The Role of Grape Seed Extract in the Effect of Swimming Exercises on Epilepsy

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Abstract: Grape seed extract (GSE) is one of the strongest known antioxidants. GSE actively prevents the formation of free radicals and assists in their elimination. Our aim was to investigate the effects on epileptiform activity of GSE administered concurrently with swimming exercises. A total of 35 male albino Wistar rats were used in this study. Epileptiform activity was induced in rats through the injection of penicillin (500 IU) into the left cerebral cortex. Thirty minutes after the application of penicillin, 200 mg/kg of GSE dissolved in normal saline was administered intraperitoneally. Based on the results of the statistical analysis, a significant decrease in spike frequency was observed after 60 minutes in the 15-minute group (67%), after 40 minutes in the 30-minute group (43%), and after 40 minutes in the 60-minute group (42%), while no significant decrease was identified in the amplitude values of the groups. According to the study results, in rats performing short-, medium-, and long-term swimming exercises, GSE administration allowed epileptiform activity to decrease within a shorter period of time. Thus, patients with epilepsy can potentially perform swimming exercises more safely by regularly using antioxidant substances.

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1. Introduction

Antioxidants are molecules, which through their reactions with free radicals, inhibit or eliminate the chain reactions that lead to the formation of free radicals, thus preventing vital compounds within our bodies from becoming damaged (Kiyici and Kishali, 2010). Antioxidants are known to have corrective effects on neurodegenerative diseases as well as free radicals formed during exercise. Antioxidant substances such as α-tocopherol (vitamin E), β-carotene, ascorbic acid (vitamin C), and folic acid prevent oxidation-induced cellular damage by preventing the formation of active oxygen, or by scavenging any active oxygen that is formed. These substances are thus capable of suppressing the development of degenerative diseases as well (Smith et al., 1995; Cooper and Storer, 2002; Sivritepe, 2002).Grape seed extract (GSE) is one of the strongest known antioxidants. The antioxidant effect of GSE is approximately fifty times greater than that of vitamin C and vitamin E (Belviranli et al., 2012). Grapes are members of the Vitaceae family, and the most important phenolic compounds that are found in them are phenolic acids, anthocyanidins, flavonols, glycosides, cinnamic acid derivatives, catechins and proanthocyanidins (Uylaser and Ince, 2008; Balu et al., 2006; Devi et al., 2006; Shao et al., 2009; Feng et al., 2005).

Regular training increases the effectiveness of the endogenous antioxidant mechanisms so that even extreme exercise (long-distance triathlon) may not cause any indications of oxidative damage in well-trained athletes (Margaritis et al., 1997). In contrast, short periods of modest exercise (8 weeks of training: 3 sessions of 35 min/week) do not result in any signs of increased capacity to neutralize free radicals (Tiidus et al., 1996). During the exercises the need for antioxidants and vitamins increase due to the reduction in oxidative stress (Cases et al., 2006). Physical activities give way to some changes in enzymatic antioxidant activity or non-enzymatic antioxidant activity in blood and tissue increases in both human and animals (Clarkson, 1995; Ji, 1993; Leeuwenburgh et al., 1997).

Many researchers have changed their views and realized that physical activity does not affect seizure frequency and may, in fact, protect against seizures. In spite of the positive results in most studies, some contradictory findings have also been observed. The positive effects of exercise in epilepsy are mostly based on questionnaires and/or clinical studies (Roth et al., 1994; Steinhoff et al., 1996) and through

standardized tests of physical endurance (Steinhoff et al., 1996; Jalava et al., 1997).

Some studies further evaluated the impact of antioxidant effects on performance or endurance in rats; the results, however, differed largely depending on the type of supplementation or protocols. Per (2010) administered GSE to rats at different doses, and determined that 200 mg/kg GSE was an effective dose. It was also determined that rats with penicillin-induced epilepsy demonstrated a decreased frequency of penicillin-associated epileptic activity when administered with Vitamin E at 500 mg/kg and Vitamin C at 100 mg/kg (Ayyildiz, et al., 2006; Ayyildiz et al., 2007). In Tutkun et al.'s (2010) study, where rats were subjected to swimming exercises for different periods of time (15, 30, and 60 minutes) for 90 days, a significant decrease in spike frequency in comparison to the control group was identified only among rats in the 15-minute swimming group.

