

# Effects of Melatonin at Different Doses on Experimental Epilepsy Model Induced By Pentylene-tetrazole

## *Pentilenetetrazol ile Oluşturulan Deneysel Epilepsi Modelinde Farklı Dozlardaki Melatoninin Etkilerinin İncelenmesi*

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### Keywords

Antioxidant, experimental epilepsy, melatonin, oxidative stress, PTZ, sıçan

### Anahtar Kelimeler

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### Abstract

**Objective:** This study aimed to evaluate the effect of melatonin (MEL) treatment on rats with experimental epilepsy induced by pentylene-tetrazole (PTZ). Changes in the control, epilepsy and two treatment groups (25 mg/kg and 100 mg/kg) were monitored as intragroup and intergroup changes.

**Materials and Methods:** Forty male Wistar albino rats (12-14 weeks old) were divided into control, PTZ, MEL25 and MEL100 groups, with 10 rats in each group. Only solvent was injected in the control group, and PTZ at a dose of 35 mg/kg was administered intraperitoneal 12 times in a total of 23 days in the PTZ group. MEL25 and 100 mg/kg were administered in the MEL25 and MEL100 groups, respectively. Parameters tested during and after the experiment were behavioural tests (elevated plus maze), biochemical tests in brain tissue [after decapitation; malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), total antioxidant status (TAS) and total oxidant status (TOS)] and epileptic seizure severity scale scores (every injection day).

**Results:** Significant differences were observed among the epilepsy, control and treatment groups, except for the hiding times, seizure scores and biochemical tests ( $p<0.05$ ). As a result of biochemical tests applied to the homogenised brain tissue after decapitation, dose-dependent results were found to be related to the different doses of MEL applied in MDA, CAT, SOD, TAS and TOS levels ( $p<0.05$ ).

**Conclusion:** In this study, MEL showed a protective and healing role against physiological changes caused by experimental epilepsy, through its capacity to reduce oxidative damage and increase antioxidant potency.

### Öz

**Amaç:** Bu çalışmada, pentilenetetrazol (PTZ) ile indüklenen deneysel epilepsi oluşturulmuş sıçanlarda melatonin (MEL) tedavisinin etkisinin değerlendirilmesi amaçlanmıştır. Kontrol grubu, epilepsi grubu ve iki tedavi grubundaki değişiklikler (25 mg/kg ile 100 mg/kg) grup içi ve gruplar arası olarak değerlendirilmiştir.

**Gereç ve Yöntemler:** Kırk adet erkek Wistar albino sıçan (12-14 haftalık) 4 farklı gruba (kontrol, PTZ, MEL25, MEL100) ayrıldı. Kontrol grubuna sadece çözünen enjekte edildi ve PTZ grubuna 35 mg/kg dozunda PTZ intraperitoneal toplam 23 gün süresince 12 kez günde bir uygulandı. Melatonin, MEL25 grubu için 25 mg/kg ve MEL100 grubu için 100 mg/kg uygulandı. Deney sırasında ve sonrasında test edilen

parametreler davranış testi (yükseltilmiş artı labirent), beyin dokusunda biyokimyasal testler [dekapitasyon sonrası; malondialdehit (MDA), katalaz (CAT), süperoksit dismutaz (SOD), toplam antioksidan durumu (TAS) ve toplam oksidan durumu (TOS)] ve epileptik nöbet şiddeti ölçek skorları (her enjeksiyon günü) idi.

**Bulgular:** Epilepsi grubu ile kontrol ve tedavi grupları arasında kaçış ve saklanma süreleri, nöbet skorları ve biyokimyasal testler açısından anlamlı farklılıklar bulundu ( $p<0,05$ ). Dekapitasyon sonrası homojenize beyin dokusuna uygulanan biyokimyasal testler sonucunda MDA, CAT, SOD, TAS ve TOS düzeylerinde farklı dozlarda uygulanan MEL ile ilgili doza bağlı iyileşmeler saptandı ( $p<0,05$ ).

**Sonuç:** Çalışma sonucunda MEL'nin deneysel epilepsinin neden olduğu fizyolojik değişikliklere karşı koruyucu ve iyileştirici rolü olduğu ve oksidatif hasarı azaltıcı ve antioksidan potansiyeli artırıcı etki gösterdiği tespit edilmiştir.

## Introduction

Epilepsy is a neurological disorder, characterized by unpredictable seizures occurring repeatedly. Epileptic seizures affect nearly 50 million people, approximately %1 of the world's population (1). These seizures are associated with cognitive and behavioral disorders that have a significant impact on patients' quality of life. Epileptic seizures may cause cognitive function defects. Abnormal electrical activities of neurons cause changes on behavioral and cognitive processes (2). Patients affected by epileptic seizures often show impaired spatial memory and emotional learning. These changes may be also associated with different comorbidities such as anxiety, sleep, disorders and depression (3,4).

