation sessions (5 min) in the arena with an interval of 30 min without any object. The training was conducted 30 min after habituation. During training phase, the wooden open box included two identical metal cubies (height: ~ 8.5 cm, width: 3.5 cm, length: 7 cm).

The retention test was performed 24 h later, in which a novel (unfamiliar) object (~ 6.5 cm in height × 5.5 cm in width × 4 cm in length) was replaced with one of the objects. Animal behaviors were recorded using a video camera, and the time spent near each object was measured for 10 min. The object exploration process was defined as sniffing or placing the nose within 1 cm of the object and orienting the nose towards the object. The discrimination index was defined as time spent in exploring the novel object, divided by the total exploration time[44,45]. After each session, the box and objects were cleaned with wet tissue paper (10% ethanol solution) to eliminate the remaining odors.

Passive avoidance learning (PAL) apparatus

The apparatus was similar to that we used in previous studies[44,46]. Briefly, the passive avoidance apparatus is made of transparent plastic. A rectangular opening guillotine door (6 cm× 8 cm) separated a light chamber (20 cm× 20 cm× 30 cm) from a dark chamber (20 cm× 20 cm×30 cm). The floor of the dark chamber consisted of stainless steel rods (3 mm diameter) spaced 1 cm apart. The electrified floor of the dark chamber was linked to a shock generator.

Training

The groups were subjected to two trials to habituate to the apparatus. The rats were placed in the light compartment of the apparatus facing away from the door, and 15 s later, the guillotine door was raised. Rats have a natural preference for the dark environment. Once the rats entered the dark compartment, the door was closed. The rats were kept in the dark compartment for 30 s and returned to their home cages. After 30 min, the habituation trial was repeated using the same protocol. After 30 min, the acquisition trial was carried out. When the animals were placed with all their four paws in the dark compartment, the step-through latency to enter the dark compartment during acquisition (STLa) was measured. Then, a mild electrical shock was applied (0.2 mA) for 0.5 s. After 30 s, the rats were returned to their home cage and after 2 min, the procedure was repeated. Once the rats placed all four paws in the dark compartment, they received a foot shock. The training was terminated once the rats remained in the light compartment for 120 consecutive seconds. The number of trials to achieve acquisition was recorded.

Retention test

The retention test was performed 24 h after PAL acquisition trials. The rats were placed in the light compartment (similar to training) and 5 s later, the guillotine door was raised. The step-through latency during retention (STLr) and the time spent in the dark compartment (TDC) were recorded for up to 300 s. The retention test was terminated once the rats did not enter the dark chamber during 300 s, and STLr and TDC values were recorded up to 0 s and 300 s, respectively[45].

Morris Water Maze (MWM)

The spatial memory was evaluated using the Morris water maze (MWM) test[47]. The apparatus comprised a black circular pool (180 cm in diameter, 60 cm in height), filled to a depth of 25 cm

with water (22 ± 1 °C), and it was placed in a soundproof and dimly lighted room. The room offered several visual cues to aid the formation of the spatial map for escape learning[48].

The pool had four quadrants with four starting lines: north, east, south, and west, and an invisible platform (10 cm in diameter) centrally located 1 cm beneath the water in the north quadrant. Animal training lasted for 5 days at nearly the same time, and each animal completed two blocks of four trials each day (90 s). An interval of 30 s was considered between two trials, and the resting time of 5 min was regarded between two consecutive blocks. Each trial was started by placing the rat facing the wall of the maze in one of four designated locations. The rat was allowed to explore the maze and the time spent to find the hidden platform was defined as the escape latency time within 60 s. The escape latency was recorded by a video camera connected directly to a computer. One day after the spatial acquisition phase (on day 6), in the retention phase, a probe trial was done. The platform was removed from the pool and the rat was allowed to swim. The time spent in the target quadrant and the swimming speed were recorded for 60 s.

Statistical analysis

Data were analyzed using SPSS 13.0 software and one-way analysis of variance (ANOVA) and Tukey's post-hoc test. Statistical significance was set at $P < 0.05$. Data are presented as mean ± SEM.

Results

NOR test

Figure 2A exhibits the discrimination index of the young rats in the NOR test. There were significant differences in the discrimination index between all young groups ($p < 0.001$). The discrimination index of the AD rats was significantly lower than that of the control rats ($p < 0.01$). The discrimination index of AD+ tadalafil rats was significantly higher than that of rats in the AD group ($p < 0.01$). There were no significant differences between the discrimination index of the control and the AD+ tadalafil groups.

Figure 2B shows the discrimination index of the aged rats. There was a significant difference in the discrimination index between the control (72.97±1.18), AD (62.30±2.96), and AD+ tadalafil (70.42±2.95) groups ($p < 0.01$). The discrimination index of the AD rats was significantly lower than that of the control rats ($p < 0.01$). The discrimination index of AD+ tadalafil rats was significantly higher than that of the rats in the AD group ($p < 0.01$).

PAL test

No significant difference was found in STLa in the first acquisition trial between the experimental groups of young rats ($p >0.98$) (data not shown). Fig. 3A shows significant differences in the number of trials to acquisition among the experimental groups ($p < 0.05$). The number of trials of the AD group was significantly higher than that of the AD groups ($p < 0.05$). There was not a significant difference between the control and AD+ tadalafil groups.

There was a significant difference in the STLr between the groups ($p < 0.001$; Fig. 3B). According to the results of Tukey's post-hoc test, the AD group had lower STLr than the control group ($p < 0.001$). Also, the control group showed a higher STLr than the AD+ tadalafil group (p

< 0.001). The AD+ tadalafil group was found with a longer STLr (63.6±27.86); however, there was not a significant difference between tadalafil-treated rats and AD groups (20.3±1.90).

In addition, there was a significant difference in TDC among the young groups ($p < 0.001$, Fig. 3C). AD rats spent more time in the dark compartment compared with the control group ($p < 0.01$). AD+ tadalafil rats spent more time in the dark compartment than the control group ($p < 0.01$). There was no significant difference between AD and AD+ tadalafil groups (Fig. 2C).

No significant difference was found in the STLa among old experimental groups ($p = 0.135$; data not shown). In addition, there was no significant difference in the number of trials to acquisition among the old groups ($p = 0.06$; Fig. 4A).

