Title: Histone Deacetylase Class IIb Inhibition Improves Amyloid-B –Induced Learning and Memory Deficits in Male Rats

Running Title: Neuroprotection of Histone Deacetylase Class IIb Inhibition

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Abstract

Alzheimer's disease (AD) is a kind of neurodegenerative disease that is associated with progressive impairment of cognitive function. The primary pathological features of AD include the aggregated amyloid-β (Aβ) and hyperphosphorylation of tau protein. Histone deacetylase enzymes (HDAC) contribute in pathophysiology of neurodegenerative diseases. This study has investigated the possible neuroprotective effects of HDAC6 and HDAC10 inhibition in a rodent-AD model.

Learning and memory deficits were induced by bilateral intra-hippocampal Aβ injection in the male Wistar rats. The Tubacin (HDAC6 inhibitor) and Bufexamac (HDAC6 and10 inhibitor) were microinjected 30 min following Aβ injection. The possible molecular changes in the hippocampus following Aβ injection, were also assessed by western blotting analysis of pCREB/CREB and Pp70/P70 ratios.

Our results revealed that Bufexamac significantly recovered learning and memory impairments induced by Aβ in the MWM task. Meanwhile, Tubacin improved memory decline without affecting learning. Bilateral intra-hippocampal injection of each of HDAC inhibitors significantly increased the pCREB/CREB and Pp70/p70 ratios compared to the Aβ group that were concurrent with behavioral alterations.

HDAC IIb treatment may be a promising strategy for improving the learning and memory impairments in an animal model of AD, suggesting that HDAC targeting is valuable strategy for further investigation.

Key words: Alzheimer's disease, Bufexamac, Cyclic adenosine monophosphate response element binding protein, Histone deacetylase enzymes, Ribosomal protein S6 kinase, Tubacin

1. Introduction

Alzheimer's disease (AD) is characterized by a slow decline in hippocampal-related functions, including cognitive impairment, memory loss, behavioral and functional disorders (Cooper and MJ, 2017). It has been shown that epigenetic modification has a role in AD pathogenesis. Epigenetics is a relationship between genetics and the environment. The histone acetylation by histone acetyltransferases (HATs) and histone deacetylation by histone deacetylases (HDACs) are the most studied histone posttranslational modifications applicable in neurocognitive disorders (Peixoto & Abel, 2013).

Generally, histone acetylation activates gene transcription, whereas histone deacetylation is closely associated with gene transcriptional repression (Lu et al., 2015). It has been reported that acetylation and deacetylation hemostasis are disturbed in the neurodegenerative states (d'Ydewalle et al., 2011, Gibson and Murphy, 2010, Gräff and Tsai, 2013). Much evidence has indicated that histone acetylation plays a vital role in rescuing learning and memory impairments (Liu et al., 2009).

Furthermore, treatment with HDAC inhibitor, the sodium butyrate could stimulate hippocampal axonal regeneration, and improve learning and memory in CK-p25 mice model of AD (Fischer et al., 2007). In addition, the HADC inhibitor, phenylbutyrate, increased the density of axons, and reduced tau hyperphosphorylation (Ricobaraza et al., 2009). Finally, decreased histone acetylation levels have been observed in the hippocampus and cerebral cortex in the aged rats (Peleg et al., 2010, Walker et al., 2013).

In the HDAC family proteins, class IIb includes HDACs 6 and 10 (Xu et al., 2007, Sartor et al., 2015). The HDAC6's role in AD has been explained previously (Zhang et al., 2013, Simões-Pires et al., 2013). It is found in both the cytoplasm and nucleus and significantly increases during AD progression. Studies have shown that acetylation activity and expression

of HDAC6 increase in the cortex and hippocampus of AD patients (Gräff et al., 2012, Ding et al., 2008). Although the function of HDAC6 in the cytoplasm has been verified in several studies, the role of another cytoplasmic deacetylase, HDAC10, a class IIb histone deacetylase (Tong et al., 2002), has not been clarified (Fischer et al., 2002, Kao et al., 2002). HDAC10 is closely related to HDAC6 (Guardiola and Yao, 2002) , and can be shuttled between the nucleus and the cytoplasm. HDAC10 also can recruit many other HDACs, indicating that it may act as a recruiter rather than a deacetylase. However, when HDAC10 is expressed by recombination, it shows deacetylation activity (Guardiola and Yao, 2002, Fischer et al., 2002, Kao et al., 2002, Tong et al., 2002). HDAC10 regulates reactive oxygen species (ROS) in gastric cancer cells. It has to be mentioned that there is substantial HDAC10 expression in different regions of the hippocampus including CA1, CA3, and dentate gyrus (Broide et al., 2007). However, its function in the hippocampus has not been studied so far. The goal of current study, was to investigate the outcome of inhibition of class IIb HDACs on learning and memory deficits induced by Aβ injection. It is reported that the cyclic adenosine monophosphate response element binding protein (CREB) and ribosomal protein S6 kinase phosphorylation are crucial in spatial learning and memory formation (Mizuno, 2002). In order to evaluate the possible molecular changes in the hippocampus due to Aβ injection, pCREB/CREB and the 70 kD ribosomal protein S6 kinase (Pp70/P70) ratio were assessed.

2. Materials and methods

2.1 Animals

In this study, adult male Wistar rats weighing 250-300 g were kept, 2–3 per each plexiglas cages. The room temperature was 22±2^{o} C and 12 h light–dark cycle (lights on at 07:00) with free access to water and food. All experiments were performed in accordance with the guide for the care and use of laboratory animals (National Institutes of Health Publication No. 80-23, revised 1996) and approval of the research committee of Tehran University of Medical Sciences (96-03-87-36403). A total of 64 male rats (n = 8 per group) divided randomly to

eight groups (Intact, saline, DMSO, Aβ, Bufexamac, Tubacin, Bufexamac+Aβ, Tubacin+ Aβ).

