Effects of the Combination of High-intensity Interval Training and Ecdysterone on Learning and Memory Abilities, Antioxidant Enzymes Activities, and Neuronal Population in an Amyloid-beta-induced Rat Model of Alzheimer's Disease

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Research Article

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Abstract

Aims: Oxidative stress and neuronal death are the primary reasons for the progression of amyloid-beta (Aβ) deposition and cognitive deficits in Alzheimer's disease (AD). Ecdysterone (Ecdy), a common derivative of ecdysteroids, possesses free radical scavenging and cognitive-improving effects. High-intensity interval training (HIIT) may be a therapeutic strategy for improving cognitive decline and oxidative stress. The present study was aimed to evaluate the effect of HIIT alone and its combination with Ecdysterone on the changes in learning and memory functions, hippocampal antioxidant enzymes activities, and neuronal population after AD induced by Aβ in male rats.

Materials and methods: Following ten days of Aβ-injection, HIIT exercise and Ecdysterone treatment (10 mg/kg/day; P.O.) were initiated and continued for eight consecutive weeks in rats. At the end of the treatment period, rat's learning and memory functions were assessed using water-maze and passive-avoidance tests. Moreover, the activity of superoxide dismutase (SOD), catalase (CAT), Glutathione Peroxidase (GPx), Glutathione Reductase (GRx) and neuronal population were evaluated in rat's brains.

Results: The results indicated that Aβ injection disrupted spatial/passive avoidance learning and memory in both water-maze and passive-avoidance paradigms, accompanied by a decrease in the superoxide dismutase and catalase (as endogenous antioxidants) in rat hippocampus. Additionally, Aβ injection resulted in neuronal loss in the cerebral cortex and hippocampus. Although consumption of Ecdysterone separately improved spatial/passive avoidance learning and memory impairments, recovered hippocampal activity of SOD, CAT, GRx, GRx and prevented the hippocampal neuronal loss, its combination with HIIT resulted in a more powerful and effective amelioration in all the above-mentioned Aβ-neuropathological changes.

Conclusion: The current work's data confirms that a combination of HIIT exercise and Ecdysterone treatment could be a promising potential therapeutic agent against AD-associated cognitive decline, owing to their free radical scavenging and neuroprotective properties.

Introduction

It is proven that Alzheimer's disease (AD) is the most potent neurodegenerative ailment (1) that includes about 6% or 7% of the elderly population over 65 years old (more than forty million people worldwide) that chance of prevalence after this age becomes doubles every five years (2). AD is characterized clinically by progressive cognitive dysfunctions and behavioral disorders related to the lack or decrease of synaptic connections and, of course, neuronal apoptosis as a result of progressive aggregation of amyloid-β (Aβ) and hyperphosphorylation of Tau protein in the hippocampus and cerebral cortex that makes patients confused and occasionally they have difficulty in their routine life. Also, oxidative stress is a critical component of pathogenesis in AD (3, 4), so that there are studies that heretofore have reported the rise of oxidative stress markers in the brain tissue of AD patients, parameters such as lipid peroxidation, protein oxidation, and formation of reactive oxygen species (ROS) (5, 6). meanwhile, the

hippocampus area has a crucial role in learning and memory (7). It is one of the most vulnerable regions in the brain, which is prone to succumb to oxidative stress derided by dementia (8). Nowadays, it is unclear whether oxidative stress is a significant reason or rises from mitochondrial dysfunctions associated with AD (9).

It is reported that supplementations with antioxidant properties are helpful, particularly in the early stages of disease (10). At the same time, an array of natural antioxidants have been drawn from plants, including phytoestrogens, which can alleviate oxidative stress (11, 12). Ecdysterone (Ecdy) (Fig.1) is one of the components of phytoestrogen that is an insect hormone and provides regulating with the molting, metamorphosis and reproduction of arthropods (13). Besides, it is widespread among a considerable number of plants with a high concentration in some certain plants such as; (Achyranthes bidentate) and (Cyanotis arachnoidea) (14, 15). In addition, evidence investigates that Ecdy may have substantially positive pharmacological impacts in mammals, such as; stimulating protein synthesis (16, 17), promoting carbohydrate and lipid metabolism (18), preserving apoptosis (19), affecting the CNS as well as enhancing immunomodulation. In other words, Ecdy is found by increasing the number of medical worth, also, the anti-oxidation profits of Ecdy have been reported (20). Besides, previous studies suggested that Ecdy can promote learning, memory and orally treat cognitive dysfunctions of rats induced by beta-amyloid peptide fragment$_{25\text{-}35}$ (A$\beta_{25\text{-}35}$) and raise the expression of C-fos. This gene is an indicator for neuronal activity and firmly has relation with learning and memory in cortex and hippocampus (21). Likewise, earlier studies in terms of behavioral investigations have indicated that Ecdy caused shorter latency and searching distance in Morris water maze test between Aβ-induced rats that means Ecdy could improve impaired spatial memory (22).

On the other hand, surveys have shown that the administration of exercise training (ET) can cause an improvement of passive avoidance learning and spatial memory in rodents (23, 24). Furthermore, ET accomplishment increases hippocampal Neurogenesis, synaptic plasticity, as well, neurotransmission in the hippocampus that on their own can contribute to cognitive advancement (25, 26). However, never should it be forgotten that there is no supporting evidence to illustrate which combination of time, frequency, intensity, and generally type of exercise may have a more significant effect on cognitive niches. Based on studies, High-Intensity Interval Training (HIIT) through increases in H_2O_2 and TNF-α can lead to a significant improvement in BDNF (Brain-Derived Neurotrophic Factor) and GDNF (Glial Derived Neurotrophic Factor) concentrations (scientists notice these factors as indexes for memory and learning). Thus, training with interval bouts that need maximum effort will result in better neurotrophic gain (27). Additionally, this training method regulates hippocampal oxidative stress, BDNF, and inflammatory mediators in laboratory animals. At the behavioral level, it has been shown that HIIT can reduce learning decline in the Morris water maze test in rats with AD (28, 29).

Although many remedial methods, including consuming chemical and herbal medicine and different ways of physical activity to inhibit AD progress, are available, an effective treatment against AD still does not exist (30, 31). So, in the present study, we decided to determine the synchronous effect of HIIT

accompanied by Ecdy in memory, learning, and cognitive functions in male Wistar rats induced by beta-amyloid.

Methods

2.1. Animal study

Before starting the experiment, seventy-two adult male Wistar rats (weighing 175-200 gr) were purchased from the breeding institute of the animal house of Hamadan University of Medical Sciences (UMSHA). The animals were housed tree in a cage in a temperature-controlled room with 23-25 °C and 50–70% relative humidity under a 12:12 light-dark cycle from 19 till seven that treatment protocol and behavioral tests started in light cycles. Also, every animal accessed to food (rodent pellets consisted of 23% protein, 47% carbohydrate, 5% lipids, 5% cellulose, 20% water, and vitamins and minerals with a caloric density of approximately 3.0 kcal/g) (32) and water freely. All experimental processes and animal care procedures were confirmed by the Veterinary Ethics Committee of UMSHA and were confirmed with the Guidelines of the principles of laboratory animal care in the National Center of Health (code: IR.UMSHA.REC.1400.458).

