



## The Effect of 6-Week Interval and Continuous Training with *Zizphus Vulgaris* Extract Supplementation on Hippocampus's BDNF Male Wistar Rats

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### Abstract

**Background:** The aim of the study was to compare the effect of 6-week interval and continuous training with *Zizphus Vulgaris* extract supplementation on the hippocampus's BDNF male Wistar rats.

**Methods:** Among 36 male rats (250-350 gr), randomly after adjusting the body weight, 6 rats were separated as the control group. 30 rats were divided into 5 groups: Continuous group (n=6), interval group (n=6), continuous with *Zizphus Vulgaris* extract group (n=6), interval with *Zizphus Vulgaris* extract group (n=6) and *Zizphus Vulgaris* extract group (n=6). The training groups completed 8 weeks of the training program, 5 days/week according to protocol. The endurance continuous protocol includes running exercise on a treadmill for, 10 m/min, 10 min/day to up 16 m/min, 40 min/ day. Endurance interval protocol includes running exercise on a treadmill for, 5\*4 min, with intensity 10 m/min to up 23 m/min, 52 min/ day. The *Zizphus Vulgaris* extract group, every 6 weeks, each rat consumed 400 mg/kg.day and 1.5 mL. At the end of the intervention, the animals were euthanized and the hippocampus's BDNF was measured. Data were analyzed using one way ANOVA tests (Pvalue<0.05).

**Results:** Interval with the *Zizphus Vulgaris* extract group had significantly increased hippocampus's BDNF compared to control and *Zizphus Vulgaris* extract groups (respectively p=0.01, p=0.02). Other comparisons were not significant.

**Conclusions:** Interval with *Zizphus Vulgaris* extract induce more effective favorable changes in the hippocampus's BDNF in male rats. Likely, that be the best Strategy to prevent negative effects on the hippocampus's BDNF decrease.

**Keywords:** Interval training, Continuous training, *Zizphus Vulgaris* extract supplementation, Hippocampus's BDNF, Male wistar rats.

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the most important trophic factors in the nervous system and play an important role in regulating the proliferation, differentiation, maintenance, morphogenesis, and function of neuronal cells in the central and peripheral nervous systems.<sup>4,5</sup> Another prominent role of the brain-derived neurogenic factor (BDNF) is derived from its antioxidant activity conferring resistance to oxidative stress.<sup>6</sup> Among all brain neurotrophins, the binding of BDNF to its specific receptor, TrkB, which has a high affinity for this protein, initiates the only signaling pathway to divulge common cellular and molecular interactions in different regions of the hippocampus.<sup>7</sup> After binding to TrkB, BDNF activates several intracellular signaling pathways including Res MAPK to promote cellular growth and survival.<sup>8</sup>

Although exercise activities have several positive effects such as delaying the aging process, increasing the lifespan, and improving the brain function and some age-related neurological diseases,<sup>9</sup> they also increase the production of reactive molecular (oxygen, etc.) species and induce oxidative stress inflicting various tissues of the body. In these conditions, the key to maintain health is to balance the production of free radicals and antioxidant agents.<sup>10</sup> While there are many pharmacological agents available for the inhibit oxidative stress induced by physical activity, widespread and long term use of these agents, it is limited due to its side-effects, costs, and poor long term compliance. Therefore, it is essential that non-pharmacological strategies to continue to be evaluated. Keeping a healthy diet and using anti-oxidant supplements are probably among the best strategies to protect against the unwanted effects of exercise-induced oxidative stress.<sup>11</sup>

Evidences show that *Zizphus Vulgaris* is a plant with antioxidative properties due to its high amounts of antioxidant compounds such as polyphenols including tannins and flavonoids.<sup>12-14</sup> These compounds, due to their hydroxyl groups, can neutralize and scavenge free radicals, and in this way play an important role in maintaining human health.<sup>15</sup> Despite that *Zizphus Vulgaris* fruit is currently used for pharmaceutical and other medicinal purposes, no comprehensive research has been done on its antioxidant properties. Due to its potent antioxidant properties, *Zizphus Vulgaris* fruit can probably inhibit oxidative stress induced by physical activity and augment antioxidative systems by inducing the production of BDNF. *Zizphus Vulgaris* due to its antioxidant compounds such as polyphenols,<sup>12-14</sup> can prevent

