TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2015) 45: 842-849
© TÜBİTAK
doi:10.3906/sag-1406-35

Effects of melatonin and theanine administration on pentylenetetrazole-induced seizures and brain tissue oxidative damage in ovariectomized rats

Shadi CHOOPANKAREH[1], Farzaneh VAFAEE[1], Mohammad Naser SHAFEI[2], Hamid Reza SADEGHNIA[3], Reza SALARINIA[4], Leila ZAREPOOR[5], Mahmoud HOSSEINI[1,*]

[1]Neurocognitive Research Center & Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[2]Neurogenic Inflammation Research Center & Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[3]Pharmacological Research Center of Medicinal Plants & Department of Pharmacology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[4]Department of Modern Sciences and Technologies, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[5]Department of Human Health and Nutritional Sciences, College of Biological Science, University of Guelph, Guelph, Canada

Received: 09.06.2014 • **Accepted/Published Online:** 26.11.2014 • **Printed:** 30.07.2015

Background/aim: The effects of coadministration of melatonin and theanine (Mel/Thea) on pentylenetetrazole (PTZ)-induced seizures and brain tissue oxidative damage were investigated in ovariectomized (OVX) and sham-operated rats.

Materials and methods: The rats were divided into the following groups: 1) sham, 2) ovariectomized (OVX), 3) sham-PTZ, 4) OVX-PTZ, 5) sham-Mel/Thea-PTZ, and 6) OVX-Mel/Thea-PTZ. Groups 1–4 received saline, while groups 5 and 6 received a combination of Mel/Thea for 6 weeks. All animals except for those in groups 1 and 2 received a single injection of PTZ.

Results: The OVX-PTZ group had higher generalized tonic-clonic seizure (GTCS) latency compared to the sham-PTZ group. Administration of Mel/Thea increased minimal clonic seizure and GTCS latencies in both the sham-Mel/Thea-PTZ and OVX-Mel/Thea-PTZ groups compared to the controls. Additionally, PTZ exposure increased malondialdehyde levels and reduced thiol concentrations in brain tissues of both the sham-PTZ and OVX-PTZ groups. Mel/Thea pretreatment resulted in MDA reduction and thiol increase in brain tissues.

Conclusion: The results of this study demonstrated the antioxidant and anticonvulsant activities of Mel/Thea despite the presence or absence of ovarian hormones.

Key words: Melatonin, theanine, pentylenetetrazole, seizures, oxidative stress, brain, rat

1. Introduction

Epilepsy is a common neurological disease that affects approximately 1% of the population (1). It is characterized by abnormal episodic bursts of electrical activity in neurons, which significantly impact the cognitive processes and behavior of the affected patients (2). The central nervous system (CNS) is very susceptible to oxidative injury due to high levels of membrane lipid constituents (3,4). Accumulating evidence has revealed an important role for oxidative stress, both as a consequence and as a cause of epileptic seizures (5). It has been found that prolonged seizures increase the production of free radicals (6) and result in oxidative damage to lipids, DNA, and susceptible proteins (7). Moreover, it has been shown that reactive oxygen species (ROS) may underlie the neurotoxic effects of some convulsant agents, such as pentylenetetrazole (PTZ) (8). Furthermore, it has been demonstrated that the antioxidative properties of melatonin, vineatrol, trans-resveratrol, and alpha-lipoic acid are associated with their anticonvulsant effects (6,9). Collectively, it appears that using antioxidants is a promising approach in the development of new therapeutic agents with neuroprotective effects against epileptic seizures (5,7).

Additionally, it has been well documented that female sex hormones play a role in epilepsy (10,11). There are also close communications and connections between the hypothalamus and pituitary gland, which regulate sex hormone secretion, and the temporal lobes, which

* Correspondence: hosseinim@mums.ac.ir

are important areas in seizures (12). Female epileptics frequently show exacerbated symptoms at specific points during their menstrual cycle, for example during periods of low progesterone levels (10). It has also been found that progesterone reduces neuronal activity, while estrogen increases neuronal excitability (11). In fact, previous studies demonstrated that low progesterone levels are associated with increased seizure frequency in women (13). Elevated estrogen levels during perimenopause also appear to exacerbate epilepsy (13). It has also has been observed that testosterone and its metabolites have antiseizure effects in men (14). These findings collectively suggest the potential influence of sexual hormones on seizure susceptibility.