2.2. Experimental design

At first, Rats for seven days were domesticated using handling and accustoming to circumstances. Then they were randomly assigned into nine groups(n=8 per group): (group1) control health (Control); they received saline 9% (as Ecdy dissolvent) for eight weeks through oral gavage (33). (group2) Sham; they received a five µL vehicle of beta-amyloid (phosphate-buffered saline (PBS)) via an intrahippocampal injection after stereotaxic surgery. (group3) Ecdy; they received Ecdy (10mg/kg/day) for eight weeks through oral gavage(33). (group4) HIIT; they performed HIIT on a special treadmill for eight weeks (5 sessions per week) (34). (group5) Ecdy + HIIT; they received Ecdy (10mg/kg/day) for eight weeks through oral gavage also performed HIIT on a treadmill for eight weeks. (group6) control Alzheimer's (Alzheimer's); they received five µL beta-amyloid via an intrahippocampal injection after stereotaxic surgery, then received saline 9% for eight weeks through oral gavage (35). (group7) Alzheimer + Ecdy; they received Ecdy (10mg/kg/day) for eight weeks through oral gavage after receiving five µL beta-amyloid via intrahippocampal injection. (group8) Alzheimer + HIIT; they performed HIIT on a special treadmill for eight weeks after receiving five µL beta-amyloid via intrahippocampal injection. (9) Alzheimer + Ecdy + HIIT; they received Ecdy (10mg/kg/day) for eight weeks through oral gavage also performed HIIT on a treadmill for eight weeks after receiving five µL beta-amyloid via intrahippocampal injection (Fig.2). Although studies also have reported some other dosages such as 1 and 100 mg/kg/day, with due attention to studies, we decided to use 10mg/kg/day of Ecdy as an efficient dosage (33).

2.3. Aβ preparation, injection and stereotaxic surgery

The Amyloid-$\beta_{1\text{-}42}$ peptide (100 µL) (cat number SCP0038, bought from Sigma Aldrich, USA) dissolved in 100 µL PBS (as vehicle solution) then incubated at 37 °C for seven days before in vivo utilizing. This action leads to fibril formation, which has neurotoxic properties (36). After preparing $A\beta_{1\text{-}42}$ to induce AD

model, the rats were anesthetized with intraperitoneal (i.p) injection of ketamine (100mg/kg) and xylzine (10mg/kg). Afterward, rats were stuck in the stereotaxic apparatus (Stoelting Co., Wood Dale, IL, USA), and their scalp was split. Then Bregma and Lambda adjusted to balance level in terms of the horizontal plane and a hole drilled above the ventricular area in the skull surface (coordinates: AP: -1.2 mm posterior to the Bregma, ML, ±2 mm lateral, and DV: 4.0 mm below the dura)(37). The injection was conducted with a five µL microsyringe (Hamilton Laboratory Products, Reno, NV, USA). Aβ solution (5 µL) was gradually injected into the region on the right side at a rate of 0.5 µL/min. Then the syringe was left in place for 5 min after the injection before being dislodged to allow diffusion of Aβ. The next scalp was stitched. Also, Sham group rats operated like this surgical protocol with the same injection volume, but they received PBS (vehicle solution) instead of Aβ. Animals are allowed to have one week of recovery after surgery before beginning treatment (37).

2.4. Preparation and gavage of Ecdy

The dry powder of Ecdysterone supplementation (purchased from Amazon company, USA) dissolved in distilled water at a concentration of 10 mg/cc and then gavaged orally with an insulin syringe accompanied by a gavage needle in the amount of 10 mg/kg. This protocol was carried out every day for eight weeks (33).

2.5. HIIT exercise protocol

One week after injection of Aβ, the exercise group rats were trained on a motorized rodent treadmill apparatus (Tajhiz Gostare Omide Iranian, Iran) (38). All rats were initially familiarized with the treadmill environment through walking at a speed between 5 to 10 m/min for 10 minutes 5 days. After animals adapted to the environment, Rats ran on a treadmill with a 15° inclination until exhaustion to measure their maximal oxygen uptake ($\dot{V}o_{2\,max}$). They started the protocol at speed of 6 m/min and increased by 3 m/min every 3 min until rats were unable to run (39). After the determination of $\dot{V}o_{2\,max}$,the main period of exercise began. The animals every day ran 1min intervals at 90% of maximal exercise capacity, followed by 1min intervals at 50% of maximal exercise capacity alternately at no incline (0%) for 30 minutes in the first week and every week, 5 minutes added to the time of exercise until the time reached to 60 minutes in the seventh week (39). This protocol lasted for eight weeks. Besides, the main period of exercise included a 5 min warm-up and a 5 min cool-down at 40% VO_2max before and after the exercise period (40). At the same time, animals trained for five days over a week (they rested on Fridays and Mondays) between 9 AM and 2 PM.

2.6. Morris Water Maze (MWM)

2.6.1. Morris water maze apparatus

Spital memory and cognitive performance were assayed using a five days Morris water maze (MWM) test. The water maze device (180 cm in diameter and 60 cm in height) was full of 25 ± 1 °C water to a depth of 45 cm located in a room containing a variety of visual cues that was allocated into four equal

quadrants. In addition, an invisible circular platform (10 cm diameter) was submerged in a stable position at 1.5 cm beneath the water's surface in the center of the Northwest quadrant of the pool. Low lights were used for illumination, and the room was sound insulated. On the other hand, the pool was separated into four specific start points as the East (E), West (W), North (N), and South (S). These adjustments remained consistent for all rats across the training trials (41).

2.6.2. Habituation

Before starting the first training session, rats were put in the Morris water maze pool to swim without any platform for habituation to the environment for 60 seconds.

2.6.3. Hidden platform training

The training sessions were performed between 9 AM and 12 PM for four days, including. Eight trials were divided equally into two blocks (every block was four trials), that there was a five-minute break between every block for every rat. For each trial, the animals in all groups were placed in the pool (facing the pool wall) to start the trial at one of the four starting points in a different order in every trial and allowed to swim for 60 seconds from a start point (E, W, N, and S) in the pool to find the invisible platform. If animals could detect the platform in 60 seconds, they were let to remain on the platform to rest and discover the environment for 30 seconds, but if they failed to find it, they were guided to the invisible platform by a technician and then were let to rest and discovery for 30 seconds. A video camera (Nikon, Melville, NY) linked to a tracking system in the computer was directly installed above the pool to record specific parameters, such as Distance moved by swimming and time spent to reach the platform (Scape latency) from the training sessions (41).

2.6.4. Probe test

On the fifth day, a probe test was conducted to evaluate spatial memory retention twenty-four hours after the training phase. On this day, the platform was eliminated, and each rat was placed in the pool like the training method and was allowed freely to swim for 60 seconds. Then, as an assay of spatial memory retention, the number of entrances to the target quadrant, time spent in the target quadrant, distance traveled in the target quadrant, and Average Speed was analyzed (42).

2.6.5. visual test

Forty-five minutes after the probe test, the platform elevated above the water surface and also was made apparent by a piece of bright sponge and located in the SE quadrant, and rats were let to swim and explore the visible platform for 60 seconds in order to evince their visual ability (43).

2.7. Passive Avoidance Task (PAT)

2.7.1. Passive avoidance apparatus

In the survey, a PAL device and a procedure (step-through method) were identical to the previous studies used to evaluate passive avoidance memory and learning (44-46). The device contained a bright cubical space with a dimension of (22_22_32 cm3) made of limpid plastic and a dark cubical space with dark opaque plastic walls (22_22_32 cm^3). Also, the space floors were made of stainless steel shafts (3mm in diameter), placed at a 1-cm distance from each other. Besides, a shock generator was used for electrifying the dark chamber floor, and a rectangular opening (6_8 cm^2) was placed between the two bright and dark spaces, which could be closed by an opaque guillotine door (41).

2.7.2. Passive avoidance training

In order to adapt the rats to the device, they were given two trials opportunity to adapt. At first, the rats entered into the bright section of the apparatus; then, after 30 seconds, the guillotine door picked up. With due attention to the natural trend of rats to the dark environment, they tried to enter the dark compartment (entrance defined as all the body entering the dark compartment). The guillotine door closed as the rats entered the dark compartment. After spending 30 seconds, they were deleted from the dark compartment and placed in their cages; the second trial repeated after 30min precisely in the same way, and the experiment was pursued after the same pause by the first acquisition trial. In the acquisition trial, the guillotine door was picked up immediately after locating rats in the bright compartment, the entrance delay to the dark compartment or step-through latency to acquisition (STLa) was evaluated, and after entering rat to the dark compartment, the door closed, an electrical shock used (0.4 mA) for 1.5 seconds. Then the rats were returned to a separate cage after 30 seconds; this protocol repeated for two more minutes after 120 seconds. Whenever the rats reentered in the dark compartment, they have received a foot shock, and the protocol was repeated, but by remaining them in the light compartment for all two minutes continuously, training interrupted, and the number of trials was recorded as the number of acquisitions. Also, the step-through latency in the acquisition trial (STLa) was recorded (41).

