Effects of *Cornus mas* L. and *Morus rubra* L. extracts on penicillin-induced epileptiform activity: an electrophysiological and biochemical study#

 # This study was conducted at Erciyes University

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Traditionally, *Morus rubra* L. (Moraceae) (red mulberry) and *Cornus mas* L. (Cornacea) (cornelian cherry) fruits are eaten fresh and are also used in marmalades, juices, jam, natural dyes in Turkey and are believed to have beneficial effects in case of multiple health issues such as antipyretic, diarrhea and intestinal parasites. However, the effects of *M. rubra* and *C. mas* on epilepsy has not been known. This study evaluates the effects of *M. rubra* and *C. mas* extracts on penicillin-induced epileptiform activity. Sixty Wistar rats randomly divided into ten groups (n=6): control, sham, penicillin, penicillin+*M. rubra* extract (2.5, 5, 10, 20 mg/kg) and penicillin+*C. mas* extract (2.5, 5, 10 mg/kg). Epileptiform activity was induced by using penicillin (500 IU, i.c.) and electrocorticogram records (150 min) were obtained. Also, biochemical analysis in blood samples were evaluated. According to the electrocorticogram analysis, the effective dose was detected as 10 mg/kg for both *C. mas* and *M. rubra*. This dose decreased the spike frequencies of convulsions while amplitude wasn't changed by both substances. In erythrocyte studies, there were significant differences regarding nitric oxide in the control, sham and penicillin groups. There were significant differences regarding malondialdehyde in all groups. In the plasma, there were significant differences among groups regarding xanthine oxidase in the penicillin-*C. mas* and penicillin-*M. rubra* groups. There were differences regarding malondialdehyde in the penicillin-*C. mas* and *M. rubra-C. mas* groups. Both extracts reduced the frequency of epileptiform activity. After administration of the extracts malondialdehyde levels decreased also in both erythrocytes and plasma.

Key words: cornelian cherry, red mulberry, antioxidant, biochemistry, experimental epilepsy

INTRODUCTION

Epilepsy is one of the most common neurologic diseases that has detrimental and physical effects. The prevalence of the disease is known to be 0.5–1%. It has been shown in experimental studies that some materials have antiepileptic effects but they could not be administered to humans. The development of new molecules and antiepileptic drugs for the treatment of epilepsy is important for the mortality and morbidity of this disease (Devi et al. 2008).

The underlying pathophysiological mechanisms in epilepsy are still unclear. No benefit is established in 1/3 of epileptic episodes in patients receiving treatment with appropriate antiepileptic drugs, and it has been impossible to prevent progressive epileptogenic alteration (Cockerell 1996). Novel and safe anticonvulsant drugs are therefore needed for the treatment of this disease. Studies show that oxidative stress plays a role in the pathophysiology of epilepsy (Bruce and Baudry 1995, Devi et al. 2008, Eraković et al. 2001, Rauca et al. 1999, Ilhan et al. 2005, Royes et al. 2007, Sudha et al. 2001). Recently, the high levels of 4-hydroxy-2-nonenal protein adducts, a by-product of lipid peroxidation, and the activation of NADPH oxidase 2 was demonstrated in the surgically resected epileptic hippocampus of drug-resistant patients. Furthermore,

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negativity for aquaporin-4 was observed in neurons in degeneration. The authors suggest that seizure induces oxidative damage as well as neuronal loss, thereby promoting neuronal hyperexcitability, also affecting the water and ion balance by AQP4 modulation, and thus generating a vicious cycle (Pecorelli 2015).

Malondialdehyde (MDA) is considered as the sign of lipid peroxidation, because it is the end product of membrane fatty acid peroxidation. Nitric oxide (NO) is also one of the most abundant free radicals in the body, and its rapid reaction with other free radicals, such as superoxide, generates a highly reactive molecule, namely the peroxynitrite anion. It is able to induce lipid oxidation and nitration of proteins, and this leads to neuronal toxicity and epileptic attack (Beckman et al. 1990, Dawson et al. 1991, Nowicki 1991).

Flavonoids are polyphenolic compounds with known antioxidant properties and they are found in herbal teas, fruits and vegetables (Asano et al. 2001, Seeram 2002). Some studies report that flavonoids can inhibit enzymes such as xanthine oxidase (XO), glutathione reductase, NADH-oxidase and protein kinase (Elliott et al. 1992, Hodnick et al. 1987, Lin et al. 2015).

Some studies indicate that *Cornus mas* L. (Fam: Cornaceae) (cornelian cherry) and *Morus rubra* L. (Fam: Moraceae) (red mulberry) are rich in phenolic compounds (Asano et al. 2001, Seeram 2002) and their extract is used against health problems in Turkey (Fakir et al. 2009). Cornelian cherry fruits have high levels of natural antioxidants such as ascorbic acid, anthocyanin, and phenolic contents (Yilmaz et al. 2009a). The protective effect of cornelian cherry fruit was demonstrated in the process of oxidation of proteins and lipoproteins by decreasing the levels of protein carbonyl and thiol groups and increasing the activity of paraoxonase-1 in the brain tissue of rats (Francik et al. 2014).

Therefore, these compounds have been considered as promising candidates as potential protectors against lipid and protein oxidation and as neuroprotective agents of the brain or as potential supplements for glutamate excitotoxicity related neurologic disorders such as epilepsy (Per et al. 2013).

Experimental epilepsy models can be performed by using convulsant drugs, GABAergic antagonists, neurotoxins, genetic and lesion applications (Xu et al. 2016). The penicillin-induced epilepsy model is one of the commonest models used to cause epilepsy. Antioxidants have been shown to have a reducing effect on the severity and frequency of seizures in many experimental models (Tutkun et al. 2015).

There is no research about the effects of red mulberry and cornelian cherry on epilepsy in the literature. In this study, epileptic activity was induced by penicillin and then cornelian cherry and red mulberry extracts were given intraperitoneally to Wistar rats. After these procedures the enzymatic antioxidants which are superoxide dismutase (SOD), catalase (CAT) and NO, the oxidant stress indicators XO and MDA and non-enzymatic antioxidants, namely flavonoids and phenolic compounds were evaluated. This study evaluates the role of enzymatic antioxidants, oxidants and non-enzymatic antioxidants on epilepsy.

METHODS

In this study, randomized controlled experimental studies were done with the aim of evaluating the effects of *Cornus mas* (cornelian cherry) and *Morus rubra* (red mulberry) extracts on epileptiform activity and oxidative stress (nitric oxide, superoxide dismutase, catalase, xanthine oxidase and MDA).

Ethics statement

Animal care and use were conducted in accordance with the Animal Research Institute Committee guidelines of Erciyes University, Turkey. This study was specifically approved by the Scientific Research Projects Unit (Project code: TSU-12-3901) of Erciyes University, Turkey.

