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RESEARCH ARTICLE

Swimming exercise and dietary supplementation of *Hemidesmus indicus* modulates cognitive decline by enhancing brain-derived neurotrophic factor expression in rats

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ABSTRACT

Background: The progressive decline in learning and memory is an inevitable effect of aging. Numerous experimental evidences suggest that diet and physical exercise prevent cognitive deficits with age. **Aims and Objectives:** The aim of this study was designed to explore the influence of dietary supplementation of *Hemidesmus indicus* (HI) extract and swimming exercise on cognitive ability and brain-derived neurotrophic factor (BDNF) expression in rats. **Materials and Methods:** Male Wistar rats received oral supplementation of HI extract (50 and 100 mg/kg BW) and swim trained for 84 days, 30 min/day, 6 days/week. The rats were subjected to behavioral studies by T-maze followed by western blotting for BDNF expression. **Results:** The synergistic intervention was found to be more effective in improving cognition in terms of acquisition and retention. Training and supplementation of 100 mg/kg BW HI extract enhanced BDNF expression in the different regions of the brain, cerebral cortex, hippocampus, and cerebellum regions of the brain. **Conclusion:** Our findings suggest that both interventions improve cognitive ability and promote neurogenesis and can be used as a therapeutic strategy in preventing age-associated neurological disorders.

KEY WORDS: *Hemidesmus indicus*; Dietary Supplementation; Swimming Exercise; Cognition; Brain-derived Neurotrophic Factor

INTRODUCTION

Cognitive deficits are due to enhanced susceptibility of the brain cells to oxidative stress (OS).[1] The previous studies from our laboratory have shown that free radicals are key elements involved in the decline of learning and memory performance with age.[2,3] Hippocampus (HC) is an important brain region associated with learning and memory, exhibits decreased neurogenesis, with age.[4]

Exercise is an important non-invasive strategy which improves brain health and reduces the occurrence of dementia and cognitive impairment.[5] Studies have reported that exercise increases behavior and cognitive function as in animals[6] and humans.[7,8] Exercise enhances resistance to brain insults, stimulates neurogenesis, and improves learning and memory function.[9]

The impact of exercise on the cognitive function has been linked to the expression of brain-derived neurotrophic factor (BDNF)[10] plays a major role in modulating cognition and synaptic plasticity.[11] Recent studies by Maejima *et al*.[12]

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demonstrated that exercise improves cognitive function by upregulating BDNF expression in the HC. Exercise and diet both are important factors that manipulate the brain function. Several reports on humans and animals have described the influence of exercise and nutrition on the health and synaptic plasticity.[13]

Polyphenols are of immense interest in nutrition and medicine due to their strong antioxidant activity and play an important role in neuroprotection.[14] *Hemidesmus indicus* (HI), an important medicinal plant belonging to the family Apocynaceae, is used in Indian traditional medicine from time immemorial. Root and stem extracts of this plant are used to treat laxative, diuretic, diaphoretic, and as a tonic for cough. It is also used to cure leukoderma, syphilis, and asthma. The roots are rich source of several compounds including flavonoids, phenolic compounds, steroids, terpenoids, saponins, tannins, insulin, lignins, proteins, carbohydrates, and cardiac glycosides.[15] Recent reports by Penumala *et al*.[16] demonstrated anticholinesterase activity of HI.

However, limited reports are available on the synergistic effects of these two interventions in neuroprotection. Based on the above facts, our aim was to study the individual and the synergistic impact of swimming exercise and dietary supplementation of HI on learning and memory performance in rats. Further, we also explored the mechanisms underlying the effect of these two interventions by evaluating the BDNF expression in different regions of the brain.

MATERIALS AND METHODS

Animals

Male Wistar rats of 2 months old were procured from the Venkateshwara Enterprises, Bengaluru, and were maintained up to 12 months of age. Three animals were maintained per cage at 28°C ± 1°C temperature, humidity of 77% ± 1%, and 12 h-dark/light cycle. All the animals were fed with laboratory chow (Amruth Feeds, India) and tap water *ad libitum*. The protocol of the study was permitted by the Institutional Animal Ethical Committee (BUB/IAEC/TRK/05/2015), Bangalore University, Bengaluru, and pertaining to the regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines.