2.2 Surgery

Rats (n = 8 per group) were deeply anesthetized by i.p. injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). During the stereotaxic surgery, the stainless steel guide cannulas were fixed bilaterally into the dorsal hippocampus (AP: -3.8, ML: ± 2.2, DV: -2.7) according to the rat atlas (Paxinos and Watson, 2009) and were fixed by acrylic cement. The guide cannula was one mm above the injection site and injection needle was one mm longer than guide cannula.

2.3 Drugs preparation and administration

The amyloid-β 25-35 peptide (Sigma, USA) was dissolved and stored at -20ºC. Aggregation of Aβ25–35 was performed by in-vitro incubation at 37ºC for 4 days. Bufexamac and Tubacin (Selleckchem, USA) stocks were dissolved in dimethyl sulfoxide (DMSO).

For intra-hippocampal injection of Aβ (5μg/2.5μl each side) a 5 μl Hamilton syringe was used. The same intra-hippocampal injection volume was used previously (Ghorbandaiepour, et al., 2024). The injection needle was 30 gauge. Animals were allowed to move freely in their standard box during all injections. The Tubacin (20 μg /rat) and Bufexamac (20 μg /rat) or DMSO (2.5%) as their vehicle were microinjected 30 min following Aβ injection.

All microinjections were performed at the speed of 0.5 μl/min and the injection needle was left in place for additional 2 min to allow the solution to completely diffuse from the cannula tip and minimize the drug backflow. The Morris Water Maze (MWM) test, was performed ten days after surgery. The experimental procedure has been shown in Fig (1).

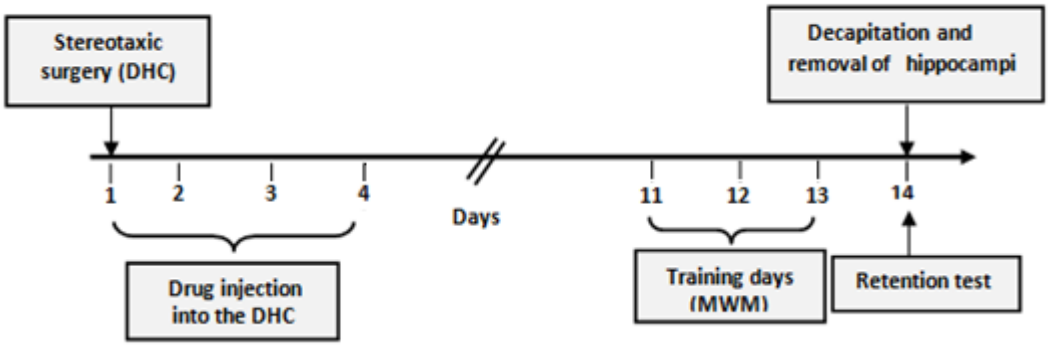

Fig.1: The time line and design of experimental procedures of the study.

Animals experienced a week handling period before the beginning of the study.

DHC: dorsal hippocampus, MWM: Morris water maze

2.4 Behavioral testing

The water maze was a black circular pool (150 cm ×60 cm). The water temperature was (20 ± 1◦C), and filled to a depth of 25 cm. Previously, the ambient temperatures (19–22 °C) has been used for rats. The behavior outcome showed no report of fatigue or hypothermia (Lindner & Ribkoff , 1991). Four distinct quadrants were considered in the tank and release points were named 1, 2, 3 and 4 zones. A circular platform (11 cm in diameter), was positioned in the center of the first quadrant, 1.5 cm below water's surface. Extra maze visual cues including computers, bookshelves, and posters on the wall were located and kept fixed in position during all experiments. The animal's behavior was recorded by a digital camera positioned above the maze's center. The path of animals swimming, latency to find the platform, traveled distance, and time in the target quadrant were recorded.

Animals are experienced three days of training sessions. In the first 3 days, a hidden platform was placed in one fixed location. In learning sessions, four trials with various starting

locations were considered. In each trial, animals were released from one of the four different starting zones. The allowed time of swimming to find the hidden platform was 90 seconds.

Upon finding the platform, the animals were allowed to stay there for 20 s until the start of the subsequent trial. The probe trial was performed without a platform, and the released point was in the opposite zone (Naderi, S 2023). Finally, for the assessment of animal visual ability and sensory-motor coordination, the visible platform test was done. In order to assess whether the surgical procedure or drug treatments over the time have not had any adverse impact on vision, the visible platform test was conducted on day 4 after the probe trial in all animals to avoid habituation (Paul, et al., 2009).

2.5 Tissue preparation

After completion of the behavioral tests, the rats were immediately sacrificed by CO2 inhalation and decapitated and their hippocampi were isolated on ice and stored in liquid nitrogen for 24 h and then stored at -80°C until the molecular analysis.

2.6 Molecular assessment: Western blot analysis

The hippocampi were homogenized on ice. The RIPA lysis buffer was consisted of Tris-HCl (50 mM, pH8.0); NaCl (150 mM); Triton X-100 (1%); Na-Deoxycholate (0.5%); and SDS (sodium dodecyl sulfate, 0.1%). The cocktail was also supplemented with protease and phosphatase inhibitor. The lysates were centrifuged (14,000 rpm, 30 min, 4°C) to remove the debris. The protein content of samples was quantified by Lowry method, and equal amounts of protein (50 μg) were separated by 12% polyacrylamide gel electrophoresis and transferred to PVDF membranes. Then, membranes after incubation with blocking buffer (5% BSA) were probed overnight at 4°C with primary antibodies. Then, washed in TBS-T, and were incubated for 1:30 h with horseradish peroxidase antibody. Immunoreactivity was visualized by ECL kit (Amersham UK). At the end, the radiographic films were scanned and protein band density of blots were calculated by Image-J software (Mohammadi, et al., 2023).

2.7 Statistical analysis

Data analysis was performed using GraphPad Prism 7.01. Data obtained from MWM training days were analyzed by Two-way analysis of variance (ANOVA). The post hoc was Tukey's test. For the probe test and molecular data analysis, the one-way ANOVA analysis was used. The results were shown as means ± S.EM and $p<0.05$ was considered significant.