Oxidative stress has an important role at epileptic stage. Reactive oxygen derivatives are mainly responsible for oxidative stress. These can be oxygen-centered radicals that have unpaired electrons or covalent molecules (such as hydrogen peroxide) (5). Reactive oxygen species;  $H_2O_2$ ,  $O_2^-$ ,  $OH^-$ ; are produced during and after epileptic seizures, may contribute brain damage. The brain is vulnerable to free radical damage due to its high oxygen consumption, high lipid content, and also less antioxidant enzymes compared to other tissues. There are several experimental models that show the relation between epileptic seizures and oxidative damages. Moreover, excessive free radical production is associated with injury to cell structures, including lipid structure disruption of cells, enzyme inactivation, and DNA damage (6).

There are various experimental models to find out the mechanisms of acute and chronic epilepsy. For these models of epilepsy are induced by administration of convulsive drugs or electrical stimulation. It is possible to initiate partial and generalized seizures by using various chemicals, such as pentylenetetrazole (PTZ), penicillin, kainic acid, etc.

(7). One of the most common experimental model is a chemical inducing method, the PTZ-induced seizures (8). PTZ is a tetrazole compound that is an agent for generalized tonic clonic epileptic seizure induction. It was demonstrated that PTZ affects the disruption of gamma amino butyric acid (GABA) (9). The formation of free radicals has been shown during and after seizures in PTZ-induced models. Especially in chronic PTZ exposure, formation of free radicals significantly increased and oxidative stress damage was found to occur. The PTZ model is a useful model for detecting post-seizure dysfunctions that serve as a screen for possible treatments, offering the possibility to study animal models for cognitive, physical and emotional deficits in human epilepsy (10).

Melatonin (MEL) is produced by the pineal gland and is an indolamine derivative of serotonin. It has long been described as a reproductively active hormone that regulates the sexual physiology seasonally (11). MEL has been shown to have various antioxidant and anticonvulsive effects on the central nervous system of mammals (12,13). Experiments reported that MEL exert proconvulsant effects in humans especially via protecting cortical GABA levels (13). As an antioxidant, MEL is effective in protecting nuclear DNA, membrane lipids, and cytosolic proteins from oxidative damage. It is also shown that MEL has role to suppress brain excitability and prevent seizures. MEL blocks glutamatergic-dependent brain excitability and thus functions as an anti-excitotoxic compound (5,13).

The objective of the present study was to evaluate the effects of different doses of MEL application on the seizure levels and oxidative status of the brain tissues in a rat model of PTZ-induced epilepsy. In addition, together with biochemical and histological evaluations, it was aimed to determine the behavioral changes caused by PTZ with the elevated plus maze test.

## Materials and Methods

**Drugs and chemicals:** PTZ and MEL were purchased from Sigma-Aldrich (St. Louis, MO, USA). The dose of PTZ and MEL were calculated from the corresponding experimental doses (13-15). All injections and experiments were performed between July to August, at the time from 02.00 pm to 07.00 pm.

**Animals:** Forty male Wistar Albino rats (220±20 g in weight) were maintained under controlled conditions, including 12-hour (h) light/dark cycle, 22-24 °C temperature, and appropriate humidity, with laboratory chow and water provided ad libitum. All animal experiments were carried out in accordance with the approval of the Animal Use Adnan Menderes University Ethical Committee (decision no: 64583101/2014/022, date: 27.02.2014).

**Groups:** The animals were acclimatized for 15 days before starting experiments. Forty rats were randomly divided in four groups with ten animals in each group: Control: Sham-control, PTZ: PTZ-epileptic, 35 mg/kg PTZ administrated PTZ+MEL25: 25 mg/kg MEL treatment group before the induction of epileptic seizures by the injection of 35 mg/kg PTZ, PTZ+MEL100: 100 mg/kg MEL treatment group before the induction of epileptic seizures by the injection of 35 mg/kg PTZ.