2.7.3. Retention test

Long-term memory as a retention trial 24 hours after the PAL acquisition trial was carried out. Like the PAL training session, the animals took in the light compartment and immediately opened the door. Then, the step-through latency in the retention trial (STLr) and the time spent in the dark compartment (TDC) were recorded for 10 minutes. Through the retention test, there were no electrical shocks. If animals did not enter the dark compartment within 300 seconds, the retention test terminated, and a ceiling score of 300 seconds was recorded (43).

2.8. Nissl staining protocol

A day after experiments finished, three rats in every group chose and were deeply anesthetized by ketamine (100 mg/kg) and xylazine (10 mg/kg) and perfused at first with normal saline and then 10% formalin for fixation through the heart and post-fixed in the same solution. After some months, formalin-fixed brain samples were placed in a tissue processor in a 21 hours protocol and washed with tap water. Ethanol dilution (70%,80%,90%, and 99% respectively) was used for dehydration, cleared in xylene,

embedded in paraffin wax (60 °C) till being cold, and next sectioned with a microtome (Leitz GmBH, Wetzlar, Germany) to obtain five µm thickness section and finally collected on glass slides. In staining protocol, brain sample slides deparaffinized in xylene (20 minutes), rehydrated in descending alcohol (96%, 90%, 80%, and 70%, 5 minutes each solution), washed in tap water, stained with cresyl violet solution for 5 minutes, rewashed in tap water and destained in ascending alcohol solutions (70%, 80%, 90%, and 96%, (just washing)), and finally cleared in xylene. The number of intact hippocampal CA1 pyramidal cells in 1mm length counted.

2.9. Assay of hippocampus biochemical parameters

At the end of the study, after behavioral tests, five rats in every group chose and were deeply anesthetized by ketamine (100 mg/kg) and xylazine (10 mg/kg) and brain them extracted from the skull, then hippocampus separated and froze at −80 °C after washing in PBS. Next, samples were sent for biochemistry measurements. Finally, hippocampus measurements were performed for Super Oxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and Glutathione Reductase (GRx) as antioxidant enzymes concerning the current protocols (47-49).

2.10. Statistical analysis

Data expressed as mean ± standard error of the mean (SEM) and The Graph Pad Prism version 8.0 (Graph Pad Software, San Diego, CA, USA) applied for statistical analysis. All data were analyzed by two-way and one-way analyses of variance (ANOVA) followed by Tukey's post hoc test. Results considered significantly different if $P < 0.05$.

Results

There was no significant difference between the control group, sham group, and positive control groups (Ecdy, HIIT, Ecdy+HIIT) ($P > 0.05$). Thus, in this survey, sham and positive control groups were ignored, and the Alzheimer group also treatment groups were just assessed with the control group.

3.1. Effect of HIIT and Ecdy on body weights

Bodyweight in all groups was controlled throughout the study. Differences between the Initial weights and final weights of groups are shown in (Fig.3). At the beginning of the survey, all groups were at the same weight; after approximately 12 weeks of the experiment, there was no significant difference between groups ($F_{8,63} = 2.712$; $P = 0.9860$).

3.2. Effect of HIIT and Ecdy treatment on the Marris water maze Test

The results of two-way ANOVA with repeated measures indicated the statistically significant effect of days of training ($F_{3, 165} = 127.2$; $P < 0.001$) and treatment ($F_{8, 63} = 3.491$; $P = 0.0021$) in swimming distance in four days of training. Besides, swimming distance by rats in the Alzheimer group on the second ($P= 0.0341$), third ($P < 0.001$), and fourth day ($P = 0.007$) of the training days significantly climbed

in comparison to the control group. On the other hand, the Ecdy treatment group on the third (P= 0.0411) and fourth day (P = 0.0232) and the treatment group of Ecdy+HIIT on the third (P < 0.001) and fourth day (P = 0.0034) significantly decreased it (Fig.4B).

Also, the results of two-way repeated measures ANOVA showed that the escape latency to the hidden platform in the Alzheimer group compared to the control group in the third (P = 0.0033) and forth (P < 0.001) days of training significantly increased [treatment factor ($F_{8, 63}$ = 3.121; P = 0.0049), day factor ($F_{3,157}$ = 120; P < 0.001)]. While, Ecdy (fourth day: P = 0.0253) and Ecdy+HIIT treatment groups (third day: P = 0.0019, fourth day: P = 0.0014) decreased it significantly (Fig.4C).

However, the one-way ANOVA results showed no significant difference between Alzheimer's and control groups ($F_{8, 63}$ = 0.9980; P = 0.4467) in the percentage of target quadrant entry in probe day. Also, no treatment group had a noticeable difference compared with the Alzheimer group (P > 0.05) (Fig.4D).

In another valuable, One-way ANOVA repeated measures illustrated that there was a significant difference between Alzheimer and control groups ($F_{8, 63}$ = 1.757; P = 0.0054) also, a significant difference between the Ecdy treatment group (P = 0.0340) and Ecdy+HIIT treatment group (P = 0.0392) with the Alzheimer group in the swimming distance in the target quadrant in probe day (Fig.4E).

Another data that was measured from the Morris water maze was the time spent in the target quadrant. Results showed the time that rats in the Alzheimer group spent in the target quadrant dropped significantly ($F_{8, 63}$ = 5.008; P < 0.001), although the Ecdy+HIIT treatment group caused an increase in it noticeably (P = 0.0026) (Fig.4F).

Likewise, the one-way ANOVA Tukey test indicated that there was not any significant difference in the average speed in probe trial between Alzheimer and control groups ($F_{8, 63}$ = 1.533; P = 0.5741), also among treatment and Alzheimer groups (P > 0.05) (Fig.4G).

3.3. Effect of HIIT and Ecdy treatment on the Passive Avoidance Task

Concerning one-way ANOVA results, there was no significant difference among initial latency or step-through latency in acquisition trial ($F_{8, 63}$ = 0.7217; P > 0.05) (Fig.5A).

In addition, one-way ANOVA analysis illustrates that step-through latency in the retention test in the passive avoidance task decreased in the Alzheimer group compared with the control group ($F_{8, 63}$ = 37; P < 0.001). On the other side, Ecdy (P = 0.0251) and Ecdy+HIIT (P < 0.001) treatment groups increased noticeably compared to the Alzheimer group (Fig.5A).

Moreover, counting time in the dark compartment by one-way ANOVA showed a significant difference between Alzheimer's and control groups ($F_{8, 63}$ = 47.96; P < 0.001). Moreover, there were significant differences between Ecdy treatment and Alzheimer group (P = 0.0132) also, Ecdy+HIIT treatment and Alzheimer group (P < 0.001) (Fig.5B).

Also, concerning the results, it was clear that there were not any significant differences between groups in the number of trials to acquisition in the passive avoidance task ($F_{8, 63}$ = 2.681; P > 0.05) (Fig.5C).