3. Results

3.1.1 Intrahippocampal injection of Bufexamac improved spatial learning and memory impairments induced by Aβ injection

As shown in (Fig 2A) there was a main effect of days [$F (2, 14) = 63.56$, $p< 0.0001$] and of groups [$F (3, 21) = 5.952$, $p <0.01$] on the traveled distance to find the hidden platform during all training days. However, the interaction between days and groups was not significant [$F (6, 42) = 0.8386$, $p = 0.5472$]. Multiple comparisons by Tukey's post-test showed that Bufexamac significantly could reverse the effects of Aβ learning impairment in the second and third training days ($p<0.01$ and $p<0.001$, respectively).

Also, as shown in Fig. 2B, Bufexamac could significantly reduce the escape latency to the platform during the second and third days of training compared to the Aβ treatment. Two way –ANOVA with repeated measures revealed that there is a main effect of days [$F (2, 14) = 55.90$, $p < 0.0001$] and of groups [$F (3, 21) = 5.012$, $p < 0.001$] on the escape latency to the hidden platform during the second and third days of training. However, interaction between days and groups was insignificant [$F (6, 42) = 0.8205$, $p = 0.2461$]. Multiple comparisons by Tukey's test revealed a significant difference in this parameter between the Aβ and Aβ+Bufexamac group in the second and third training days ($p<0.01$ and $p<0.001$, respectively) (Fig 2B).

Data analysis by one-way ANOVA in the probe test showed a statistically significant difference in the time in target quadrant between the Aβ group compared with Saline, DMSO and Aβ+Bufexamac groups ($p<0.0001$, $p<0.01$ and $p<0.001$, respectively) (Fig 2C).

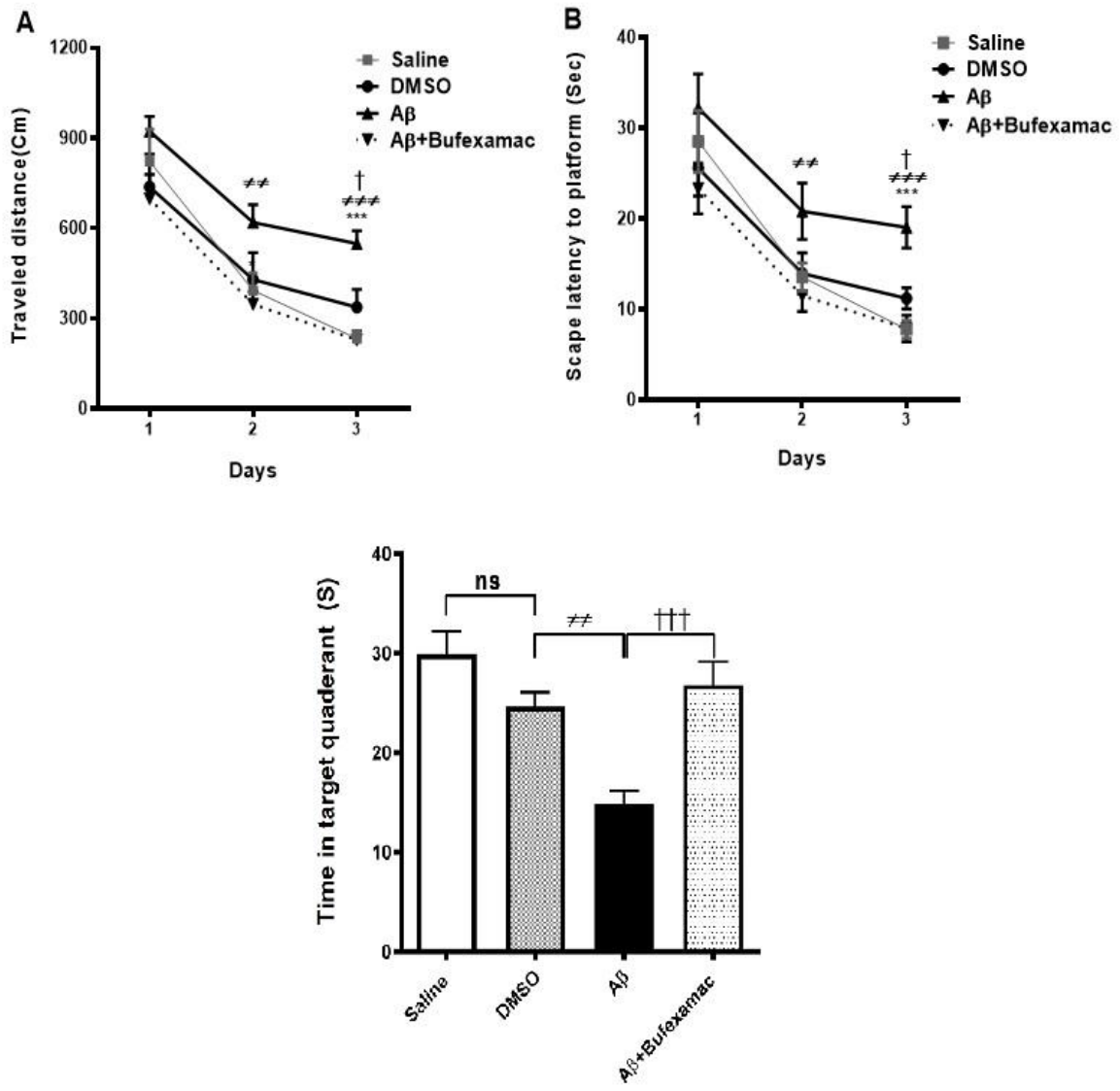

Fig.2. Effect of intra-hippocampal injection of Bufexamac on spatial learning and memory impairments induced by Aβ. Injection of Bufexamac strongly improved the impaired animals' performance during the training days in the MWM task. Rats receiving Bufexamac (A) traveled shorter distances and (B) spent less time (escape latency) to find the hidden platform compared to the Aβ group during the second and third days of training. (C) Following Bufexamac intra-hippocampal injection, the time spent in the target quadrant on the probe day significantly increased in comparison with the Aβ group. Data has been shown as mean ± SEM. *** p <0.001 compared to the saline group and ## p <0.01, ###P<0.001 compared to the DMSO group on the training days, and ***P<0.001 compared to the saline group, ## P<0.01 compared to the DMSO group and ††† p <0.001 compared to the Aβ group on the probe day (n=8 per each group).