In the current study, we investigated, for the first time in the literature, the changes induced by GSE, a strong antioxidant, on the effects of 15-, 30-, and 60-minute swimming exercises in epileptic activity. To this end, we administered 200 mg/kg GSE every other day by gavage to rats performing daily swimming exercises of 15, 30, and 60 minutes for a period of three months, and investigated how this affected epileptiform activity.

2. Material and Methods

Adult male Wistar rats weighing 180–220 g (Ondokuz Mayis University, Turkey) were used throughout this study after at least one week of acclimatization. All described procedures were approved by the local ethics committee. Animals were housed in groups of 3–4 and were allowed free access to food and water, except for the short time that the animals were taken out of their cages for the experiments. All animals were kept in a temperature controlled (22±1ºC) environment on a 12-h light/dark cycle. Rats were assigned into one of the following experiments and groups: control (Group 1); 15 minutes-trained for 90 days + 200 mg/ kg GSE (Group 2); 30 minutes-trained for 90 days + 200 mg/ kg GSE (Group 3); 60 minutes- trained for 90 days + 200 mg/ kg GSE (Group 4);adapted to the water (sham) (Group 5). Each group was consisted of seven rats.

2.1. Adaptation to the water

All animals were adapted to the water before the beginning of the experiment. The rats were kept in shallow water at 32ºC for seven days/week, from 10:00 A.M. to 12:00 A.M. for adaptation. The adaptation to the water proceeded further during the experimental period. The purpose of the adaptation to water was to reduce stress without promoting a physical training adaptation (Souza et al., 2009).

2.2. Exercise training program

Training period lasted for 90 days and consisted of daily sessions of 15, 30, and 60 minutes for seven days/week without workload. Exercise performed by swimming in two training glass tanks (100x50x50 cm LxWxH) containing tap water. GSE (200 mg/kg) was given to rats, every other day by using gavage as the route of exposure. After 90 days swimming training, rats were prepared for induction of epileptiform activity in the next training session.

2.3. Induction of epileptiform activity

The animals were anesthetized with urethane (1.25 g/kg, i.p.) and placed in a stereotaxic frame. Rectal temperature was maintained between 36 ºC and 37 ºC by using a feedback-controlled heating system. A polyethylene cannula was introduced into the right femoral artery to monitor blood pressure, which was kept above 110 mmHg during the experiments. All contact and incision points were infiltrated with procaine hydrochloride to minimize possible sources of pain. The left cerebral cortex was exposed by craniotomy (5 mm posterior to the bregma and 3 mm lateral to the sagittal sutures). The epileptic focus was produced by 500 units of penicillin G potassium injection (1 mm beneath the brain surface by a Hamilton microsyringe type 701N; infusion rate 0.5 µl/min) (Kozan et al., 2009). Penicillin was prepared in the sterile distilled water and administered intracortically in a volume of 2.5 µl.

2.4. Electrocorticography recordings

Two Ag–AgCl ball electrodes were placed over the left somatomotor cortex (electrode coordinates: first electrode, 2 mm lateral to the sagittal suture and 1 mm anterior to the bregma; second electrode, 2 mm lateral to the sagittal suture 5 mm posterior to the bregma). The common reference electrode was fixed on the pinna. The electrocorticography electrodes (ECoG) activity was continuously monitored on a four-channel recorder (powerLab, 4/SP, AD Instruments, Castle Hill, Australia). All recordings were made under anesthesia and stored on a computer. The frequency and amplitude of epileptiform ECoG activity was analyzed off-line.