**Experimental procedures:** After acclimation, experiments started with the first injection of PTZ and finished after the 12<sup>th</sup> injection of PTZ on the 23<sup>rd</sup> day. MEL, purchased in powder form, was prepared to be dissolved in 10% dimethyl sulfoxide (DMSO) and given at the appropriate dose (16,17). Control group (Sham-control group) received an intraperitoneal (i.p.) injection of 10% DMSO solution prepared in distilled water every other day (3.5 mL/kg, 12 injections total). PTZ group (PTZ-epileptic group) was administered with PTZ (35 mg/kg, i.p., 12 injections total) every other day. MEL25 group (PTZ+MEL25 group) was injected by 25 mg/kg MEL half an h before the administration of PTZ (35 mg/kg PTZ, 25 mg/kg MEL, i.p., 12 injections total). MEL100 group (PTZ+MEL100 group) was injected by 100 mg/kg MEL half an h before the administration of PTZ (35 mg/kg PTZ, 100 mg/kg MEL, i.p., 12 injections total). Groups were monitored for 1 h after PTZ injection by 2 blind-researchers. At the end of experiments rats were anesthetized by ketamine (80 mg/kg) and xylazine (4 mg/kg) intraperitoneally and

sacrificed by decapitation. The brains were collected for biochemical measurements.

**Epileptic seizures monitoring:** Animals were continuously monitored for 12 h/day at light period for 7 days before experiments and 23 days during experiments. Seizure severity was scored using the Racine scale (18) by direct observation and indirect observation based on the video records. Recordings were analyzed and scored by two independent, blind investigators. The scores of Racine scale are; 0 for no convulsive behavior; 1 for myoclonic jerks; 2 for bilateral forelimb clonus lasting less than 3 s; 3 for bilateral forelimb clonus lasting more than 3 s; 4 for tonic-clonic seizure under 10 minutes (min); 5 for tonic-clonic seizure over 10 min; 6 for death. At the end of the experiments, the scores from both investigators were collected by another researcher and the medians of these scores were used as the final scores.

**Elevated plus maze test:** The elevated T maze was made of transparent glass and consisted of two open arms, (50x10 cm) two enclosed arms (50x10x40 cm) and a central platform (10x10 cm). The plus-maze was 50 cm above the floor. The researcher observed elevated plus-maze and recorded the data by chronometer behind the closed arms. Pellow's (19) method was used as experimental procedure. Rats were placed individually in a new cage that had same conditions as home cage for 5 min before the test. Every rat was then placed in the center of the plus-maze one by one, that was facing one of the closed arms. After familiarization repeats to test platform, for experiment, measurements were taken by a researcher. Main object was the time spent on open arm enclosed arms. A rat was taken to have entered an arm when all four legs were on the arm. To measure time spent on enclosed arms, open arm time period was recorded and difference to total time was calculated. At the end of the test, the total measurement time for every rat was recorded. The duration of analysis time was kept constant as 5 min-300 seconds in accordance with the protocol in the literature (20).

**Tissue samples:** On the last day of experiment, 90 min after last PTZ administration, the animals were killed by decapitation and their brains were dissected. Ten milliliters of 140 mM KCl solution/gram of tissue were added and all tissue were then homogenized

in a motor-driven homogenizer (Ultra Turrax, IKA-WERKE, Germany) (21). Then samples centrifuged at 12,000 rpm for 10 min. The supernatant was used for biochemical measurements.

**Determination of brain lipid peroxidation:** Lipid peroxidation was determined in tissue samples by measuring malondialdehyde (MDA) levels as thiobarbituric acid reactive substances (TBARS) (22). Trichloroacetic acid and TBARS reagent were added to the tissue homogenates, then mixed and incubated at 100 °C for 60 min. After cooling on ice, the samples were centrifuged at 3000 rpm for 20 min and the absorbance of the supernatant was read at 535 nm. MDA levels were calculated from the standard calibration curve using tetraethoxypropane and expressed as nmol/gr tissue.

**Determination of brain superoxide dismutase (SOD) activity:** SOD activity was determined according to the method of Sun et al. (23). Tissue should be perfused with 150 mM KCl to remove any red blood cells. The tissue samples homogenized in ice-cold 0.1 M Tris/HCl, pH 7.4 containing 0.5% Triton X-100, 5 mM  $\beta$ -ME, 0.1 mg/mL phenylmethylsulfonyl fluoride. After centrifugation the supernatant contains total SOD activity from cytosolic and mitochondria. The absorbance of the supernatant was read at 450 nm. SOD activity was given as miliunits per milliliter homogenate (mU/mL).

**Determination of brain catalase (CAT) activity:** CAT activity was assayed according to the method described by Maehly and Chance (24), based on the disappearance of  $H_2O_2$  at 240 nm. 10  $\mu$ L of homogenate was added to 180  $\mu$ L of 20 mM potassium phosphate buffer pH 7.2. Subsequently, 10  $\mu$ L of 5 mM  $H_2O_2$  was added and absorbance was immediately recorded every 30 s for 10 min, using a ELx800™ Microplate Reader (Biotek Instruments Inc. Winooski, USA). One CAT unit was defined as one  $\mu$ mol of hydrogen peroxide consumed per min and the specific activity was calculated as CAT mU/mL homogenate.