3.1.2 Intra hippocampal injection of Tubacin did not affect learning but improved memory impairment due to Aβ treatment

As shown in (Fig 3A) Tubacin could not affect the learning impairment due to Aβ treatment in both traveled distance and escape latency to the hidden platform. Two way –ANOVA with

repeated measures revealed that there is a main effect of days [F (2, 14) = 36.08, P < 0.0001] and of groups [F (3, 21) = 3.012, P < 0.05] on the escape latency to the hidden platform during the first, second and third days of training. However, interaction between days and groups was not statistically significant [F (6, 42) = 1.551, P = 0.1854]. However, multiple comparisons by Tukey's test showed no difference in this parameter between the Aβ and Aβ+Tubacin group in all training days (Fig 3B).

The results of the One-way ANOVA analysis in the retrieval test revealed a statistically significant difference in the time in target quadrant between the Aβ group compared with the Saline, DMSO and Aβ+Tubacin groups (p <0.0001, P<0.01 and P<0.001, respectively) (Fig 3 C).

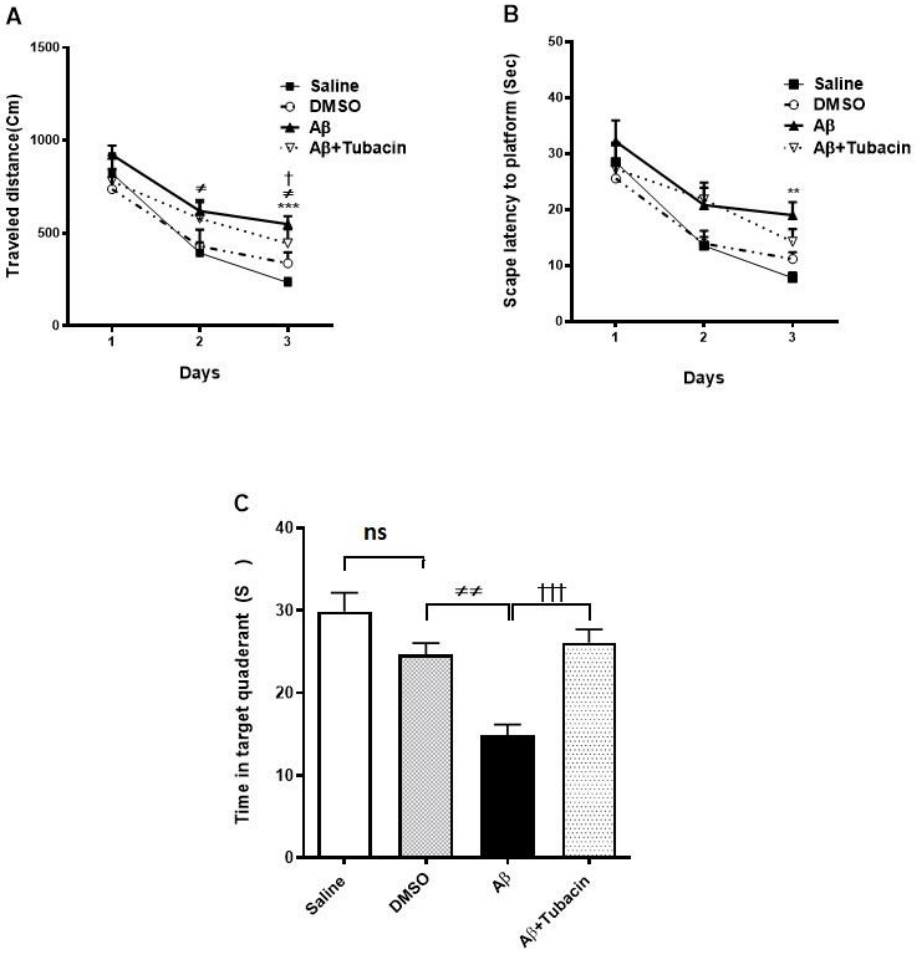

Fig. 3. Effect of intra-hippocampal injection of Tubacin on spatial learning and memory impairments induced by Aβ. Injection of Tubacin strongly improved Aβ-treated animals' performance during the training days in the MWM task. This treatment significantly decreased (A) traveled distance and (B) latency to find the hidden platform (escape latency) compared to the Aβ (25-35) group during the second and third days of training. (C) Tubacin intra-hippocampal injection, significantly increased time spent in the target quadrant compared with the Aβ group on the probe day. The data is shown as mean±SEM. ** p <0.01, *** p <0.001 compared to the saline group, and # P<0.05 compared to the DMSO group on the training days. *** p <0.001 compared to the saline group, ## p <0.01 compared to the DMSO group and ††† p <0.001 compared to the Aβ group (n=8 per each group).

3.2 Visuomotor activity did not change during the experiments

A visible platform test on day 4 after the probe trial was performed in all experimental groups to evaluate the vision of animals. The results of One-way ANOVA followed by Tukey's test did not show any significant differences among groups ($F(7, 56) = 0.8300$, $p=0.5669$, Fig4).