## Introduction

Today, the positive effects of regular exercise and physical activity have been confirmed in various studies.<sup>1</sup> Exercise increases blood flow throughout the body and in the brain.<sup>2</sup> It seems that increased blood flow delays the loss of brain neurons, which generally begins around the age of 40 years, and in this way improves cerebral functions.<sup>3</sup> Exercise increases the level of neurotrophin, which can be one of the reasons for increased hippocampal volume. Neurotrophins are

some diseases in which free radicals are produced as a result of oxidative stress. It seems that Due to its potent antioxidant properties, *Zizphus Vulgaris* can probably cover and neutralize oxidative stress induced by physical activity and exercise, and inhibit exercise-induced oxidative stress so that on the one hand, it is possible to benefit from the positive effects of physical activity such as delaying the aging process and improving the brain function<sup>9</sup> and, on the other hand, probably it is possible to increase the production of BDNF protein with antioxidant activity by using the antioxidant properties of *Zizphus Vulgaris* and augment antioxidative systems. In addition, increasing BDNF levels will not only increase the maintenance and function of neuronal cells<sup>4,5</sup> but also increase resistance to oxidative stress.<sup>6</sup>

Since no research has been done on the effects of *Zizphus Vulgaris* extract along with regular interval and continuous training on the alternation of BDNF levels, we here aimed to investigate this issue.

## Materials and Methods

The present experimental study was enrolled on male albino Wistar rats of 8 weeks old. Thirty-six rats (weighing 250 -350 grams) were purchased from the laboratory animal breeding center of Birjand university of medical sciences and transferred to the Birjand college of agriculture where they were kept under 20-22°C temperature, 40-60% humidity, 12-hour dark/light cycle, and free access to water and food.

Initially and after matching for bodyweight, 6 rats were randomly selected as the control group, and then the remained animals (n=30) were randomly allocated into 5 groups including continuous exercise group (n=6), interval exercise group (n=6), continuous exercise+*Zizphus Vulgaris* extract group (n=6), interval exercise+*Zizphus Vulgaris* extract group (n=6) and *Zizphus Vulgaris* extract group (n=6). The training groups completed 8 weeks of the training program, 5 days /week according to protocol. The endurance continuous protocol includes running exercise on a treadmill for, 10 m/min, 10 min/day to up 16 m/min, 40 min/ day. The endurance interval protocol includes running exercise on a treadmill for, 5\*4 min, with intensity 10 m/min to up 23 m/min, 52 min/ day. Except for the control and the first two exercise groups, the other groups received *Zizphus Vulgaris* extract at a dose of 400 mg/kg (1.5 mL per day) by gavage. Exercises began when the rats were at the age of 10-week. To match the animals' body weights, the rats in the control group consumed an average amount of the food consumed by the exercise groups during the prior day. Bodyweight and food intake were daily determined before exercises.

For histological examinations, tissue biopsies were obtained 24 hours after the end of the 6-week interventions. For this purpose, the rats were anesthetized by injection of an anesthetic agent (combination of ketamine and xylazine with the doses of 7 and 10 mg/kg, respectively). The animals were beheaded using special scissors, and then the entire brain was removed from the skull. After that, the hippocampus was separated from the rest of the brain, washed in normal saline, and rapidly inserted into liquid nitrogen. After being frozen, the tissues were stored in a special refrigerator at -80°C. All the tissue biopsies were performed in a similar procedure in the morning. To extract the hippocampus proteins, the brain tissue was first homogenized (powdered) using liquid nitrogen. Then a protease inhibitor buffer (Stebiofarm, China) was added to each sample, and the samples were centrifuged (14000 rpm, 4°C for 15 minutes). Total protein concentration was determined by the Bradford test. Finally, the BDNF level was measured in all the samples by a specific ELISA kit (Stebiofarm, China) with a sensitivity of 0.01 ng/mg. All the laboratory tests and methods of the present study were approved by the experts of the research council of university of Sistan and Baluchestan (approval ID: 962/806/2813, 24 June, 2018).