2.5. Data analysis

The results are given as the means±standard error of the mean (SEM). Statistical differences between all groups were first checked by the Kruskal-Wallis nonparametric ANOVA test. After confirmation of significance, statistical differences between groups were tested by the non-parametric Mann-Whitney U test with the Bonferroni adjustment. The level of significance was corrected according to Bonferroni in multiple comparisons among four groups ($p<.05/4 = .0125$). Statistical analyses were performed by using SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA).

3. Results

In the current study, the effect of single-dose GSE on penicillin-induced epileptiform activity was investigated in rats subjected to swimming exercises for 90 days and administered with 200 mg/kg GSE every other day.

Thirty minutes after the application of penicillin, GSE was injected intraperitoneally at a dose of 200 mg/kg. In the 15-minute group, a significant decrease in comparison to the control group was observed in the spike frequency percentage of epileptiform activity at minute 60 following the administration of 200 mg/kg GSE. No significant decrease was identified in the change in amplitude percentage (78.1±13.9 µV).

In the 30-minute group, a significant decrease in comparison to the control group was observed in the spike frequency percentage of epileptiform activity at minute 40 following the administration of 200 mg/kg GSE; and by the end of the recording period ($p<.05$; Figure 1), a spike change of 43% was identified after minute 40 (Figure 1). No significant decrease was identified in the change in amplitude percentage (86.6±5.2 µV).

In the 60-minute group, a significant decrease in comparison to the control group was observed in the spike frequency percentage of epileptiform activity at minute 40 following the administration of 200 mg/kg GSE; and by the end of the recording period ($p<.05$; Figure 1), a spike change percentage of 42% was identified after minute 40 (Figure 1). No significant decrease was identified in the change in amplitude percentage (81.4±9.3 µV).

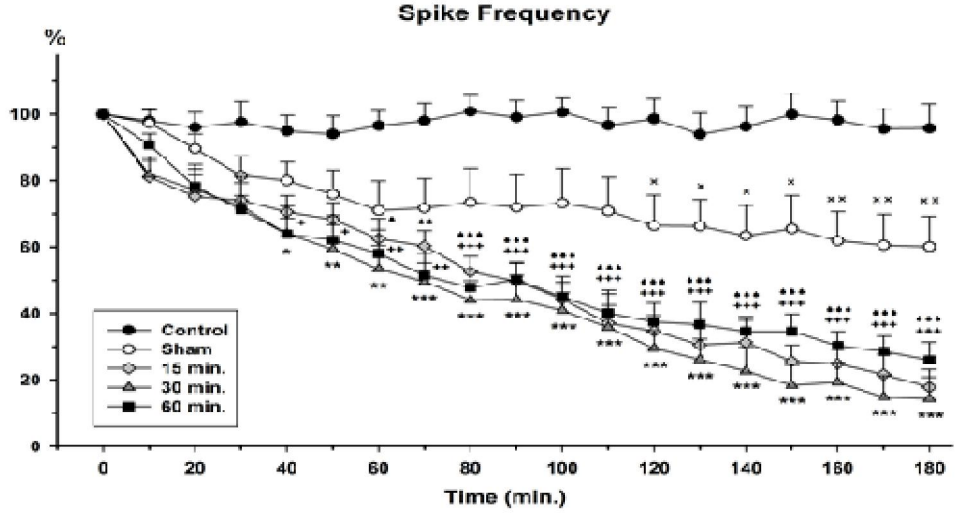

Figure 1. The effects of swimming exercises on the mean spike frequency of penicillin-induced epileptiform ECoG activity.

