

¹Serkan Çakır*, ¹Hayriye Orallar, ²Ayhan Cetinkaya, ³Yıldırım Kayacan, ⁴Ali Can Önal, ⁵Arzu Yildirim, ⁶Ersin Beyazcicek, ⁷Selim Benek, ⁸Murat Özkan, ⁹Ömer Barış İnce and ¹Nezih Okur

¹Poultry Science Department, Faculty of Agriculture and Natural Sciences, Abant İzzet Baysal University, 14280 Bolu, Turkey; ²Experimental Animals Research and Application Center, Abant İzzet Baysal University, 14280 Bolu, Turkey; ³Sports Sciences, Faculty of Yasar Dogu, Ondokuz Mayıs University, Samsun Turkey; ⁴Bolu Koroğlu State Hospital, 14200, Bolu Turkey; ⁵Department of Field Crops, Faculty of Agriculture and Natural Sciences, Abant İzzet Baysal University, 14280 Bolu, Turkey, ⁶Department of Physiology, Faculty of Medicine, Düzce University, 14280 Düzce, Turkey; ⁷Department of Physiology, Faculty of Medicine, Abant İzzet Baysal University, 14280 Bolu, Turkey; ⁸Electricity and Energy Department, Bolu Vocational High School, Abant İzzet Baysal University, 14280 Bolu, Turkey; ⁹Agriculture and Rural Development Support Institute, Afyon, Turkey

*Corresponding author: Serkan Çakır: E-mail: cakirsf@yahoo.com

Abstract

Background: The aim of the present study was to assess the effect of Hawthorn (*Crataegus oxyacantha*) and physical activity. We studied its effect on penicillin induced epilepsy. in gerbils.

Maternal and Methods: Epilepsy was induced by administration of peniciline G (500 IU, ip). The gerbils were divided randomly in four groups (6 animals per each group) and studied as described below: 1) Control group 2) Exercise group (30 min/each day for 8 weeks) (Eg) 3) Extract group, 50mg/kg/day/animal in 1 ml saline, 3 h prior to exercise (Exe) 4) Exercise+Extract + (Exe+Ex). The severity of epilepsy was observed and recorded.

Results: The means of latencies (Mean±SE) were 236±45, 369±36, 386±58 and 433±37 ms in groups of control, Exe, Ex, and Exe+Ex respectively. The mean spike latency significantly (P=0,033 F=3,560) decreased in Exe, Ex and Exe+Ex when compared control. Although spike frequency significantly (P<0.05) diminished in groups of Exe and Ex, no significant decrease was observed in control and Exe+Ex. Similar trend was seen for amplitude values. Spike amplitude values were determined to be significantly (P<0.05) lower than those of control and Exe+Ex.

Conclusion: Crataegus oxyacantha extract has shown positive affect to ameliorate on some seizure parameters in this study. However, further more advanced physiologic and neurochemical studies are required to determine the mechanisms involved.

Key words: *Crataegus oxyacantha*, physical activity, epilepsy, gerbil,

Introduction

Epilepsy is one of the most serious neurological disorders which are induced by a sudden increment of stimulatory factors in the cortical neurons. About 0.5-3.0% of people experience it during their lifetime (Theodore and Fisher, 2007). The neurophysiological basis of epileptic seizures has been associated with an imbalance between excitatory and inhibitory processes in the central nervous system (Badawy et al., 2009) which is manifested as a unique form of electrical activity that has a characteristic EEG and clinical spectrum. An additional contributing factor in process of epileptogenesis is oxidative stress, a disproportion between reactive oxygen species generation and clearance (Cardenas-Rodriguez et al., 2013). It has been known that during epileptic attack, oxidative stress occurs, free radicals are produced and membrane lipid peroxidation happens, all of which cause tissue damage (İlhan et al., 2005). Nevertheless, the brain is susceptible to free radical damage, considering the large lipid content of myelin sheaths and the high rate of brain oxidative metabolism.

Modern drug therapy of epilepsy is complicated by the inability of drugs to control seizure in some patients and side effects that range in severity from minimal impairment of the central nervous system. Therefore, there is a growing interest in natural antioxidants and their application in nutrition and medical treatments since they contribute to the prevention of oxidative stress.