On the other hand, some dietary components with antioxidative properties have shown neuroprotective effects. For instance, L-theanine (gamma-glutamylethylamide), a unique amino acid present almost exclusively in tea, has demonstrated antioxidative and neuroprotective effects (15,16). Previous studies have shown that L-theanine increases brain serotonin, dopamine, and gamma amino butyric acid (GABA) levels; improves memory function; and has a neuroprotective effect in several animal models of neurological disorders (17,18).

Additionally, some neuroagents with antioxidative properties, such as melatonin, were demonstrated to have neuroprotective effects in previous studies (19–23). Melatonin, an indoleamine derivative of serotonin produced by the pineal gland, has shown antioxidative, sedative, anticonvulsive, and hypnotic effects in the CNS of mammals (19,21–23). In rats, melatonin has anticonvulsant and inhibitory effects on the glutamate-mediated response of the striatum to motor cortex stimulation (20). Melatonin also depresses brain excitability and prevents seizures (6). It has been well documented that melatonin influences reproductive hormones both in males and females (24,25). It has been shown that melatonin decreases estrogen levels while increasing the progesterone concentrations in rats (26).

The objective of the present study was to evaluate the effects of the administration of a combination of melatonin and theanine on seizures and oxidative brain tissue damage induced by PTZ in ovariectomized (OVX) rats.

2. Materials and methods

2.1. Drugs and chemicals

PTZ, melatonin, and theanine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemical compounds such as thiobarbituric acid (TBA), trichloroacetic acid (TCA), 2,2'-dinitro-5,5'-dithiodibenzoic acid (DTNB), ethylenediaminetetraacetic acid (EDTA), and hydrochloric acid (HCl) were bought from Merck (Kenilworth, NJ, USA).

2.2. Animal groups

Forty-eight virgin female Wistar rats (230 ± 20 g in weight) were maintained in an animal house under controlled conditions, including 12-h light/dark cycle, 22–24 °C temperature, and appropriate humidity, with laboratory chow and water provided ad libitum. The study protocol using the laboratory rats complied with the general guidelines of animal care of Mashhad University of Medical Sciences, Iran.

The animals were acclimatized for 15 days and then were ovariectomized under ketamine anesthesia (150 mg/kg, i.p.) (27–30). Anesthesia was confirmed by a reduced respiratory rate and a lack of response to the gentle pinching of the foot pad. Abdominal incisions were made through the skin of the flank of the rats, and the ovaries and ovarian fats were removed. Ovaries were isolated by ligation of the most proximal portion of the oviduct before removal. The same procedure was performed on the rats in the sham group, except that the wound was closed without removing the ovaries (31–33).

Animals were divided into the following 6 groups (n = 8): 1) sham, 2) OVX, 3) sham-PTZ, 4) OVX-PTZ, 5) sham-Mel/Thea-PTZ, and 6) OVX-Mel/Thea-PTZ. The animals in groups 1–4 were treated with saline daily (2 mL/kg) for 6 weeks, while groups 5 and 6 received a combination of melatonin and theanine (Mel/Thea; 3 mg and 25 mg/kg) intraperitoneally daily for 6 weeks. After 6 weeks of saline or Mel/Thea injections, animals in groups 3–6 received a single injection of PTZ (90 mg/kg, i.p.). Minimal clonic seizure (MCS) and generalized tonic-clonic seizure (GTCS) latencies, as ictal behaviors, were then measured (34–37). Finally, the animals were sacrificed, and the cortical and hippocampal tissues were removed for biochemical analyses.

2.3. PTZ-induced seizures

In order to assess the ictal behavior, animals were placed in a Plexiglas arena (30 cm × 30 cm × 30 cm) after PTZ injection and were observed for 60 min after PTZ administration. Behavioral responses of the animals to PTZ were evaluated using the following criteria: latency to the first MCS, incidence of MCS, latency to the first GTCS, incidence of GTCS, and mortality percentage (34–36).