**Total antioxidant status (TAS) and total oxidant status (TOS) levels:** TAS measurement is based on the principle that the color formed by the 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) radical changes with the antioxidants in the samples added to the medium, and the measurement was performed using the biochemical kit. Antioxidants in the sample reduce dark blue-green colored ABTS

radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample. In order to perform TOS measurement, after decapitation, the brain tissue was homogenized as indicated. Oxidants present in the sample oxidize the ferrous ion-chelator complex to ferric ion. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter ( $\mu$ mol  $H_2O_2$  Equiv./L).

#### Statistical Analysis

All data were analyzed by GraphPad 7 statistical program (GraphPad Software, Inc., CA, USA). Elevated Plus Maze durations and Epileptic Seizure scores were compared by the Kruskal-Wallis non-parametric test-Dunn's multiple comparison test and other parameters were compared by One-way ANOVA-Dunnett's multiple comparison test. D'agostino pearson normality test was applied to determine normality. Values represent the mean  $\pm$  standard deviation of n=10 animals per group. ns= non-significant,  $p>0.05$ ,  $*p\leq 0.05$ ,  $**p\leq 0.01$ ,  $***p\leq 0.001$ . The control, MEL25 and MEL100 groups were compared with the PTZ group, and the treatment groups were compared among themselves. In the graphs, the arrows at the top show this comparison. In group comparisons, PTZ group was considered as the focus of comparison. The deteriorations in the control group and changes in the treatment groups were examined in this way.

#### Results

**Epileptic seizure scores:** To estimate the severity of seizures, Racine scale was used. Seizure severity was grouped in 3 ways: 1. No effect, 2. Minimal clonic seizure (MCS), 3. Generalized tonic-clonic seizure (GTCS). For 23 days, 12 injections, animals from the groups were monitored and scored based on Racine scale by two blind researchers. 0 severity for Racine scale means no effect, from 1 to 3 severity for Racine scale means MCS and 4<sup>th</sup> and 5<sup>th</sup> severity for Racine scale means GTCS. There was no rat to die as a result of PTZ injection, so no animal was grouped 6 severity for Racine scale. For the first injection of PTZ there is no difference significantly between the groups since

35 mg/kg PTZ is not acute dosage for rats to show difference. PTZ injections were associated with both minimal and generalized seizure incidences in all PTZ-exposed groups. For the first MEL injection, there was no significantly different alteration in between the groups (Figure 1). However after first time, MEL injections for both doses showed a protective effect in PTZ-induced seizures. It was showed that, without treatment, PTZ-induced rats had severe GTCs. However, the animals that were treated by MEL showed MCS with low severity. 100 mg/kg MEL treatment had a protective effect on rats, to increase the MCS and GTCs both for severity and latency compared to the PTZ group and MEL25 group. 25 mg/kg MEL treatment had also positive effects to protect from seizures; however compared to MEL100 group, MEL25 had lower significance against PTZ group. Total results of epileptic seizure scores are shown in Table 1 and summarized in Figure 1 and Figure 2a and 2b.

**Biochemical results:** In order to investigate whether the antioxidant properties of MEL against PTZ were mediated by an increase in antioxidant

enzymes, SOD and catalase activities were measured. Biochemical results; MDA levels, SOD activities and CAT activities are shown in Table 2.

**MDA levels:** There were significantly increased MDA levels the animals that were grouped as PTZ compared to control group animals. Administration of 25 mg/kg MEL did make a significant difference in brain MDA concentrations between the MEL25 and PTZ groups ( $p < 0.05$ ). MEL100 group, that was administrated 100 mg/kg MEL, had more significant difference compared MEL25 group against PTZ group. The results are summarized in Figure 3. The MDA levels in the brains of PTZ group were observed to

|         | Seizure scores (Median) |
|---------|-------------------------|
| Control | 0.0                     |
| PTZ     | 4.036±0.062             |
| MEL25   | 2.600±0.121             |
| MEL100  | 1.618±0.121             |

PTZ: Pentylenetetrazole, MEL: Melatonin

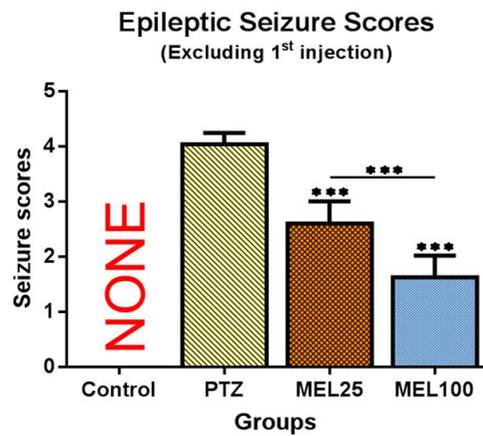


Figure 2a. Effects of different doses of melatonin on total epileptic seizures except 1<sup>st</sup> day. Group comparisons are: PTZ/MEL25; \*\*\*, PTZ/MEL100; \*\*\*, MEL25/MEL100; \*\*\*