Several species of the genus *Crataegus* have been reported to possess a wide range of pharmacological actions, especially on cardiovascular system (Wang et al., 2013). The antioxidant activity of *Crataegus* preparations contributes significantly to its therapeutic profile with its flavonoid and procyanidin content. Gou et al. (2003) noted that of 28 fruit pulps tested, the hawthorn pulp (Chinese hawthorn) produced the highest measure of antioxidant activity. A similarly high antioxidant activity in *Crataegus aronia*, a hawthorn indigenous to Israel, Jordan, and the Palestine, has been found (Zhang, 2000). Despite its high level antioxidant property, the plant has not been reported to have antiepileptic effect yet. However, some studies were undertaken showing its affect on nervous system. A large randomized controlled trial found that a combination of *Crataegus oxyacantha*, *Eschscholzia californica* and magnesium was more effective than placebo in reducing anxiety in 264 individuals with generalised anxiety disorder (Zapfe Jun, 2001). Another study evaluated for the central effects of the phytotherapeutic product-CPV (dry extract of *Crataegus oxyacantha*, *Passiflora incarnata* and *Valeriana officinalis*) in animals models. Evaluation of anxiolytic effect of this extract on the elevated plus-maze (EPM) was carried out in order to investigate the psychopharmacological profile of CPV extract and the result indicated anti-

anxiety potential of extract (Ljubuncic, 2005). Mild to moderate sedative effect has been demonstrated in humans and animal studies with hawthorn constituents (Zhang, 2000).

Lack of physical activity is a well-known risk factor for numerous disorders. The relationship between physical exercise and epilepsy, especially the underlying mechanism involved therein is still intriguing. Physical activity can play a favourable role in reducing the frequency and intensity of some seizures, as is evident from recent experimental and clinical studies (Souza et al, 2009). According to some clinical studies, aerobic exercise training programs could either decrease (Eriksen et al., 1994) or have no effect on seizure frequency (McAuley et al, 2001). However, an exercise-induced increase in epileptiform EEG activity has also been reported (Horyd et al., 1981; Nakken et al., 1997). Treadmill running training is considered as the most frequently used aerobic exercise type in the experimental studies (Contarteze et al., 2009).

Stigmatization, social isolation and other related psychological problems are common among patients with epilepsy. There were established beliefs that patients suffering from epilepsy should avoid physical activity and involvement in sports due to paroxysmal nature of seizure attacks and the possibility of injury. Therefore, increased sedentary lifestyle among epileptic patients is observed in population-based studies (Dubow and Kelly, 2003). On the other hand, physical exercise is known to improve physical and mental health, as well as to contribute to better social integration. Hence, the beneficial aspects of physical exercise are lost for epileptic patients (Fountain and May, 2003).

Pharmacodynamics profile of hawthorn extracts has been examined in animal studies (Chang et al., 2005). But no studies of *Crataegus oxyacantha* extract and physical activity appear to have been performed on Peniciline G induced epilepsy. The present experiment was originally undertaken using Mongolian gerbil which exhibits spontaneous seizures in response to sensory stimulation and forced exploratory behaviour. The aim of the present study is to show possible ameliorative effects of *Crataegus oxyacantha* extract alone and combined with physical activity due to its high antioxidant property on experimentally induced epilepsy.

Materials and Methods

Plant Material

Fruits of *Crataegus oxyacantha* were collected from Trabzon/Turkey in September. The identity of plant was confirmed by specimen at Abant İzzet Baysal University by anatomical examination and comparison with plants in herbarium.

Preparation of Extract

The identified fruits were washed 2-3 times with tap water and finally with distilled water and shade dried at room temperature for about two weeks. Dried plants were ground with a blender. 330 g of powdered plant material was extracted with 900 mL of ethanol in water bath at 45°C for 18 h then filtered with filter paper (Whatman No. 1). Filtrates were evaporated under vacuum using rotary evaporator at 65°C, dissolved in 20 ml distilled water and then lyophilized. *Crataegus oxyacantha* extract was stored at -20°C. Just prior administration, stock solution was prepared by dissolving the extract powder in saline and the working concentrations of the extract were made by diluting the stock solution.

Animals

A total 24 male Mongolian gerbils weighing 100-140 grams were kept and maintained under standard environmental conditions of temperature (21.5±2 °C), humidity (60±1%) and 12:12 hour light/dark cycle in a well-ventilated room; and were allowed free access to standard pellet diet and water *ad libitum*. The experimental protocol was approved by the Animal Ethics Committee of Abant İzzet Baysal University, Bolu, Turkey.