2.4. Biochemical assessment

After behavioral assessments, the animals were sacrificed and the brains were removed. The cortical and hippocampal regions were dissected on an ice-cold surface and homogenized in ice-cold phosphate-buffered saline to give 10% homogeny. Total SH groups were measured using DTNB as the reagent. This reagent reacts with the thiol groups to produce a yellow complex that has peak absorbance at 412 nm. Briefly, 1 mL of Tris-EDTA buffer (pH 8.6) was added to 50 µL of brain homogenate in 1-mL cuvettes, and sample absorbance was read at 412

nm against Tris-EDTA buffer alone (A_1). Then 20 µL of DTNB reagent (10 mM in methanol) was added to the mixture. The mixture was incubated at room temperature for 15 min, and then the sample absorbance was read again (A_2). The absorbance of DTNB reagent was also read as the blank (B). Total thiol concentration (mM) was calculated using the following equation (37–40):

total thiol concentration (mM) = $(A_2 - A_1 - B) \times 1.07/(0.05 \times 13.6)$.

Malondialdehyde (MDA) levels, as an index of lipid peroxidation, were measured in cortical and hippocampal regions. MDA reacts with TBA as a thiobarbituric acid reactive substance to produce a red-colored complex that has peak absorbance at 535 nm. The TBA/TCA/HCL reagent was added for homogenization, and the solution was incubated in a boiling water bath for 40 min. After cooling, the whole solution was centrifuged at 1000 × *g* for 10 min. The absorbance was measured at 535 nm (37–40). The MDA concentration was calculated as follows: C (M) = absorbance/(1.56×10^5).

2.5. Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM) and were analyzed using one-way ANOVA followed by Tukey's post hoc comparison test. Comparisons between two groups were done using an unpaired t-test. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Behavioral results

PTZ exposure was associated with seizure incidence (MCS and GTCS) in all PTZ-exposed groups. Ovariectomy showed a protective effect in PTZ-induced seizures by increasing the GTCS latency in the OVX-PTZ group compared to sham-PTZ group ($P < 0.01$, Table 1). However, it did not affect the MCS latency ($P > 0.05$, Table 1). Additionally, Mel/Thea administration significantly increased both MCS and GTCS latencies in the sham-Mel/Thea-PTZ group compared to the sham-PTZ group ($P < 0.05$, Table 1). Mel/Thea treatment had a similar effect on OVX rats, as it increased the MCS and GTCS latencies in the OVX-Mel/Thea-PTZ group compared to the OVX-PTZ group ($P < 0.01$, Table 1).

3.2. Biochemical results

Ovariectomy was associated with biochemical changes in brain tissue, which was seen in the reduced thiol contents and increased MDA levels in the hippocampal regions of the OVX group compared to the sham group ($P < 0.001$, Table 2). PTZ exposure also resulted in a significant reduction in thiol levels and a significant increase in MDA concentrations in the hippocampal tissues of the sham-PTZ group compared to the sham group ($P < 0.01$ and $P < 0.001$, respectively, Table 2). Additionally, PTZ exposure aggravated the adverse effects of ovariectomy on hippocampal thiol concentrations by reducing the hippocampal thiol levels of the OVX-PTZ group compared to the OVX group ($P < 0.01$); however, it did not cause a significant difference in MDA concentrations ($P > 0.05$, Table 2).

Administration of Mel/Thea improved the hippocampal thiol levels in the sham-Mel/Thea-PTZ group ($P < 0.05$), but it did not make a significant difference in hippocampal MDA concentrations between the sham-Mel/Thea-PTZ and sham-PTZ groups ($P > 0.05$, Table 2). Furthermore, Mel/Thea administration was associated with the alteration of both MDA and thiol levels in the hippocampal area of the OVX-Mel/Thea-PTZ group by reducing MDA levels ($P < 0.05$, Table 2) and increasing thiol concentrations compared to the OVX-PTZ group ($P < 0.001$, Table 2).

In addition to biochemical alterations in the hippocampal area, ovariectomy also caused significant changes in MDA and thiol levels in cortical regions. OVX rats showed reduced thiol and increased MDA levels in cortical areas compared to the sham group ($P < 0.05$ and $P < 0.001$, respectively, Table 3). Similarly, PTZ exposure was associated with an increased level of cortical MDA and a reduced level of cortical thiol in both the sham-PTZ and OVX-PTZ groups compared to their control groups (sham and OVX groups, respectively) ($P < 0.05$, Table 3).

Table 1. Comparison of MCS and GTCS latencies between the study groups.