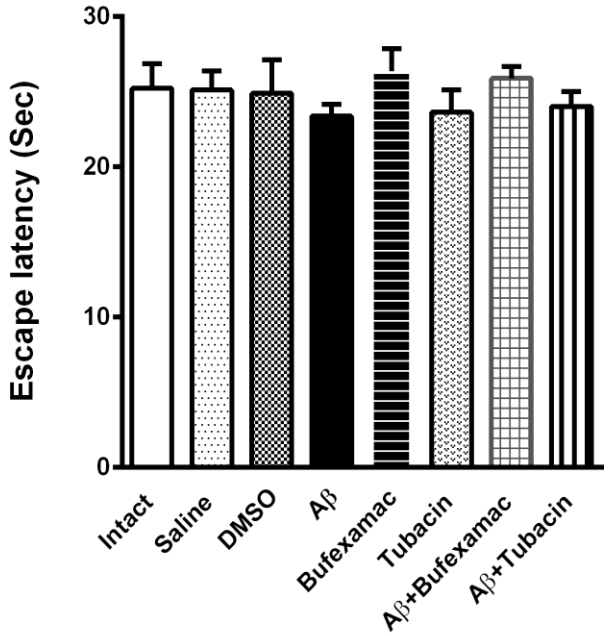

Fig. 4. Comparison of visuomotor activity between different experimental groups. There was no significant difference in ability of the animals of different experimental groups to find the visible platform in the visible test.

3.3 Molecular assessment

3.3.1 Aβ treatment decreased Pp70/p70 ratio in the hippocampal area which was recovered by Bufexamac and Tubacin treatment

The phosphorylation of P70 (S6K1), a kinase that is involved in the enhancement of protein synthesis processes, was measured after MWM. The ratio of the phosphorylated to the total form of Pp70/P70 density bands showed that Aβ injection caused a significant decrease in Pp70/p70 compared with the saline group ($F(7, 32) = 0.3300$, $p<0.05$). Bilateral intra-

hippocampal injection of HDAC inhibitor Bufexamac significantly increased the Pp70/p70 ratio compared to the Aβ group (F (7, 32) = 0.3300, p<0.05). Tubacin significantly increased the Pp70/p70 ratio compared to the Aβ group (F (7, 32) = 0. 0.3300, p <0.05, Fig .5).

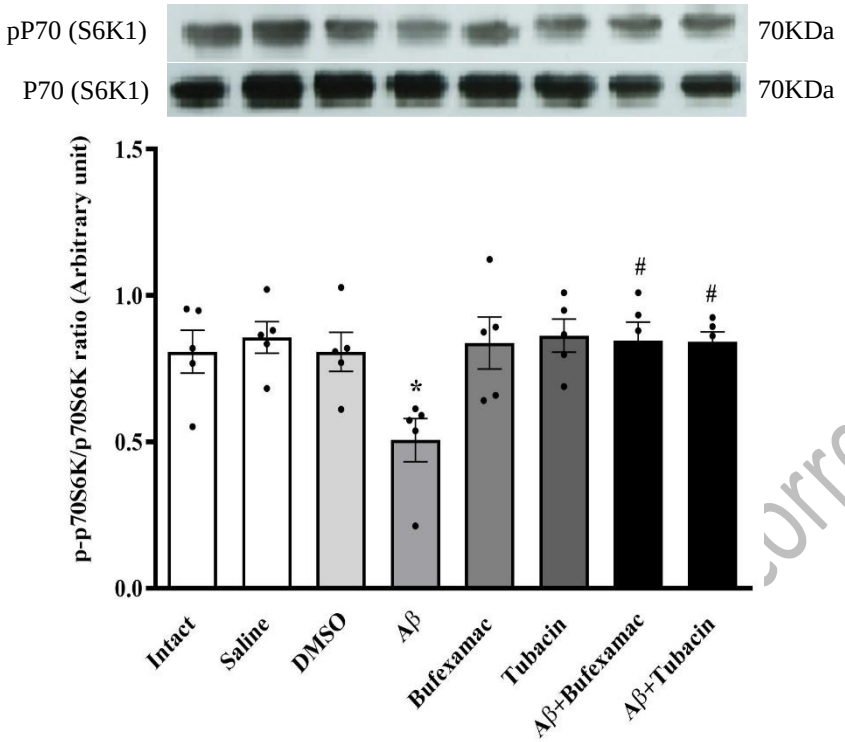

Fig. 5. The densities of Pp70 and their ratios to p70 (Pp70/p70 ratio) were measured in all experimental groups.
One-way ANOVA revealed a significant decrease in the Pp70/p70 ratio in the Aβ group in comparison with the control group. A significant increase in the ratio was detected in the Aβ-treated group receiving Bufexamac (Aβ+Bufexamac) or Tubacin (Aβ+Tubacin) compared to the Aβ group. B: The densities of Pp70 and p70 bands and their ratios in all experimental groups were evaluated. The data is shown as mean ± SEM.* p <0.05 compared with the control in the Aβ group and # p <0.05 compared to the Aβ group in the Aβ+Bufexamac and Aβ+Tubacin groups (n=5 per each group). The phosphorylation amounts of proteins and its level has been calculated in ratio to total amount, so the effect of β-actin has been neutralized.

3.3.2 Aβ treatment decreased pCREB/CREB ratio in the hippocampal area which was recovered by Bufexamac and Tubacin treatment

CREB, a transcription factor that is related to learning and memory processes, was measured after MWM. The ratio of the phosphorylated to the total form of pCREB / CREB density

bands showed that intra CA1 bilateral injection (5 µg) of Aβ caused a significant decrease in pCREB / CREB compared to the saline group (F (7, 32) = 0.5049, p<0.05).

Bilateral intra-hippocampal injection of HDAC 6 and 10 inhibitor Bufexamac (20 µg) significantly increased the pCREB/CREB ratio compared to the Aβ group (F (7, 32) = 0. 5049, p <0.001). Also, HDAC 6 inhibitor Tubacin (20 µg) significantly increased the pCREB/CREB ratio compared to the Aβ group (F (7, 32) = 0. 5049, p <0.001, Fig.6).