PTZ: Pentylenetetrazole, MEL: Melatonin

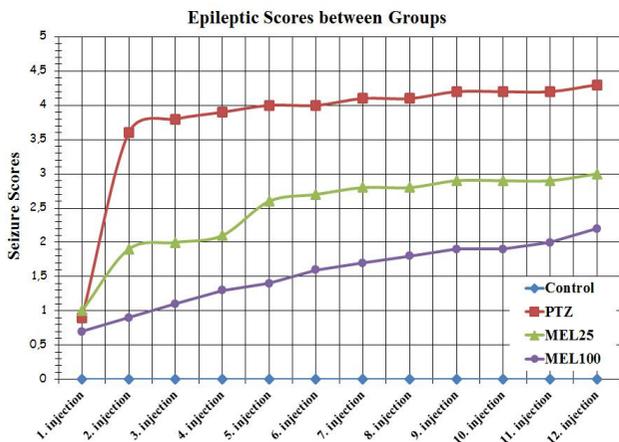


Figure 1. Effects of different doses of melatonin on total epileptic seizures

PTZ: Pentylenetetrazole, MEL: Melatonin

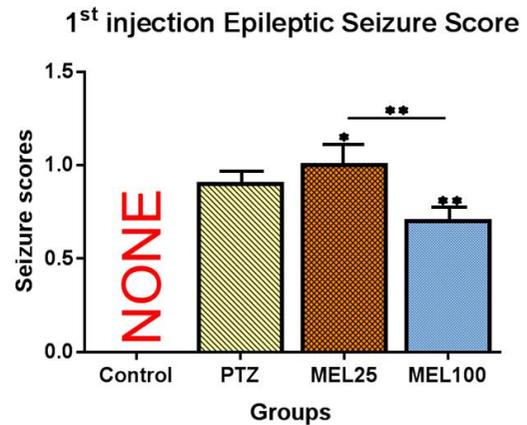


Figure 2b. Effects of different doses of melatonin on first day epileptic seizures. Group comparisons are: MEL25/PTZ; \*, PTZ/MEL100; \*\*, MEL25/MEL100; \*\*

PTZ: Pentylenetetrazole, MEL: Melatonin

be significantly increased in comparison to that of control group. It was also observed that, a significant decrease of the MDA levels in comparison to that of PTZ group were more prominently in MEL100 group.

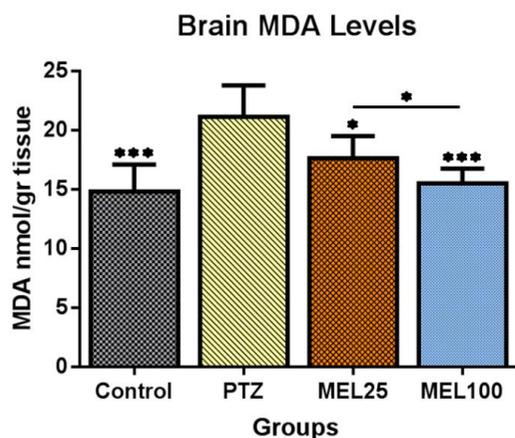
**SOD activity:** SOD activity was found significantly low at PTZ group compared to control, MEL25 and MEL100 groups ( $p < 0.05$ ). As a result of treatment with 25 and 100 mg/kg MEL, SOD activities are found significantly higher compared to PTZ group ( $p < 0.05$ ). There is also dose dependent difference between MEL25 and MEL100 groups, demonstrating that MEL administration supports antioxidant activity in a dose-dependent manner. The results are summarized in Figure 4.

**CAT activity:** The results revealed that CAT activity was observed to be decreased in the PTZ group in comparison to that of control group. The results of MEL25 and MEL100 groups demonstrated that MEL has supported the CAT activity in the brain in a

dose-dependent manner. The highest CAT activity was observed to be in the MEL100 group, while the CAT activity of MEL25 group were observed to be higher than that of PTZ group, although not as higher as MEL100 group. The results are summarized in Figure 5.