Groups	MCS latency (s)	GTCS latency (s)
Sham-PTZ	59.4 ± 4.05	67.6 ± 5.48
Sham-Mel/Thea-PTZ	135 ± 24.09*	227.66 ± 77.73*
OVX-PTZ	105.88 ± 24.8	144.33 ± 32.75**
OVX-Mel/Thea-PTZ	262.2 ± 67.109++	416.2 ± 120.41++

All data were expressed as mean ± SEM (n= 8 in each group).
*$P < 0.05$, **$P < 0.01$ compared to sham-PTZ.
++$P < 0.01$ compared to OVX-PTZ.

Table 2. Comparison of the MDA and total thiol concentrations in hippocampal tissues between the study groups.

Groups	MDA concentration in hippocampal tissues (nmol/g tissue)	Thiol contents in hippocampal tissues (mM)
Sham	9.139 ± 0.57	25.35 ± 2.27
Sham-PTZ	16.2 ± 0.75***	14.17 ± 2.08**
Sham-Mel/Thea-PTZ	13.21 ± 1.41	24.94 ± 3.52#
OVX	15.14 ± 1.42***	14.55 ± 1.87***
OVX-PTZ	14.34 ± 0.68	5.65 ± 1.52++
OVX-Mel/Thea-PTZ	10.01 ± 0.48$	22.41 ± 2.28$$$

All data were expressed as mean ± SEM (n= 8 in each group).
P < 0.01,*P < 0.001 compared to the sham group.
++P < 0.01 compared to the OVX group.
#P < 0.05 compared to the sham-PTZ group.
$P < 0.05, $$$P < 0.001 compared to the OVX-PTZ group.

Table 3. Comparison of the MDA and total thiol concentrations in cortical tissues between the study groups.

Groups	MDA concentration in cortical tissues (nmol/g tissue)	Thiol contents in cortical tissues (mM)
Sham	8.17 ± 0.5	21.13 ± 4.94
Sham-PTZ	16.09 ± 0.51***	3.73 ± 0.83***
Sham-Mel/Thea-PTZ	10.08 ± 0.3###	16.1 ± 3.96#
OVX	12.13 ± 0.49***	11.23 ± 2.13*
OVX-PTZ	15.79 ± 0.53+++	6.26 ± 0.66+
OVX-Mel/Thea-PTZ	10.67 ± 0.56$$$	11.44 ± 1.18$

All data were expressed as mean ± SEM (n= 8 in each group).
*P < 0.05, ***P < 0.001 compared to the sham group.
+P < 0.05, +++P < 0.001 compared to the OVX group.
#P < 0.05, ###P < 0.001 compared to the sham-PTZ group.
$P < 0.05, $$$P < 0.001 compared to the OVX-PTZ group.

Mel/Thea administration improved both MDA and thiol levels in the cortical regions of the sham-Mel/Thea-PTZ group compared to the sham-PTZ group (P < 0.001 and P < 0.05, respectively, Table 3). Similarly, the Mel/Thea combination reduced the cortical MDA concentrations while increasing thiol levels in the OVX-Mel/Thea-PTZ group compared to the OVX-PTZ group (P < 0.05 and P < 0.001, Table 3).

4. Discussion

Oxidative stress is involved in many neurological and neurodegenerative disorders. Previous studies have demonstrated an increase in free radical levels during seizures (6), suggesting the important role of oxidative stress in the pathogenesis of epileptic seizures (5). Similarly, in the present study, we observed an increase in MDA levels and a reduction in total thiol groups in the brain

tissues of animals that had PTZ-induced seizures. It has been well demonstrated that production of ROS, including superoxide anions, hydroxyl radicals, and hydrogen peroxide, increases in the brains of animals subjected to seizures (41,42). Oxidative damage of brain tissue by free radicals may lead to psychiatric or cognitive problems such as depression, anxiety, and memory loss (43,44). The reduction of the life span observed in epileptic patients may also be partly related to neuronal oxidative damages (45). Furthermore, oxidative stress has been suggested as a link between aging and seizures (46). The results of the present study also demonstrate a link between seizures and brain tissue oxidative damage in a PTZ-induced seizure model. This model has been frequently used to examine the potential anticonvulsant properties of drugs or natural compounds (34–36). PTZ decreases GABA system function and increases the activity of the glutamate neurotransmission system (47). One of the suggested mechanisms for the neurotoxic effects of PTZ is the ROS-induced oxidative damage in the CNS (48,49).