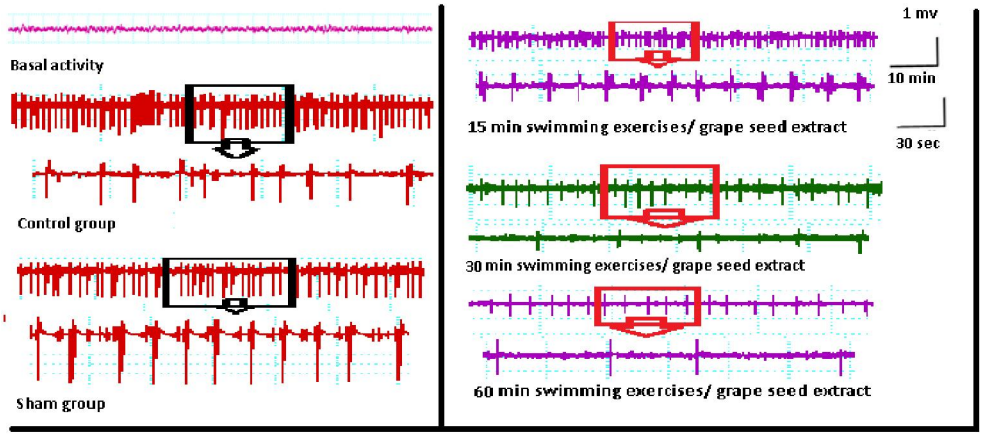

Figure 2. Average frequency of 200 mg/kg GSE after penicillin in 110 min. **a.** Basal activity prior to the injection of any substance into the cortex. **b.** Induction of epileptiform activity in the cortex following the injection of penicillin (50 IU i.c.). **c.** The decrease in the mean frequency of epileptiform activity after the injection of 200mg/kg GSE i.p. **d.** The decrease in the mean frequency of epileptiform activity following the injection of 200 mg/kg GSE i.p. **e.** The decrease in the mean frequency of epileptiform activity following the injection of 200 mg/kg GSE i.p.

The mean frequency of ECoG epileptiform activity significantly decreased in the 60th minute after GSE (200 kg/mg) application in the 15-minute swimming training group. The mean frequency of ECoG epileptiform activity significantly decreased 40 min after GSE (200 kg/mg) application in the 30-minute and 60-minute swimming training groups ($p<.05$).

The percentage frequency of epileptiform ECoG activity value (%FV) depends on both the frequency of epileptiform ECoG activity before and after 30 minutes as it is defined as:

$$\%FV=(MSF_{A30}/MSF_{B30}) \times 100$$

where MSF_{A30} is the he mean spike frequency after 30 minutes and MSF_{B30} is the mean spike frequency in 30 minutes.

4. Discussion

In our study, the effect of GSE on the epileptiform activity of rats subjected to swimming exercises was investigated. Although numerous clinical and experimental studies have demonstrated the effects of antioxidants and exercise in decreasing epileptiform activity, no studies regarding the concurrent application of GSE and swimming exercises of varying durations was encountered in the literature.

In this study, epileptiform activity was evaluated through electrophysiological methods in rats that exercised for a period of 90 days, and received 200 mg/kg of GSE through gavage every other day. Following the induction of epileptiform activity with penicillin in the group subjected to 15-minute swimming exercises, a single dose GSE (200 mg/kg, i.p.) was administered. A significant decrease (67%) was then identified in the frequency of epileptiform activity at minute 60 after GSE administration. In the 30- and 60-minute swimming exercise groups, on the other hand, significant decreases in the frequency of epileptiform activity were identified at minute 40 after GSE administration (43% and 42%, respectively).

There are various studies demonstrating the effectiveness of exercise in preventing epilepsy. Within the context of epilepsy attacks induced after long-term running exercises, Arida et al. (1999-b) identified a significant decrease in the epileptic attacks of groups that exercised.

In their study, Tutkun et al. (2010) determined that only short-term (15-minute) swimming exercises led to a decrease in penicillin-induced epileptiform activity, while medium- (30-minute) and long-term (60-minute) exercises had no effect on either the frequency or the amplitude of the induced epileptiform activity. In our study, it was determined that GSE administration also led to a significant decrease in the frequency of penicillin-induced epileptiform activity in rats subjected to medium- and long-term swimming exercises. These data clearly illustrate that GSE is able to scavenge the free radicals caused by medium- and long-term exercise.