3.4. Effect of HIIT and Ecdy treatment on the histological changes in the cerebral cortex and hippocampus

Based on the results shown in Fig. 6, histological changes in the hippocampal CA1, CA3 and DG areas and also cerebral cortex measured by Nissl staining. A significant decrease found in the number of intact neurons of the hippocampal CA1 ($F_{4, 10 =}$ 16.88; P < 0.001), CA3 ($F_{4, 10}$ = 49.25; P < 0.001), and DG ($F_{4, 10}$ = 44.42; P < 0.001) areas and CC ($F_{4, 10}$ = 6.308; P = 0.0072) of the Alzheimer group compared to the Control groups. While, Ecdy treatment group could increase number of intact neurons significantly in hippocampal CA1, CA3, DG areas of the brain compare to the Alzheimer group (P = 0.0205; P = 0.0171; P = 0.0186 respectively). Meanwhile, Ecdy+HIIT treated group markedly culminated in improvement of neuronal distraction in the hippocampal CA1 (P = 0.0022), CA3 (P < 0.001), DG (P < 0.001) areas and also in CC (P = 0.0407) compared with the Alzheimer group (Fig.6).

3.5. Effect of HIIT and Ecdy treatment on hippocampus oxidative stress parameters

ANOVA one-way repeated measures indicated Superoxide Dismutase (SOD) that is known as an antioxidant enzyme and prevents oxidative stress using superoxide ion (O_2^-) neutralization in the Alzheimer group dropped significantly ($F_{8, 36}$ = 5.173; P < 0.001) and treatment groups of Ecdy and HIIT+Ecdy increased it noticeably near to the normal range (P = 0.0288, P = 0.0039 respectively) (Fig.7A).

On the other hand, results revealed that CAT, as one of the crucial enzymes to neutralize Hydrogen peroxide (H_2O_2), decreased significantly in the Alzheimer group compare to the control group ($F_{8, 36}$ = 8.815; P < 0.001). In comparison, Ecdy and group of HIIT+Ecdy could promote the amount of SOD considerably in the Alzheimer group (P = 0.0276, P < 0.001 respectively) (Fig.7B).

Besides, Glutathione Peroxidase (GPx), that is a helpful enzyme in avoiding stress oxidative notably decreased in the Alzheimer group compare to the control group ($F_{8, 36}$ = 6.753; P = 0.0059). In comparison, HIIT+Ecdy could refine the amount of GPx significantly in the Alzheimer group (P = 0.0199) (Fig.7C).

Also, results showed Glutathione Reductase (GRx), as an antioxidant enzyme that converts GSSG to GSH, declined significantly in the Alzheimer group compare to the control group ($F_{8, 36}$ = 18.89; P = 0.0015). In comparison, Ecdy and group of HIIT+Ecdy could promote the amount of SOD considerably in the Alzheimer group (P = 0.0019, P = 0.0079 respectively) (Fig.7D).

Discussion

In the present survey, Aβ-induced experimental Alzheimer produced a wide array of disorders such as spatial memory impairments in MWM and decreased learning and memory in PAT. These behavioral

inadequacies were related to the Aβ concentration in the hippocampus and cerebral cortex and oxidative stress that is one of the fundamental causes for Aβ-induced tissue damage. Under such a condition, extra amounts of reactive oxygen species (ROS) lead to oxidative damage, including lipid peroxidation and protein oxidation, culminating in programmed cell death and neuronal loss in the hippocampus and cerebral cortex.

Conversely, eight weeks HIIT as a new training method did not alter any behavioral alteration separately in the Morris water maze in the Aβ-induced Alzheimer rats. While Ecdy as a natural hormone decreased swimming distance and escape latency to the hidden platform in training days, it also caused an improvement in total swimming distance in the probe day. Additionally, eight weeks of HIIT accompanied by Ecdy could help decrease swimming distance and escape latency to the hidden platform in training days, and also, they caused an improvement in total swimming distance and total time spent in the target quadrant in the probe day. Besides, it was specified that eight weeks of performing HIIT did not alleviate learning and memory impairments as a result of Aβ concentration in the hippocampus in none of the valuables in the passive avoidance task. Nevertheless, eight weeks of consumption of Ecdy and a combination of both HIIT and Ecdy could promote latency time to the dark compartment (STLr) and time spent in the dark compartment (TDC). Histological studies through Nissl staining demonstrated that eight weeks of Ecdy separately increased the number of intact neurons in the hippocampal DG, CA1, CA3 areas but not CC. In addition, although HIIT exercise separately did not have any impact on the neuronal population, a combination of eight weeks consumption of Ecdy accompanied with eight weeks HIIT culminated in an increase in intact neurons of the hippocampal DG, CA1, CA3 hippocampus areas, and CC that were declined as a result of Aβ injection. Also, biomechanical experiments revealed that consumption of Ecdy separate (except GPx) and in combination with HIIT exercise augmented to the Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and Glutathione Reductase (GRx) enzymes in the hippocampus tissue that were dropped because of Aβ injection. In contrary, HIIT exercise did not alter content of antioxidant enzymes in the hippocampus after subtraction due to Aβ injection.

Furthermore, previous studies have reported the beneficial effects of Ecdy and HIIT alone in advancing learning and memory dysfunctions. A study indicated that HIIT for eight minutes per day promotes memory and learning impairment in Aβ-induced rats; it was due to an increase in antioxidant enzymes of Super Oxide Dismutase and Catalase and decreased Lipid peroxidation in the hippocampus. Also, the study showed gene expression levels of BDNF that were dropped because of A injection after HIIT exercise improved (50). The other survey reported that six weeks of HIIT exercise (6 sessions per week) caused a significant increase in Super Oxide Dismutase antioxidant enzyme and Total Antioxidant Capacity in the cerebellum but not in the cerebral cortex. In addition, in this study, there was a significant difference between anxiety behaviors and recognition memory before and after HIIT exercise (51). A study conducted on traumatic brain injury illustrated HIIT caused an improvement in recognition performance in novel object recognition tests and anxiety behaviors in the elevated plus-maze that were impaired as a to culminate of trauma. Moreover, after biochemical assays were declared trauma increased MDA content and decreased Super Oxid Dismutase, Catalase, and glutathione in the

hippocampus, treatment with HIIT could alleviate all the above factors near the normal range (52). Furthermore, according to another survey, Parkinson's disease demolished short-term memory in the Y maze test and decreased gene expression levels of BDNF in the hippocampus, but HIIT exercise treated short-term memory and increased gene expression levels of BDNF (53).

On the other side, a survey showed that a particular dosage of Ecdy promoted spatial memory and learning in the rats that faced a memory impairment after $A\beta_{(35-25)}$ injection. Also, after $A\beta_{(32-25)}$ injection, the gene expression level of C-fos is an indicator for neuronal activity in the brain and has a close relation with memory and learning decreased but, consumption of Ecdy increased it near to the average amount (54). The study with Shihao et al. on traumatic brain injury (TBI) in rats reported that peritoneal injection of Ecdy (16 mg/kg) for seven days resulted in significant decreases in the neuronal death rate, brain water content, MDA content, and a noticeable increase in SOD enzymes in the hippocampus compared with TBI group that illustrated therapeutic effects of Ecdy on TBI through inhibiting free radical damage and brain edema (55). According to XichaoXia et al., type 1 diabetes damaged the hippocampus CA1 area, caused higher expression levels of NF-κB as an inflammation factor, and exhibited significant memory loss. Moreover, the expression levels of Super Oxide Dismutase, catalase, GSH-Px Glutathione Reductase, and BDNF were significantly decreased in the diabetic rats. Despite this, the treatment with Ecdy in three dosages (1,10,100 mg/kg/day), especially in higher dosage for 12 weeks, orally reversed the conditions mentioned above caused by diabetes (33).

In addition, never should it be forgotten that this survey is the first research in which the protective effects of HIIT and Ecdy next to each other on memory deficits, stress oxidative enzymes, and neuronal loss in the rat models of Alzheimer’s disease are investigated.

Conclusion

The present survey results (Fig.8) displayed that, although Ecdysterone has antioxidant properties separately and could help alleviate neuronal loss and behavioral deficits a little bit, a treatment included combination of Ecdysterone with HIIT exercise has a substantial antioxidant effect on Aβ-induced tissues. Hence, a combination of both causes synaptic refinement and behavioral improvement concerning the prepared study. However, further and more fundamental researches are required to investigate the involved mechanisms in detail.