Experimental Design

The gerbils were divided randomly in four groups (6 animals per group) and studied as described below: 1) Control group 2) Exercise group (30 min/each day for 8 weeks) (Exe) 3) Extract, 50 mg/kg/day/animal, in 1 ml saline, 3 h prior to exercise (Ex) 4) Exercise+Extract (Exe+Ex). Gerbils were fasted for 12 h before gavage procedure. *Crataegus oxyacantha* extract was dissolved in saline and given to the animals for 3 h prior to the each exercise. The volume of administration was kept at 1ml to the animal. A gavage tube was used to deliver the substance by the oral route, which is the clinically expected route of administration of extract.

Exercise Protocol

Treadmill adaptation period was performed to animals to accustom the animals to exercise. The CE certified four lane animal treadmill [May Time 0804, Animal Treadmill] with adjustable settings for rate, distance, running time, speed, inclination and a built-in memory to store data was used for the physical exercise. In order to avoid any stress during the exercise, all gerbils were subjected to a conditioning exercise at the lowest treadmill speed for 5 min along 10 consecutive days. To prevent the escape of exercise, incremental electrical shock was given (1-6 mA) to continue physical activity. After the treadmill adaptation period, all gerbils continued to exercise for 8 weeks, 30 min once a day according to treadmill exercise. All animals in the experiment were performed between the hours 10 and 12 in the morning.

Induction of Epilepsy

After 8 weeks gavaging period, 3 h after the last administration of the test material, epilepsy was induced. Gerbils were anesthetized with urethane (1.25 g/kg, ip) and placed in a stereotaxic frame under spontaneous respiration. Incision region was infiltrated with prilokain hydrochloride to prevent possible pain. After shaving, 3 cm incision was created on the skull in the rostro-

caudal direction. The soft tissue on the skull was removed and the bregma (reference point) was identified. Under stereotaxic guidance, two stainless steel screws were placed over the left somatomotor cortex (first screw 3 mm lateral and 4 mm rostral to bregma; second screw 3 mm lateral and 4 mm caudal to bregma) and a well conductor bipolar electrode was connected to the screws. After seeing the brains basal activity with Power Lab data acquisition system, a 1 mm hole was opened (1.5 mm left lateral and 1.5 mm caudal to the bregma) with a hand drill for penicillin G potassium (Sigma Chemical Co., St. Louis, MO, USA) injection dissolved in sterile physiological saline. The epileptic focus was produced by single dose of 500 IU intracortical penicillin G potassium (1 mm vertical direction to the brain surface) by a Hamilton micro syringe (type 701N, 22s gauge, bevel tip) with a volume of 2.5 μ l.

Monitoring, Recording and Ecog Waveform Analysis

Animals were observed for a period of 2 h post penicilline G administration. Induction protocol for seizures described below was followed. Bipolar Ag/AgCl top electrodes were implanted into the anaesthetized gerbil motor cortex for ECoG recording stereotaxically. Ag/AgCl clamp electrode was used for grounding: first electrode; 2mm lateral to sagittal suture and 1 mm anterior to bregma second electrode; 2 mm lateral to sagittal suture and 5 mm posterior to bregma and the grounding electrode which increases the contact and reduces the incidence of fatalities were placed on left ear for recording. The ECoG of the gerbils was monitored and recorded from the beginning of the stabilization period using commercial equipment Bio Amp (AD Instruments, Australia) and Power Lab 8/SP (AD Instruments, Australia). The recorded raw data were viewed and evaluated using the commercial software by Chart 5.1.1 (AD Instruments, Australia). The duration of various phases of epilepsy were observed. The parameters noted were mean onset time of epileptic activity (latency), duration of epileptic activity, epileptic frequency and altitude values due to Penicillin G induced epilepsy due to Penicillin G induced epilepsy.

Statistical Analysis

All data were expressed as mean standard error of mean (SEM). Statistical difference between various groups were analysed by one-way analysis of variance test (ANOVA), followed by post-hoc Tukey's test using SPSS version 21 software. P values less than 0.05 were considered as significance.

Results

As already observed in previous experiments, intracortical injection of penicillin (500 IU) induced an epileptiform ECoG activity characterized by bilateral spikes and spike-wave complexes. Epileptiform activity began within 4-5 min. It reached a constant level as to the frequency and amplitude in the 30 min and lasted for 2 h. Figure 1 shows the effect of extract tested and physical training on latency of penicillin induced epileptiform activity.