Animals

In this study, 60 male (8–12 weeks old, selected by the random sampling method) Wistar rats weighing 225–260 g were used. The rats were kept at a constant temperature (18–26°C) in a 12 h light/dark cycle with unrestricted access to food and water. Ten days before the studies started the rats were transferred into Erciyes University, Animal Physiology Research Laboratory which is located in the Biology Department of the Faculty of Science.

Plant material

The "44-01" type of cornelian cherry (*Cornus mas* L.) and "R7 and R8" types of mulberry (*Morus rubra* L.) species were harvested when the fruits were fully ripe (in August 2012). Each type of fruit (1 kg) was collected and brought to the laboratory in an ice-box. After cleaning by washing (cornelian cherry washed after the stones were removed), the fruit samples were ground with a juice mill until a heavy viscous juice was obtained. The juices were maintained at –80°C until analysis. Studies have shown that *Cornus mas* and *Morus rubra* has been shown to cross the blood brain barrier (Francik et al. 2014, Lee et al. 2015, Ming et al. 2011).

Determination of phenolic compounds of fruits

Extract of Cornus mas

Gallic acid was used as a standard and the results were expressed as mg gallic acid equivalents per g dry weight basis. The total antioxidant capacity of samples was determined by hydrogen atom transfer reactions (β-carotene bleaching assay) and assay based on electron transfer (ferric reducing antioxidant power, FRAP assay). According to this study in which ascorbic acid content and total anthocyanidin equal to that found in 100 g fruit was given in mg, the 44-01 genotype contained 65.73 mg of gallic acid equivalents/gram fruit weight (GAE/g FW) as phenolic content and caused 88.9% β-carotene bleaching assay. L-Ascorbic acid standard solution/gram dry weight fruit was calculated as 109 micromole (μmol) in FRAP analysis. The ascorbic acid content was 37 mg/100 g. Total anthocyanidins were found to be 172 mg for 100 g of fruit weight. The total phenolic content was calculated based on this study (Yilmaz et al. 2009a).

Extract of Morus rubra

In the studies conducted on red mulberry, the total phenolic content calculated for R7 was 1005±87 mg GAE/g FW and for R8 it was 2388±87 mg GAE/g FW. The total monomeric anthocyanin content (TMA) for R7 was found as 3±1 and for R8 it was 200±5 mg cyanidin-3-glucoside/equivalents of fw. The trolox equivalent antioxidant capacity (TEAC) calculated for R7 was 5.1±1.6 and for R8 it was 7.1±0.2 mg (EC_{50}). The FRAP value for R7 was 3.7±0.2 and for R8 was 6.7±1.1 μm for m TE/g FW. The total phenolic content was calculated based on this study (Dugo et al. 2001, Yilmaz et al. 2009b).

Procedure

Surgical procedures

The animals were anesthetized with urethane (1.25 g/kg, i.p.) and placed in a stereotaxic frame. Rectal temperature was maintained between 36.0°C and 37.0°C using a feedback controlled heating system. The left cerebral cortex was exposed by craniotomy (5 mm anterior and posterior to bregma and 3 mm lateral to sagittal sutures). Two Ag-AgCl ball electrodes were placed over the left somatomotor cortex.

Induction of epileptiform activity

The epileptic focus was produced by a 500 IU penicillin G potassium injection (1 mm beneath the brain surface by a Hamilton microsyringe type 701N, (22 s ga, bevel tip); infusion rate 0.5 μl/min). Epileptiform activity was observed after 2–4 min. Epileptiform activity or spike that has been defined as highly synchronized bursting activity with clear trains of voltage fluctuations and persistent for at least 2 seconds.

Test groups

The experimental groups below were created to show the relationship between red mulberry and cornelian cherry extracts and epilepsy. After creating epileptic activity, the effective dose to reduce epileptic activity was found. SOD, NO, CAT, XO, and MDA values were evaluated in the groups. The rats were assigned to the following experimental groups:

Group 1. Control group (n=6): minimum 150 min was recorded after opening the cerebral cortex without any item given. After, blood samples were taken.

Group 2. Sham group (n=6): surgical stress was applied *via* intraperitoneal SF (serum physiological) after opening the cerebral cortex. Electrophysiological recording was performed for 150 min.

Group 3. Penicillin group (n=6): minimum 150 min was recorded after 500 IU (2.5 μL i.c) penicillin was applied intracortically. Blood samples were taken. This group assigned as control group for the evaluation of epileptiform activity for the other groups.

According to this, basal activity records were done without injection of any substance. In the second group, it was seen that intraperitoneally serum physiological injection (2.5 μl, i.c) had no significant effect on brain activity. After injection of the penicillin (500 IU, i.c) epileptiform activity occurred after 2–4 min and epileptiform activity was determined to become stable after 30 min.

According to findings obtained in the first stage, in the second stage of the research, *Cornus mas* doses of 2.5, 5, 10 mg and *Morus rubra* doses of 2.5, 5, 10 and 20 mg was applied after 30 min penicillin (500 IU) administered.

Group 4. Penicillin+2.5 mg/kg red mulberry extract group (n=6): minimum 120 min was recorded 30 min after the administration of 500 IU penicillin intracortically and then 2.5 mg/kg red mulberry extract was given intraperitoneally. After, blood samples were taken.

Group 5. Penicillin+5 mg/kg red mulberry extract group (n=6): minimum 120 min was recorded 30 min after the administration of 500 IU penicillin intracortically and then 5 mg/kg red mulberry extract was given intraperitoneally. After, blood samples were taken.

Group 6. Penicillin+10 mg/kg red mulberry extract group (n=6): minimum 120 min was recorded 30 min after the administration of 500 IU penicillin intracortically and then 10 mg/kg red mulberry extract was given intraperitoneally. After, blood samples were taken.

Group 7. Penicillin+20 mg/kg red mulberry extract group (n=6): minimum 120 min was recorded 30 min after the administration of 500 IU penicillin intracortically and then 20 mg/kg red mulberry extract was given intraperitoneally. After, blood samples were taken.

Group 8. Penicillin and 2.5 mg/kg of cornelian cherry extract group (n=6): minimum 120 min was recorded 30 min after the administration of 500 IU penicillin intracortically and then 2.5 mg/kg cornelian cherry extract was given intraperitoneally. After, blood samples were taken.

Group 9. Penicillin+5 mg/kg cornelian cherry extract group (n=6): minimum 120 min was recorded 30 min after the administration of 500 IU penicillin intracortically and then 5 mg/kg cornelian cherry extract was given intraperitoneally. After, blood samples were taken.

Group 10. Penicillin+10 mg/kg of cornelian cherry extract group (n=6): minimum 120 min was recorded 30 min after the administration of 500 IU penicillin intracortically and then 10 mg/kg cornelian cherry extract was given intraperitoneally. After, blood samples were taken.

