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The effect of regulatory aerobic exercise on lipoproteins index of plasma in epileptic male rat

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SUMMARY

There is no report on the evaluation aerobic exercise on lipoproteins and Atherogenic Index of Plasma (AIP) in epileptic male rats. In the present study our purpose was to elucidate the effect of regulatory aerobic exercise on Lipoproteins and (AIP) in epileptic male rat by pentylentetrazol. In this experimental study, 40 male rats randomly divided into 2 main different epileptic (kindling) and non-epileptic groups (n=20). The epileptic groups were kindled by intraperitoneal injection of 40 mg/kg pentylentetrazol (PTZ), then divided into two epileptic subgroups, including the aerobic exercise and without exercise. The non-epileptic groups were divided into two subgroups including aerobic exercise and without exercise. After 6-week exercise rats were deep anesthetized by ketamine, and then blood was taken. Data were analyzed by t-test and ANOVA.

The epileptic male rats that received aerobic exercise increased significantly the seizure of parameters S2L and S4L, compared with the epileptic group that did not receive regular exercise $P<0.05$. Also, in the aerobic exercise groups decreased the parameters S5D and SD compared with the control group $P<0.001$. The exercise significantly decreased total cholesterol (TC), triglyceride (TG) and low density lipoprotein (LDLP) as compared with the epileptic group without exercise $P<0.001$. The present study indicates that the aerobic regularly exercise not only decreased the duration of seizures, but also reduce the vascular risk factor in epileptic group rats.

Key words: Aerobic exercise – Lipoproteins – Pentylentetrazol – Rats – Epilepsy

INTRODUCTION

Epilepsy is one of the major disorders of the central nervous system in humans, which a small area or a large part of the brain spontaneously activates. These abnormal activities can lead to both increased irritability and coordination impairment that ultimately causes seizures (Espinosa-Jovel et al., 2018). Globally, about one percent of the population suffers from epilepsy, while more than one percent of people experience epilepsy during their lives, and this might be due to various causes (Frantz et al., 2017). There are many methods to induce epilepsy in animals (kindling), including chemical and electrical. Chemically, pentylentetrazole (PTZ) drug can be injected, which leads to epilepsy in the temporal lobe of the brain (Jutila et al., 2002). Previous studies have shown that regular sports activities will improve different systems of the human body, such as the central nervous and cardiovascular systems (Houser, 1990). In fact, regular exercise will improve the function of

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the body by reducing the level of Cholesterol, Triglyceride (TG), low-density lipoprotein (LDL-C), and very low-density lipoprotein (VLDL) (Cavalheiro et al., 1991; Leclerc, 1992; Banz et al., 2003). Besides, it has been demonstrated that aerobic exercise may increase the level of High Density Lipoprotein (HDL), which is considered as a useful lipoprotein that is able to reduce the risk of cardiovascular disease (Leclerc, 1992). O'Donovan et al. (2005) reported that aerobic training in male rats by treadmill led to reduction of Total Cholesterol, Triglyceride, and increases the biogenesis of HDL and Reverse Cholesterol Transport (RCT). RCT can keep cholesterol at a normal level, thus preventing atherosclerosis in the vessels. Other reports indicate that cardiovascular risk factors, including plasma cholesterol and lipoproteins, are higher in epileptic populations than in non-epileptic populations. The main reason is unclear and needs further studies (Rahmati-Ahmadabad et al., 2018). Atherogenic Index of Plasma (AIPs) and Lipoproteins are important factors that are influenced by aerobic exercises. Many studies have demonstrated the beneficial effects of regular aerobic exercise on the risk factors in either cardiovascular patients or non-epileptic animals, suggesting a reduction in those factors and enhancement of Metabolic syndrome due to loss of weight, lower blood pressure and increase insulin sensitivity (Elliott et al., 2007). However, the effects of regular aerobic activities on the amount of altered lipoprotein levels in Kindled epileptic animals by injecting pentylentetrazole (PTZ) has not been reported. Therefore, this study was designed to evaluate the impact of aerobic activity on AIPs and plasma lipoproteins in epileptic male rats.