There was a significant difference in the STLr among old groups ($p < 0.05$; 4B). STLr in the AD groups was significantly shorter than the control groups ($p < 0.01$). There was no significant difference in the STLr between the control and AD+ tadalafil groups.

A significant difference was found in TDC among old experimental groups ($p < 0.01$; Fig. 4C). The TDC of the AD groups was significantly longer than the control rats ($p < 0.01$). The control groups showed a longer TDC than the AD+ tadalafil groups ($p < 0.05$). There was no significant difference between the control and AD+ tadalafil groups.

MWM test

There was a significant difference in the latency and distance traveled in the retention trials among young groups (Fig. 5). AD groups spent more time to find the hidden platform compared with the control and AD+ tadalafil groups ($p < 0.01$ and $p < 0.05$ respectively, Fig. 5A). In addition, AD groups traveled a longer distance to find the hidden platform compared with the control and AD+ tadalafil groups ($p < 0.001$ and $p < 0.001$, respectively, Fig. 5B).

There was a significant difference between the old experimental groups in the escape latency time (Fig. 6A) and distance traveled in training days (Fig. 6B). Old AD rats were found with more time spent and longer distance traveled to find the hidden platform compared with the control groups ($p < 0.001$ and $p < 0.01$, respectively). The mean distance traveled and escape latency showed an increase in the AD+ tadalafil groups than the control groups ($p < 0.001$ and $p < 0.01$, respectively). There was no significant difference between the AD+ tadalafil and AD groups in escape latency and distance traveled.

There was not a significant difference between young rats regarding time spent in the target quadrant to find the detectable platform in the visible test ($P = 0.5012$; data not shown). A significant difference was observed in the time spent in the target quadrant in the probe test between young rats (Fig. 7A). AD groups compared with the control groups spent less time in the target quadrant ($p < 0.001$). AD+ tadalafil rats spent more time in the target quadrant than the AD groups ($p < 0.001$).

No significant difference was observed between old rats in the escape latency to find the observable platform in the visible test ($P = 0.3307$; data not shown). The time spent in the target quadrant significantly decreased in the AD young compared with the control and AD+ tadalafil young groups ($p < 0.001$; Fig. 7B). Old rats were found with a significant difference in time spent in the target quadrant in the probe test ($P < 0.001$; Fig. 7B). The time spent in the target quadrant significantly decreased in the AD and AD+ tadalafil groups compared with the control groups ($p < 0.001$; Fig. 7B). There were no significant differences in the time spent in the target quadrant between the AD and AD+ tadalafil groups

Discussion

According to the main finding of the present study, the –STZ (ICV) injection impaired cognition and caused a decrease in learning and memory in both old and young rats. Treatment with tadalafil for 40 consecutive days improved the memory deficiency resulted from the STZ (ICV) administration in young rats. In addition, tadalafil ameliorated the deteriorative effects of STZ (ICV) on the recognition, learning, and memory in young rats after 40 days of treatment.

The rats subjected to STZ (ICV) administration exhibited a decline in cognition, learning, and memory. Our results are consistent with other data reporting STZ (ICV)-induced memory deficits[44]. . In the MWM test, –STZ (ICV)-treated rats were unable to memorize the platform location. This observation indicates the inability of rats receiving ICV administration of STZ, particularly aged animals to remember spatial information, a characteristic of cognitive failure due to hippocampal impairment[49]. The microinjection of STZ, at a sub-diabetogenic dose, in rodents leads to prolonged impairment of brain insulin resistance, glucose uptake, and metabolism [28,29,50,51]. STZ causes damage to the cholinergic system[30,52] and nucleotide signaling[53]. This impairment caused the learning and memory dysfunctions [40,54,55], aggregation of Aβ peptides, tau hyperphosphorylation, and neuroinflammation[8]. A reduction in cognition found in STZ (ICV)-treated young and aged AD rats is correlated with the neuronal loss in the sporadic form of AD[56]. However, in the present study, we only considered behavioral study and the immunohistological and biochemical study is necessary for better understanding.

Tadalafil belongs to the PDE-5 inhibitors, and crosses the blood-brain barrier, and reverses cognitive dysfunction in mice[57]. It promotes an increase in cGMP in cortical and hippocampal neurons and enhances working memory in gerbils[58] in both young and aged mice[34]. PDE5

inhibitors antagonize age-associated memory deficits, such as dementia[59]. Sildenafil, the PDE5 inhibitor, can reverse cognitive impairments in mouse models[60-62], and inhibit scopolamine-induced cognitive impairments in the T-maze test in rats[63]. Tadalafil has been used for the treatment of nervous system diseases. It improves memory in aged animals[34]. Administration of tadalafil for ten weeks improved the cognitive function in the MWM test in J20 mice [57], which was possibly due to a decrease in the phosphorylation of Tau proteins in the hippocampus [57].

The learning and memory impairment is more severe in old rats compared with young ones. Aging is associated with a considerable decline in performance in many memory tasks [3,5,64]. It has been shown that the aged rats have spatial memory deficits than young rats[65]. A reduction in information processing is associated with difficulties in retention [66]. Old animals utilized non-spatial strategies to solve the maze than young animals [67]. Aged STZ (ICV)-treated rats were found with more difficultly in MWM, which may be due to a decrease in the speed of processing spatial information[68].

We also found that tadalafil was more effective in young rats than aged rats in MWM. Aging is also associated with a reduction in cGMP levels in the hippocampus and an increase in PDE activity[69,70]. Cyclic nucleotides are intracellular signaling molecules and are catalyzed by PDEs via hydrolysis[71]. Decreased levels of cGMP in cerebrospinal fluid are associated with cognitive decline and amyloid-beta pathology in neurodegenerative diseases[15]. On the other hand, AD is associated with increased neuronal expression of PDE5 mRNA[13,15,24]. PDE5 enzyme levels are increased in the hippocampus of STZ rats[72]. PDE-5 inhibitors induce vasodilation through cGMP, increased blood flow, glucose metabolism, and ultimately improving memory[34]. Protein kinase G activity is also decreased in aged rat brains consistent with a reduction in cGMP levels[73]. PDE5

inhibitors reverse age-related deficits in cAMP-response element-binding protein, which is crucial for synaptic plasticity and memory[59,74]. Daily administration of PDE5 inhibitors, such as tadalafil can control the cGMP homeostasis via hydrolysis of cGMP[75]. The increased cGMP level in the brain might be responsible for memory enhancement in STZ (ICV)-treated rats.