Data collection tools

Electrophysiological recordings

After the cortex was exposed, the animals were fixed in a stereotaxic apparatus. 37°C vaseline pool was created by 4 scissors from 4 corners with the scalp of the animals to prevent loss of liquid from the brain and other tissues, and interference records, maintain the temperature. The body temperature of the animal was maintained at 37°C with a homeothermic blanket (Harvard Instrument). For electrophysiological recordings, two silver (Ag)/silver chloride (AgCl) ball electrodes were used. The positive ball electrode was placed 1 mm anterior to bregma and 2 mm lateral to the sagittal suture. The negative one was placed 5 mm posterior to bregma and 2 mm lateral to the sagittal suture. For grounding,

an Ag/AgCl clamp was fixed to the right auricle by rubbing the electrode with recording gel. All EEG signals were filtered. The recording parameters were as follows: 0.3–100 Hz low and high frequency filter, 50 Hz notch filter.

Activity received through the electrodes that was boosted in the Bioamp (ADInstruments, Australia) amplifier was then immediately transferred to the PowerLab 16/SP (ADInstruments, Australia) data acquisition unit. Analog signals obtained from the cortex with PowerLab were then converted into numerical data and transferred to the computer. Brain activity Chart v7.0 (ADInstruments, Australia) software was used and data displayed on the computer were recorded for post-test analysis. All experimental procedures are shown in the Fig. 1.

Biochemical samples

Blood samples were taken from heart of the rat because a large amount of blood is needed for biochemical analyses so it was taken at the end of the procedure. During electrophysiological recordings, whole blood samples were taken from the groups and placed into citrated tubes. Blood samples were taken from the sham and control groups. From the penicillin group, blood samples were taken after obtaining stable spike amplitude and frequency level. During recordings, after determining the effective dose with electrocorticogram, when a significant difference was determined in the spike frequency and amplitude of convulsions, blood samples were taken. After completing the process, subjects underwent cervical dislocation. Whole blood samples were centrifuged for 10 min at 4°C at 1000 rpm. Plasma was pipetted at the top. Afterwards, erythrocytes were washed twice with saline. Erythrocyte samples were stored at -80°C. In order to assess lipid peroxidation in the plasma and erythrocytes (for plasma nmol/ml for erythrocytes nmol/gHgb) MDA levels were measured. MDA evaluation was performed using the Immunodiagnostic brand kit (Catalog No. KC1900) HPLC technique. In order to assess oxidative stress, NO, CAT, XO
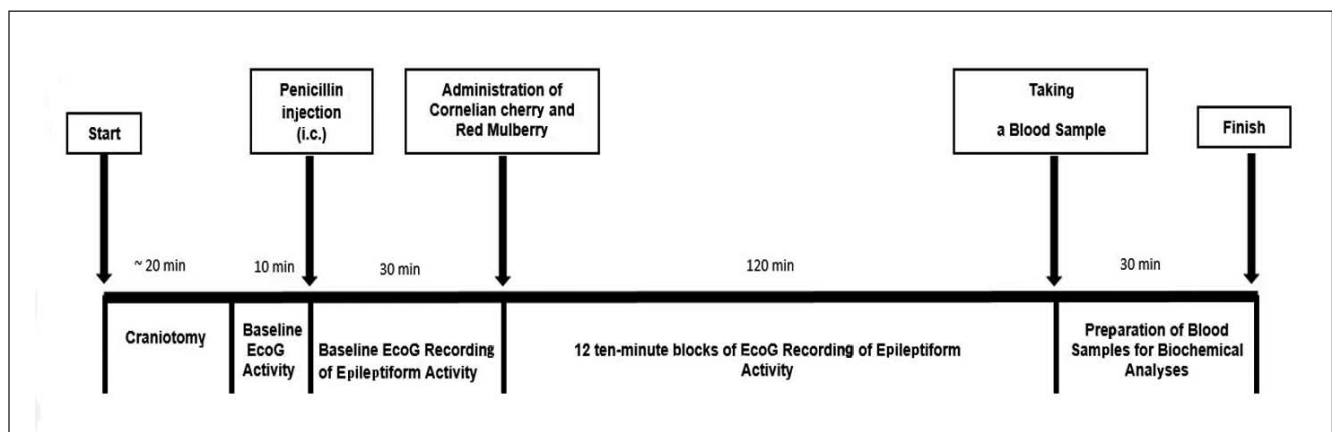

Fig. 1. The time scheme of experimental procedures.

and SOD levels were measured. NO (mol/L) was measured by the ELISA technique in the erythrocytes and plasma with a CAYMAN brand kit (Catalog No. 780001). XO [microunit (μU)/ml] was measured by the ELISA technique in the erythrocytes and plasma with CAYMAN brand kit (Catalog No. 10010895). SOD (U/mL) was measured in red blood cells and plasma by the ELISA technique with a CAYMAN brand kit (Catalog No. 706002). CAT (nmol/min/ml) was measured by the ELISA technique in the erythrocytes and plasma with a CAYMAN brand kit (Catalog No. 707002).

Statistical analysis

All statistical analyses were performed using the IBM SPSS Statistics 22.0 package program (IBM Corp., Armonk, New York, USA). Data are expressed as frequencies, mean ±standard deviation and median (min–max). Shapiro-Wilk's test was used and a histogram and q-q plot were examined to assess the data normality. Levene's test was used to assess the variance homogeneity. A two-sided one-way analysis of variance was applied to compare the differences between groups for continuous variables and Tukey tests were applied for multiple comparisons when data were normal. When data weren't normal the Kruskal-Wallis test was applied to compare the differences between groups and Mann-Whitney U test with Bonferroni Correction was applied for multiple comparisons. The value of $P<0.05$ denoted statistical significance.

RESULTS

Evaluation of biochemical results

Results of erythrocytes studies

The values in Table I show the levels of XO, CAT, MDA and SOD in erythrocytes. No significant differences were

determined in the statistical analysis of the results for XO, CAT and SOD in erythrocytes ($P=0.087$, $P=0.134$, $P=0.835$ respectively).

The values in Fig. 2A show the levels of NO in erythrocytes. There was a statistically significant difference between the control group and the sham group in terms of NO ($P=0.001$). The mean value in the control group was 101.233 μmol/L, while in the sham group it was 67.626 μmol/L. There was a significant difference between the penicillin group and the control group ($P=0.008$). The mean value in the penicillin group was 52.313 μmol/L. There was no statistically significant difference between the penicillin-red mulberry and penicillin-cornelian cherry groups ($P>0.05$). The mean of the red mulberry group was 48.763 μmol/L, while the mean of the cornelian cherry group was 52.317 μmol/L.

The values in Table I show the levels of MDA in erythrocytes. There were statistically significant differences among the sham-control ($P<0.001$), penicillin-control ($P<0.001$), penicillin-sham ($P<0.001$), penicillin-cornelian cherry ($P<0.001$), penicillin-red mulberry ($P<0.001$), red mulberry and cornelian cherry ($P<0.001$) groups in terms of MDA in erythrocytes. The MDA value was highest in the penicillin group (37.193 nmol/g Hgb). The cornelian cherry group had the lowest value (24.488 nmol/g Hgb).