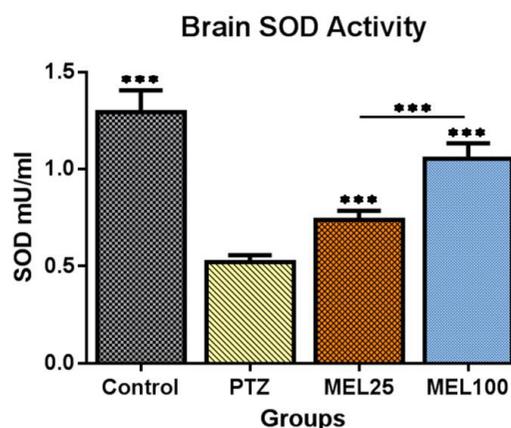
**TAS and TOS levels:** The highest antioxidant content was observed in the control group, where there was a decreased antioxidant content in the PTZ group and the antioxidant power was increased in the MEL treatment groups (Figure 6a). The highest oxidative stress content was observed in the PTZ group, and the oxidant content of the MEL treatment groups decreased in dose-dependent manner, where the oxidant content was the least in the control group (Figure 6b).

**Behavioral results:** To estimate behavioral results, epileptic seizure monitoring and elevated plus maze tests were done. Elevated plus maze test: Animals that were only exposed to PTZ, had shown significantly



**Figure 3.** The effect of melatonin administration on the MDA level, as a marker of lipid peroxidation in the rat brain. Group comparisons are: Control/PTZ; \*\*\*, PTZ/MEL25; \*\*\*, PTZ/MEL100 \*\*\*, MEL25/MEL100; \*\*\*

PTZ: Pentylene tetrazole, MEL: Melatonin, MDA: Malondialdehyde



**Figure 4.** The effect of melatonin administration on the activity of the antioxidant enzyme SOD in the rat brain. Group comparisons are: Control/PTZ; \*\*\*, PTZ/MEL25; \*\*\*, PTZ/MEL100; \*\*\*, MEL25/MEL100; \*\*\*

PTZ: Pentylene tetrazole, MEL: Melatonin, SOD: Superoxide dismutase

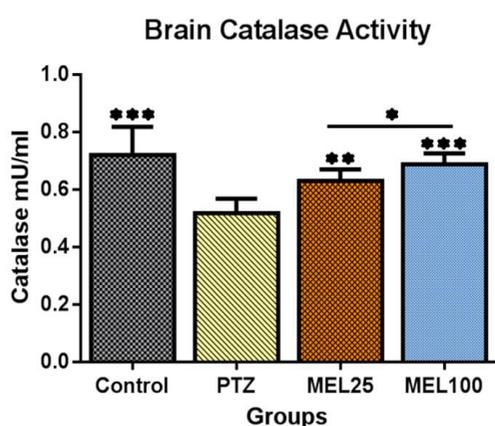
|         | MDA (nmol/gr tissue) | SOD (mU/mL) | CAT (mU/mL) | TAS (mM)   | TOS ( $\mu\text{mol H}_2\text{O}_2$ ) |
|---------|----------------------|-------------|-------------|------------|---------------------------------------|
| Control | 14.81±1.145          | 1.292±0.040 | 0.718±0.035 | 1.73±0.085 | 19.42±0.32                            |
| PTZ     | 21.10±0.948          | 0.520±0.013 | 0.516±0.018 | 1.31±0.045 | 29.84±0.03                            |
| MEL25   | 17.62±0.665          | 0.736±0.017 | 0.630±0.014 | 1.53±0.016 | 25.19±0.18                            |
| MEL100  | 15.49±0.441          | 1.053±0.028 | 0.686±0.014 | 1.70±0.098 | 20.86±0.16                            |

PTZ: Pentylene tetrazole, MEL: Melatonin, SOD: Superoxide dismutase, CAT: Catalase, TAS: Total antioxidant status, TOS: Total oxidant status, MDA: Malondialdehyde

low time spending at open arms and group escape time was lower against MEL groups. Animals that were injected MEL had spent more time at open arms compared to PTZ group and dose depended MEL effect had shown. Total escape time scores for control, PTZ, MEL25 and MEL100 groups are shown in Table 3 and summarized Figure 7 and Figure 8, and MEL100 group score is much more similar to control group compared to the other groups.

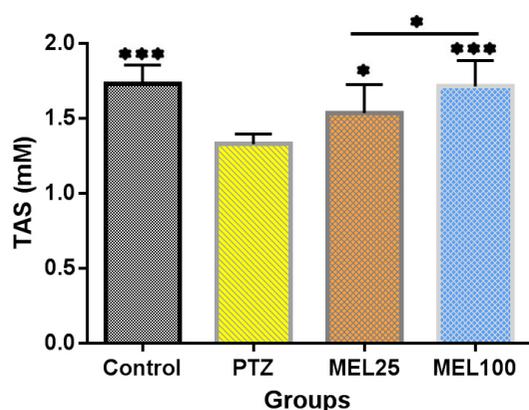
## Discussion

In the present study, we investigated the anticonvulsant activity of dose dependent MEL