Conclusions

Chronic treatment with tadalafil reverses dementia symptoms in –STZ (ICV)-treated aged rats. However, it did not improve learning in aged rats. Inhibition of PDE5 is beneficial to treat age-related memory dysfunction in a sporadic AD model in aging. It is suggested that pretreatment with tadalafil can improve memory impairment in aged AD rats.

Ethical Issues

All animal experiments were performed in accordance with the guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). The Ethical Committee of Hamedan University of Medical Sciences (No. IR.UMSHA.REC.1394.210) approved all experimental procedures. This research was supported by a grant (Grant number: 9407073542) from the Neurophysiology Research Center of Hamadan University of Medical Sciences.

Conflict of interest

The authors declare that there is no conflict of interest for any of the authors.

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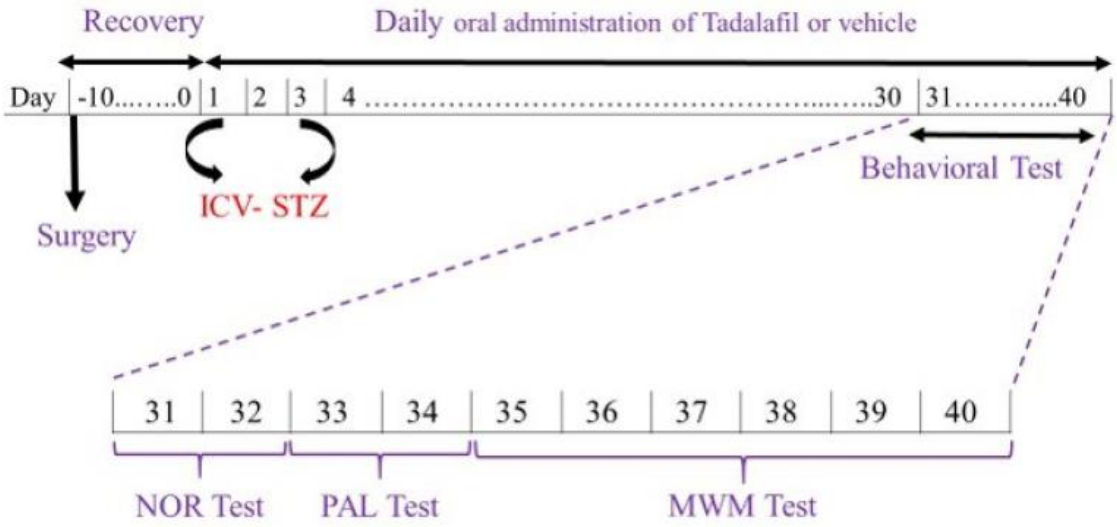
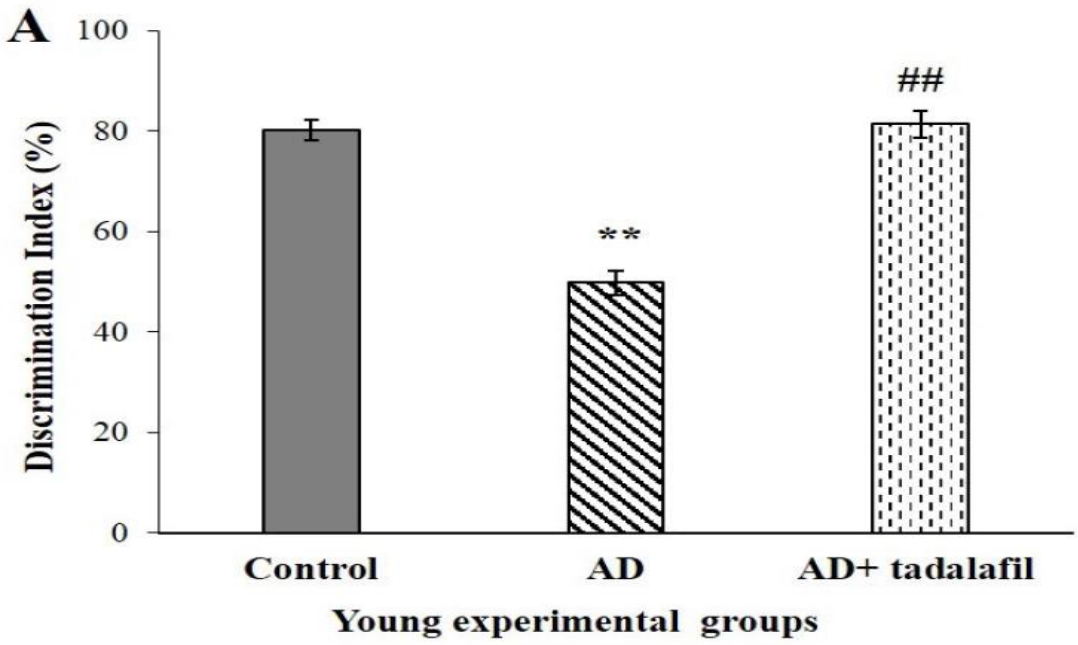


Fig .1. A schematic diagram of the experimental design and timeline.


A
100
80
60
40
20
0
Discrimination Index (%)
**
##
Control
AD
AD+ tadalafil
Young experimental groups

