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Wi-Fi decreases melatonin protective effect and increases hippocampal neuronal damage in pentylenetetrazole induced model seizures in rats

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ABSTRACT

Aim: Epilepsy is a common brain disorder in which the seizures could cause a neuronal loss in the hippocampus. Oxidative stress has an important role in the pathology of epilepsy. Some studies indicate that Wi-Fi increases oxidative stress and suppresses antioxidant systems. The aim of this study is to investigate the effect of Wi-Fi on melatonin anticonvulsive effect and oxidative damage in pentylenetetrazole-induced epileptic seizures in rats.
Methods: In our study, we used 30 male Wistar Albino rats, 230–250 grams of the body weight. The animals were divided into five groups as control, saline (1 ml/kg/day olive oil for 30 days), Wi-Fi (12 h/day for 30 days), melatonin (10 mg/kg/day for 30 days) and melatonin + Wi-Fi (10 mg/kg/day +12 h/day for 30 days). In the thirtieth day, thirty minutes after the last drugs administration at the indicated doses, PTZ in 45 mg/kg was administered to induce epileptic seizure. The animals were observed for 30 min during the seizure stages (according to the Racine Scale) and first myoclonic jerk times (FMJ). Twenty-four hours after PTZ injection, brain tissues were removed for biochemical and histopathological evaluation. The hippocampal Cornu Ammonis (CA) 1, CA3 and DG (dentate gyrus) regions were histopathologically evaluated in terms of a neuronal damage in addition that oxidative stress markers (total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI)) were measured in brain tissues.
Results: Wi-Fi was not found to affect behavioral changes associated with epilepsy ($p > 0.05$). However, Wi-Fi reduced anticonvulsive and antioxidant effect of melatonin ($p < 0.05$). Moreover, Wi-Fi increased neuronal damage in hippocampus ($p < 0.05$).
Conclusion: Wi-Fi did not directly affect epileptic seizures. Nevertheless, it inhibits the positive effects of melatonin on epilepsy and it also has negative effects on hippocampal neuronal damage. These effects of Wi-Fi may occur via oxidative pathways.

1. Introduction

Epilepsy, the most common neurological disease, is a brain disorder observed by epileptic seizures of a group of neurons in the central nervous system [1,2]. Epileptic seizures induce oxidative stress and neurodegenerative changes and cause the damage of hippocampus [2–4]. After an epileptic seizure, Reactive Oxygen Species (ROS) are produced in the hippocampus. Due to the brain tissue's high oxygen consumption and the weak antioxidant system, brain tissue could be exposed more easily to oxidative damage than the other types of tissues [5–7]. Nowadays, some studies have reported that Wi-Fi (a commercial name for IEEE 802.11-compliant wireless networking) exposure induces oxidative stress in rat brain [8]. The widespread use of Wi-Fi (2.45 GHz) in daily lives makes it important for health researches, especially on brain disorders, which are under consideration due to prolonged use of electronic media with unlimited access to internet.

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Exposure to Wi-Fi gives brain an oxidative damage in epileptic seizures, and the neuro-hormone melatonin which is secreted from the pineal gland has been considered as a potential ROS scavenger [9], antioxidant agent [10], anticonvulsant agent [11], neuroprotective agent [12]. Moreover, Melatonin reduced convulsions induced by pentetrazol, pilocarpine, L-cysteine and kainite [13,14].

There is little information about the effects of Wi-Fi and such radiations on man's health, but it is still under investigation. The adverse effects of microwaves on tumors [15,16], the effect of melatonin on Wi-Fi induced oxidative stress in rat lens [17], the effect of Wi-Fi on epileptic seizures [18], and the effect of electromagnetic radiation of mobile phones on the central nervous system [3] are some of the published studies. However, the impact of Wi-Fi on melatonin anticonvulsive effect in pentylenetetrazole-induced epileptic seizures in rats has not been investigated yet.