**Figure 5.** The effect of melatonin administration on the activity of the antioxidant enzyme Catalase in the rat brain. Group comparisons are: Control/PTZ; \*\*\*, PTZ/MEL25; \*\*, PTZ/MEL100; \*\*\*, MEL25/MEL100; \*

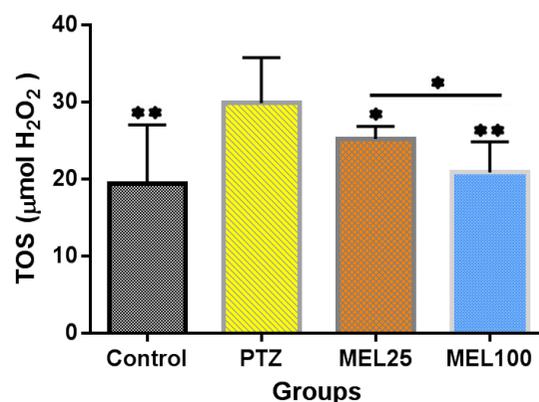
PTZ: Pentylentetrazole, MEL: Melatonin



**Figure 6a.** Comparison of “Cerebral Total Antioxidant Concentration” between groups. Group comparisons are: Control/PTZ; \*\*\*, PTZ/MEL25; \*, PTZ/MEL100 \*\*\*, MEL25/MEL100; \*

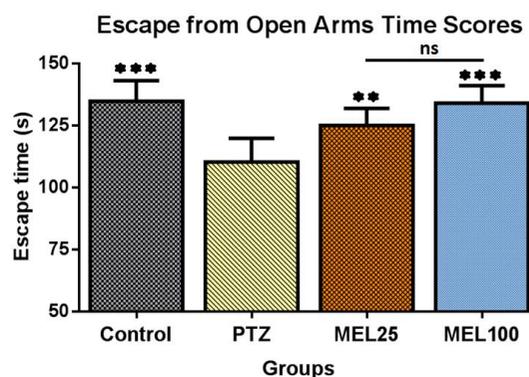
PTZ: Pentylentetrazole, MEL: Melatonin, TAS: Total antioxidant status

administration against to oxidative epileptic seizure severity that induced to PTZ. Many academic researches showed that there are different experimental models to create similar seizures that like human epilepsy (25-27). Our study also



**Figure 6b.** Comparison of “Cerebral Total Oxidant Content” between groups. Group comparisons are: Control/PTZ; \*\*, PTZ/MEL25; \*, PTZ/MEL100 \*\*\*, MEL25/MEL100; \*

PTZ: Pentylentetrazole, MEL: Melatonin, TOS: Total oxidant status

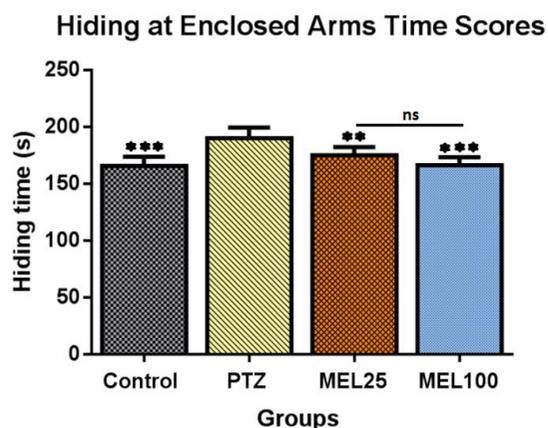


**Figure 7.** Effects of different doses of melatonin on exploratory and anti-hiding behavior in rats given a 5-minutes test in the elevated plus-maze. These results represent total time spent of open arms. Group comparisons are: Control/PTZ; \*\*\*, PTZ/MEL25; \*\*, PTZ/MEL100; \*\*\*, MEL25/MEL100; ns

PTZ: Pentylentetrazole, MEL: Melatonin, ns: Not significant

| Groups  | Escape time (s) |
|---------|-----------------|
| Control | 134.7±2.633     |
| PTZ     | 110.3±3.008     |
| MEL25   | 124.9±2.218     |
| MEL100  | 133.9±2.258     |

PTZ: Pentylentetrazole, MEL: Melatonin



**Figure 8.** Effects of different doses of melatonin on hiding behavior at enclosed arms in rats given a 5-minutes test in the elevated plus-maze. These results represent total time spent of enclosed arms. Group comparisons are: Control/PTZ; \*\*\*, PTZ/MEL25; \*\*, PTZ/MEL100 \*\*\*, MEL25/MEL100; ns

PTZ: Pentylenetetrazole, MEL: Melatonin

showed that, 35 mg/kg PTZ injection created tonic-clonic seizures and this created different conditions; like oxidative damage, prevent normal activity of antioxidant system and depressive behaviors of animals. Uyanikgil et al. (15), Wada and Fukuda (28) and Frantz et al. (6) demonstrated these effects on animals that are manipulated by injected PTZ.