The means of latencies (Mean \pm SE) were 236 \pm 45, 369 \pm 36, 386 \pm 58 and 433 \pm 37 ms in groups of control, Exe, Ex, and Exe+Ex respectively. The mean spike latency significantly (P=0,033 F=3,560) decreased in Exe, Ex and Exe+Ex when compared control. The shortest delay to onset of first spike was observed in control.

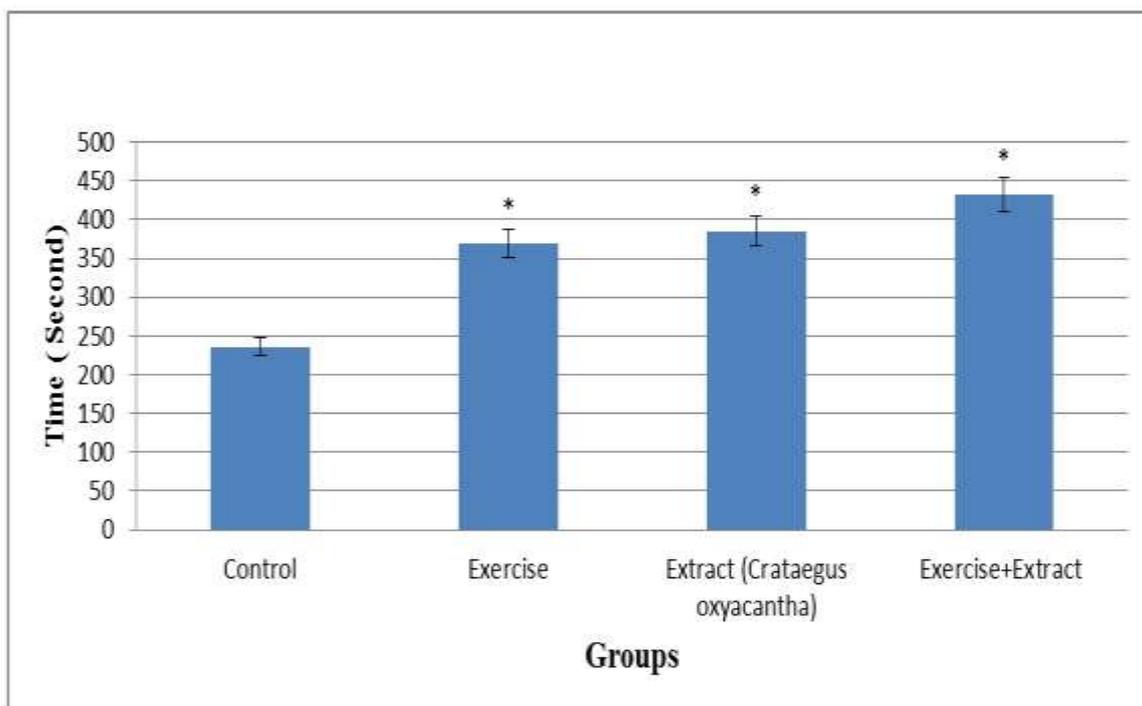


Figure 1: The latency of first epileptic activity (Data are means \pm SE*P<0.05: compared to control)

As seen in Figure 2, although spike frequency significantly (P<0.05) diminished in groups of Exe and Ex, no significant decrease was observed in control and Exe+Ex. Similar trend was seen for amplitude values. Spike amplitude value was determined to be significantly (P<0.05) lower than those of control and Exe+Ex.