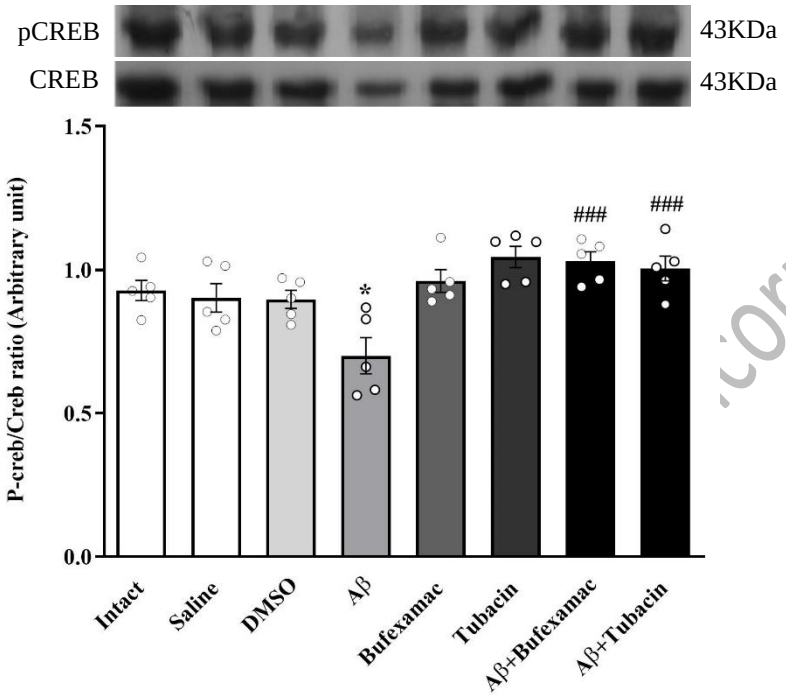

Fig.6.pCREB/CREB ratio and pCREB and CREB levels in the control, Aβ and treatment groups. A: The densities of pCREB and their ratios to CREB (pCREB/CREB ratio) were measured in all experimental groups. The data analysis by one-way ANOVA showed a significant decrease in the pCREB/CREB ratio in the Aβ group in comparison with the control groups as well as a significant increase in this ratio in Aβ+Bufexamac and Aβ+Tubacin groups compared to the Aβ group. B: The densities of pCREB and CREB bands and their ratios in all experimental groups were evaluated. The data has been shown as mean ± SEM. *P<0.05 compared with the control in Aβ group and ### p <0.001 compared to the Aβ group in the Aβ+Bufexamac and Aβ+Tubacin groups (n=5 per each group).

Discussion

In the present study, the Aβ administration induced neurotoxicity demonstrated by spatial memory impairment, and a significant decrease in the pCREB/CREB, and the Pp70/p70 ratio.

Our data was consistent with previous published studies that reported the memory decline in rodent Aβ models (Aminyavari, 2019, Karimi-Zandi, 2022). The CREB is responsible for various neurophysiological phenomena such as plasticity and has been implicated in cognitive decline (Tanis KQ, 2008). In the present study, a decrease in the pCREB/CREB, and the Pp70/p70 ratio were used as molecular confirmation criteria for cognitive alteration. Tubacin as a specific HDAC 6 inhibitor significantly recovered memory impairments induced by Aβ in the MWM task. The up-regulation of HDAC6 in AD and its correlation with tau hyperphosphorylation has been reported. Therefore, the beneficial effect of Tubacin may be related to modifying the mentioned effect (Ding et al., 2008). Evidence shows that HDAC6 localized within the cytosol regulates the acetylation of non-histone proteins such as p53,FOXP3, heat shock protein 90, tubulin, Tau, cortactin and peroxiredoxin (Li et al., 2013, Hubbert et al., 2002, Zhang et al., 2007). Since these proteins have a role in learning and memory processes, their acetylation by HDAC6 inhibitors can be effective in the improvement process of memory impairments resulting from AD (Selenica et al., 2014). Reduction or inhibition of HDAC6 decreased Aβ plaques in AβPPswe/PS1ΔE9 mice, ameliorated tau pathologies in rTg4510 mice and primary cultured neurons and finally improved cognitive deficits. In addition, tau acetylation has been reported to compete with tau phosphorylation at several HDAC6-regulated sites and thus inhibit tau aggregation (Carlomagno et al., 2017, Cook et al., 2014, Guardiola and Yao, 2002).

Among all parameters that play role in occurrence and development of AD, epigenetic factors should be considered carefully, as it is interaction of multiple factors that may be resulted in epigenetic changes. Even it has been proposed that disbalance in histone acetylation takes place at very early stage of AD, before decline in cognition (Marinho, et al., 2023).

There are no reports that compare the selective inhibitors of members of class IIb in this context. Furthermore, although the effect of HDAC6 inhibition on AD development has been studied by different labs, working on HDAC10 inhibition has been neglected.

In the present investigation, Bufexamac as a class IIb-specific HDAC inhibitor significantly increased the Pp70/p70, and pCREB/CREB ratios compared to the Aβ group. These alterations were parallel with memory improvement. Besides, its usage, resulted in learning recovery, the characteristic that did not ensue upon injection of Tubacin. An explanation for this extra positive effect could be its broader inhibitory effects, including HDAC 6 and 10. Tubacin selectively inhibits just HDAC 6, while Bufexamac has selective inhibitory effect for HDAC 6 and 10 (Bantscheff, et al., 2011). Using Tubastatin, as another inhibitor of class IIb HDAC, that can inhibit HDAC10 with higher affinity compared to HDAC6 may be one of the possible proposals to find out the value of this assumption. On this way the point of interference can be limited. (Oehme, et al., 2013).

Second hypothesis for this outcome may be related to its anti-inflammatory property that is of the main features of bufexamac and could not be rule out. In an investigated by Oehme et al. the possibility of compensatory effect of HDAC6 for HDAC10 has been rejected (Bantscheff, et al., 2011). Therefore, it seems that the members of class IIb have an individual and perhaps different role in the context of neurodegenerative diseases.

Despite all these possibilities, the efficacy of HDACs may change based on the context (tissue and pathology condition), and concentration of these components. Unknown mechanism of their action and also main target and pathway of them, are of the major issue with HDACs that need to be solved. It should be kept in mind that these inhibitors due to their inherent characteristics can affect multiple pathways. Answering these questions may lead to their broader, safer, and perhaps more practical.