Declarations

Statements and declarations

Parsa Gholipour: Conceptualization, Writing - review & editing, Data curation, Visualization, Project administration, Funding acquisition, Formal analysis. **Alireza Komaki:** Methodology, Writing - review & editing. **Mahdi Ramezani:** Supervision, Conceptualization, Writing - review & editing, Data curation, Visualization, Project administration, Funding acquisition, Formal analysis.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

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Figures

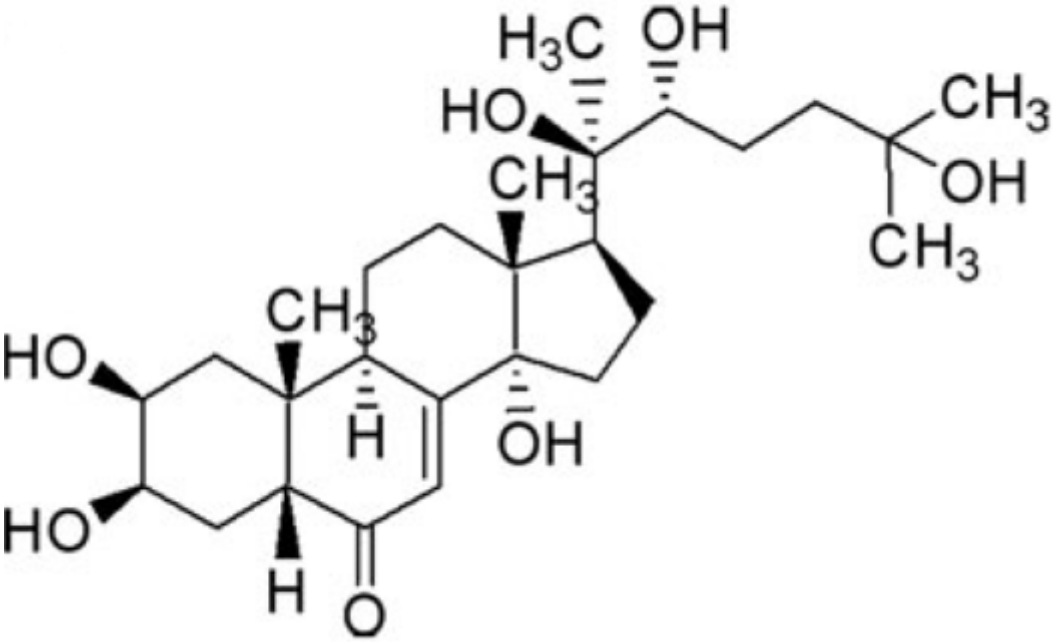

Figure 1

Chemical structures of Ecdy.

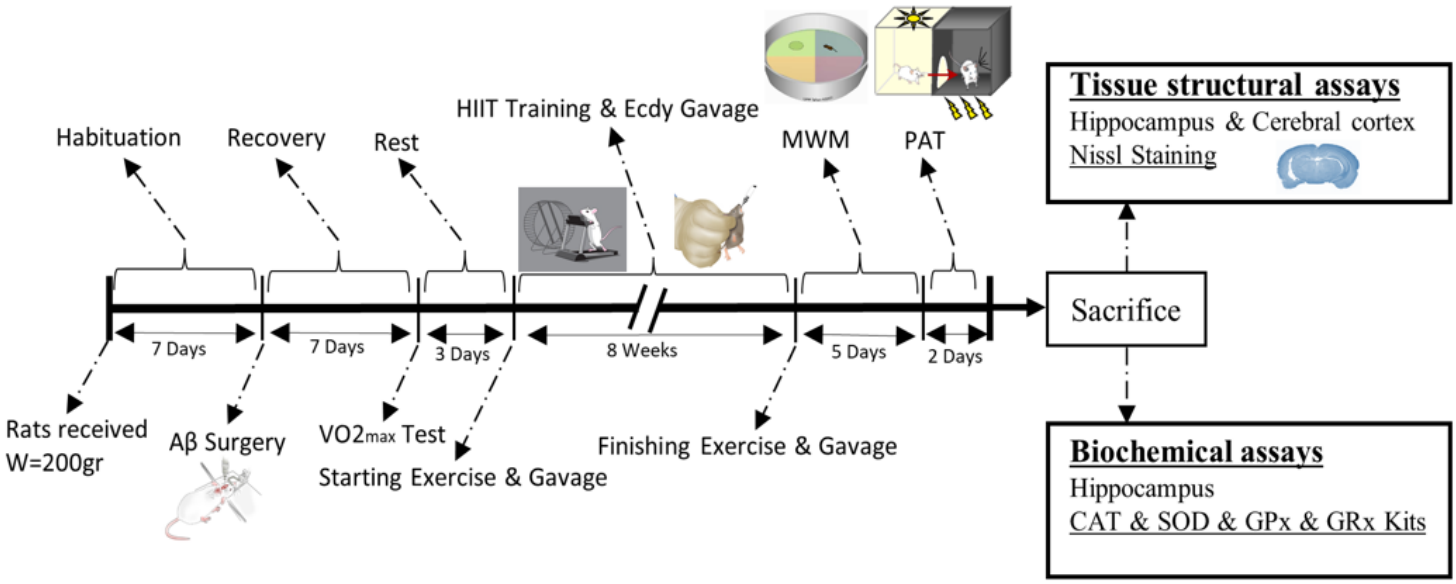

Figure 2

schematic of the experimental design and timeline.