Results of plasma studies

The values in Table II show the levels of CAT, SOD, MDA and NO in plasma. No significant differences were determined in the statistical analysis of the results for CAT, SOD and NO in plasma ($P=0.115$, $P=0.37$, $P=0.066$ respectively).

The values in Table II show the levels of XO in plasma. There were statistically significant differences between the cornelian cherry and penicillin groups

Table I. Mean values in erythrocyte for XO, CAT, SOD, and MDA (±SEM)

group name	mean values in erythrocyte			
	for XO (μU/mL)	for CAT (nmol/min/mL)	for SOD (U/ml)	for MDA (nmol/g Hgb)
control	7.898±0.60	75.502±12.98	48.397±10.65	24.360±0.09[a]
sham	7.758±3.01	98.646±15.45	42.272±26.06	29.740±1.46[b]
penicillin	11.283±4.41	89.687±26.48	45.100±15.68	37.193±0.72[c]
r. mulberry	7.017±2.92	103.972±9.27	44.743±15.71	27.722±0.73[d]
c. cherry	4.802±3.33	94.573±18.65	35.588±17.42	24.488±0.78[a]
P values between groups	*0.087*	*0.134*	*0.835*	<0.001

a, b, c, d: different upper letters above the columns indicate significant difference between the groups at $P<0.05$ (used test: one-way ANOVA, *post-hoc* Tukey).
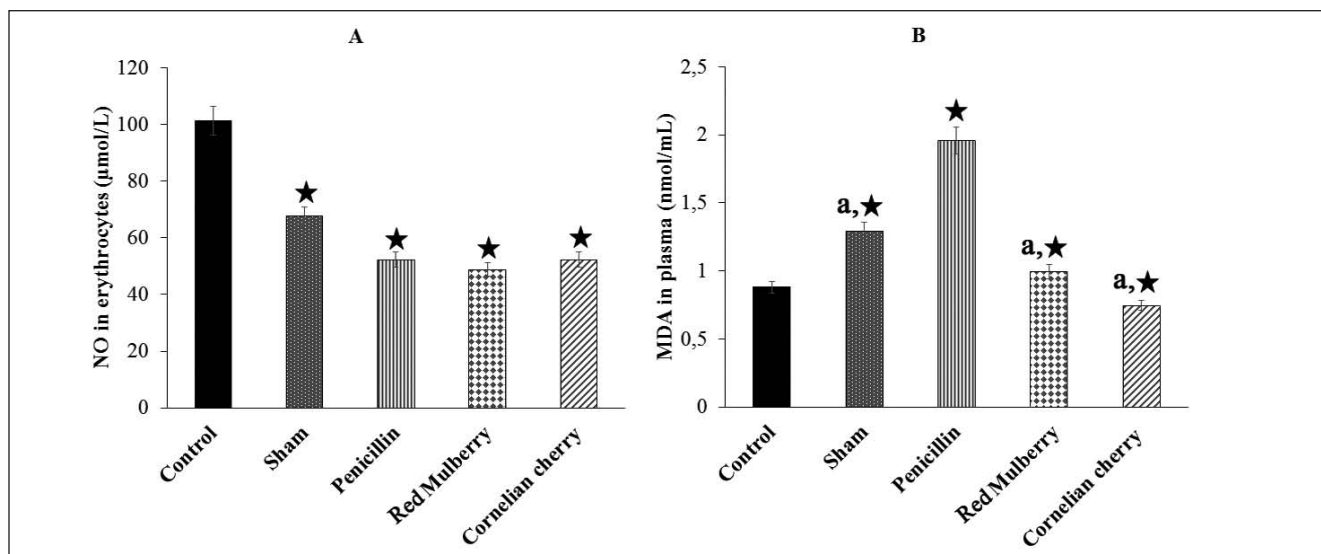
Fig. 2. Mean values in erythrocytes for NO (A) (*P*=0.001 for control-sham, *P*=0.008 for control-penicillin, *P*=0.001 control-cornelian cherry, *P*=0.77 for penicillin-red mulberry, cornelian cherry-red mulberry for *P*=0.93, sham-penicillin for *P*=0.46) Median values for MDA in plasma. (B) (*P*<0.001 for red mulberry-cornelian cherry, *P*<0.001 for cornelian cherry-penicillin). [a]*P*<0.05 compared to normal control group and **P<0.05 compared to penicillin group [used test: (A) Kruskal-Wallis and Mann-Whitney U test with Bonferroni corrected; (B) one-way ANOVA and *post-hoc* Tukey].

(*P*=0.008) and penicilin and red mulberry groups (*P*=0.02) in terms of XO. While the mean of the penicillin group was 191.057 µU/ml, the mean value of the cornelian cherry group (354.753 µU/ml) was significantly higher. After the cornelian cherry group, the mean value of the red mulberry group 328.172 µU/ml was found to be the second highest. The cornelian cherry group had the highest mean in all groups.

The values in Fig. 2B show the levels of MDA in plasma. There were statistically significant differences between the penicillin-cornelian cherry (*P*<0.001) and red mulberry-cornelian cherry (*P*<0.001) groups in terms of MDA. While the penicillin group had the highest median value (1.96 nmol/mL), the lowest value was found in the cornelian cherry group (0.745 nmol/mL). The median value in the control group was the

second lowest value after the cornelian cherry group (0.880 nmol/mL).

Evaluation of electrocorticogram results

Assessments for cornelian cherry

Evaluation of amplitude

For the evaluation of amplitude, 2.5 mg/kg, 5 mg/kg, 10 mg/kg cornelian cherry extracts were given to the penicillin group and recorded. The values presented in Fig. 3 were obtained by evaluating 10-minute data. There were no statistically significant differences between groups in terms of amplitude (time, *P*=0.422; drug, *P*=0.362, TimexDrug, *P*= 0.128).

Table II. Median values for SOD and NO (min–max) and mean values in plasma for CAT and XO (±SEM)

group name	median values in plasma		mean values in plasma	
	for SOD (U/ml)	for NO (µmol/L)	for CAT (nmol/min/ml)	for XO (µU/ml)
control	11.15 (5.8–11.8)	13.43 (12.1–24.1)	10.72±2.02	263.23±33.03[ab]
sham	5.81 (4.3–6.1)	56.95 (12.1–85.2)	40.79±23.74	242.36±91.21[ab]
penicillin	6.12 (0.5–6.4)	59.72 (17.1–78.2)	59.88±26.02	191.06±110.23[a]
r. mulberry	7.11 (1.3–10.3)	48.38 (15.3–67.1)	43.12±21.29	328.17±31.93[b]
c. cherry	8.72 (1.6–11.8)	71.76 (46.8–98.6)	32.43±22.67	354.75±33.82[b]
P values between groups	*P=0.370*	*P=0.066*	*P=0.115*	*P=0.006*