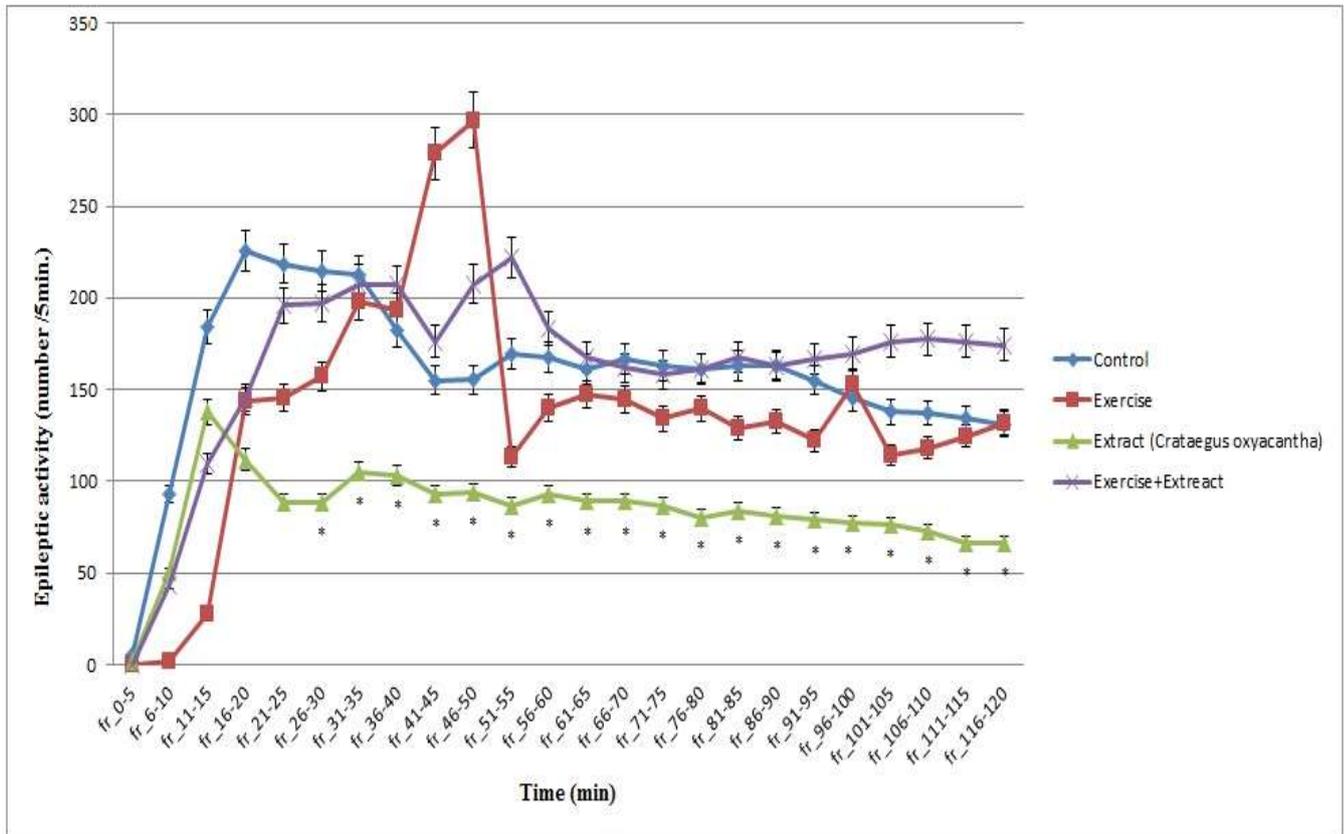


Figure 2: The number of spike wave (epileptic activity) during 120 minutes ECoG records in all groups (Data are means \pm SE *P<0.05: compared to control).

Discussion

Several clinical and experimental studies have been performed to ameliorate seizures induced by different methods. Physical exercise and herbal extracts are often used tools to decrease epileptiform activity in the animal studies. A variety of animal seizure models have been designed to evaluate the relationship between exercise and epilepsy (Arida et al., 2009; Souza et al., 2009). However, there has been no study examining the effect of *Crataegus oxyacantha* extract on seizures. In the present experiment, originally, ameliorative effect of combined with physical exercise and *Crataegus oxyacantha* extract was also evaluated.

The extract material tested in the present trial contains a range of pharmacologically active substances, of which the most widespread compounds reported are flavonoids, triterpenic acids and phenol (Chang et al., 2002). All of these active substances are the most important constituents for its antioxidant activity. Guo (2003) noted that of 28 fruit pulps tested, the hawthorn pulp (Chinese hawthorn) produced the highest measure of antioxidant activity. The *Crataegus oxyacantha* extract showed ameliorative impact on epileptic activity in this study. Despite lack of study used *Crataegus oxyacantha* in epilepsy, helpful affects have been observed on seizures using different herbal extracts. The antioxidant activity of *Crataegus oxyacantha* contributes significantly to its ameliorative profile on seizures. It has been known that during epileptic attack, oxidative stress occurs, free radicals are produced and membrane lipid peroxidation happens, all of which cause tissue damage (Ilhan et al., 2005). Although antioxidant affect has not been evaluated in the study, ameliorative effect of *Crataegus oxyacantha* might be associated with its high antioxidant level. Furthermore, Tsuda et al. (1994) suggested that penicillin exerts its proconvulsant effect by inhibiting GABA-gated chloride ion influx. Alterations in neurotransmitter systems induced extract should be taken into account for inhibitory/excitatory balance to reduce seizure frequency. Despite the data given above, preventive effects of extract on epilepsy are still unclear.