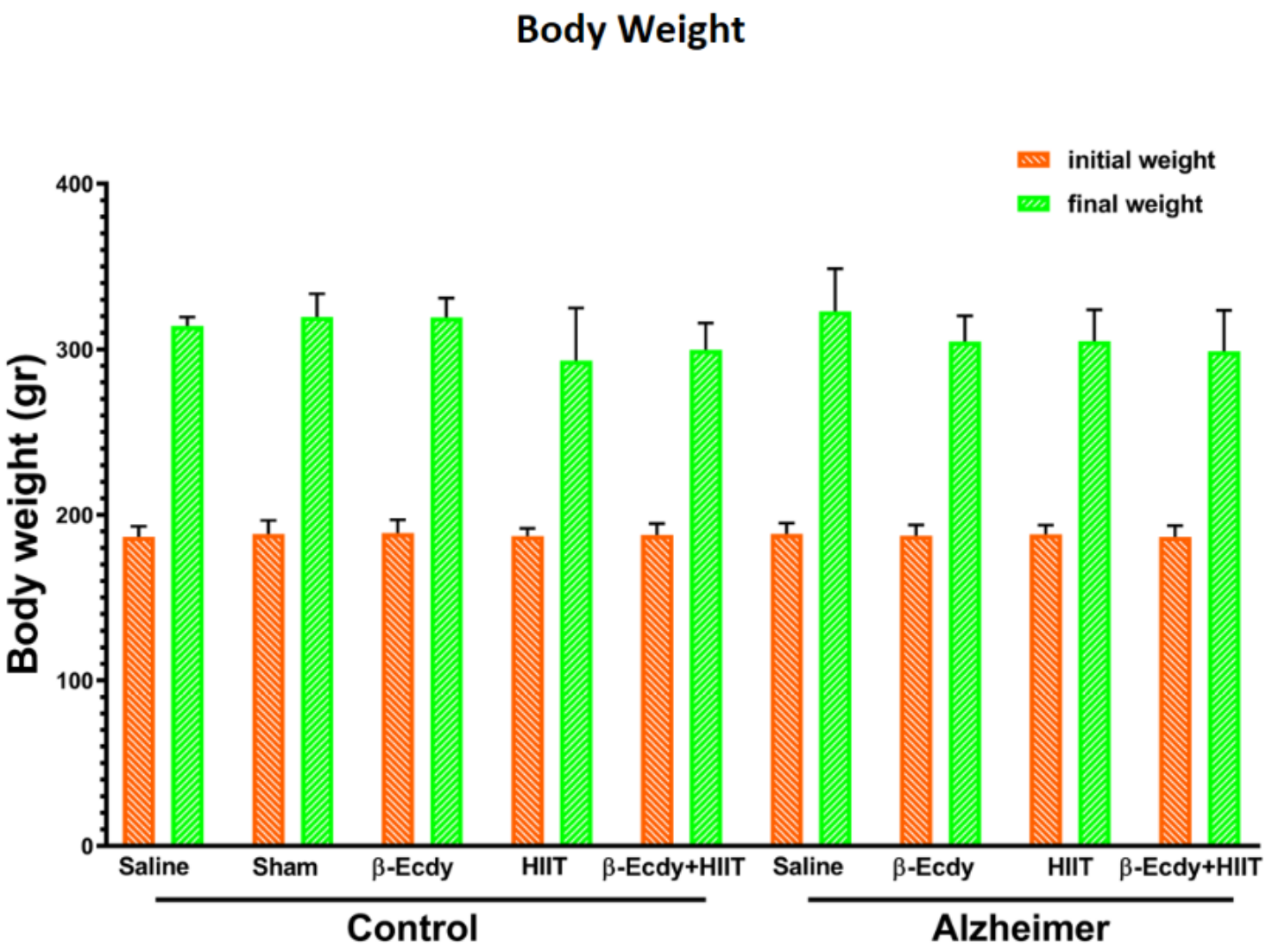

Figure 3

Initial weight and final weights.

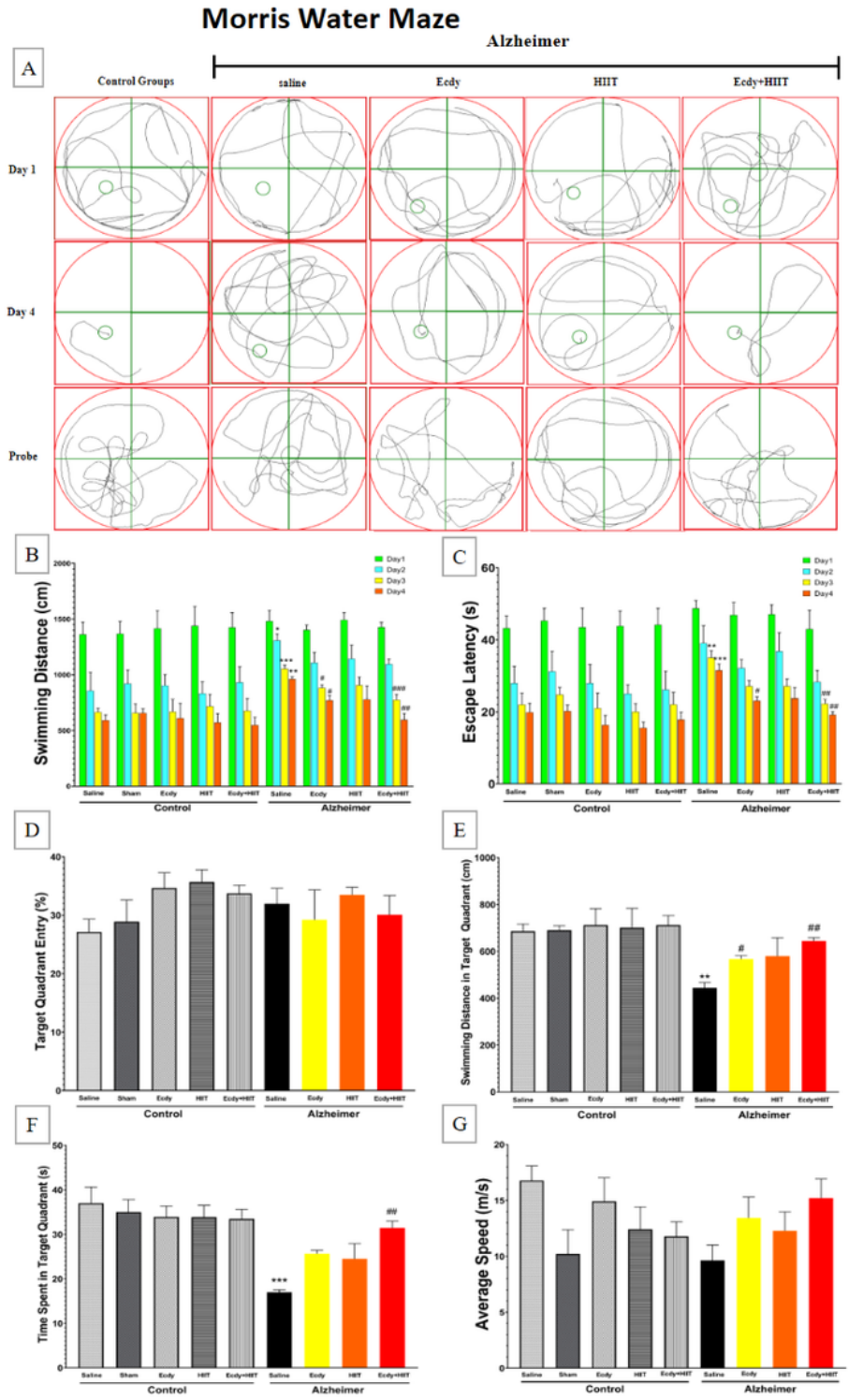

Figure 4

effect of HIIT and Ecdy treatment on swimming distance and escape latency to the hidden platform in the training trials and percentage of target quadrant entry, swimming distance in target quadrant, time spent in target quadrant and average speed in the probe trial in the Barnes Maze Test. Each bar represents mean ± SEM of 8 animals per group. *: P < 0.05, **: P < 0.01, ***: P < 0.001 vs. control group; #: P < 0.01, ##: P < 0.01, ###: P < 0.001 vs. the Alzheimer group.