In conclusion, the present study showed that HDAC IIb inhibition may present a promising opportunity for developing new therapeutic strategies for learning and memory impairments in rodent models of Alzheimer's like disease.

Contributors

ZM, contributed to investigation and data curation, methodology, software, formal analysis, writing the original draft of manuscript. FM, was responsible for conceptualization, methodology, supervision, project administration, and writing – review & editing. FKh, contributed to methodology, validation, formal molecular analysis, writing – review & editing. MZ, was responsible for conceptualization, methodology, supervision, project administration, resources, funding acquisition, and writing – review & editing.

Conflict of Interest Statement

The authors declare no conflict of interest.

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References

Bantscheff, M., Hopf, C., Savitski, M. M., Dittmann, A., Grandi, P., Michon, A. M., Schlegl, J., Abraham, Y., Becher, I., Bergamini, G., Boesche, M., Delling, M., Dümpelfeld, B., Eberhard, D., Huthmacher, C., Mathieson, T., Poeckel, D., Reader, V., Strunk, K., Sweetman, G., Drewes, G. (2011). Chemoproteomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes. Nature biotechnology, 29(3), 255–265. https://doi.org/10.1038/nbt.1759

BROIDE, R. S., REDWINE, J. M., AFTAHI, N., YOUNG, W., BLOOM, F. E. & WINROW, C. J. 2007. Distribution of histone deacetylases 1–11 in the rat brain. Journal of Molecular Neuroscience, 31, 47-58.

CARLOMAGNO, Y., CHUNG, D.-E. C., YUE, M., CASTANEDES-CASEY, M., MADDEN, B. J., DUNMORE, J., TONG, J., DETURE, M., DICKSON, D. W. & PETRUCELLI, L. 2017. An acetylation–phosphorylation switch that regulates tau aggregation propensity and function. Journal of Biological Chemistry, 292, 15277-15286.

COOK, C., CARLOMAGNO, Y., GENDRON, T. F., DUNMORE, J., SCHEFFEL, K., STETLER, C., DAVIS, M., DICKSON, D., JARPE, M. & DETURE, M. 2014. Acetylation of the KXGS motifs in tau is a critical determinant in modulation of tau aggregation and clearance. Human molecular genetics, 23, 104-116.

D'YDEWALLE, C., KRISHNAN, J., CHIHEB, D. M., VAN DAMME, P., IROBI, J., KOZIKOWSKI, A. P., BERGHE, P. V., TIMMERMAN, V., ROBBERECHT, W. & VAN DEN BOSCH, L. 2011. HDAC6 inhibitors reverse axonal loss in a mouse model of mutant HSPB1–induced Charcot-Marie-Tooth disease. Nature medicine, 17, 968.

DING, H., DOLAN, P. J. & JOHNSON, G. V. 2008. Histone deacetylase 6 interacts with the microtubule-associated protein tau. Journal of neurochemistry, 106, 2119-2130.

FISCHER, A., SANANBENESI, F., WANG, X., DOBBIN, M. & TSAI, L.-H. 2007. Recovery of learning and memory is associated with chromatin remodelling. Nature, 447, 178-182.

FISCHER, D. D., CAI, R., BHATIA, U., ASSELBERGS, F. A., SONG, C., TERRY, R., TROGANI, N., WIDMER, R., ATADJA, P. & COHEN, D. 2002. Isolation and characterization of a novel class II histone deacetylase, HDAC10. Journal of Biological Chemistry, 277, 6656-6666.

Ghorbandaiepour, T., Sadroddiny, E., Zahmatkesh, M., & Hassanzadeh, G. 2024. Inhibition of hippocampal melatonin synthesis by siRNA induced learning and memory deficits in male rats. Hormones and behavior, 164, 105599.

GIBSON, C. L. & MURPHY, S. P. 2010. Benefits of histone deacetylase inhibitors for acute brain injury: a systematic review of animal studies. Journal of neurochemistry, 115, 806-813.

GRÄFF, J., REI, D., GUAN, J.-S., WANG, W.-Y., SEO, J., HENNIG, K. M., NIELAND, T. J., FASS, D. M., KAO, P. F. & KAHN, M. 2012. An epigenetic blockade of cognitive functions in the neurodegenerating brain. Nature, 483, 222-226.

GRÄFF, J. & TSAI, L.-H. 2013. The potential of HDAC inhibitors as cognitive enhancers. Annual review of pharmacology and toxicology, 53, 311-330.

GUARDIOLA, A. R. & YAO, T.-P. 2002. Molecular cloning and characterization of a novel histone deacetylase HDAC10. Journal of Biological Chemistry, 277, 3350-3356.

HUBBERT, C., GUARDIOLA, A., SHAO, R., KAWAGUCHI, Y., ITO, A., NIXON, A., YOSHIDA, M., WANG, X.-F. & YAO, T.-P. 2002. HDAC6 is a microtubule-associated deacetylase. Nature, 417, 455-458.

KAO, H.-Y., LEE, C.-H., KOMAROV, A., HAN, C. C. & EVANS, R. M. 2002. Isolation and characterization of mammalian HDAC10, a novel histone deacetylase. Journal of Biological Chemistry, 277, 187-193.

KARIMI-ZANDI, L. E. A. 2022. Increment of CSF fractalkine-positive microvesicles preceded the spatial memory impairment in amyloid beta neurotoxicity. Cytokine 160, 156050.

LI, Y., SHIN, D. & KWON, S. H. 2013. Histone deacetylase 6 plays a role as a distinct regulator of diverse cellular processes. The FEBS journal, 280, 775-793.

Lindner MD, Ribkoff VK. 1991. Relationship between performance in the Morris water task, visual acuity, and thermoregulatory function in aged F-344 rats. Behav Brain Res, 45:45–55.

LIU, R.-T., ZOU, L.-B. & LÜ, Q.-J. 2009. Liquiritigenin inhibits Aβ 25–35-induced neurotoxicity and secretion of Aβ 1–40 in rat hippocampal neurons. Acta Pharmacologica Sinica, 30, 899-906.