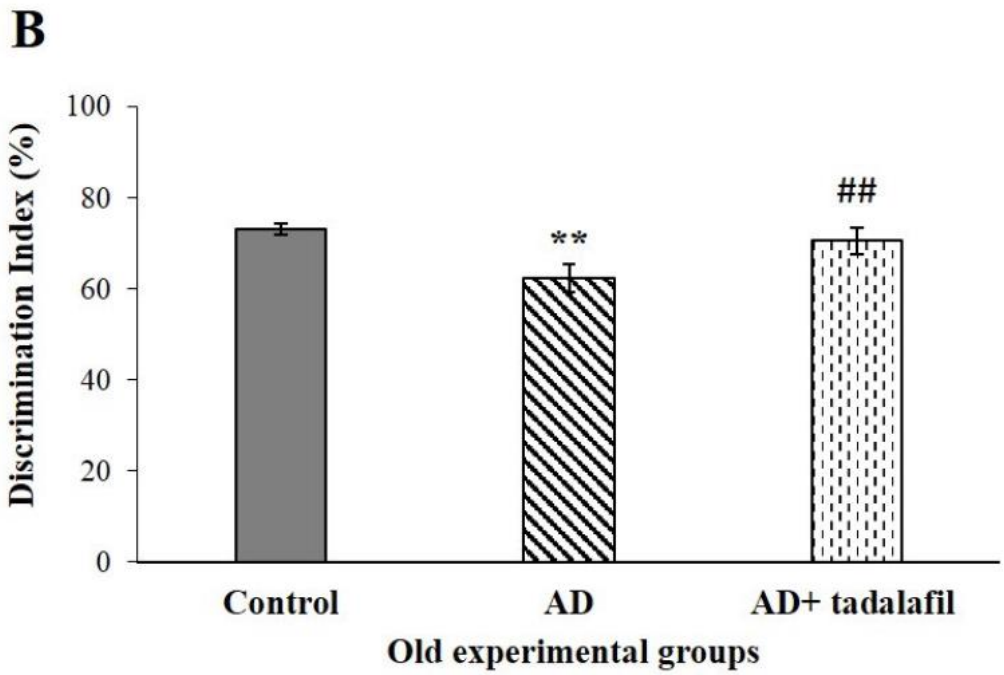
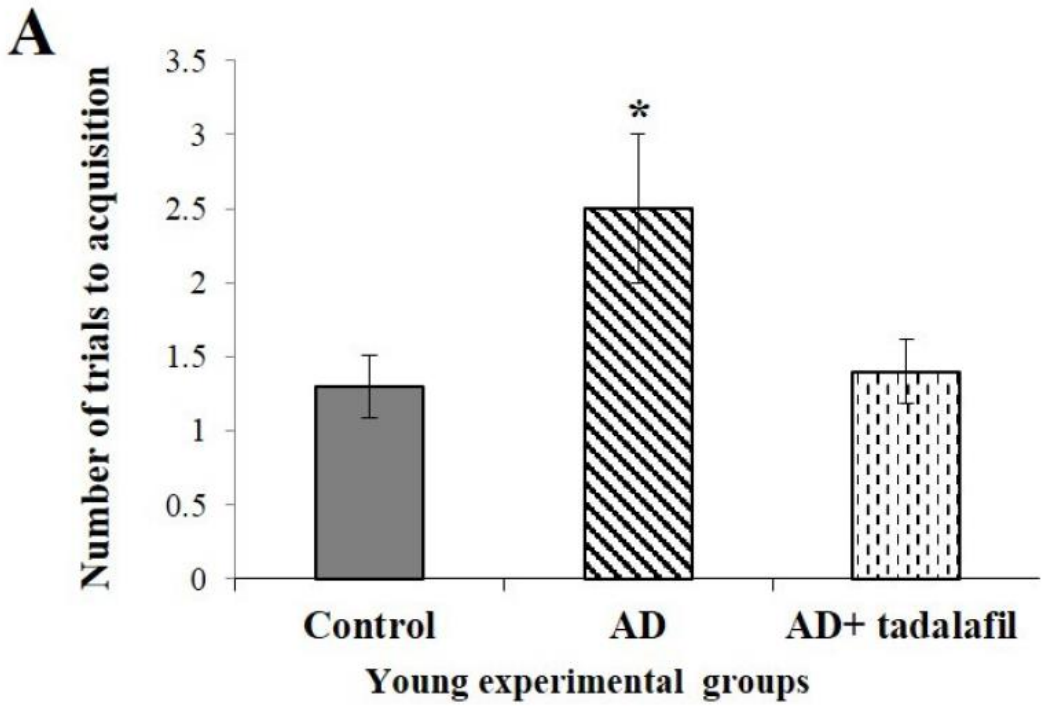
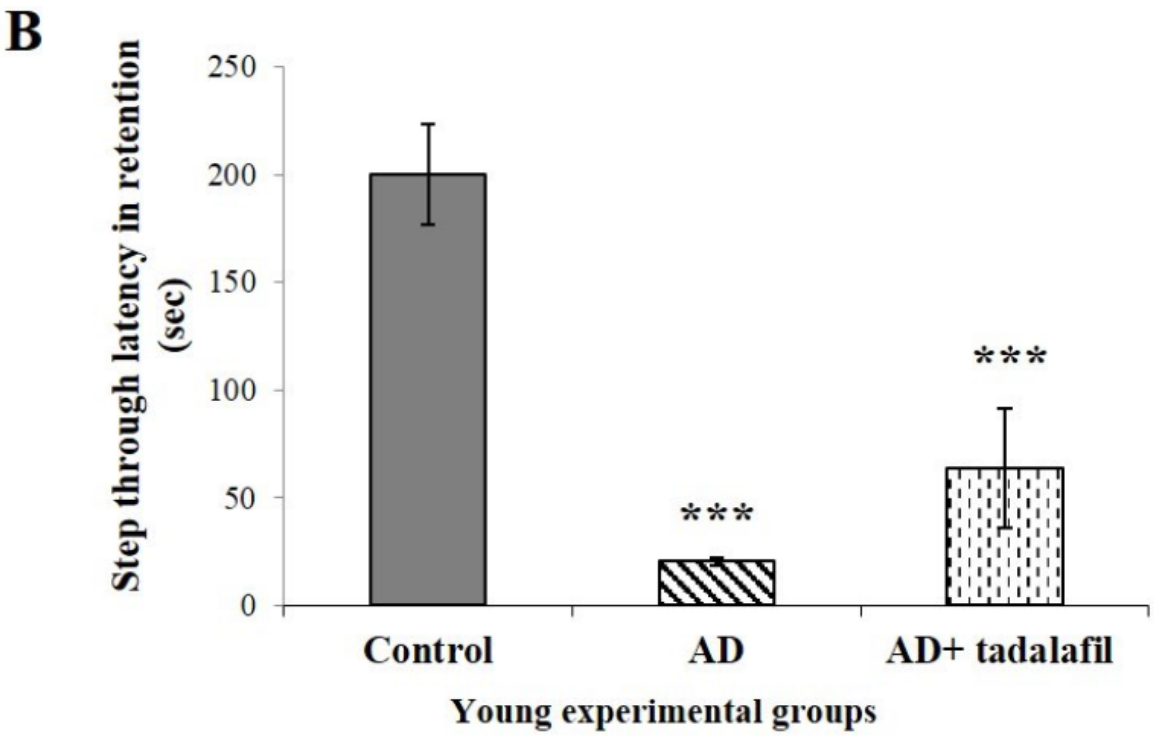


Fig. 2. Effect of chronic treatment with Tadalafil on the young (A), and old (B) rats in the discrimination index in the novel object recognition test. ** $p < 0.01$ in compared with the control group; ## $p < 0.01$ in compared with the AD group (n = 10, in each group). Each column represents the mean ±SEM.