Preparation of HI Aqueous Root Extract

The HI roots were procured from B.R. Hills, Karnataka, India. The authentication of plant material was done in the Department of Botany, Bangalore University. The roots were washed in the tap water; outer fleshy part was separated, dried (40°C), and finely powdered. The extract was prepared by dissolving 100 g of powder in 1 l of water (50°C). After 24 h, the obtained solution was filtered through Whatman no. 1 paper and was concentrated using lyophilization, weighed (16% yield), and preserved at 4°C until further use.[10]

Experimental Design

The rats were divided into six groups ($n = 8$) – (i) swim trainees on a standard diet (SW-T[N]), swim trainees supplemented with 50 mg/kg BW, (ii) (SW-T [+HI_1]) and 100 mg/kg BW, (iii) (SW-T [+HI_2]) of HI extract, (iv) sedentaries on a standard diet (SE-C [N]), sedentaries supplemented with 50 mg/kg BW, (v) (SE-C [+HI_1]) and 100 mg/kg BW, and (vi) (SE-C [+HI_2]) of HI extract.

Rats were supplemented orally using the intragastric tube for a total period of 84 days.

Exercise Training

Swimming exercise training was done in a rectangular glass tank (77 cm × 38 cm × 39 cm) according to the procedure of Anand *et al*.[11] The tank is filled with water up to a height of 22 cm and maintained at 32°C ± 1°C. Rats were swim trained with 3% load of their body weight tied to their tails for 5 min/day with a gradual increment to 30 min/day. After the pre-training, rats were swim trained for 30 min/day, 6 days/week for a total period of 84 days. SE-Cs were limited to cage activity.

Assessment of Spatial Working Memory

After the accomplishment of training regimen, T-maze studies were carried out to assess the spatial learning and memory performance following the protocol of Jolitha *et al*.[1] Rats were acclimatized to T-maze for 1 day for 10 min and to eat food pellet in both the arms as a reward. Following this, rats were habituated to the T-maze for 2 days and were placed in start box for 60 s, and the sliding door was opened. Rats were allowed to locate the food pellet in a pseudorandom sequence between the two-goal arms. During the acquisition, the same procedure was followed except that one arm was baited per trial. Further, they were practiced to evade the earlier visited arm to receive the reward. Rats underwent 10 trials/day. After each trial, the device was washed with 70% ethanol to remove any odor cues. After the completion of 2 weeks of acquisition, memory retention test was performed once in a week for 28 days with an intertrial interval of 60 s. The number of correct choice in 10 trials was noted.

Tissue Preparation

After the experimental protocol, the rats were subjected to CO_2 asphyxiation. The cerebral cortex (CC), HC, and cerebellum (CB) regions of the brain were separated and washed in cold saline, weighed, and stored at −80°C until use. The homogenates were centrifuged at 2000 ×g for 10 min at 4°C (Superspin-RV/FM, Plastocrafts). The supernatant obtained was used to estimate BDNF levels.

Estimation of Protein

Protein was measured by Lowry *et al*.[12] method using bovine serum albumin as a standard.

Western Blot Analysis

BDNF expression was determined by western blotting according to the procedure of Radhika *et al*.[13] Sixty microgram of protein were electrophoresed on 12% SDS-PAGE and blotted to a polyvinylidene fluoride (Merck Millipore) membrane through the semi-dry blotting apparatus. Membranes were blocked for 1 h at room temperature (RT) with 5% skimmed milk powder in Tris buffer saline comprising 0.05% Tween 20 and then incubated overnight with the primary antibodies at 4°C against BDNF (1:1000, Santa Cruz Biotechnology, USA, MW 14 kDa) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:2000, Cell Signaling Technology, MW 37 kDa), respectively. After washing the membrane with buffer, they were incubated with anti-rabbit (1:2000, GE Healthcare, Buckinghamshire, UK) and anti-mouse (1:2000, GE Healthcare, UK) secondary antibodies conjugated to HRP antibodies for 60 min at RT. All the steps of blocking and incubation were followed by washing (5 min) the membranes thrice with TBS-T (Tris 10 mM, Tween-20 0.05%, NaCl 150 mM, pH 7.5). Chemiluminescence detection was performed using the ECL solution (BIO-RAD). The band intensities were measured using gel documentation system (Eastman Kodak Co., New York, USA). The protein expression levels were normalized to the signal intensities of GAPDH.