a, b: different upper letters above the columns indicate significant difference between the groups at *P*<0.05 (used test: Kruskal-Wallis for SOD and NO; one-way ANOVA, *post-hoc* Tukey for CAT and XO).
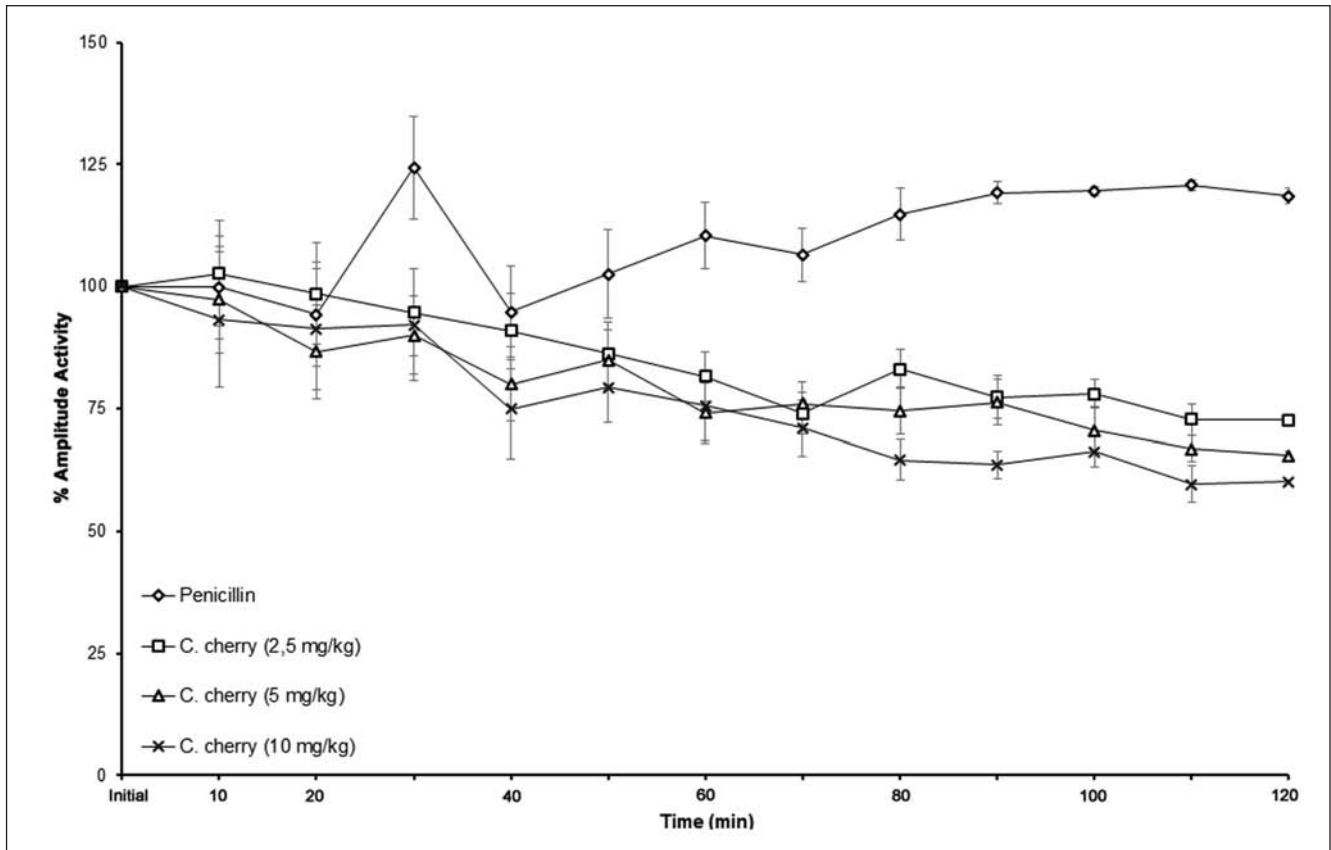
Fig. 3. The effects of the cornelian cherry extracts on amplitude (expressed as percent of baseline value) of penicillin-induced epileptiform activity. There is a trend for a decrease in amplitudes, however is not statistically significant. Whisker lines indicate ±SEM; (time, P=0.422; drug, P=0.362, TimexDrug, P=0.128; used tests: two-way repeated measures anova; amplitude=mV, ±SEM).

Evaluation of spike frequency

In studies among the cornelian cherry groups, there were no statistically significant differences between the penicillin group and the 2.5 mg/kg cornelian cherry extract group.

There were statistically significant differences between the penicillin group and the 5 mg/kg cornelian cherry extract group at the 110th mins but this difference did not persist at the 120th min (P=0.012 and P=0.032, respectively).

There were statistically significant differences between the penicillin group and the 10 mg/kg cornelian cherry extract group at the 50th min (P=0.007). At the 50th min and all after, there were significant differences in spike frequency (P=0.004 for 60th min, P=0.015 for the 70th min, P=0.008 for the 80th min, P=0.004 for 90th min, P=0.004 for the 100th min, P=0.008 for the 100th min, P=0.003 for the 110th min and P=0.003 for the 120th min). There were no differences between the penicillin group and other groups. The spike frequencies of the cornelian cherry groups are shown in Fig. 4.

Assessments for red mulberry

Evaluation of amplitude

For the evaluation of the amplitude, 2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg red mulberry extracts were given to penicillin group and recorded. The values given in Fig. 5 were obtained by evaluating 10-minute data. There were no statistically significant differences between groups in terms of amplitude (time, P=0.517; drug, P=0.362, TimexDrug, P= 0.964).

Evaluation of spike frequency

In studies among red mulberry groups, there were no statistically significant differences between the penicillin group and the 2.5 mg/kg red mulberry extract group.

There were statistically significant differences between the penicillin group and the 5 mg/kg red mulberry extract group at the 100th and 110th mins but this difference did not persist at the 120th min (P values 0.0125 for the 100th and 110th mins).

There were statistically significant differences between the penicillin group and the 10 mg/kg red mulberry extract group at the 20th min (*P*=0.0125). This difference did not persist at the 30th, 40th and 50th mins. At the 60th min, a significant difference (*P*=0.0125) was seen again but this difference disappeared at the 70th min. The 80th min and all after, there were significant differences in spike frequency (*P*=0.0125 for 80th–120th mins).

There were statistically significant differences between the penicillin group and the 20 mg/kg red mulberry extract group at the 100th min (*P*=0.0125) and at the 110th min (*P*=0.0125). There were no differences between the control group and other groups. The spike frequencies of the red mulberry extract groups are shown in Fig. 6.

DISCUSSION

In this study, and in agreement with Ilhan and others (2005), the MDA levels were highest in the penicillin group. The mean values in the red mulberry and cornelian cherry

groups were lower than those in the penicillin group. The difference between the cornelian cherry and red mulberry groups (*P*<0.01) was statistically significant. The decrease in both erythrocyte and plasma MDA levels in the cornelian cherry group was greater than that in the red mulberry group. This suggests that cornelian cherry reduces lipid peroxidation in a more pronounced manner than red mulberry, although both have a negative effect on lipid peroxidation.