The aim of this study is to investigate the impact of Wi-Fi on anticonvulsive, antioxidant, and neuroprotective effects of melatonin in PTZ-induced epileptic seizures in rats.

2. Materials and methods

2.1. Animals

All experimental protocols were performed in accordance with the guidelines of Ethical Committee for the Purpose of Control and Supervision of Experiments on Animals (65202830-050.04.04-145). All rats were housed and bred for 12 h in light and 12 h in dark in polypropylene cages and at a temperature of 20–22 °C with free access to both food and water.

2.2. Drugs

Melatonin and pentylentetrazol were dissolved in physiological saline. The drugs were purchased from SigmaAldrich Co., St Louis, MO, USA. Solutions were freshly prepared on the days of the experiments. All the treatments were performed by intraperitoneal (i.p.) route.

2.3. Experimental design

In our study, we used 30 male Wistar Albino rats, 230–250 grams of the body weight and we used long term exposure of melatonin and Wi-Fi (for 30 days). Animals were divided into five groups as Group1: control, Group 2: saline (1 ml/kg/day serum physiologic for 30 days), Group 3: Wi-Fi (12 h/day for 30 days), Group 4: melatonin (10 mg/kg/day for 30 days) and Group 5: melatonin + Wi-Fi (10 mg/kg/day +12 h/day for 30 days). Melatonin preparation and dose selection were performed according to the literature [19]. On thirtieth day, thirty minutes after the last administration of the drugs in indicated doses, PTZ as 45 mg/kg was administered to induce epileptic seizure. The animals were observed for 30 min for resulting epileptic seizures and the first myoclonic jerk times (FMJ) were recorded. Seizure stages were evaluated by the Modified Racine Scale (0 No response 1 Sudden behavioral arrest and/or motionless staring, 2 Facial jerks with muzzle or muzzle and eye, 3 Neck jerks, 4 Clonic seizures in a sitting position, 5 Convulsions including clonic and/or tonic–clonic seizures while lying on their bellies and/or pure tonic seizures, 6 Convulsions including clonic and/or tonic–clonic seizures while lying on either side and/or wild jumps [20]. Twenty-four hours after PTZ injection, brain tissues were removed for biochemical and histopathological evaluations.

2.4. Wi-Fi exposure system

Radio frequency generator is a regular wireless internet router (Zyxel NBG-418NV2 wireless Access Point/Router) which was used in previous studies [27]. It generates radio frequency electromagnetic fields with a frequency of 2.4 GHz and works at a speed up to 300 Mbps. The rats were exposed to Wi-Fi at a distance of average 60 cm from a commercial Wireless Internet router. The maximum output power was 2 W. The electric field density was set at 11 V/m. Zyxel wireless internet router and rat cages were situated in a Faraday Cage for preventing pollution from external electromagnetic fields. In the control group (Group 1 and Group 2), rat cages were situated in a Faraday Cage but they were not exposed to any electromagnetic fields. The other groups (Group 3 and Group 5) were exposed to 2.4 GHz of radio frequency electromagnetic fields for 12 h/day for 14 days.

2.5. Biochemistry

Brain tissue samples from each group were homogenized within 10 volumes of the ice-cold homogenization buffer and centrifuged at 12,000 × g for 10 min at 4 °C. The supernatant was collected for protein concentration determination by a Bradford protein assay kit (Merck, Germany) and for TAS and TOS determination by using TAS, TOS kit (Total Oxidant Status Assay Kit, sample code: RL0024, Rel Assay Diagnostics® Mega Tıp Ltd., Gaziantep, Turkey).

2.6. Histopathology

Histopathologic investigations were performed similar to previous studies [21]. After 36-h fixation with 10 % neutral-buffered formalin (NBF), all the brain tissues were embedded in paraffin blocks and cut into 5-μm-thick serial sections in order to examine histopathological assessment. The coronal hippocampus sections of the rat were stained with toluidine blue stain to quantify the number of dark neurons. All sections were examined and were photographed by Olympus C-5050 digital camera at Olympus BX51 microscope. In the hippocampal CA1, CA3 (Cornu Ammonis) and DG (Dentate gyrus) regions, dark neurons and survival neurons were counted in five sections per each studied animal (n = 5 for each group). The numbers of dark neurons were given by percentage (toluidine blue stained neurons*100/survival neuron). The blind observers of the study groups accomplished all histological assessments.