MATERIALS AND METHODS

Animals

In this study 40 male Wistar rats (150-250 g) were obtained from the Laboratory Animal of the Center for Medical Science of Sabzevar University (Sabzevar, Iran). The rats were kept in a standard environment (12-h light/12-h dark) without food or water restriction. Rats were randomly divided into two groups of 20 including epileptic and non-epileptic. To induce epilepsy in rats 40 mg/kg, PTZ was injected intraperitoneally till they experienced 5 steps of seizures 3 or 4 times. It should be noticed that all the process of the experiment was confirmed by the Ethics Committee of Medical Sciences of Sabzevar University (code. IR.REC.92.34).

According to Racine's Scale, by inducing convulsions in rats they will show some behavioral symptoms that are divided into 5 phases: phase 1: movements of mouth and facial muscles; phase 2: head nodding; phase 3: accruing clonus on the opposite forelimb; phase 4: rearing with clonus; Stage 5: both rearing and falling with clonus of forelimbs (Elliott et al., 2007). Seizures stages during kindling by PTZ are as follows: (1) period of delay time to starting phase II convulsions (Stage 2 Latency; S2L). (2) period of delay time to starting phase IV convulsions (Stage 4 Latency; S4L). (3) period of delay time to starting phase V convulsions (Stage 5 Latency; S5L). (4) duration of phase 5 seizures (Stage 5 Duration; S5D). (5) Duration of seizure attack (SD) (Bartsch et al., 2014). The non-epileptic group was divided into two subgroups of with and without regular aerobic exercise.

Regular aerobic exercise with treadmill

To familiarize the animals with the treadmill and reduce their stress, the rats were trained with treadmill 3 days before starting the protocol (3 days, 10 minutes, speed 12 m/min). The rats which did not run on the treadmill were eliminated during the familiarization phase and replaced with new ones. After that, the training group performed aerobic exercise by treadmill six times in a week for 6 weeks. During the first week, rats were running on the treadmill for 30 minutes at 18 m/min. In the second week, the speed and time of training were increased to 20 m/min for 45 minutes. The rats were then run for one hour at a speed of 26-24 m/min every day for two weeks. This training was constant during the last two weeks of the exercise program. Animals were monitored immediately after each injection for 20 minutes and the duration of the behavioral steps was recorded (Dishman et al., 1988; Arida et al., 2003).

Measurement of lipoproteins

At the end of the last exercise session, rats were anesthetized by ketamine and their blood samples were centrifuged at 5000 rpm for 5 minutes at 30° C in order to measure some factors such as cholesterol, triglyceride, LDL, HDL.

Histopathological process

After sacrificing the rats by ketamine and collecting their blood, their brains (n=10) were removed for the purpose of histological study. The brains were fixed in formalin 10% for 2 days. Then the samples were prepared as paraffin blocks. The blocks were cut with microtome (with 5 µm thickness). The sections were stained by hematoxylin and eosin. At the end of procedure the sections were assessed by light microscope (Raoofi et al., 2015).

Statistical analyses

One-way analysis of variance (ANOVA) and T-test were applied by SPSS software. Then, differences were analyzed via Tukey's multiple comparison test. Charts were plotted using the EXCEL software. The statistical significances were achieved when $P < 0.05$.