Experimental studies have demonstrated a positive effect of physical exercise in animals with epilepsy (Souza et al. 2009, Pietrelli et al., 2012). Although there is convincing evidence indicating that physical exercise has the positive role in reducing the frequency and severity of seizures in several models of experimental epilepsy, this subject is still a matter of controversy. The present study demonstrated that exercise alone decreased the mean frequency and amplitude of epileptiform activity. However, the same result was not obtained in Exe+Ex group.

Rats with pilocarpine induced epilepsy submitted to a physical training program presented a lower frequency of spontaneous seizures than controls (Arida et al., 2013). Other animal models of epilepsy (penicillin, pentylentetrazol, kainic acid) have shown similar positive results (Tutkun et al., 2010). Several evidences have been come up with reducing frequency and amplitude values of seizures seen here. During exercise, not only aerobic but intensive (anaerobic) physical activity increases serum lactate content and causes metabolic acidosis. Reduced seizure activity during exercise might be caused by an increase in GABA concentration as a consequence of metabolic acidosis. Threshold for epileptic activity is another subject to start first spike. Arida et al. (1999) reported that the physical training did not induce epileptic seizures in the model of temporal lobe epilepsy in rats and they also showed that chronic exercise increased the threshold in the model of kindling development. Some changes of transmitter's level could be reason to start epileptic activity. We did not evaluate the level of neurotransmitters level such as GABA, glutamate, dopamine, serotonin

and noradrenalin in the homogenised brain samples which might be important to interpret the results obtained in our study. For example, a regular physical activity program increases serotonin, noradrenaline, dopamine synthesis and release, up-regulates neurotrophins, reduces stress and therefore, decreases hypothalamic–pituitary–adrenal activity and adrenal glucocorticoids (Park et al., 2005), which consequently may diminish seizure frequency and amplitude variables. Arida et al. (1998) demonstrated that exercise retarded amygdala kindling development in rats. One of the mechanisms suggested for this effect is the involvement of neurotransmitters. The inhibitory influence of noradrenaline on seizure development is well established. Noradrenaline appears to be increased in rats submitted to physical training; it has an inhibitory effect on the development of seizure, and its depletion facilitates the propagation of epileptiform activity of hippocampal kindling (Arida et al., 2008).

As said before, epilepsy plays an important role in increasing free radicals in the brain. Perhaps, the most possible mechanism to decrease seizure frequency and altitude by exercise could be related to stress-exercise relationship. Stress has been considered one of most frequent precipitants of seizures in animals with epilepsy. Today among the use of stress reduction therapies, regular physical activity has been proposed for the treatment of seizures (Mensah et al., 2003). The role of neuroactive steroids to reduce hypothalamic–pituitary–adrenal axis activation may play an important function in returning to seizure activity following exercise. Besides, this physical activity may ameliorate antioxidant mechanism damaged during epilepsy. Souza et al. (2009) suggested that the increase of antioxidant defences and reduction of basal production of oxidants elicited by physical training may protect against Na⁺, K⁺-ATPase inhibition induced by pentylentetrazole. In this, complicated mechanism opioids are said to be another pathway to cause seizures. For example, β-endorphin is significantly activated depending on the type of stress (intensive exercise). Based on information of opioid system involvement in seizure control (Hammers et al., 2007), exercise might affect seizure susceptibility also via the opioid system.

Another interesting point to be considered is the exercise-induced stress. Physical and especially psychic stress is sometimes accepted as factor(s) that precipitate seizures (Nakken et al., 2005). However, it was well established that physical activity has anticonvulsant properties in various experimental models of epilepsy, including electrically - and chemically-induced seizures tests.

Latency was also delayed by both exercise and *Crataegus oxyacantha* extract in the present study. In some studies, significant delays of the first spike have also been determined for induced seizures. Although increased latency might occur in the result of increase of threshold of first spike, many factors may have played a role in complicated pathways.