2.7. Statistical analysis

The results were expressed as a mean ± standard deviation of mean (SEM). The data analyses were performed by SPSS Version 25.0 for Windows. The RCS score, FMJ time and dark neurons were evaluated using a one-way analysis of variance (ANOVA). A posthoc Tukey test was utilized to identify the differences between the experimental groups, and it was a value of $p < 0.05$ which is accepted as statistically significant.

3. Results

3.1. Evaluation of groups in terms of RCS and FMJ onset times

When the Racine scores were evaluated between the groups, there were statistically significant differences between the Group 2: saline (1 ml/kg/day serum physiologic for 30 days) and Group 4: melatonin (10 mg/kg/day for 30 days). The highest RCS score and the lowest FMJ values were detected in Group 2: saline (1 ml/kg/day serum physiologic for 30 days). The lowest RCS score and the highest FMJ values were detected in Group 4: melatonin (10 mg/kg/day

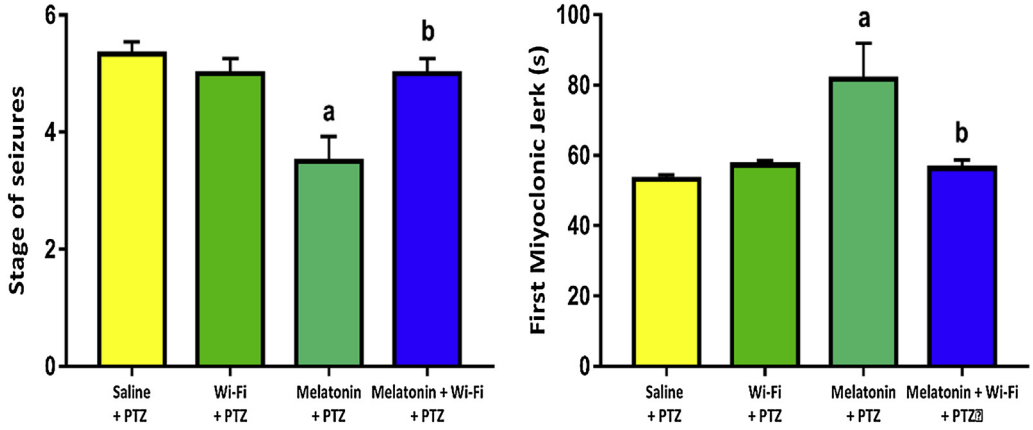

Fig. 1. The Effect of Wi-Fi and melatonin on RCS and FMJ in PTZ-induced seizures. The values are presented as mean ± SEM. It is [a]p < 0.05 in comparison with saline group and it is [b]p < 0.05 in comparison with melatonin group.

Table 1
The Effect of Wi-Fi and Melatonin on TAS, TOS and OSI in rat brain after PTZ induced seizures. Values are presented as mean ± SEM. [a]p < 0.05 in comparison with the control group, [b]p < 0.05 in comparison with PTZ group and [c]p < 0.05 in comparison with melatonin group.

Group	TAS (μmol/mg protein)	TOS (μmol/mg protein)	OSI
Control (Group1)	0.17 ± 0.01	0.63 ± 0.02	361.08 ± 13.53
Saline (PTZ 45 mg/kg, i.p.) (Group 2)	0.16 ± 0.00	0.78 ± 0.02[a]	473.64 ± 17.00[a]
Wi-Fi (Group 3)	0.18 ± 0.01	0.77 ± 0.03[a]	424.65 ± 22.26[c]
Melatonin (10 mg/kg i.p) (Group 4)	0.20 ± 0.00[b]	0.73 ± 0.01	351.65 ± 10.95[b]
Melatonin + Wi-Fi (Group 5)	0.18 ± 0.00	0.77 ± 0.01[a]	423.99 ± 10.72[c]