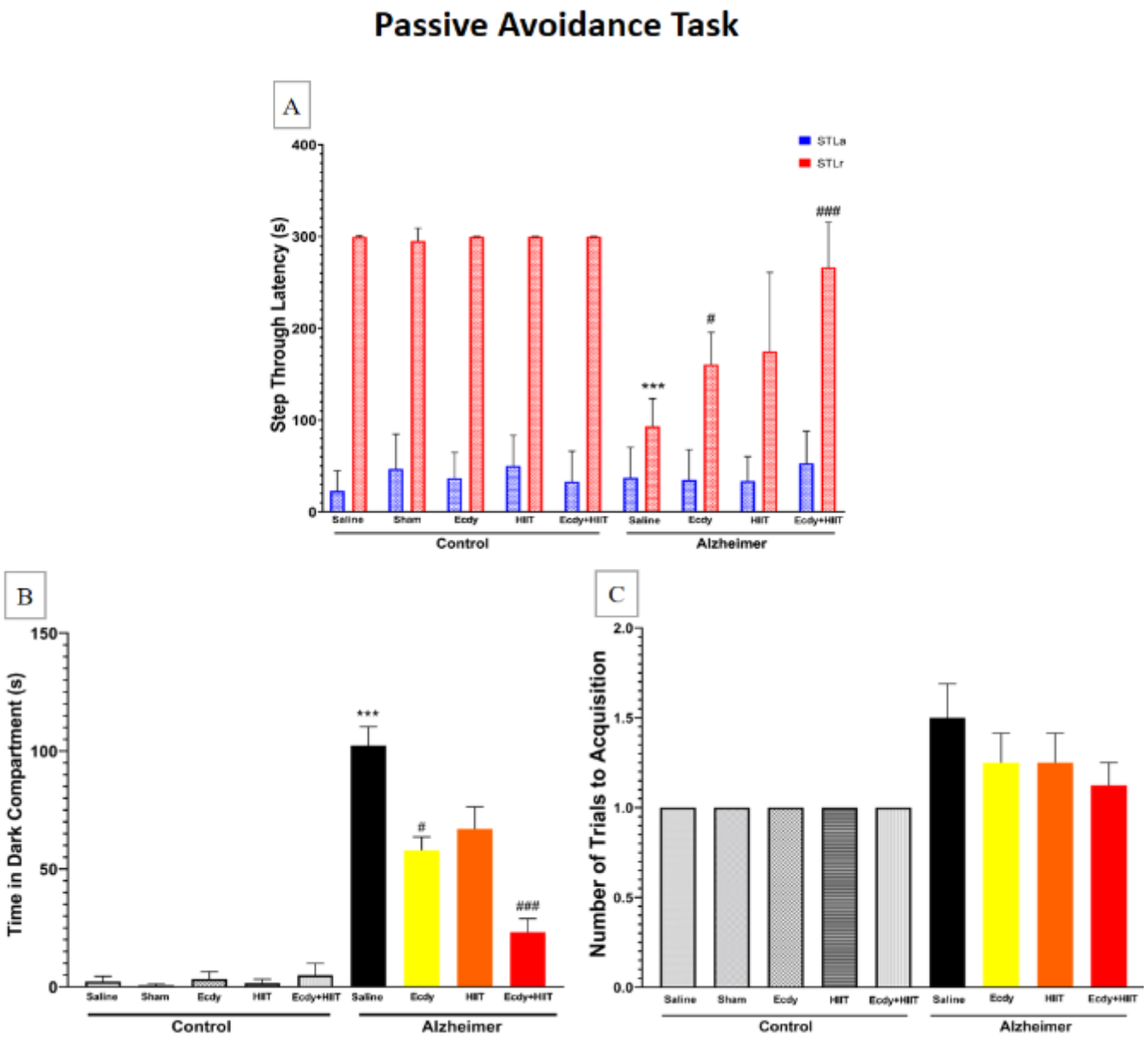

Figure 5

effect of HIIT and Ecdy treatment on the step through latency in acquisition trial and retention test separately, time in dark compartment and number of trials to acquisition in the passive avoidance task. Each bar represents mean ± SEM of 8 animals per group. ***: P < 0.001 vs. control group; #: P < 0.01, ###: P < 0.001 vs. the Alzheimer group.

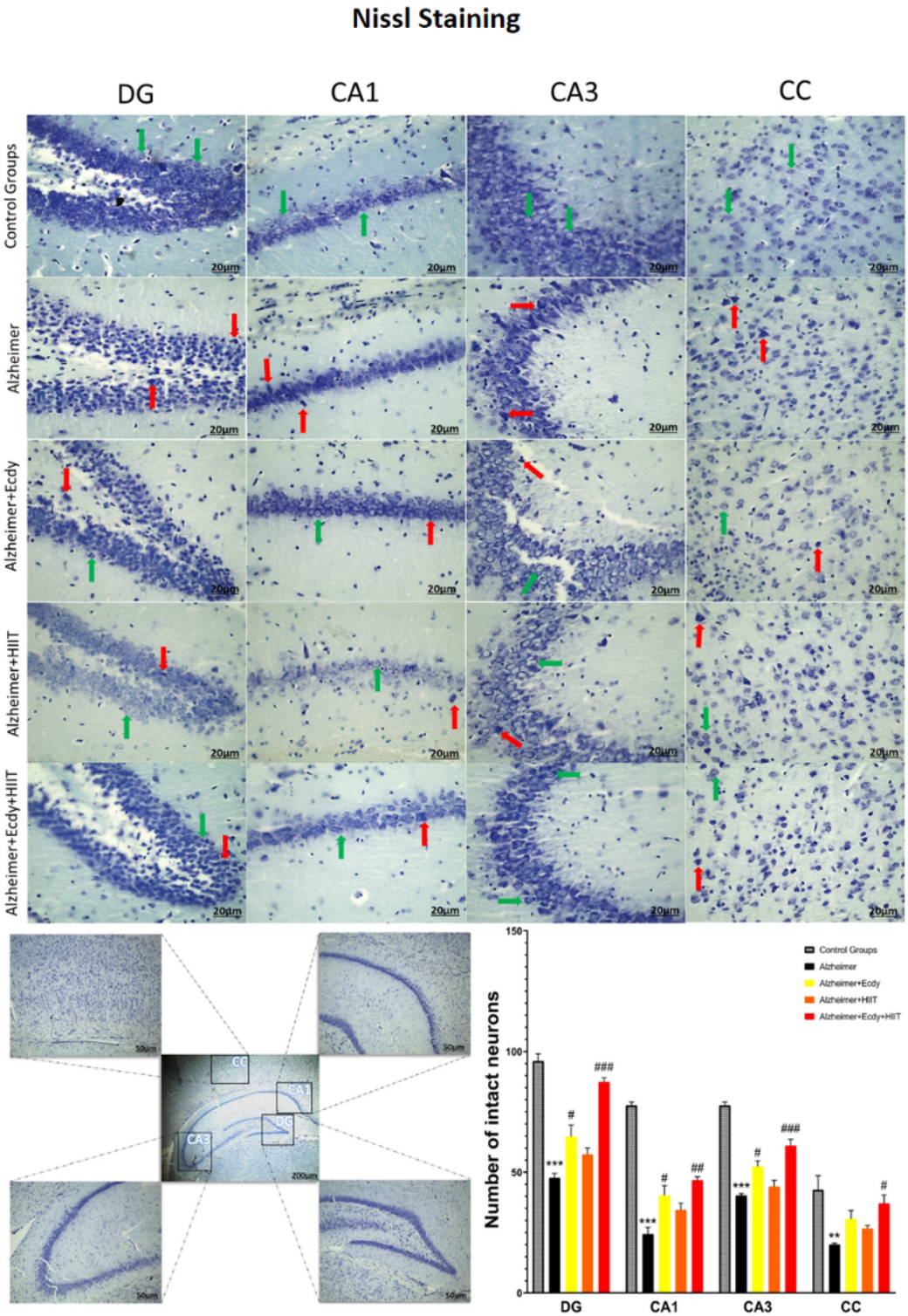

Figure 6

Effects of HIIT and Ecdy treatment on histological changes in the cerebral cortex (CC) and hippocampal CA1, CA3, and DG regions (cresyl violet (Nissl) stain, scale bar 200,50,20 μm) of Aβ-induced rats. The 20 μm photographs in figure illustrates intact neurons (clear cells with distinct round nuclei, identified by green arrows) and dark neurons (shrinkage of cells with pyknotic nuclei, identified by red arrows), the 200 μm photographs represents photomicrograph of cerebral cortex and hippocampal regions of the rat (Nissl

stain, 40×magnification), also the figure includes the quantitative data of the number of intact neurons. Each bar represents mean ± SEM of 3 animals per group. **: P < 0.01 vs. control group; #: P < 0.01, ##: P < 0.01, ###: P < 0.001 vs. the Alzheimer group.