Statistical Analysis

Results were expressed as mean ± standard error. The data were analyzed by two-way analysis of variance followed by Tukey's test using GraphPad Prism statistical software. $P < 0.05$ was considered statistically significant.

RESULTS

Learning

The learning potential in terms of the acquisition was significantly increased in all the experimental groups. Training improved the learning ability in supplemented trainees and unsupplemented trainees over their sedentary counterparts in all the trials. As shown in Figure 1a, on the last trial, supplemented sedentaries (HI_1 and HI_2) showed 76% and 83% correct choices, respectively, over the sedentary controls. Among the swim trainees, SW-T ($+HI_2$) group rats made 96% correct choices over their sedentary counterparts.

Memory

The memory retention was measured in terms of percentage latency. The percentage of retention was significant in all the experimental groups even after 28 days compared to the SE-C (N) [Figure 1b]. Swimming exercise and dietary supplementation of HI extract ($+HI_2$) was more effective in improving the memory of the learned task by 90%, 83%, 70%, and 60% with respect to the unsupplemented sedentaries at 7, 14, 21, and 28 days, respectively.

BDNF Levels

BDNF which plays a significant role in synaptic plasticity was estimated by western blot. The relative protein level of BDNF was significantly enhanced in supplemented sedentaries and trainees, over unsupplemented sedentaries. The levels were more enhanced in HC compared to the CC and CB regions. SW-T ($+HI_2$) group exhibited higher expression by 66% (CC), 68% (HC), and 56% (CB), respectively, over their SE-C (N) [Figure 2].

DISCUSSION

In our study, dietary supplementation of HI extract and swimming exercise showed a significant increase in acquisition and memory retention. Further, the molecular mechanisms underlying these benefits were estimated through the expression of BDNF levels in discrete regions of the brain. The BDNF expression is significantly enhanced in combination of exercise and dietary supplementation in the HC compared to CC and CB.

Swimming training is considered to be a model for exercise performance to improve muscle oxidative ability and

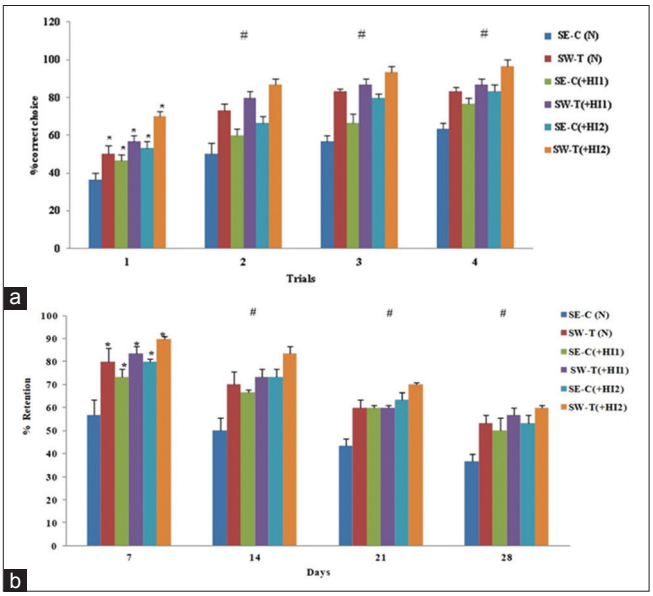

Figure 1: Percentage correct choices during (a) learning and (b) memory retention of the rats. Values are expressed as mean ± standard error of eight animals/group and were analyzed by two-way analysis of variance followed by Tukey's test. $^{*,\#}P < 0.05$ was considered statistically significant. *The comparison of sedentary control with experimental groups. #The comparison of the 1st trail/7th day with other trials/days