In a study demonstrating the strong antioxidant effect of grapes, Per (2010) administered GSE to rats at different doses (50, 100, 200, and 400 mg/kg) 30 minutes after the induction of epileptiform activity with penicillin, and determined that 200 mg/kg was the most effective dose. In Per's (2010) study, a significant decrease in epileptiform activity in comparison to the control group was identified at minute 80 following the administration of 50 mg/kg i.p. GSE (with a 49.03% decrease in activity); at minute 40 following the administration of 100 mg/kg i.p. GSE (with a 44.06% decrease in activity); and at minute 20 following the administration of 200 mg/kg i.p. GSE (with a 36.21% decrease in activity). In the group administered with 400 mg/kg i.p. GSE 30 minutes after the administration of penicillin, no significant decrease was identified in comparison to the control group in the frequency or amplitude of epileptiform activity until the end of the experiment (mean spike frequency 29±2 spike/min, amplitude 1007±193 μV). The results obtained in the current study are consistent with Per's (2010) findings regarding the decrease in epileptiform activity caused by GSE use.

Arida et al. (1999-a,b) identified that exercise had beneficial effects during the epileptogenesis period. They applied the pilocarpine epilepsy model in their study, and staged three separate experimental periods of 45 days each. During the first period, the number of epilepsy attacks experienced by rats prior to any exercise was recorded. During the second period, the number of epilepsy attacks that occurred after the rats were subjected to an exercise program of 10 minutes every seven days of the week at 12 m/min speed, 0 inclination and 60% VO_2max. In the last period, the frequency of epilepsy attacks following the exercise period was recorded. At the end of the study, a significant decrease was identified in the epilepsy frequency of rats that were subjected to exercise.

In their study, Kiran et al. (2004) subjected rats to 20- and 40-minute swimming exercises every day at low, medium, and high intensities (with a 2%, 3%, and 5% addition to their body weight during the exercise, respectively). The exercise program was continued for a period of four months. At the end of the study, it was determined that rats performing low intensity exercises for 20 minutes every day benefited from a stronger protective effect against oxidative stress than rats performing medium and high intensity exercises.

Feng et al. (2005) pathologically investigated the effects of 50 mg/kg i.p. GSE, administered every two days to newborn rats 5 minutes before hypoxia and 4 hours after reoxygenation, on the loss of weight in the right cerebral hemisphere for a period of 22 days. At the end of their study, they determined that GSE significantly reduced the loss of cerebral weight in newborn rats caused by lipid peroxidation and hypoxic damage ($p < .05$).

In their study investigating the effects of GSE on oxidative stress induced by cisplatin in rats, Yousef et al. (2009) determined that cisplatin led to oxidative stress in the plasma, heart, kidneys, and liver, and that GSE reduced the oxidative damage that resulted.

There are also studies demonstrating that the administration of 400 mg/kg vitamin C (another type of antioxidant) to rats reduced lipid peroxidation and free radical formation; that exercise increases the formation of free radicals in rat heart tissue; and that the presence of free radicals in heart tissue was reduced significantly through the supplementation of vitamin E during rest and after exercise (Kanter,1998).

Furthermore, Ayyildiz et al. (2006) determined that the administration of 500 mg/kg vitamin E to rats with epilepsy reduced the frequency of penicillin-associated epileptic activity. Ayyildiz et al. (2007) also identified that 100 mg/kg was the most effective vitamin C dose for reducing epileptic activity 30 minutes after penicillin application, and that doses of 50, 100, 200, and 400 mg/kg significantly reduced the frequency of epileptiform activity while causing no changes in amplitude.

5. Conclusion

GSE use has the effect of reducing epileptic activity in rats performing medium and long-term swimming exercises. It can be seen that GSE has the potential to prevent epileptic activity by preventing oxidative processes that occurs during exercise, and that epileptic patients can potentially swim and perform other exercises with greater safety after taking antioxidants such as GSE.

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