A
Number of trials to acquisition
3.5
3
2.5
2
1.5
1
0.5
0
*
Control
AD
AD+ tadalafil
Young experimental groups


B
Step through latency in retention (sec)
250
200
150
100
50
0
***
***
Control
AD
AD+ tadalafil
Young experimental groups

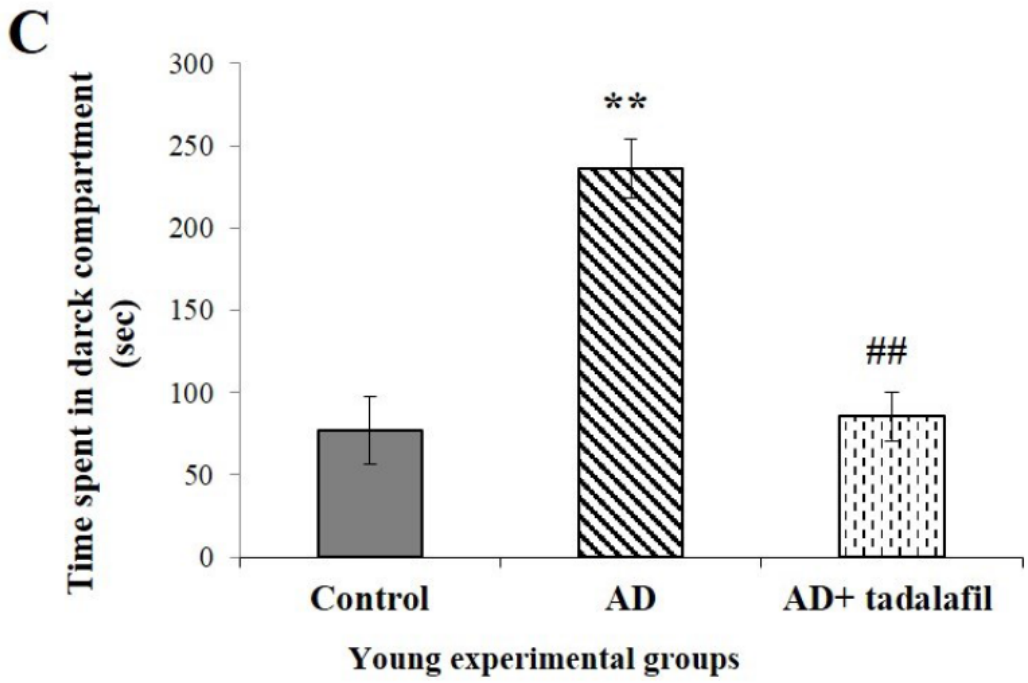
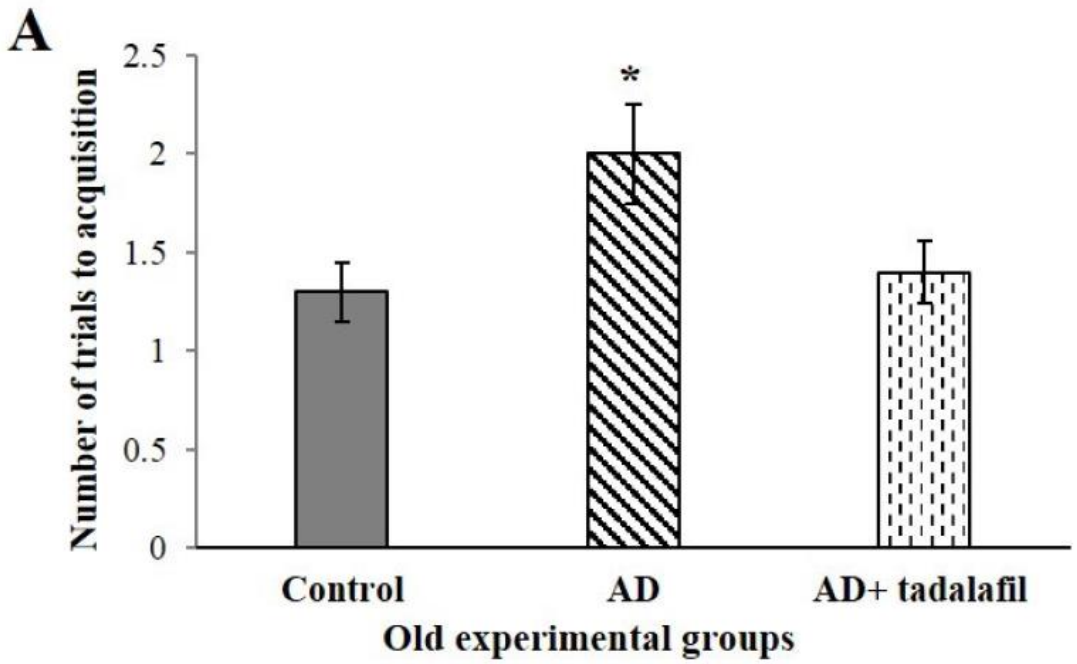
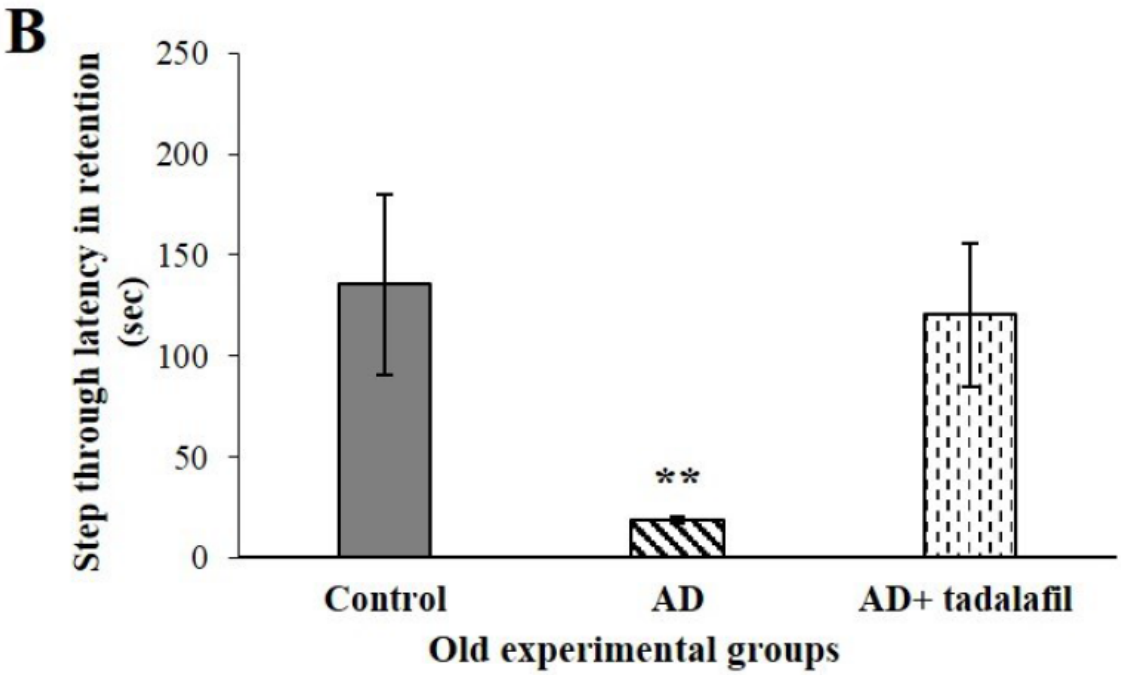