Mean MDA values in both plasma and erythrocyte investigation in this study were low in the control group but higher in the sham group. These findings suggest that surgical stress also increases lipid peroxidation. Yıldırım and colleagues (2007) reported an increase in serum MDA levels following trauma. In agreement with previous studies, that study also suggested that this was associated with increased oxidative stress.

Sudha and others (2001) compared 50 normal subjects and 29 patients with epilepsy and observed increased erythrocyte lipid peroxidation in the patient group. No significant difference was determined in SOD and CAT
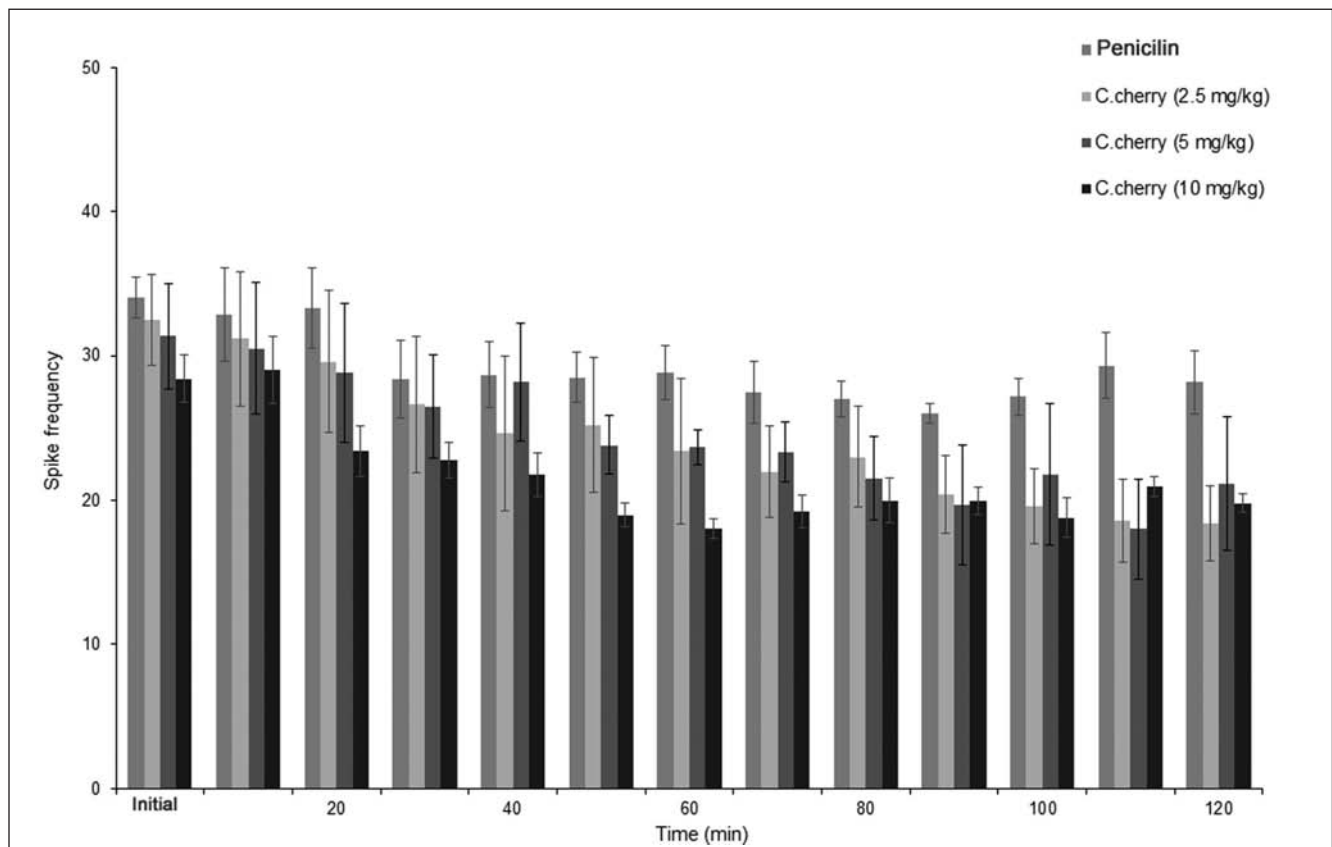

Fig. 4. The effects of the cornelian cherry extracts on spike frequency of penicillin-induced epileptiform activity. Cornelian cherry extracts 2.5, 5, and 10 mg/kg (i.p.) administered after penicillin (500 I.U.) injection (i.c.). Cornelian cherry at the dose of 10 mg/kg, significantly decreased the mean frequency of epileptiform activity. The earlier effects appeared at the 50th min (*P*=0.007). At the 50th min and all after, there were significant differences in spike frequency (*P*=0.004 for 60th min, *P*=0.015 for the 70th min, *P*=0.008 for the 80th min, *P*=0.004 for the 90th min, *P*=0.004 for the 100th min *P*=0.003 for the 110th min, *P*=0.003 for the 120th min). Whisker lines indicate ±SEM (used tests: Kruskal-Wallis and Mann-Whitney U with Bonferroni corrected; the value of *P*<0.0166 denoted statistical significance).

concentrations between the epileptic patient group and the normal group. Similarly, to previous studies, no difference was observed in SOD and CAT levels. Erol and colleagues (2010) examined the relation between oxidative stress and migraine, in agreement with this study, no significant difference between the groups in terms of SOD. CAT was also examined in that study, and was significantly higher in patients with chronic headache. Arhan and others (2011) examined NO, lipid peroxidation and the XO system in children who were diagnosed with idiopathic epilepsy and newly had started on antiepileptics. No difference was determined in terms of XO and MDA, while NO levels were higher before treatment. That suggested that NO might be involved in the pathogenesis of epilepsy.

Royes and colleagues (2007) reported that endogenous NO might have a neuroprotective role. Several studies have described NO as both neuroprotective and neurodestructive (Dawson et al. 1991, Dowson et al. 1992, Lei et al. 1992, Moncada et al. 1992, Nowicki et al. 1991, Wallis et al. 1992). The paradoxical effect on neurons has been attributed to changes in NO redox. NO may be present in the form of nitric oxide (NO) and nitrosonium ions (NO^+) (Lipton et al. 1993). The NO^+ form is thought to be neuroprotective. It

is thought to perform downregulation in NMDA receptor activity (by performing S-nitrosylation *via* thiol groups). In contrast, peroxynitrite forms from thereaction between NO and superoxide, and thus NO itself exhibits a toxic effect (Beckman et al. 1990).

In this study, NO levels were higher in erythrocytes in the control group, while decreasing significantly in the sham group. This decrease was more pronounced in the penicillin group. There was no change in NO levels with the administration of cornelian cherry and red mulberry compared to the penicillin group. No significant difference was determined (P=1 and P=0.776, respectively).