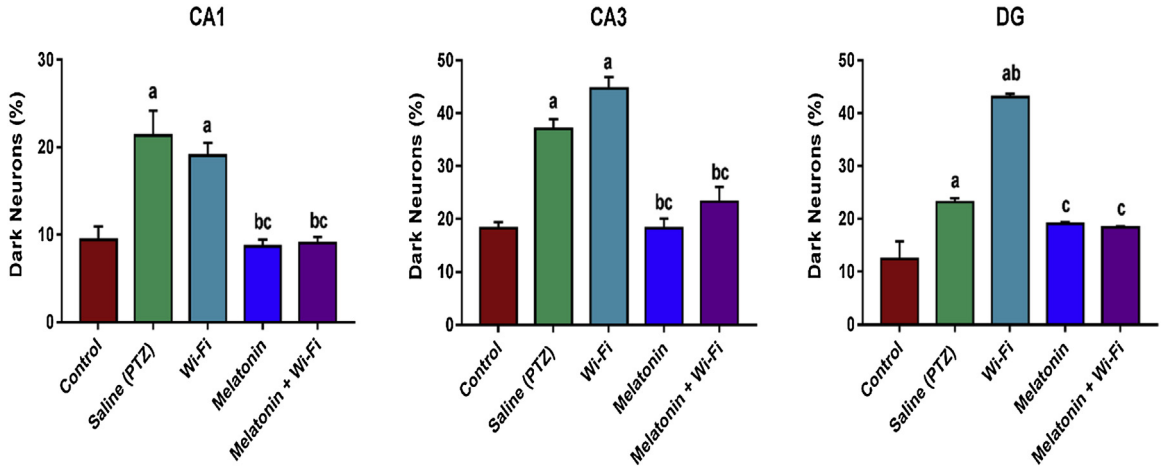

Fig. 2. The Effect of Wi-Fi and Melatonin on dark neuron in CA1, CA3, and DG hippocampal regions after PTZ induced seizures. The values are presented as mean ± SEM. [a]p < 0.05 in comparison with the control group and [b]p < 0.05 in comparison with PTZ group and [c]p < 0.05 in comparison with Wi-Fi group.

for 30 days) that means melatonin affects RCS and FMJ values (p > 0.05) (Fig. 1). Wi-Fi did not have a significant effect on epileptic seizures according to RCS and FMJ results. In addition, Wi-Fi suppressed positive effects of melatonin as seen in Group 5: melatonin + Wi-Fi (10 mg/kg/day +12 h/day for 30 days).

3.2. Biochemical results

TAS and TOS were used as markers for demonstrating the antioxidant and the oxidative effects of melatonin, respectively. We observed some increased TAS and decreased OSI values (p < 0.05) in melatonin (Group 4) in comparison with PTZ group (Group 2), the increased TOS and OSI values in PTZ(Group 2), melatonin (Group 4) and Wi-Fi + melatonin (Group 5) in comparison with the control group (Group 1) (Table 1). However, total antioxidant status (TAS) was affected by melatonin supplementation and was not affected by Wi-Fi exposure.

3.3. Histopathological assessments

As seen in Figs. 2 and 3, compared to the control group, PTZ and Wi-Fi exposure significantly increased the percentage of dark neurons in CA1, CA3, and DG regions. Hippocampal DG region (Wi-Fi affected) increased the dark neuron numbers as compared to the control group and PTZ. Melatonin significantly decreased the production of the dark neurons in the hippocampal CA1, CA3, and DG areas as compared to PTZ group.