LU, X., WANG, L., YU, C., YU, D. & YU, G. 2015. Histone acetylation modifiers in the pathogenesis of Alzheimer's disease. Frontiers in cellular neuroscience, 9, 226.

Marinho D, Ferreira IL, Lorenzoni R, Cardoso SM, Santana I, Rego AC. Reduction of class I histone deacetylases ameliorates ER-mitochondria cross-talk in Alzheimer's disease. Aging Cell. 2023 Aug;22(8):e13895. doi: 10.1111/acel.13895.

Mizuno, M., Yamada, K., Maekawa, N., Saito, K., Seishima, M., & Nabeshima, T. 2002. CREB phosphorylation as a molecular marker of memory processing in the hippocampus for spatial learning. *Behavioural brain research*, *133*(2), 135–141. https://doi.org/10.1016/s0166-4328(01)00470-3

Mohammadi, S., Zahmatkesh, M., Asgari, Y., Aminyavari, S., & Hassanzadeh, G. 2023, Evaluation of hippocampal arylalkylamine N-acetyltransferase activity in amyloid-β neurotoxicity. Journal of molecular endocrinology, 71(2), e220161. https://doi.org/10.1530/JME-22-0161

Oehme I, Linke JP, Böck BC, Milde T, Lodrini M, Hartenstein B, Wiegand I, Eckert C, Roth W, Kool M, Kaden S, Gröne HJ, Schulte JH, Lindner S, Hamacher-Brady A, Brady NR, Deubzer HE, Witt O. Histone deacetylase 10 promotes autophagy-mediated cell survival. Proc Natl Acad Sci U S A. 2013 Jul 9;110(28):E2592-601. doi: 10.1073/pnas.1300113110.

Paul, C. M., Magda, G., & Abel, S. 2009. Spatial memory: Theoretical basis and comparative review on experimental methods in rodents. Behavioural brain research, 203(2), 151–164. https://doi.org/10.1016/j.bbr.2009.05.022

PAXINOS, G. & WATSON, C. 2009. The rat brain in stereotaxic coordinates: compact sixth edition. New York: Academic Press.

Peixoto, L., Abel, T. The Role of Histone Acetylation in Memory Formation and Cognitive Impairments. Neuropsychopharmacol 38, 62–76 (2013). https://doi.org/10.1038/npp.2012.86

PELEG, S., SANANBENESI, F., ZOVOILIS, A., BURKHARDT, S., BAHARI-JAVAN, S., AGIS-BALBOA, R. C., COTA, P., WITTNAM, J. L., GOGOL-DOERING, A. & OPITZ, L. 2010. Altered histone acetylation is associated with age-dependent memory impairment in mice. Science, 328, 753-756.

RICOBARAZA, A., CUADRADO-TEJEDOR, M., PÉREZ-MEDIAVILLA, A., FRECHILLA, D., DEL RÍO, J. & GARCÍA-OSTA, A. 2009. Phenylbutyrate ameliorates cognitive deficit and reduces tau pathology in an Alzheimer's disease mouse model. Neuropsychopharmacology, 34, 1721-1732.

SAMANEH AMINYAVARI, M. Z., FARIBA KHODAGHOLI, MEHDI SANATI, 2019. Anxiolytic impact of Apelin-13 in a rat model of Alzheimer's disease: Involvement of glucocorticoid receptor and FKBP5,. Peptides118, 170102.

SARTOR, G. C., POWELL, S. K., BROTHERS, S. P. & WAHLESTEDT, C. 2015. Epigenetic readers of lysine acetylation regulate cocaine-induced plasticity. Journal of Neuroscience, 35, 15062-15072.

SELENICA, M.-L., BENNER, L., HOUSLEY, S. B., MANCHEC, B., LEE, D. C., NASH, K. R., KALIN, J., BERGMAN, J. A., KOZIKOWSKI, A. & GORDON, M. N. 2014. Histone deacetylase 6 inhibition improves memory and reduces total tau levels in a mouse model of tau deposition. Alzheimer's research & therapy, 6, 12.

SIMÕES-PIRES, C., ZWICK, V., NURISSO, A., SCHENKER, E., CARRUPT, P.-A. & CUENDET, M. 2013. HDAC6 as a target for neurodegenerative diseases: what makes it different from the other HDACs? Molecular neurodegeneration, 8, 7.

Naderi, S., Khodagholi, F., Pourbadie, H. G., Naderi, N., Rafiei, S., Janahmadi, M., Sayehmiri, F., & Motamedi, F. 2023. Role of amyloid beta (25−35) neurotoxicity in the ferroptosis and necroptosis as modalities of regulated cell death in Alzheimer's disease, NeuroToxicology, 94 71-86.

Tanis, K. Q., Duman, R. S., & Newton, S. S. (2008). CREB binding and activity in brain: regional specificity and induction by electroconvulsive seizure. Biological psychiatry, *63*(7), 710–720.

TONG, J. J., LIU, J., BERTOS, N. R. & YANG, X.-J. 2002. Identification of HDAC10, a novel class II human histone deacetylase containing a leucine-rich domain. Nucleic acids research, 30, 1114-1123.

WALKER, M. P., LAFERLA, F. M., ODDO, S. S. & BREWER, G. J. 2013. Reversible epigenetic histone modifications and Bdnf expression in neurons with aging and from a mouse model of Alzheimer's disease. Age, 35, 519-531.

XU, W., PARMIGIANI, R. & MARKS, P. 2007. Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene, 26, 5541-5552.

ZHANG, L., SHENG, S. & QIN, C. 2013. The role of HDAC6 in Alzheimer's disease. Journal of Alzheimer's Disease, 33, 283-295.

ZHANG, X., YUAN, Z., ZHANG, Y., YONG, S., SALAS-BURGOS, A., KOOMEN, J., OLASHAW, N., PARSONS, J. T., YANG, X.-J. & DENT, S. R. 2007. HDAC6 modulates cell motility by altering the acetylation level of cortactin. Molecular cell, 27, 197-213.