Iadecola and others (1995) examined the relations of selective NOS inhibitors. In that study, cerebral ischemic injury decreased with the use of the nNOS inhibitors 7-nitroindazol, ARL17477 or S-methyl-iso thioureido-L-norvaline, but this did not affect eNOS. Similarly, the iNOS inhibitor aminoguanidine reduced post-ischemic iNOS activity and shrank the infarct area following middle cerebral artery occlusion (Iadecola et al. 1995, Yoshida et al. 1994, Zhang et al. 1996). In these experiments, aminoguanidine did not affect nNOS enzymatic activity, arterial pressure or cerebral blood flow.
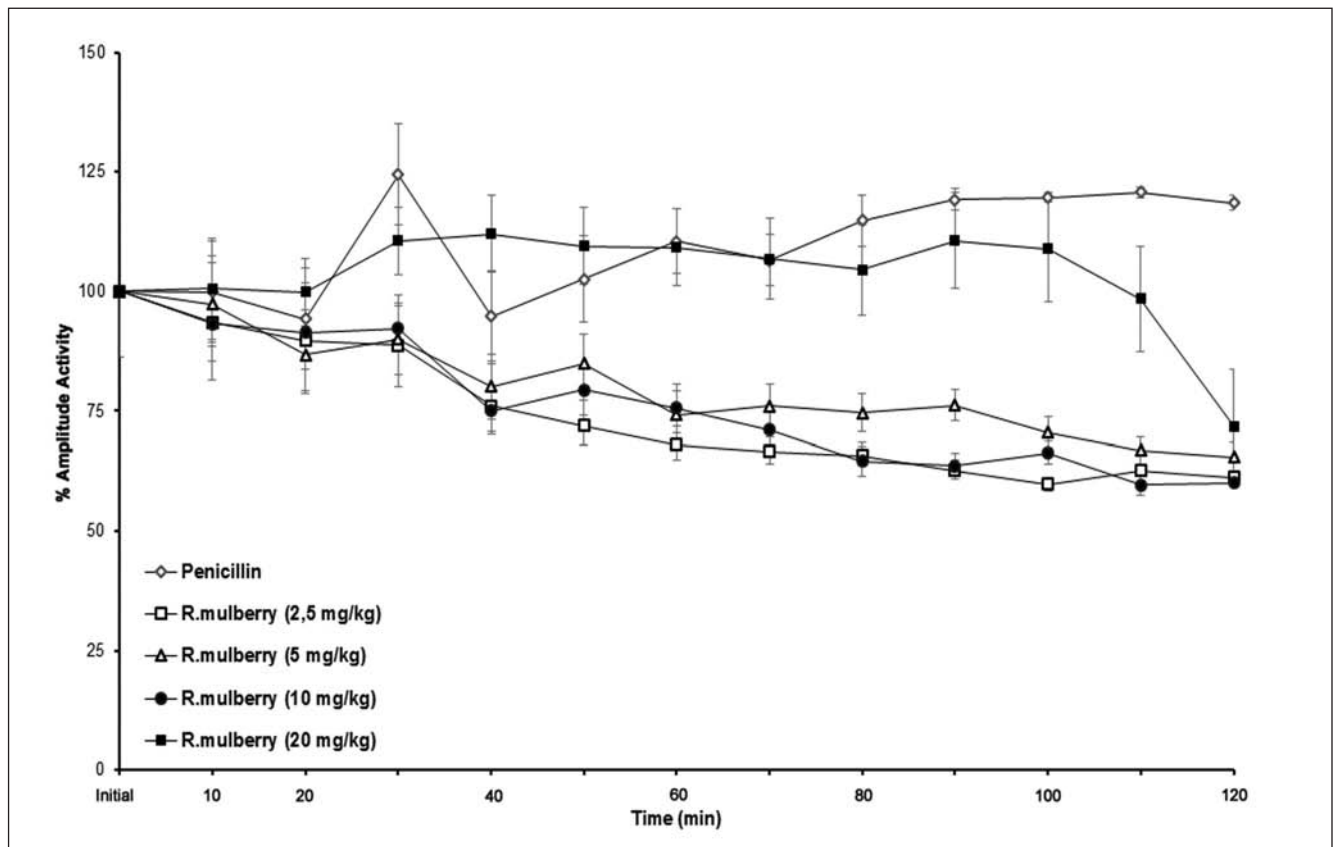

Fig. 5. The effects of the red mulberry extracts on amplitude (expressed as percent of baseline value) of penicillin-induced epileptiform activity. There is a trend for a decrease in amplitudes, however is not statistically significant. Whisker lines indicate ±SEM; (time, *P*=0.517; drug, *P*=0.362, TimexDrug, *P*=0.964; used tests: two-way repeated measures anova; amplitude=mV, ±SEM).

This suggests that nNOS and eNOS are not affected by this drug. Therefore, in contrast to studies using non-selective NOS inhibitors, a decrease was observed in infarction areas in those in which nNOS and iNOS inhibitors were used. These findings suggest that while nNOS and iNOS activity are deleterious to the ischemic brain, eNOS activity is beneficial, at least in the early period (Zhang et al. 1996).

In this study, NO levels in erythrocytes were highest in the control group, and decreased in the sham group. These values had a tendency to decrease still further in the penicillin group. There was no statistical difference between the penicillin, cornelian cherry and red mulberry groups. Analysis of Iadecola's (1997) study shows that high NO levels in the control group were equated with elevated eNOS levels since no endothelial damage was observed. The increasing fall in these values in the sham and penicillin group may suggest, in the light of the increasing MDA values, the presence of membrane and accompanying endothelial damage. According to this hypothesis, there may be a decrease in NO levels in association with decreased eNOS levels. The reason for the decrease in NO with the application of cornelian cherry and red mulberry, with their known antioxidant properties, may be an interruption in eNOS production due to the permanent nature of the changes in the cell wall. We think that the difference in erythrocytes in terms of NO, while there was no difference in plasma, supports this idea.