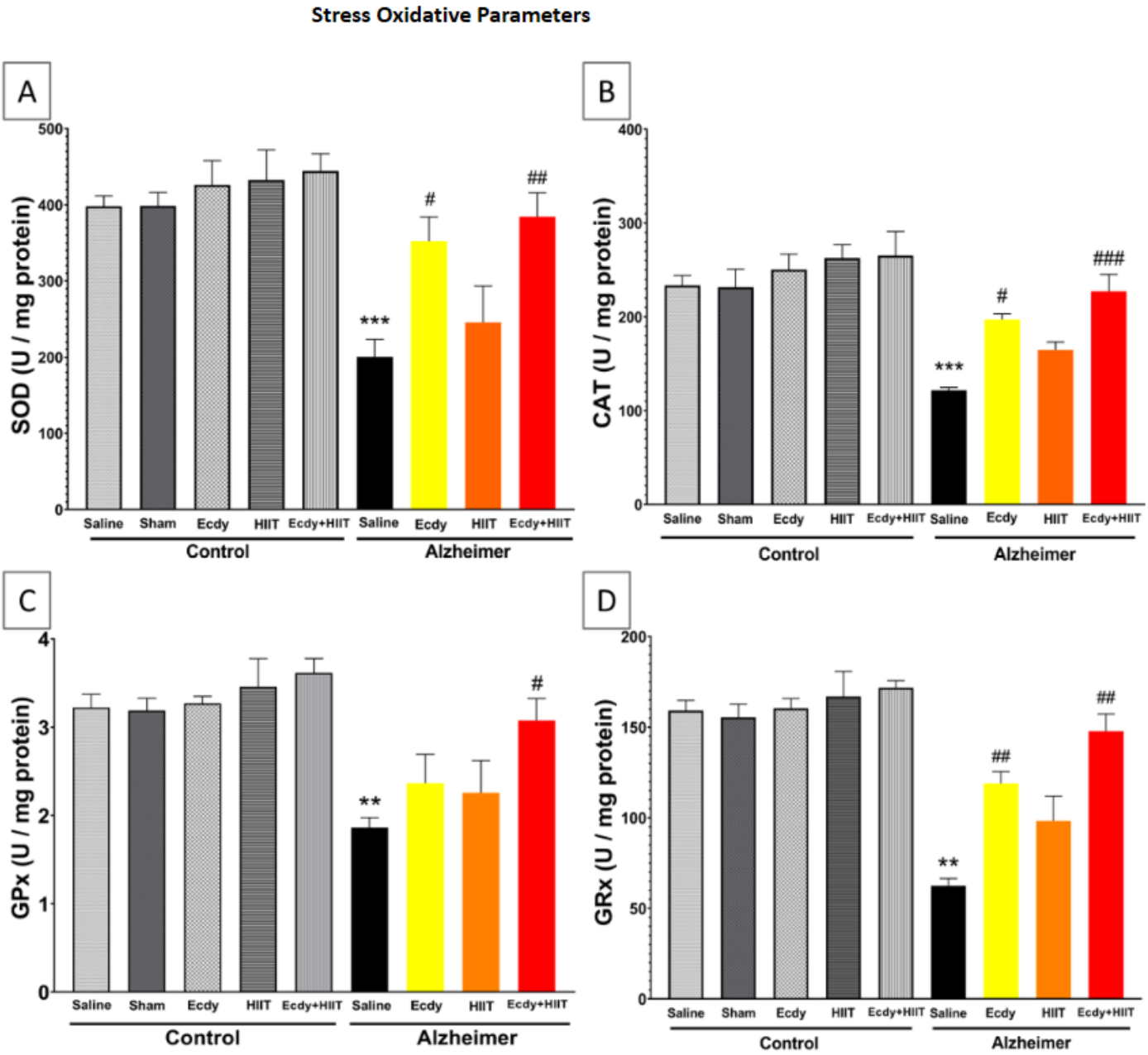

Figure 7

Effect of HIIT and Ecdy treatment on antioxidant enzymes of SOD, CAT, GPx and GRx of the hippocampus of the Aβ-induced rats. Each bar represents mean ± SEM of 5 animals per group. **: P < 0.01, ***: P < 0.001 vs. control group; #: P < 0.01, ##: P < 0.01, ###: P < 0.001 vs. the Alzheimer group.

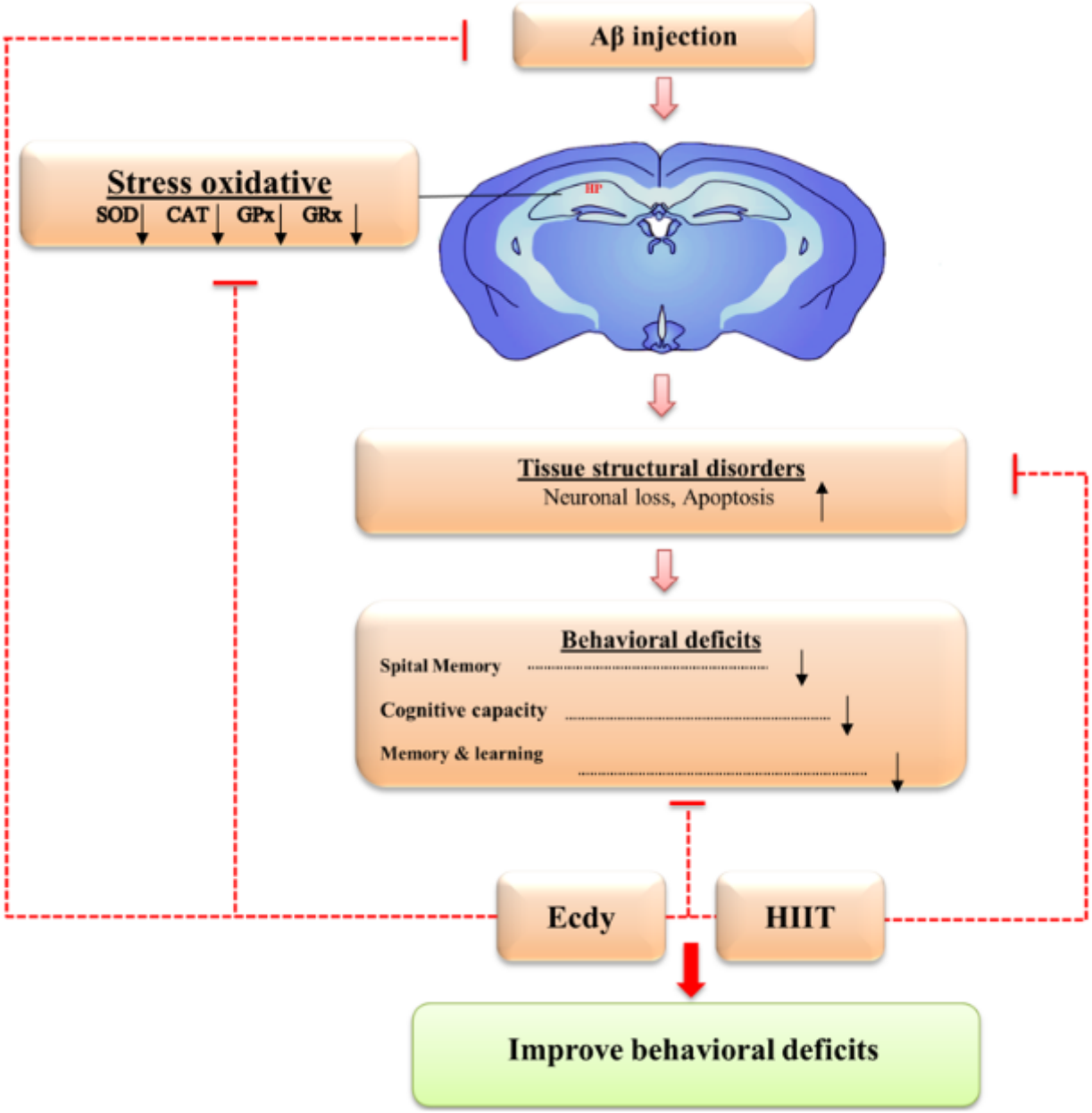

Figure 8

Schematic representation of the “protective effects of Ecdy and HIIT against AD-associated behavioral deficits in rats: Possible involved mechanisms”. HP: Hippocampus; CAT: Catalase; SOD: Superoxide Dismutase; GPx: Glutathione Peroxidase; GRx: Glutathione Reductase; Ecdy: Ecdysterone; HIIT: High Intensity Interval Training.