Fig. 3. Effect of chronic treatment with Tadalafil among the young groups on the number of trials to acquisition in the acquisition trial (A), step-through latency (B), and the time spent in the dark compartment (C) in the retention phase of passive avoidance learning task. ** $p < 0.01$, and * $p < 0.05$ in compared with the control group; ## $p < 0.01$ in compared with the AD group (n = 10, in each group). Each column represents the mean ±SEM.


A
Number of trials to acquisition
2.5
2
1.5
1
0.5
0
*
Control
AD
AD+ tadalafil
Old experimental groups


B
Step through latency in retention (sec)
250
200
150
100
50
0
**
Control
AD
AD+ tadalafil
Old experimental groups

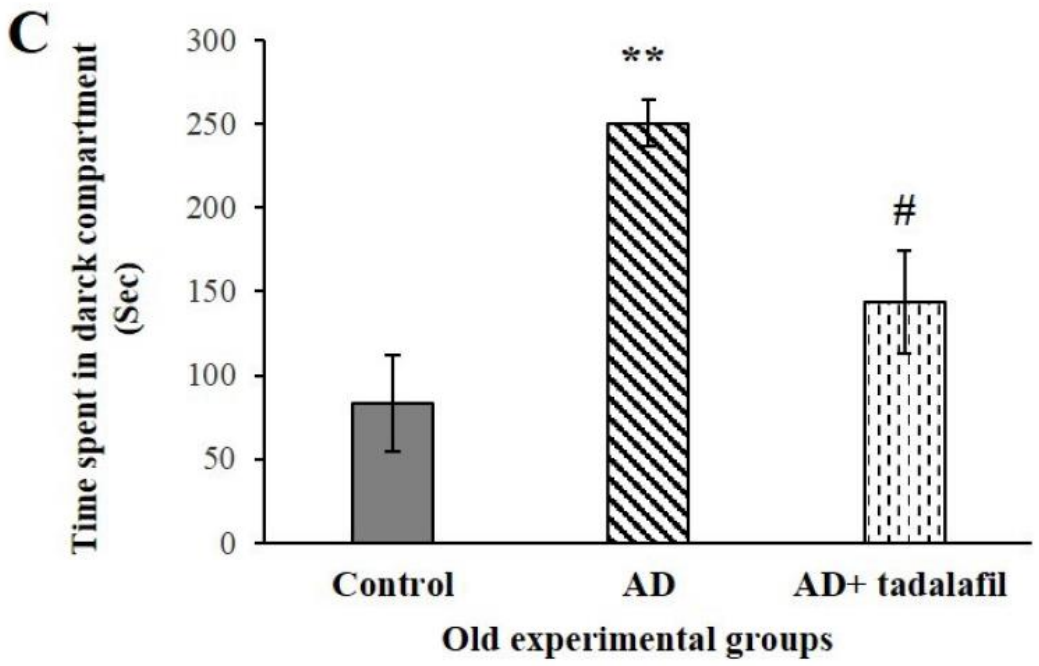


Fig. 4. Effect of chronic treatment with Tadalafil among the old groups on the number of trials to acquisition in the acquisition (A), step-through latency (B), and the time spent in the dark compartment (C) in the retention phase of passive avoidance learning task. ** $p < 0.01$ and * $p < 0.05$ in compared with the control group; # $p < 0.05$ in compared with the AD group (n = 10, in each group). Each column represents the mean ±SEM.