Nowadays, it is gaining attention to herbal extract due to their high level of antioxidant capacity or other properties. *Crataegus oxyacantha* extract has not been examined yet for its potential effect on epileptic activity. This is the first study that tested the effect of this extract. *Crataegus oxyacantha* extract has shown positive affect to ameliorate on some seizure parameters in this study. However, further and more advanced physiologic and neurochemical studies are required to determine the mechanisms involved. Whether exercise is helpful, harmful, or simply has no impact on seizure frequency has been studied in many epilepsy induced trials. There are favourable reports considering that some epileptic parameters are modulated by exercise. Similarly, the present study clearly demonstrated favourable effect of exercise. Results obtained here and from the other exercise studies could contribute to people's knowledge of the beneficial effects of exercise on epilepsy. Perhaps, under medical supervision, people with epilepsy should be encouraged to exercise and efforts should be made to remove any barriers to exercise

Conflict of interests: None of the authors has any conflict of interest to disclose.

Acknowledgements

This study was supported by Research Found of Abant İzzet Baysal University with the number of 2005.10.03.879.

References

1. Arida R.M.; Vieira, A.J. and Cavalheiro, E.A. (1998). Effect of physical exercise on kindling development. *Epilepsy Res.* 30: 127-132.
2. Arida R.M.; Scorza, F.A., Santos, N.F., Peres, C.A. and Cavalheiro, E.A. (1999). Effect of physical exercise on seizure occurrence in a model of temporal lobe epilepsy in rats. *Epilepsy Res.* 37: 45–52.
3. Arida, R.M.; Cavalheiro, E.A., Silva, A.C. and Scorza, F.A. (2008). Physical activity and epilepsy: proven and predicted benefits. *Sports Med*, 38: 607–615.
4. Arida R.M.; Scorza, F.A., Scorza, C.A. and Cavalheiro, E.A. (2009). Is physical activity beneficial for recovery in temporal lobe epilepsy? Evidences from animal studies. *Neurosci Biobehav Rev.*, 33: 422-431.
5. Arida, R.M.; Almeida, A.C., Cavalheiro E.A, and Scorza F.A. (2013). Experimental and clinical findings from physical exercise as complementary therapy for epilepsy. *Epilepsy Behav.* 26: 273–278.
6. Badawy R.A.; Harvey, A.S. and Macdonell, R.A. (2009). Cortical hyperexcitability and epileptogenesis: understanding the mechanisms of epilepsy–part 1. *J. Clin. Neurosci.* 16: 355–365
7. Cardenas-Rodriguez N, B Huerta-Gertrudis, L Rivera-Espinosa, Montesinos-Correa, H., Bandalac, Carmona-Aparicio, L. and Coballase-Urrutia, E. (2013). Role of oxidative stress in refractory epilepsy: evidence in patients and experimental models. *Int. J. Mol. Sci.* 14:1455–1476
8. Chang, Q. and Zuo, Z. (2002). Hawthorn. *J. of Clin. Pharma.* 42(3): 605-612.
9. Chang Q.; Zuo, Z., Ho, W.K. and Chow, M.S. (2005). Comparison of the pharmacokinetics of hawthorn phenolics in extract versus individual pure compound. *J of Clin. Pharma.* 45: 106–112.
10. Contarteze RV.; De Alencar Mota, C.S., De Oliveira, C.A., De Almeida Leme, J.A., Bottcher, L.B., De Mello, M.A. and Luciano, E. (2009). Exercise test and glucose homeostasis in rats treated with alloxan during the neonatal period or fed a high calorie diet. *J. Diabetes* 1:65 – 72.
11. Dubow J.S. and Kelly, (2003). Epilepsy in sports and recreation. *Sports Med.* 33: 499–516.