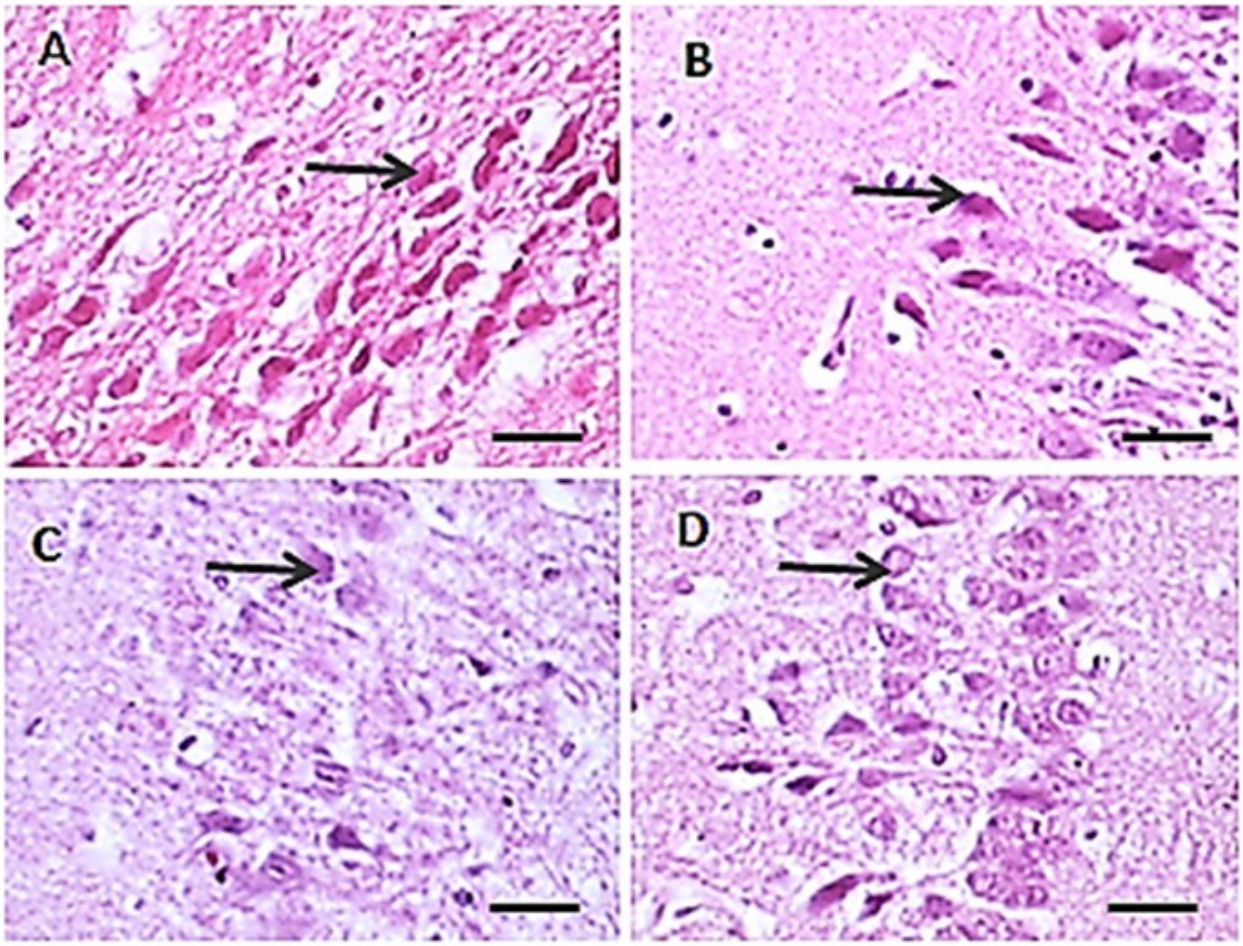

Fig 1. Histopathological features of the dentate gyrus hippocampus in the epileptic male rates of the temporal lobe treated with of the *exercise and without exercise* .Higher morphological changes to showed in the epileptic male rat without exercise, these different; included to increasing acidophilic cytoplasm and also could not differentiate margin nucleus from cytoplasm could be seen in white the arrow picture A. Decreasing acidophilic cytoplasm of the dentate gyrus cells in epileptic rat after exercising the white arrow for six weeks showed in neuronal in picture B. In control group without exercise white arrows showed the healthy neuron in picture C. Increasing in number neutral neuron in dentate gyrus were showed in filed microscopic, so at least morphological change in control groups white arrow show the healthy neuron in picture D that received exercise. H&E staining (400× magnification). More situation affect were observed in dentate gyrus neuron that received exercise in comparison the other groups. Scale Bar = 10 μm. **A**: Epileptic Without training, **B**: Epileptic With training, **C**: Control With training, **D**: Control Without training.