Data analysis was performed in SPSS 20 software. Kolmogorov-Smirnov and Levin tests were used to check the normality of data and to examine the homogeneity of variances, respectively. All the variables were expressed as means±standard deviations. One-way analysis of variance (ANOVA) was used to examine the differences among the exercise, extract-supplemented, and control groups. The statistical significant level was set at 0.05.

## Results

The result of the study showed that a significant increase in the hippocampus's BDNF was only observed in interval with *Zizphus Vulgaris* extract group compared with that control and *Zizphus Vulgaris* extract groups (table 1,2).

According to the results of Comparison of BDNF (ng/ml) after 6-week intervention between studied group with control group, (table 1), the interval with *Zizphus Vulgaris* extract group had significantly increased the hippocampus's BDNF compared to control group (Pvalue=0.01). Other comparisons were not significant (figure 1).

Comparison of BDNF (ng/ml) after 6-week intervention among the studied group (table 2), showed that only the hippocampus's BDNF in interval with *Zizphus Vulgaris* extract group was significantly greater than *Zizphus Vulgaris* extract group (p=0.02). Other comparisons were not significant (figure1).

**Table 1. Comparison of BDNF (ng/ml) after 6-week intervention between studied group with control group**

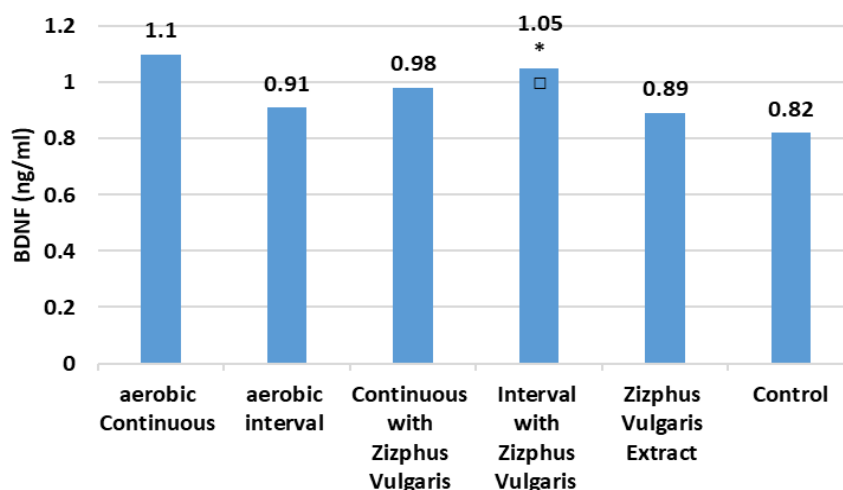
Variable	Groups	Mean±SD	Mean difference	Significance level
BDNF (ng/ml)	Aerobic Continuous	1.1±0.15	0.27	0.06
	Aerobic interval	0.91±0.17	0.08	0.98
	Control (0.82±0.09)	0.98±0.06	0.16	0.07
	Continuous+ <i>Zizphus Vulgaris</i> extract	1.05±0.07	0.23	0.01*
	Interval+ <i>Zizphus Vulgaris</i> extract	0.89±0.03	0.07	0.73

\*Significant level=0.05

**Table 2. Comparison of BDNF (ng/ml) after 6-week intervention among the studied group**

Variable	Group	Mean difference	Pvalue	
BDNF (ng/ml)	Aerobic interval	0.19	0.53	
	Continuous+Zizphus Vulgaris extract	0.11	0.73	
	Interval+Zizphus Vulgaris extract	0.04	1.00	
	Zizphus Vulgaris extract	0.21	0.16	
	Continuous+Zizphus Vulgaris extract	-0.07	0.98	
	Aerobic interval	Interval+Zizphus Vulgaris extract group	0.14	0.63
		Zizphus Vulgaris extract	0.01	1.00
	Aerobic continuous+Zizphus Vulgaris extract	Interval+Zizphus Vulgaris extract	-0.06	0.75
		Zizphus Vulgaris extract	0.09	0.15
		Aerobic interval+Zizphus Vulgaris extract	Zizphus Vulgaris extract	0.16