4. Discussion

A large number of research studies focus on the specific effects of melatonin on epileptic seizures. However, in the present study, we are mainly concerned about the effects of Wi-Fi on its anticonvulsive effect in PTZ-induced epileptic seizures in rats. In this study, we found that Wi-Fi inhibits anticonvulsant effects of melatonin on

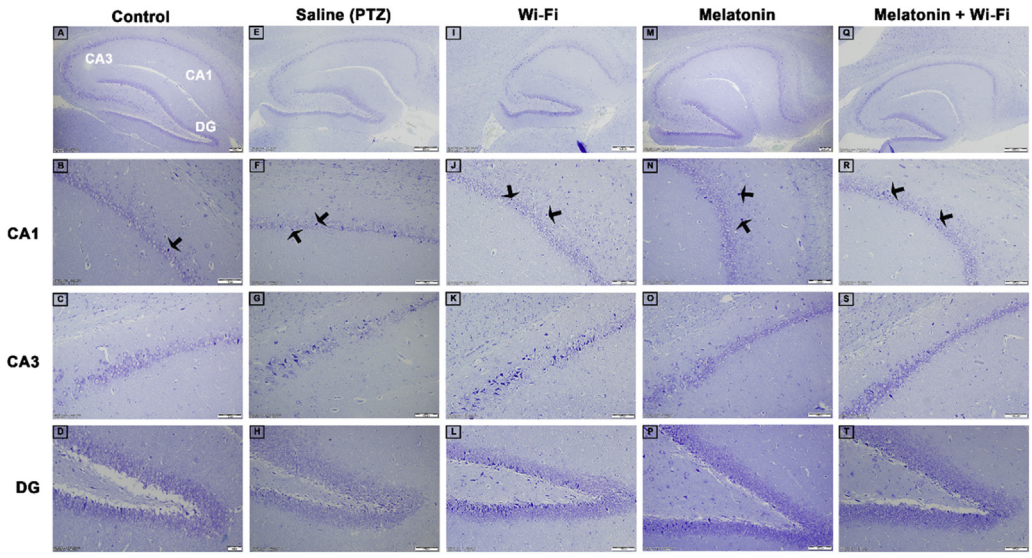

Fig. 3. The sections of the rat coronal hippocampus with toluidine blue staining. Basophilic (dark) neurons (arrow) distributed between normal pyramidal neurons. CA1region; (B) Control (F), PTZ group, (J) Wi-Fi group, (N) Melatonin group, (R) Melatonin + Wi-Fi group. Basophilic (dark) neurons distributed in the hippocampal CA3 region; (C) Control, (G) PTZ group, (K) Wi-Fi group, (O) Melatonin group, (S) Melatonin + Wi-Fi group. In the hippocampal DG (dentate gyrus) region; (D) Control, (H) PTZ group, (L) Wi-Fi group, (P) Melatonin group, (T) Melatonin + Wi-Fi group. General hippocampal images of groups A, E, I, M, and Q (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

PTZ-induced epileptic seizures in rats. The studies on epilepsy were carried out by PTZ-induced model because it is popular for testing new therapeutic targets [22,23].