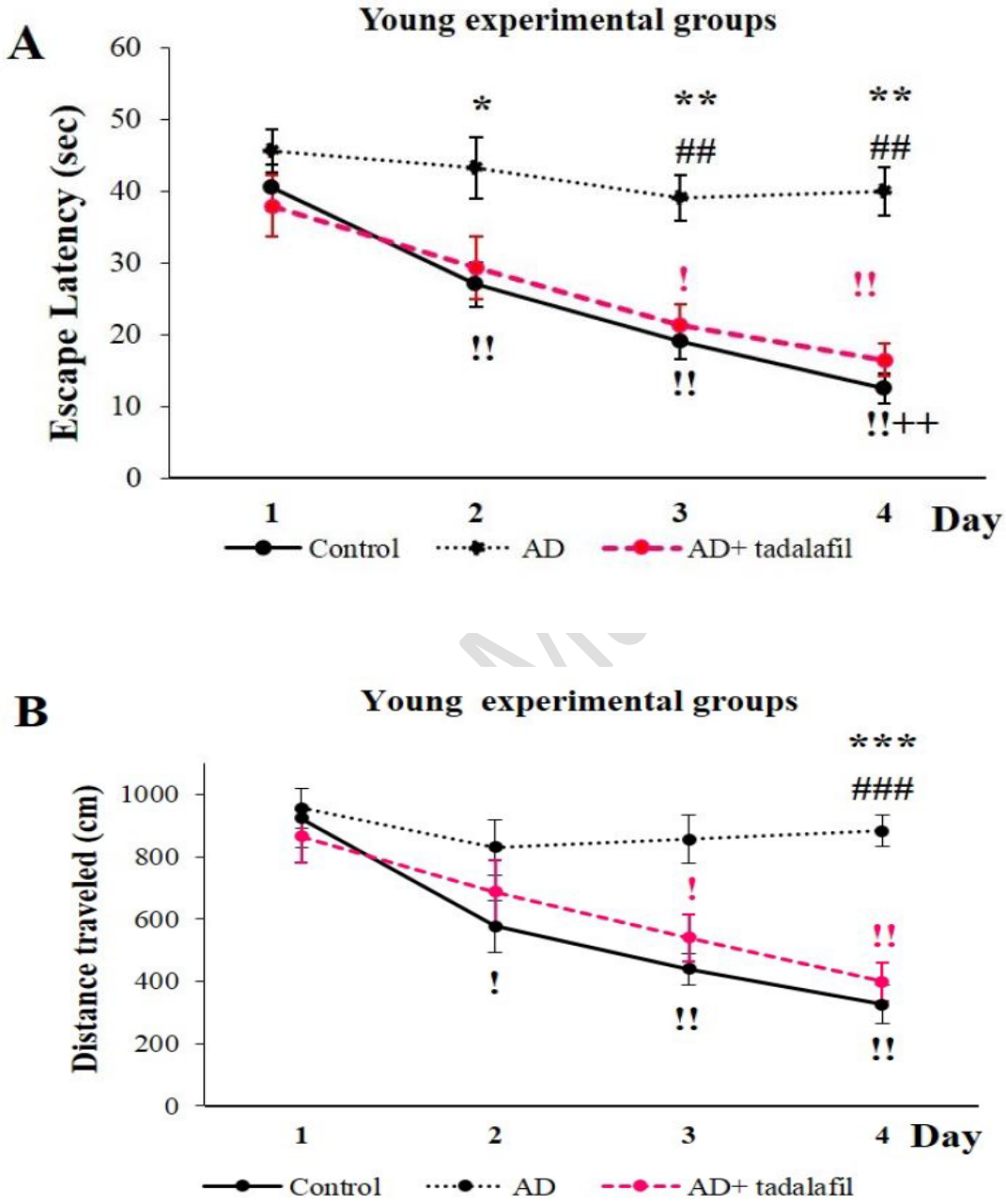


Fig. 5. Effect of chronic treatment with Tadalafil among the young groups in escape latency (A) and distance traveled (B) to find a hidden platform in the Morris Water Maze during retention day. *** $p < 0.001$, ** $p < 0.01$, and * ($p < 0.05$) in compared with the control group. ### $p < 0.001$ and ## $p < 0.01$ in compared with the AD+ Tadalafil group; !! $p < 0.01$ and ! $p < 0.05$ in compared with the first day; and ++ ($p < 0.01$) in compared with the second day (n = 10, in each group). Each column represents the mean ±SEM.

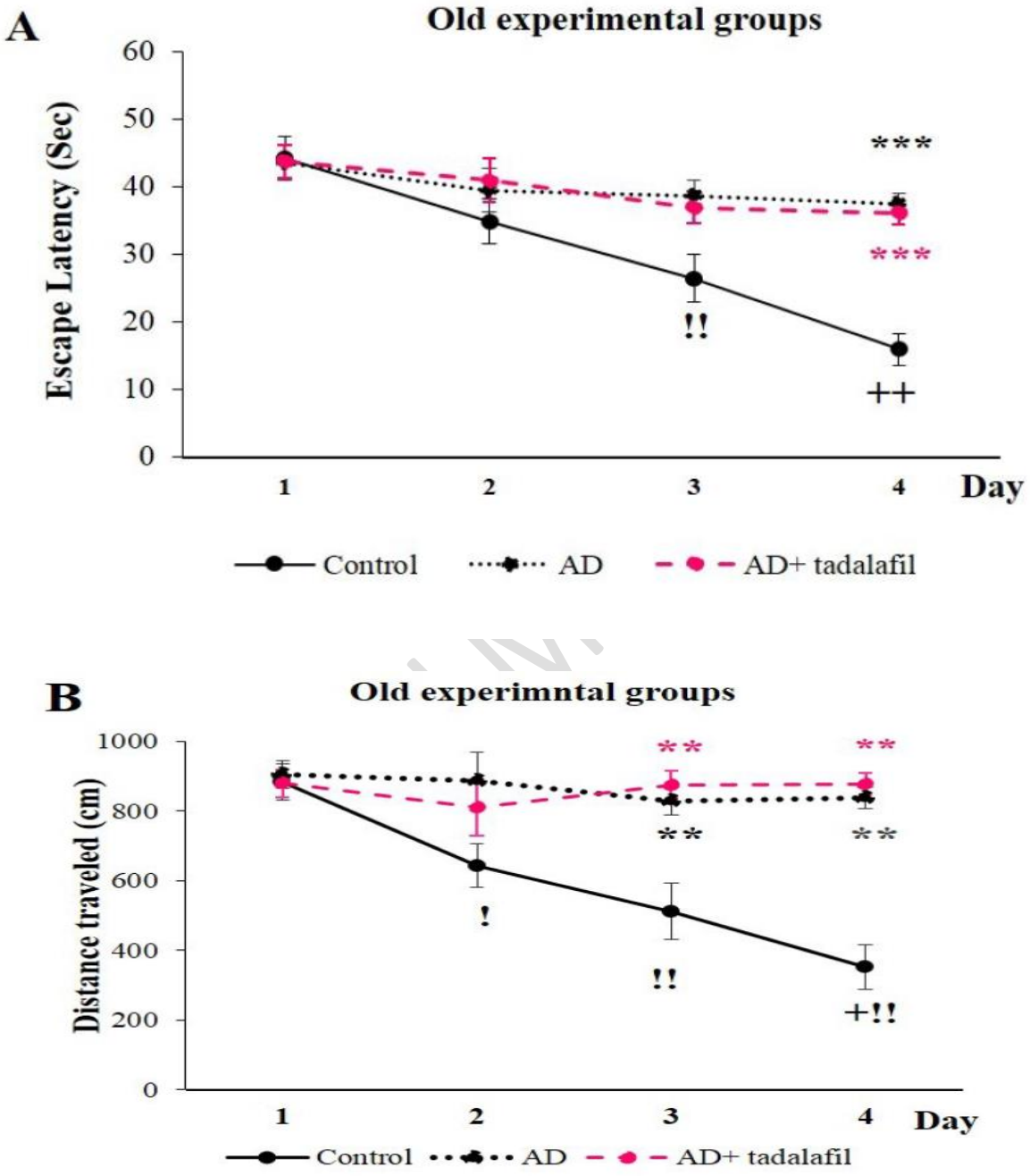
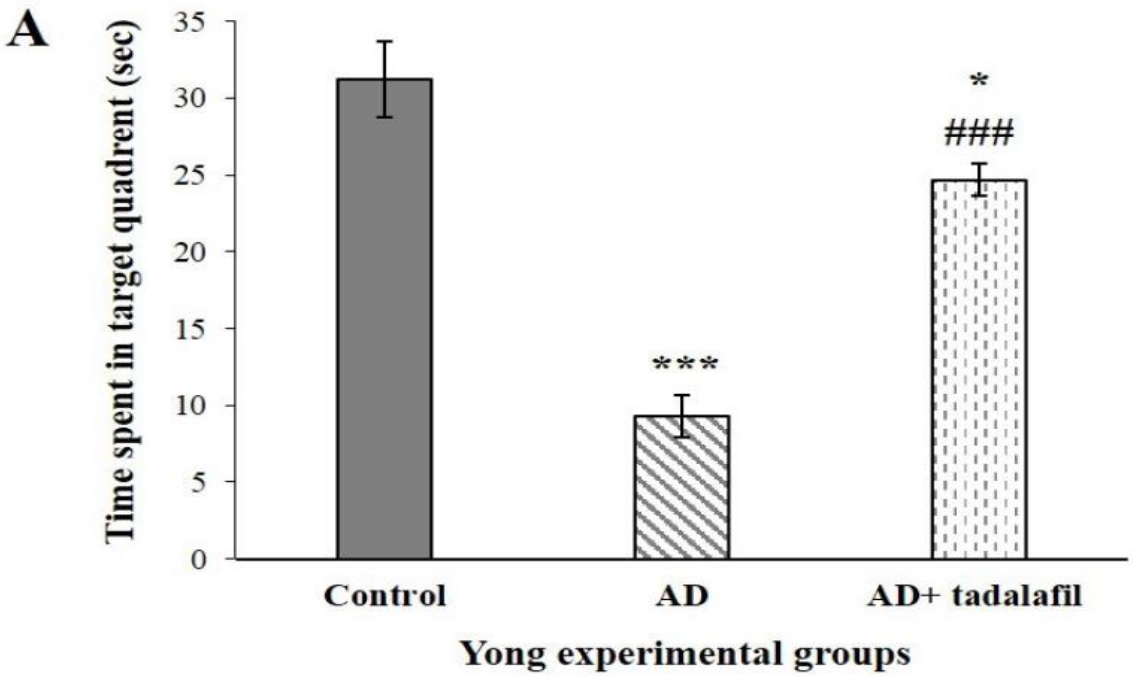