\*Significant level=0.05

**Figure 1. Hippocampus's BDNF of experimental and control groups**

## Discussion

The aim of the study was to investigate the effect of 6 weeks of interval and continuous exercises along with Zizphus Vulgaris extract supplementation on BDNF levels in the hippocampus in male Wistar rats. According to the results, the hippocampus BDNF level was significantly higher in the aerobic interval exercise+Zizphus Vulgaris extract group compared to the control and the groups received the extract alone.

Several studies show different findings regarding alternations in BDNF levels during exercises. The results of Taheri Chadorneshin et al. (2018) showed that intense interval exercise increased the content of Neurotrophins involved in memory function.<sup>16</sup> Conversely, Lezy et al. (2013) attributed the lack of change in brain BDNF level after 6 weeks of treadmill exercise to the low intensity of exercises and a lactate level lower than the required threshold.<sup>4</sup> In the studies of Azuma et al. (2015) and Schiffer et al. (2009), no significant elevations were observed in BDNF levels after 16 and 12 weeks of HIIT exercises, respectively,<sup>17,18</sup> which was consistent with the findings of the present study. However, Afzalpour et al. (2015) observed a significant increase in BDNF level in different regions of rats' brain after six weeks of HIIT training.<sup>19</sup> The reason for this difference can be variable

duration of exercise sessions. In the study of Afzalpour et al., the intensity of interval exercises (6 repeats, 3 minutes) which accompanied active rest between, that was different from the present study. Overall, researches have shown contradictory results regarding the effects of continuous and interval exercises on neurotrophins levels.

It seems that the decreasing trend in plasma BDNF levels in some studies may be due to its shift towards target tissues such as compressed muscles during physical activities.<sup>20</sup>

It is believed that one of the reasons for brain shrinkage and memory loss is the secretion of cortisol hormone which can damage the brain, particularly after stress.<sup>21</sup> Therefore, BDNF and cortisol are likely to have opposite effects on the functionality of nervous system and brain.<sup>22</sup> So, another reason for the differences observed among studies could be that forced exercises less potently induce BDNF due to associated stress, indicating the importance of exercise type as well as duration in alternations of BDNF levels. Accordingly, the reason for the inconsistency between the results of the mentioned studies and ours can be different durations and intensities of continuous and interval exercises.

As mentioned, the BDNF level increased in both continuous and interval exercise groups (with and without Zizphus Vulgaris extract supplementation); however, the

elevation was only significant in the interval exercise+Zizphus Vulgaris extract group. Regarding the effects of exercise on BDNF level, several mechanisms have been suggested. It seems that exercise induces BDNF gene expression in the brain, particularly in the hippocampus, through the tyrosine kinase B receptor.<sup>23</sup> Also, moderate-intensity exercises have shown to trigger the BDNF-tyrosine kinase pathway in the hippocampus, which subsequently exerts its effect by inhibiting the phosphorylation of P47phox.<sup>24</sup>

Zizphus Vulgaris fruit contains vitamin C and other active ingredients such as polyphenols (e.g. tannins, flavonoids, carotene, and beta carotene).<sup>25</sup> Vitamin C, on the other hand, can affect the production and secretion of brain-derived neurotrophic factors probably through increasing the volume of brain structures, especially the hippocampus.<sup>26</sup> Various studies have also reported that the consumption of sources containing polyphenolic compounds, especially flavonoids, can be effective in maintaining human health.<sup>27</sup>

Based on the results of the present study, it seems that the interval exercise along with Zizphus Vulgaris extract supplementation can effectively modulate the changes of the hippocampus BDNF level in male rats. Therefore, this can be a suitable strategy to prevent the adverse effects of reduced BDNF levels in the hippocampus.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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