RESULTS

Histomorphometric findings

A histological method was used through the H&E staining for assessing the effects of aerobic exercise on the hippocampus morphological changes (Fig. 1). Higher morphological changes in the epileptic-without-exercise group showed an increase in acidophilic cytoplasm, and could not differentiate margin nucleus from cytoplasm. Besides, epileptic rats after exercising observed an increase acidophilic cytoplasm of the dentate gyrus cells.

The effect of aerobic exercise on fully kindled seizures rats

In this work, the seizure parameters were recorded and then compared between different groups. Exercise increases S2L and S4L as compared to epileptic group ($p<0.001$, Fig. 2). The results show that the seizure duration decreases after aerobic exercise in all of seizure stages compared to the control group ($p<0.05$, Fig. 2).

Aerobic exercise reduced lipoproteins index of plasma in epileptic rats

TC, TG, HDL and LDL values were significantly decreased already after six weeks of therapy with aerobic exercise ($p < 0.001$, Fig. 3). Most notably, Lipoproteins profile values before and during aerobic exercise treatment were significantly increased in the epileptic group ($p<0.001$, Fig. 3).

DISCUSSION

The present work evaluated the effect of regulatory aerobic exercise on Lipoproteins Index of Plasma in epileptic male rats. Albeit previous studies have shown that epilepsy activity is decreased