Novel studies demonstrated that, MEL has anticonvulsive activity on the experimental animals that were manipulated and showed epileptic seizures induced by penicillin, pilocapine, kainite or PTZ (29,30). However, most studies demonstrated positive effects of MEL on acute epileptic attacks, some studies showed positive results on chronic epileptic seizures. Different from the other researches, our study focused on both chronic period and different doses of MEL (10,12,13,26). As a result of that, we uncovered antiepileptic activity of MEL, during ongoing PTZ injections and after decapitation biochemical results supported these results. Our results showed that, without any protective agents, PTZ group seizure scores are higher than dose dependent MEL administered groups. Choopankareh et al. (31) also recorded similar results that supported our study.

Previous studies showed the results of increased free radical levels during the seizures (5,31). Similarly, we demonstrated an increase in MDA levels at

PTZ administered group. On the other hand, both low and high dose MEL groups showed decrease in MDA levels, especially high dose MEL had more effective results. Reactive oxygen species, like superoxide anions, hydroxyl radicals, and hydrogen peroxide cause oxidative damage (32) and during the seizures increased oxidative damage create different neurological and psychiatric problems, like neuronal cell death, depression, anxiety etc. (33).

In this study, animals from PTZ group, without administered MEL, resulted in a significantly decrease in time spent to open arms at the test of elevated plus maze. On the other hand, with MEL treatment, time spent to open arms increased. Pellow et al. (19) who validated plus-maze as a measure of anxiety in the rat, demonstrated that, anxiety significantly reduces exploratory behaviors in open arms, since decreased time spent in open arms and increased time spent in enclosed arms are defined as high anxiety for rats. Wada and Fukuda (28) also found significantly decreasing of spent time open arms the animals that didn't take any drugs except PTZ.

It is known that, ROS both play role to disrupt physiological stages of organism and prevent to produce antioxidant system enzymes (34,35). In a result of this condition, higher MDA levels are estimated to reduce antioxidant activity. Eun et al. (36) demonstrated that, in epileptic cerebral cortices, down regulated SOD transcription occurred and protein level of SOD was lower than the samples that were not from epileptic cerebrums. Previous studies showed that, CAT activity is an essential parameter to identify effective function of antioxidant systems (37,38). In this study both SOD and CAT activity were found higher in MEL administered groups. In addition, high MEL administered group had significantly higher results than low MEL administered group.

Oruc et al. (39) compared the TAS and TOS results with some other parameters such as HIF 1 $\alpha$  after crocin treatment in cerebral ischemia, and found that TOS levels increased and TAS levels decreased in the brain tissue of cerebral ischemic rats, while crocin treatment was shown opposite effect. Koksal et al. (40) found that the increased TOS level as a result of ischemia decreased with the MEL treatment and increased TAS levels were found in rats treated with MEL. In our study, MEL caused increased TOS levels in the brain tissues of rats group in the PTZ-

induced epileptic seizures, and decreased TAS levels to increase. Statistically significant dose dependent effect was evaluated.

## Conclusion

In conclusion, our study showed that, MEL has dose depended therapeutic effects on epileptic seizure determination. We found that, different doses of MEL support antioxidant activities capacities and decrease lipid peroxidation. The antioxidant power of MEL was shown at this study. It has been determined that dose-dependent MEL administration decreases the total oxidant content by increasing the antioxidant power, and normalizes the epileptic seizures and the metabolic changes that occur. This has revealed that MEL use is an effective substance that can be used to eliminate direct or indirect negative effects that may arise due to epileptic seizure processes in the long term.

## Ethics

**Ethics Committee Approval:** All animal experiments were carried out in accordance with the approval of the Animal Use Adnan Menderes University Ethical Committee (decision no: 64583101/2014/022, date: 27.02.2014).

**Informed Consent:** An animal experiment.

**Peer-review:** Externally peer-reviewed.

## Authorship Contributions

Concept: F.Ş., R.O.E., Design: F.Ş., R.O.E., Supervision: F.Ş., R.O.E., Fundings: F.Ş., R.O.E., Materials: F.Ş., R.O.E., Data Collection or Processing: F.Ş., R.O.E., Analysis or Interpretation: F.Ş., R.O.E., Literature Search: F.Ş., R.O.E., Writing: F.Ş., R.O.E., Critical Review: F.Ş., R.O.E.

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