Fig. 6. Effect of chronic treatment with Tadalafil among the old groups in escape latency (A) and distance traveled (B) to find a hidden platform in the Morris Water Maze during retention day. **($p < 0.01$ in compared with the control group; II $p < 0.01$ and I $p < 0.05$ in compared with the first day; and ++ $p < 0.01$ and + $p < 0.05$ in compared with the second day (n = 10, in each group). Each column represents the mean ±SEM.


A
Time spent in target quadrent (sec)
35
30
25
20
15
10
5
0
***
*
###
Control
AD
AD+ tadalafil
Yong experimental groups

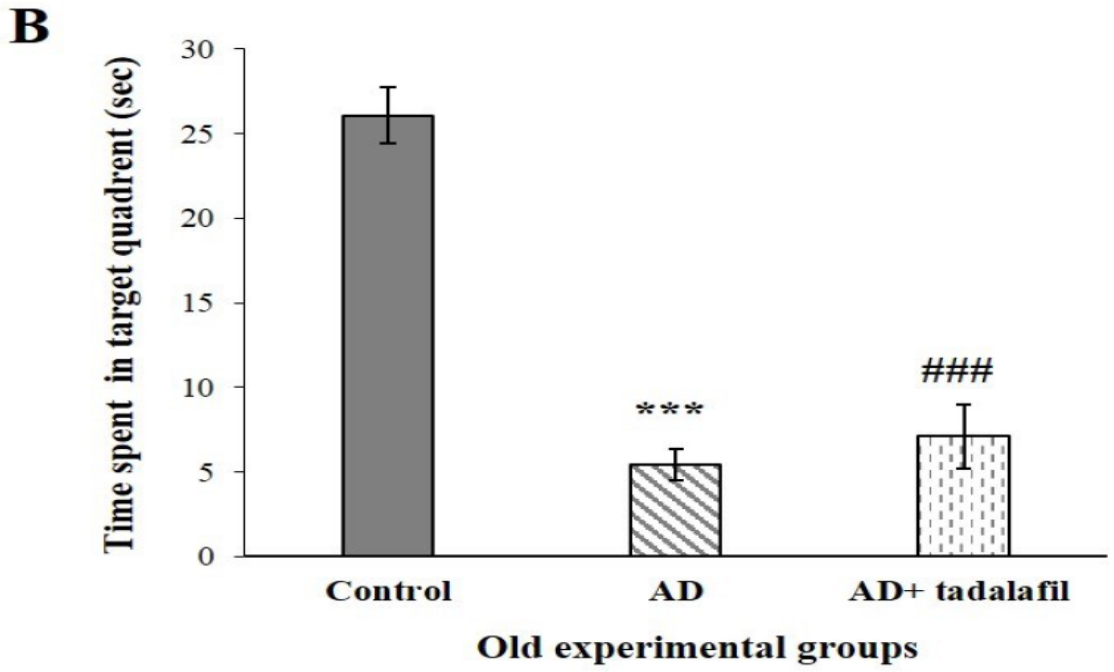


Fig. 7. Effect of chronic treatment with Tadalafil in the probe test in the Morris Water Maze test among the young (A), or old (B) groups. *** $p < 0.001$) in compared with the control group; and ### $p < 0.001$ in compared with the AD (n = 10, in each group). Each column represents the mean ±SEM.