Previously, several in vivo and in vitro studies demonstrated the potential effects of theanine and melatonin in reducing oxidative stress (16,50,51). Theanine increases the production of GABA and dopamine, while it protects the cells of the hippocampus, the major center of learning and memory, from oxidative damage (17). Moreover, in vitro studies using MDA as a marker of lipid peroxidation demonstrated the ability of theanine to inhibit the oxidation of low-density lipoprotein (51). In another study, Reiter showed that melatonin has a potent scavenging role for both ROS and reactive nitrogen species (52). Mohanan and Yamamoto also showed that melatonin has preventive effects on kainic acid (KA)-induced seizures and lipid peroxidation in mice (53). Additionally, several studies confirmed the anticonvulsant effects of melatonin (54,55). The results of the present study demonstrated that the combination of melatonin and theanine increases both GTCS and MCS latencies, indicating the anticonvulsant effects of melatonin and theanine and their ability to reduce seizure susceptibility. The administration of the Mel/Thea combination also resulted in a significant reduction in MDA and in elevated thiol concentrations in brain tissues. Consistent with our findings, some studies reported strong antioxidant activity for theanine and melatonin (16,23), suggesting their protective potential against brain tissues oxidative damage. The results of the present study suggest that the Mel/Thea combination has a protective role against seizures and seizure-related complications; however, more studies are needed.

Additionally, previous studies demonstrated a link between seizures and sex hormones. For instance, it was shown that menopause affects seizure patterns (56). Using OVX rats is a common model of hormone deprivation to study the effects of postmenopausal conditions on the CNS. In this study, ovariectomy resulted in a significant decrease in susceptibility to PTZ-induced seizures. This finding is consistent with previous reports showing that ovariectomies cause a decrease in GTCS and seizure susceptibility (36). It has been shown that estrogen has proconvulsant effects, while progesterone has inhibitory effects on seizure susceptibility (57,58). Galanopoulou et al. showed that estradiol treatment decreased the seizure threshold in female rats (59). In contrast, Hoffman et al. reported that estrogen had no effect on KA-induced seizures (60). Pereira et al. also reported that estrogen replacement therapy attenuated the frequency of seizures in a pilocarpine-induced epilepsy model (61). There is increasing evidence that sex hormones might have a role in seizure susceptibility (62,63); however, the exact mechanisms are unknown. It has been well documented that sex hormones exert their regulatory effects on neuronal excitability by modulating neurotransmitter receptors including GABA, N-methyl-D-aspartic acid (NMDA), and opioid receptors, as well as by direct and/or indirect regulation of adenosine receptors (64–67). These mechanisms may be involved in the lower susceptibility of OVX rats to PTZ-induced seizures in the present study.

Furthermore, several studies have demonstrated the effect of melatonin on the sex hormones of female rats (25,68,69). We hypothesized that the effects of Mel/Thea on seizures may be different between OVX and treatment-naive female rats. For this reason, the effects of a combination of Mel/Thea on PTZ-induced seizures in OVX and naive female rats were also investigated. The Mel/Thea combination delayed latency of PTZ-induced seizures and decreased oxidative damage in both naive female and OVX rats, without a significant difference between the two groups. It has been suggested that the anticonvulsant effects of melatonin may be due to its antioxidative function, neuroprotective effects, central GABAergic transmission, and regulation of the nitric oxide (NO) pathway (70). Mohanan and Yamamoto showed that the scavenging of hydroxyl radicals may contribute to the anticonvulsant effects of melatonin (53). In another study, Acufla-Castroviejo et al. demonstrated that melatonin increased GABA concentration in epilepsy (71). Yahyavi-Firouz-Abadi et al. also reported that the NO pathway is involved in the melatonin-induced modulation of seizure susceptibility in mice (72). These mechanisms may be involved in the anticonvulsant effects of Mel/Thea in the current study.

In conclusion, the results of this study demonstrated that the administration of a combination of Mel/Thea

increases anticonvulsant activity. These beneficial effects are accompanied by an antioxidant effect in brain tissues. We did not find any difference in the efficiency of Mel/Thea between treatment-naive and OVX rats, but further investigations are needed.

Acknowledgments
The results described in this paper were from an MSc thesis. The authors would like to thank the Vice Presidency of Research, Mashhad University of Medical Sciences, for financial support.