Dietary studies on rats have shown that only 20% of flavonoids are absorbed by the gastrointestinal system (Ameer et al. 1996). Plasma levels peak within 2 h of administration (Rice-Evans et al. 1996). In this study, a difference was determined at 100 and 110 mins after the administration of 5 mg/kg cornelian cherry to Wistar rats. That difference did not persist at 120 min. Differences were determined at 20 and 60 mins following intraperitoneal administration of cornelian cherry at 10 mg/kg, identified as the effective dose. There was no subsequent change in spike frequency until the 80th min. A difference at all periods after the 80 min (80–120 mins) supported this information. In the red mulberry group, a difference began to be observed after
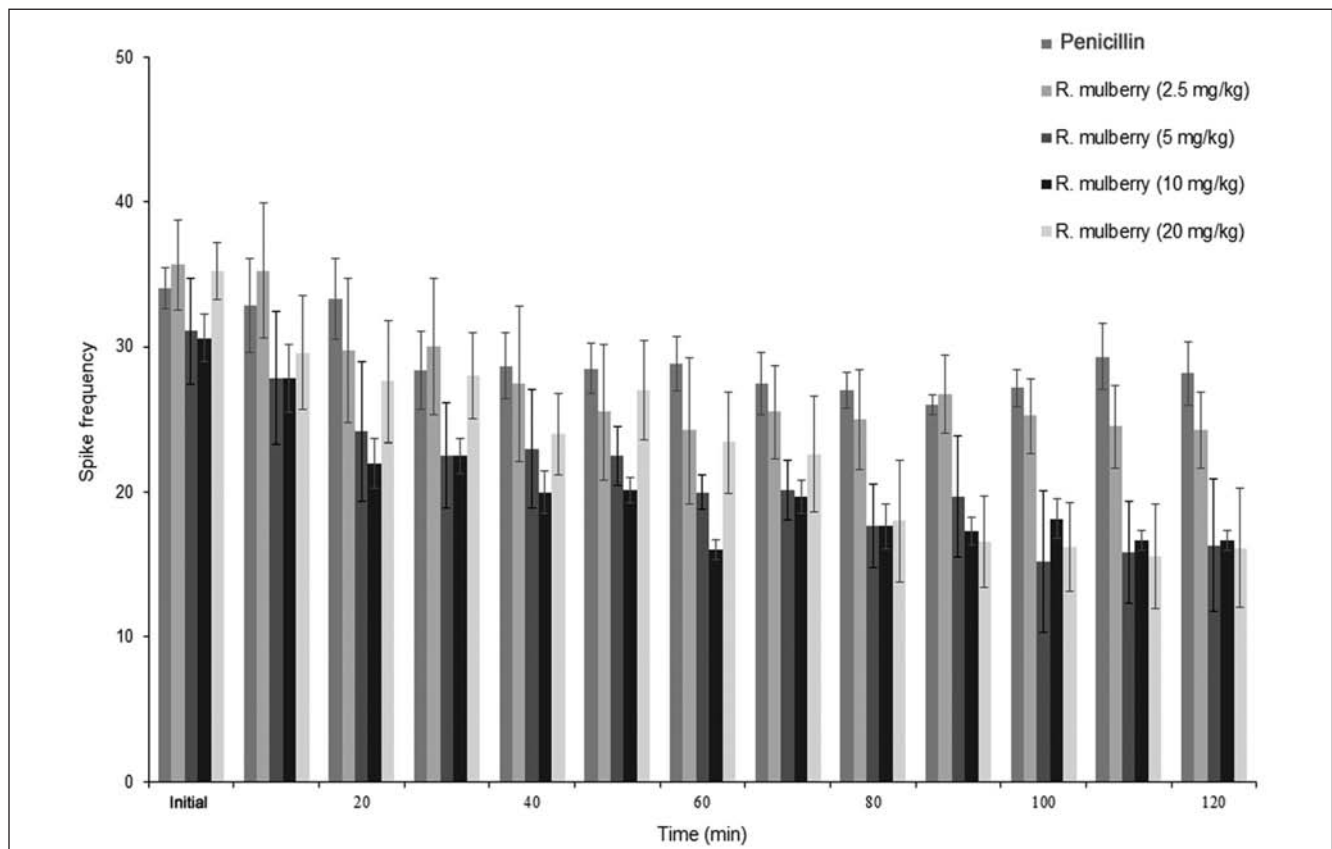

Fig. 6. The effects of the red mulberry extracts on spike frequency of penicillin-induced epileptiform activity. Red mulberry extracts 2.5, 5, 10 and 20 mg/kg (i.p.) administered after penicillin (500 I.U.) injection (i.c.). Red mulberry at the dose of 10 mg/kg, significantly decreased the mean frequency of epileptiform activity. The earlier effects appeared at the 20th min (P=0.01). This difference did not persist at the 30th, 40th and 50th min. At the 60th min, a significant difference (P=0.005) was seen again but this difference disappeared at the 70th min. The 80th min and all after, there were significant differences in spike frequency (P=0.008, 0.003, 0.008, 0.003, 0.004 for 80th–120th mins, respectively). Whisker lines indicate ±SEM (used tests: Kruskal-Wallis and Mann-Whitney U with Bonferroni corrected; the value of P<0.0125 denoted statistical significance).

80th min following intraperitoneal administration at the effective dose of 10 mg/kg essence, again persisting until the 120th min. The fact that findings in the two groups were similar suggested that decreases in spike frequencies may be associated with the antioxidant content of flavonoid compounds.

Per and colleagues (2013) administered grape seed extract (GSE), which has antioxidant properties, intraperitoneally following the induction of a penicillin model of epilepsy at 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg. Similarly, to this study, there was no change in epileptiform activity amplitude, while a significant decrease was determined in spike frequency. GSE was started at 50 mg/kg and 100 mg/kg (low) doses. The effective dose was determined as 200 mg/kg (moderate dose) in the same study. At a dose of 400 mg/kg (high) there was no change in epileptiform activity. Similarly, in this study, a relative effect began at low doses in both the cornelian cherry and red mulberry groups (2.5 and 5 mg/kg were established as low doses). No effect was observed at 2.5 mg/kg, while at 5 mg/kg there was a decrease in spike frequency at 100 and 110 mins. A dose of 10 mg/kg, determined as a medium dose in this study, caused a significant decrease in spike frequency in the cornelian cherry and red mulberry essence groups, and was therefore identified as the effective dose. In contrast to Per and others study (2013) there was a decrease in spike frequencies at the high dose of 20 mg/kg. At the same time, the amount of flavonoid in 200 mg/kg GSE, taken as the effective dose by Per and others (2013) was the same as the amount of cornelian cherry and red mulberry essence flavonoid at 10 mg/kg, the effective dose in this study. The point of agreement between these studies is that the same amount of flavonoid suppressed epileptic activity frequency.

Ayyıldız and colleagues (2006) reported no change in epileptiform activity at the effective dose following administration of vitamin E in an experimental penicillin epilepsy model, but that it resulted in a decrease in frequency. In another study by Ayyıldız and others (2007) involving vitamin C and a penicillin model, there was again no change in epileptiform activity, but a decrease in frequency was observed. This suggested that substances with antioxidant characteristics affect the spike frequency of epileptic activity.

CONCLUSION

In this study, cornelian cherry and red mulberry essences at low (2.5–5 mg/kg), medium (10 mg/kg) and high (20 mg/kg, only for red mulberry) doses did not alter the amplitude of epileptiform activity but significantly lowered the mean frequency. The dose that most effectively reduced the frequency of epileptiform activity was 10 mg/kg for both extracts. Biochemical analyses showed that both substances have a positive effect on MDA, which shows lipid peroxidation. These findings suggest that red mulberry and cornelian cherry essences exhibit an anticonvulsive effect *via* MDA.

Further studies using various experimental models are now needed to reveal how red mulberry and cornelian cherry essence affect the pathophysiology of epilepsy.

ACKNOWLEDGMENTS

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