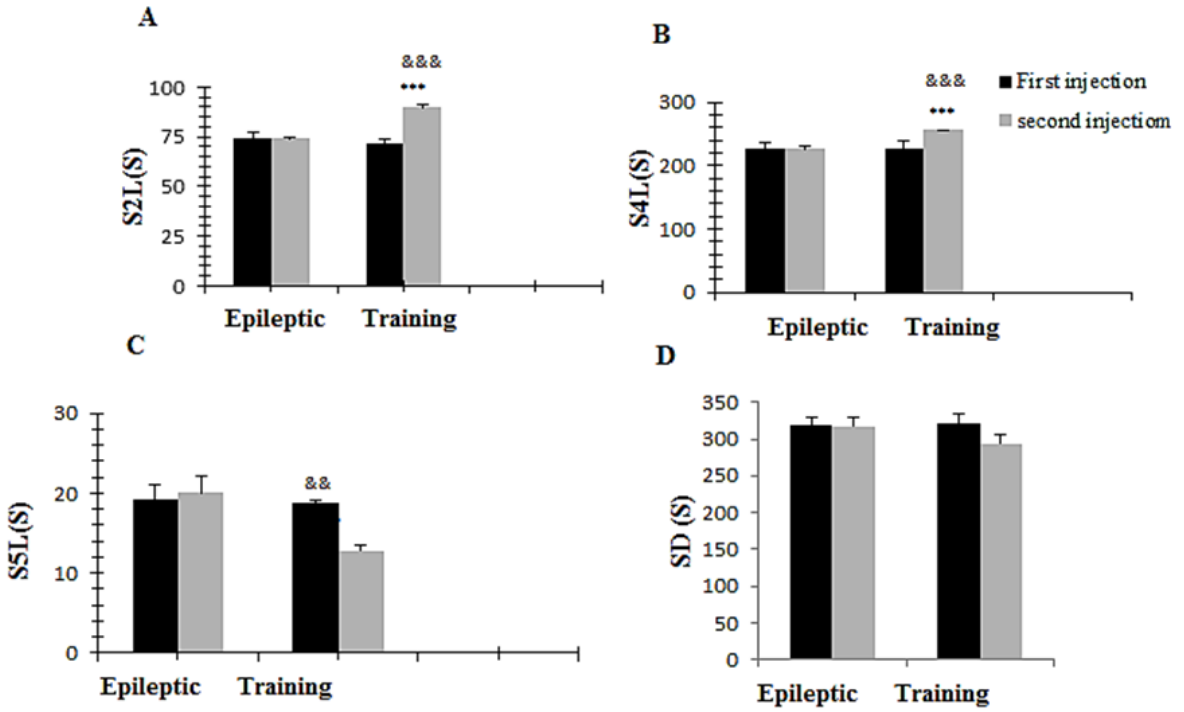

Fig 2. The effect of aerobic exercise on seizure parameters in fully kindled rats in different groups. The results show that the seizure duration decreases after aerobic exercise in all of seizure stages compared to the control group. *** p< 0.001 for comparison between the training groups after second and first injections. &&& p< 0.001 for comparison between the training group after second injection and epileptic group. && p<0.01 for comparison between training groups after second and first injections. * p< 0.05 for comparison between the training groups after second injections with other groups. S2L: Delay time until beginning of stage 2 seizure, S4L: Delay time until beginning of stage 4 seizure. S5L: Delay time until beginning of stage5 seizure, SD: Seizure duration.

by exercise (Arida et al., 2009), there are no studies about the effects of aerobic exercise on plasma Lipoproteins and Atherogenic Index of Plasma in epileptic male rats. The results obtained in this study showed that the injection of PTZ (40 mg/kg) during kindling led to a significant increase in S2L and S4L parameters in epileptic group that received aerobic exercise compared with the without exercise group. On the other hand, in the epileptic group receiving aerobic exercise, parameters S5D and SD decreased compared with the control group. According to the results obtained from assessment of lipid profile, the exercise in epileptic group significantly decreased total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) in compered with the epileptic group without exercise. Therefore, the mean frequency of epileptic activity significantly decreased in all treadmill-exercise groups. These data suggest that aerobic exercise has the Potential for Safety Improvement that does not negatively affect the lipid metabolism profile in our rats after a medium follow-up of about 6 weeks. Experimental and clinical studies are important ways to test hypotheses in medicine (Cakil et al., 2011; Kayacan et al., 2016). Exercise has been found to increase cerebral blood flow and amino acid transport across the blood-brain barrier, and alter the release of endogenous peptides and neuronal stimuli (Herholz et al., 1987; Spranger et al., 2013). Physical activity reduces seizure frequency, and also leads to improved cardiovascular and psychological health in persons with epilepsy (Nakken, 1999). It has been also observed that fewer seizures occur during both mental and physical activity compared with periods of rest. It has been suggested that reduced epileptogenic EEG activity during exercise may be caused by an increase in GABA concentration as a consequence of metabolic acidosis (Arida et al., 2009). Exercise stimulated both synapses and nerve endings, and prevented age-related degeneration in brain functions (Cotman and Berchtold, 2002). Based on these findings, exercise is anticipated to have favorable effects on epilepsy (Arida et al., 2009; 2010). Previous studies showed that the treadmill-exercise program (12 m/min speed, 0° inclination and 60% max VO2) for 10 min/day caused a significant decrease in the rate of pilocarpine-induced epileptic seizures in rats (Arida et al., 1999a, b). Eriksen et al. (1994) performed an exercise program including body building, stretching, aerobics, and dancing for 15 weeks (60 min twice a week) on a total of 15 epileptic women, and noted a significant decrease in the frequency of epileptic seizures at the end of the program. In our