12. Eriksen H.R.; Ellertsen, B., Grønningsaeter, H., Nakken, K.O., Løyning, Y. and Ursin, H. (1994). Physical exercise in women with intractable epilepsy. *Epilepsia*. 35: 1256-1264.
13. Fountain N.B. and May, A.C. (2003). Epilepsy and athletics. *Clin. Sports Med.* 22: 605-616.
14. Guo C. (2003). Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nut. Res.* 23: 1719-1726.
15. Hammers A.; Asselin, M.C., Hinz, R., Kitchen, I., Brooks, D.J., Duncan, J.S. and Koeppe, M.J. (2007). Upregulation of opioid receptor binding following spontaneous epileptic seizures. *Brain* 130(4): 1009-1016.
16. Horyd W.; Gryziak, J., Niedzielska, K. and Zieliński, J.J. (1981). Effect of physical exertion on seizure discharges in the EEG of epilepsy patients. *Neurol Neurochir. Pol.* 15: 545-552.
17. İlhan A.; Iraz, M., Gurel, A., Armutcu, F. and Kamilsı, S. (2005). Antiepileptogenic and antioxidant effects of *Nigella sativa* oil against pentylentetrazole-induced kindling in mice. *Neuropharm.* 49: 456-64.
18. Ljubuncic P.; Portnaya, I., Cogan, U., Azaizeh, H. and Bomzon, A. (2005). Antioxidant activity of *Crataegus aronia* aqueous extract used in traditional Arab medicine in Israel. *J. Ethnopharmacol.* 101: 153-161.
19. Mc Auley J.W.; Long, L., Heise, J., Kirby, T., Buckworth, J., Pitt, C., Lehman, K.J., Moore, J.L. and Reeves, A.L. (2001). A prospective evaluation of the effects of a 12-week outpatient exercise program on clinical and behavioral outcomes in patients with epilepsy. *Epilepsy Behav.* 2: 592-600.
20. Mensah S; Beavis, J., Thapar, A. and Kerr, M.A. (2007). Community study of the presence of anxiety disorder in people with epilepsy. *Epilepsy Behav.* 11(1):118-124.
21. Nakken K.O.; Løyning, A., Løyning, T., Gløersen, G. and Larsson, P.G. (1997). Does physical exercise influence the occurrence of epileptiform EEG discharges in children? *Epilepsia* 38: 279-284.
22. Nakken K.O.; Solaas, M.H., Kjeldsen, M.J., Friis, M.L., Pellock, J.M. and Corey, L.A. (2005). Which seizure-precipitating factors do patients with epilepsy most frequently report? *Epilepsy Behav.* 6: 85-89.
23. Park E.; Chan, O., Li, Q., Kiraly, M., Matthews, S.G., Vranic M. and Riddell, M.C. (2005). Changes in basal hypothalamo-pituitary adrenal activity during exercise training are centrally mediated. *Am J Physiol Regul Integr Comp Physiol*, 289(5): 1360-1371.
24. Pietrelli A.; Lopez-Costa, J. and Goni, R. (2012). Aerobic exercise prevents age-dependent cognitive decline and reduces anxiety-related behaviors in middle-aged and old rats. *Neuroscience* 202:252-266.
25. Souza M.A.; Oliveira, M.S., Furian, A.F., Rambo, L.M., Ribeiro, L.R., Lima, F.D., Dalla Corte, L.C., Silva, L.F., Retamoso, L.T., Dalla Corte, C.L., Puntel, G.O., De Avila, D.S., Soares, F.A., Figuera M.R., De Mello, C.F. and Royes, L.F. (2009). Swimming training prevents pentylentetrazol-induced inhibition of Na⁺, K⁺, -ATPase activity, seizures, and oxidative stress. *Epilepsia* 50: 811-823.
26. Theodore W.H. and Fisher, R. (2007). Brain stimulation for epilepsy. *Acta Neurochir Supp.* 97(2): 261-272.
27. Tsuda A.; Ito, M., Kishi, K., Shiraishi, H., Tsuda, H. and Mori, C. (1994). Effect of penicillin on GABA_A gated chloride ion influx. *Neurochem Res*, 19: 1-4.
28. Tutkun E.; Ayyildiz, M. and Agar, E. (2010). Short-duration swimming exercise decrease penicillin-induced epileptiform ECoG activity in rats. *Acta Neurobiol. Exp.* 70: 382-389.
29. Wang J.; Xiong, X. and Feng, B. (2013). Effect of *Crataegus* usage in cardiovascular disease prevention: an evidence-based approach. *Evid Based Complement Alternat. Med.*, 2013: 1-16.
30. Zapfe Jun G. (2001). Clinical efficacy of *crataegus* extract WS 1442 in congestive heart failure NYHA class II. *Phytomedicine* 8: 262-266.
31. Zhang Z. (2000). Characterisation of antioxidants present in Hawthorn fruits. *J. Nutr. Biol.* 12: 144-152.