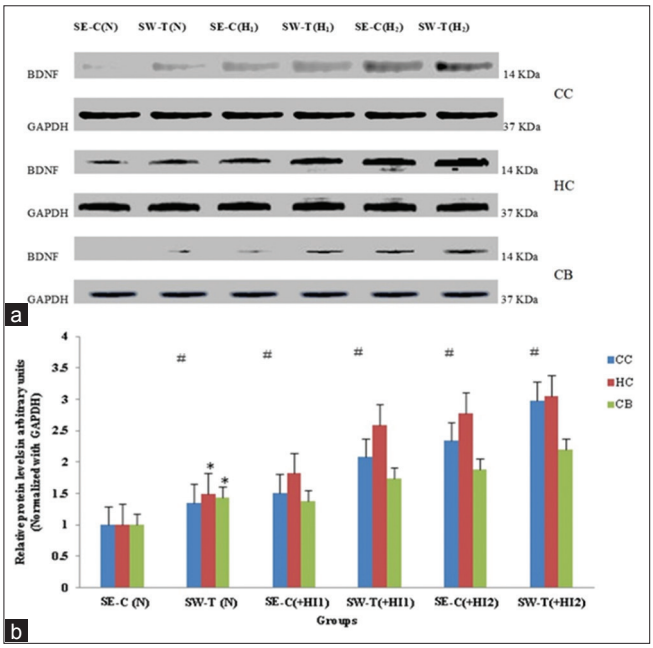

Figure 2: Effect of exercise and dietary supplementation of *Hemidesmus indicus* on brain-derived neurotrophic factor expression in discrete brain regions of the rats. (a) Representative protein immunoblots. (b) Densities of immunoblot bands. Values are expressed as mean ± standard error of eight animals/group and were analyzed by two-way analysis of variance followed by Tukey's test. *,#$P < 0.05$ was considered statistical significance. *The comparison of cerebral cortex with hippocampus and cerebellum. #The comparison of sedentary control with experimental groups

promotes adaptation to aerobic exercise. Swimming appears to be a natural behavior of rats and also does not require electric stimulus.[11,14] Studies have indicated that swimming exercise attenuates age-related spatial learning.[15] In our study, the learning and memory capabilities were assessed by T-maze test which is an appropriate method for acquisition and memory retention in rats.[16] Our results showed a significant increase in learning and memory ability in all the experimental groups. However, combined intervention of exercise and supplementation results in better spatial learning and working memory compared to either swim trained or supplemented groups. These findings may be due to additive effects of two interventions and can be correlated to the decreased OS, food intake, and energy metabolism.[17] The results are in concurrence with the studies of Abhijit *et al*.,[18] wherein the supplementation of polyphenols and swimming exercise enhanced learning and memory in rats.

Improvement in learning and memory in the trained and supplemented groups can be attributed to BDNF protein, which plays an important role in neuronal survival, differentiation, and learning process.[19] BDNF expression was detected using western blotting. HC revealed higher expression of BDNF compared to other regions in all the experimental groups, suggesting increased neurogenesis in this region following exercise and dietary supplementation and also may be due to the increased conversion of proBDNF to mBDNF.[20] Our findings are in line with several studies that have reported increased BDNF expression following exercise training and dietary supplementation.[4,21,22]

Strength and Limitations

The strength of the study is that our results showed that the diet and exercise improve learning and memory performance. These interventions can modulate the cognitive decline in the elderly. The study has several limitations. Age-related response to exercise and dietary supplementation has not been carried out. Other molecular pathways such as AKT and CRB are not included in the study.

CONCLUSION

Our results suggest that the combined effect of swimming exercise and HI supplementation improves learning and memory impairment. Our studies revealed BDNF-mediated synaptic plasticity as an important molecular mechanism for underlying these cognitive benefits. The results suggest that the synergistic intervention of exercise and diet might be used as an effective therapeutic strategy to overcome neurodegenerative diseases and in the prevention of age-related cognitive deficits.

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REFERENCES

1. Jolitha AB, Subramanyam MV, Asha Devi S. Age-related responses of the rat cerebral cortex: Influence of Vitamin E and exercise on the cholinergic system. Biogerontology 2009;10:53-63.
2. Devi A, Jolitha AB, Ishii N. Grape seed proanthocyanidin extract (GSPE) and antioxidant defense in the brain of adult rats. Med Sci Monit 2006;12:BR124-9.
3. Devi SA, Kiran TR. Regional responses in antioxidant system to exercise training and dietary Vitamin E in aging rat brain. Neurobiol Aging 2004;25:501-8.
4. Cechella JL, Leite MR, Pinton S, Zeni G, Nogueira CW. Neuroprotective benefits of aerobic exercise and organoselenium dietary supplementation in hippocampus of old rats. Mol Neurobiol 2018;55:3832-40.
5. Cheng M, Cong J, Wu Y, Xie J, Wang S, Zhao Y, *et al.* Chronic swimming exercise ameliorates low-soybean-oil diet-induced spatial memory impairment by enhancing BDNF-mediated