It is widely accepted that PTZ-induced alterations are associated with the oxidative stress. Increasing oxidative stress (ROS production) leads to the death of neurons in the brain [24,25]. So, it is clear that oxidative stress has a considerable role in epileptogenesis and increasing ROS production is believed to have damaging effects on brain by epileptic activity. This parallels our results that Wi-Fi exposure also induces oxidative damage in rat brain in PTZ-induced seizures [26,27]. In this study, the oxidative damage was measured by several parameters like TAS, TOS and OSI levels. The increased TOS and OSI (oxidative stress index, an indicator of the degree of oxidative stress) values (Table 1) proved the harmful effect of Wi-Fi on epileptic seizures. Similar to our study, radiation was found to cause oxidative stress in some studies [28,29], but in other studies, radiation was concluded not to cause oxidative stress [30]. Melatonin (10 mg/kg/day) was used due to anticonvulsive, antioxidant and neuroprotective properties [31]. Melatonin has a potential scavenger role both for Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) [10]. It also stimulates the activity of antioxidative enzymes similar to our study. Tök et al. (2014) studied the effects of melatonin on Wi-Fi-induced oxidative stress on the lens of rats and they demonstrated protective effect on the oxidant system of melatonin supplementation on the lens of rats [25]. Aynali et al. (2013) demonstrated that protective effects of melatonin on Wi-Fi induced oxidative stress in the laryngotracheal mucosa of rats [19]. In our study, it is surprising that melatonin on its own had a protective effect, however, after the exposure to Wi-Fi, the protective effect of melatonin was suppressed in PTZ-induced seizure. This finding implicates that the neuroprotective effect of melatonin in rat brain is masked by Wi-Fi exposure. Previous researches have confirmed that Wi-Fi exposure induces oxidative stress and hippocampal apoptosis [25,32]. Furthermore, the cytosolic Ca^{2+} increase in rat hippocampus was induced by Wi-Fi exposure [32]. Thus, these conditions contributing to the epileptogenesis may reduce the anticonvulsant properties of the hormone. All of these studies were performed with long-term treatment by melatonin at 10 mg/kg for 30 days. It is reported that there is a potential therapeutic significance of a long-term melatonin treatment in a KA model for neuroprotective activity of the hormone in brain regions [14]. Melatonin treatment has a significant neuroprotective effect in specific brain areas such as the CA1, CA3 and DG hippocampal regions [33]. The results of a recent study [34] showed that PTZ-induced seizures were resulted in dark neuron production in the hippocampal regions, as observed in previous studies. As well known in literature, Wi-Fi has important negative effects on human health [35,36]. On the basis of these evaluations, there are plenty of studies about long term exposure of Wi-Fi (2.45 GHz) on rat and mice [37–41]. Shahin et al. (2015) investigated the effect of short (15 days) and long-term (30 and 60 days) at a low-level of 2.45 GHz at MW radiation exposure on hippocampus with special reference to spatial learning and memory and its underlying mechanism in Swiss strain male mice, *Mus musculus*. They observed that the short-term as well as the long-term at 2.45 GHz, MW radiation exposure increases the oxidative/nitrosative stress, thus lead to enhanced apoptosis in hippocampal subfield neuronal and non-neuronal cells. The common neurodegenerative disease, epilepsy, and the increasing use of Wi-Fi made these two research areas important. Ghazizadeh, V. and Nazıroğlu, M. (2014) tested the effects of Wi-Fi (2.45 GHz) exposure on (PTZ)-induced epileptic rats [32]. They indicated that epilepsy and Wi-Fi are involved in Ca2+ influx and oxidative stress-induced hippocampal and DRG death. In our study, Wi-Fi exposure was also found to cause oxidative stress and to increase the number of dark neurons in hippocampal CA1, CA3 and DG regions. We investigated the protective effect of melatonin on Wi-Fi exposure to PTZ-induced epileptic seizures and we found that melatonin had protective effects rather than Wi-Fi exposure did. Similar to our findings, Nazıroğlu, et al. (2012) investigated melatonin effects on Wi-Fi exposure and they found the protective effects of melatonin on Ca^{2+} homeostasis in the DRG neuron [42].

Oxidative stress plays a key role in epileptogenesis after the first seizure. Through progressive neurobiological changes, the first seizure later becomes a cause for recurrent seizures in TLE. These changes override with long term exposure of melatonin. The observed effect was associated with a significant delay in the onset of the first spontaneous seizure and a decrease in seizure frequency

during the treatment and after its discontinuation. Several studies confirmed the anticonvulsive effect of melatonin [11,43,44]; where all these factors were considered epileptic behavior.

5. Conclusion

In this study, our aim is to investigate antioxidant, anticonvulsive and neuroprotective effect of melatonin on Wi-Fi exposed PTZ-induced seizures in rat brains. Wi-Fi does not directly affect epileptic seizures. Nevertheless, it inhibits melatonin positive effects on epilepsy and also it has negative effects on hippocampal neuronal damage. These effects of Wi-Fi may occur via oxidative pathways.

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