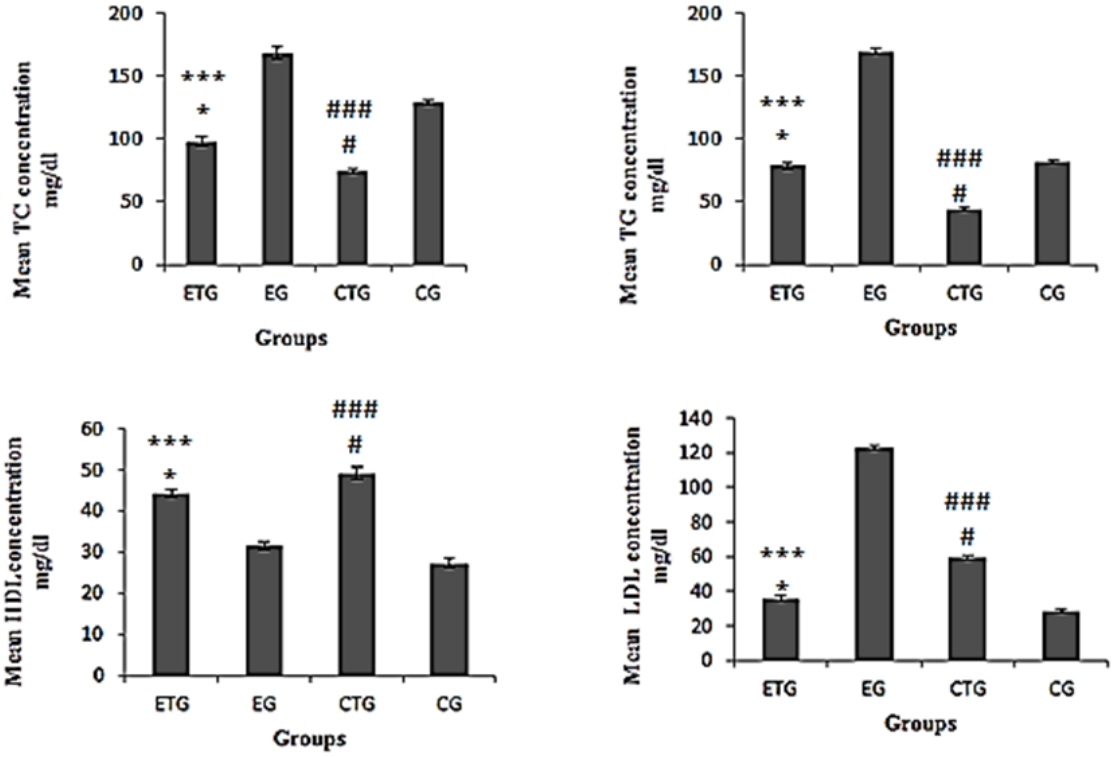

Fig 3. Comparison of Mean TG, TC, LDL and HDL concentration before and after aerobic exercise treatment. ETG: Epileptic With training, EG: Epileptic Without training, CTG: Control With training, CG: Control Without training.

* p<0.05 for comparison between the ETG and CG, # p<0.05 for comparison between the CTG and ETG, ***p<0.001 for comparison between the ETG and EG, ### p<0.001 for comparison between the CTG and CG.

study of 40 rats (n = 20 per in group) subjected to physical exercise during 6 weeks, none of the participants suffered from seizures during the exercise or at rest, or exhaustion after the exercise. We observed significant increase in mean TC, HDL-C, LDL-C and TG levels when compared with the epileptic group without exercise, in rats receiving exercise in epileptic group. Serum concentration of certain lipids and lipoproteins in young adults are important risk factors for the development of coronary heart disease in later life. Considerable data has suggested that, besides total cholesterol (TC), elevated triglyceride (TG) concentrations, increased LDL-C and decreased HDL-C contribute to cardiovascular diseases. Thus, assessing changes in serum lipid levels following aerobic exercise may be useful to prevention of cardiovascular complications in later life (Reddy, 1985; Gowtham et al., 2012).

In conclusion, the novel finding presented here reinforces the beneficial effect of exercise on epilepsy that has been demonstrated in animal studies (Ariza et al., 2007; Peixinho-Pena et al., 2012). On the basis of the available data presented and from clinical studies, seizures occurring during exercise are infrequent and reinforce the beneficial contribution of exercise to seizure reduction. Combinations of neuroprotective and anti-epileptogenic strategies have been effective for combating the disease progression. In addition, a better understanding of the dyslipidemia prevalence, associated to the use of aerobic exercise in rats with epilepsy, could facilitate the appropriate patient management to reduce the risk of vascular diseases in adult rats, especially when considering the higher incidence of cardiovascular and cerebrovascular disease in rats with epilepsy compared to general population.

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