synaptic potentiation in developing spontaneously hypertensive rats. Neurochem Res 2018;43:1047-57.
6. Jolitha AB, Subramanyam MV, Asha Devi S. Modification by Vitamin E and exercise of oxidative stress in regions of aging rat brain: Studies on superoxide dismutase isoenzymes and protein oxidation status. Exp Gerontol 2006;41:753-63.
7. Laurin D, Verreault R, Lindsay J, MacPherson K, Rockwood K. Physical activity and risk of cognitive impairment and dementia in elderly persons. Arch Neurol 2001;58:498-504.
8. Dubbert PM. Physical activity and exercise: Recent advances and current challenges. J Consult Clin Psychol 2002;70:526-36.
9. Anand S, Devi SA, Ravikiran T. Differential expression of the cerebral cortex proteome in physically trained adult rats. Brain Res Bull 2014;104:88-91.
10. Ravikiran T, Sowbhagya R, Anupama SK, Anand S, Bhagyalakshmi D. Age-related changes in the brain antioxidant status: Modulation by dietary supplementation of *Decalepis hamiltonii* and physical exercise. Mol Cell Biochem 2016;419:103-13.
11. Anand S, Rajashekharaiah V, Tekupalli R. Effect of age and physical activity on oxidative stress parameters in experimental rat model. Int J Clin Exp Physiol 2015;2:185-90.
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.
13. Radhika NS, Govindaraj V, Sarangi SK, Rao AJ. Neonatal exposure to 17β-estradiol down-regulates the expression of synaptogenesis related genes in selected brain regions of adult female rats. Life Sci 2015;141:1-7.
14. Goes AT, Souza LC, Filho CB, Del Fabbro L, De Gomes MG, Boeira SP, *et al.* Neuroprotective effects of swimming training in a mouse model of Parkinson's disease induced by 6-hydroxydopamine. Neuroscience 2014;256:61-71.
15. Cechella JL, Leite MR, Dobrachinski F, da Rocha JT, Carvalho NR, Duarte MM, *et al.* Moderate swimming exercise and caffeine supplementation reduce the levels of inflammatory cytokines without causing oxidative stress in tissues of middle-aged rats. Amino Acids 2014;46:1187-95.
16. Bizon JL, LaSarge CL, Montgomery KS, McDermott AN, Setlow B, Griffith WH. Spatial reference and working memory across the lifespan of male fischer 344 rats. Neurobiol Aging 2009;30:646-55.
17. Gomez-Pinilla F. The combined effects of exercise and foods in preventing neurological and cognitive disorders. Prev Med 2011;52 Suppl 1:S75-80.
18. Abhijit S, Subramanyam MV, Devi SA. Grape seed proanthocyanidin and swimming exercise protects against cognitive decline: A study on M1 acetylcholine receptors in aging male rat brain. Neurochem Res 2017;42:3573-86.
19. Kim H, Heo HI, Kim DH, Ko IG, Lee SS, Kim SE, *et al.* Treadmill exercise and methylphenidate ameliorate symptoms of attention deficit/hyperactivity disorder through enhancing dopamine synthesis and brain-derived neurotrophic factor expression in spontaneous hypertensive rats. Neurosci Lett 2011;504:35-9.
20. Berchtold NC, Chinn G, Chou M, Kesslak JP, Cotman CW. Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. Neuroscience 2005;133:853-61.
21. Sadeghinejad M, Soltani Z, Afzalpour ME, Khaksari M, Pourranjbar M. What is the combined effect of intense intermittent exercise and *Ginkgo biloba* plant on the brain neurotrophic factors levels, and learning and memory in young rats? Pharmacol Rep 2019;71:503-8.
22. Cechetti F, Fochesatto C, Scopel D, Nardin P, Gonçalves CA, Netto CA, *et al.* Effect of a neuroprotective exercise protocol on oxidative state and BDNF levels in the rat hippocampus. Brain Res 2008;1188:182-8.

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