References

1. Sander JW. The epidemiology of epilepsy revisited. Curr Opin Neurol 2003; 16: 165–170.
2. Meador KJ. Cognitive outcomes and predictive factors in epilepsy. Neurology 2002; 58: S21–S26.
3. Halliwell B. Oxygen radicals as key mediators in neurological disease: fact or fiction? Ann Neurol 1992; 32: S10–S15.
4. Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Science 1993; 262: 689–695.
5. Patel M. Mitochondrial dysfunction and oxidative stress: cause and consequence of epileptic seizures. Free Radic Biol Med 2004; 37: 1951–1962.
6. Gupta Y, Gupta M, Kohli K. Neuroprotective role of melatonin in oxidative stress vulnerable brain. Indian J Physiol Pharmacol 2003; 47: 373–386.
7. Kudin AP, Kudina TA, Seyfried J, Vielhaber S, Beck H, Elger CE, Elger CE, Kunz WS. Seizure-dependent modulation of mitochondrial oxidative phosphorylation in rat hippocampus. Eur J Neurosci 2002; 15: 1105–1114.
8. Aldarmaa J, Liu Z, Long J, Mo X, Ma J, Liu J. Anti-convulsant effect and mechanism of *Astragalus mongholicus* extract in vitro and in vivo: protection against oxidative damage and mitochondrial dysfunction. Neurochem Res 2010; 35: 33–41.
9. Sharma J, Sharma A, Bahadur A, Vimala N, Satyam A, Mittal S. Oxidative stress markers and antioxidant levels in normal pregnancy and pre-eclampsia. Int J Gynaecol Obstet 2006; 94: 23–27.
10. El-Khayat HA, Soliman NA, Tomoum HY, Omran MA, El-Wakad AS, Shatla RH. Reproductive hormonal changes and catamenial pattern in adolescent females with epilepsy. Epilepsia 2008; 49: 1619–1626.
11. Finocchi C, Ferrari M. Female reproductive steroids and neuronal excitability. Neurol Sci 2011; 32: 31–35.
12. Harden CL. Sexuality in men and women with epilepsy. CNS Spectr 2006; 11: 13–18.
13. Murialdo G, Magri F, Tamagno G, Ameri P, Camera A, Colnaghi S, Perucca P, Ravera G, Galimberti CA. Seizure frequency and sex steroids in women with partial epilepsy on antiepileptic therapy. Epilepsia 2009; 50: 1920–1926.
14. Rhodes ME, Frye CA. Androgens in the hippocampus can alter, and be altered by, ictal activity. Pharmacol Biochem Behav 2004; 78: 483–493.
15. Juneja LR, Chu DC, Okubo T, Nagato Y, Yokogoshi H. L-Theanine—a unique amino acid of green tea and its relaxation effect in humans. Trends Food Sci Technol 1999; 10: 199–204.
16. Kakuda T. Neuroprotective effects of the green tea components theanine and catechins. Biol Pharm Bull 2002; 25: 1513–1518.
17. Nathan PJ, Lu K, Gray M, Oliver C. The neuropharmacology of L-theanine (N-ethyl-L-glutamine) a possible neuroprotective and cognitive enhancing agent. J Herb Pharmacother 2006; 6: 21–30.
18. Park SK, Jung IC, Lee WK, Lee YS, Park HK, Go HJ, Kim K, Lim NK, Hong JT, Ly SY et al. A combination of green tea extract and l-theanine improves memory and attention in subjects with mild cognitive impairment: a double-blind placebo-controlled study. J Med Food 2011; 14: 334–343.
19. Dawson D, Encel N. Melatonin and sleep in humans. J Pineal Res 1993; 15: 1–12.
20. Banach M, Gurdziel E, Jêdrych M, Borowicz KK. Melatonin in experimental seizures and epilepsy. Pharmacol Rep 2011; 63: 1–11.
21. Bonnefont-Rousselot D, Collin F. Melatonin: action as antioxidant and potential applications in human disease and aging. Toxicology 2010; 278: 55–67.
22. Reiter RJ. Functional pleiotropy of the neurohormone melatonin: antioxidant protection and neuroendocrine regulation. Front Neuroendocrinol 1995; 16: 383–415.
23. Yamamoto HA, Tang HW. Preventive effect of melatonin against cyanide-induced seizures and lipid peroxidation in mice. Neurosci Lett 1996; 207: 89–92.
24. Luboshitzky R, Lavie P. Melatonin and sex hormone interrelationships--a review. J Pediatr Endocrinol Metab 1999; 12: 355–362.
25. Okatani Y, Morioka N, Wakatsuki A. Changes in nocturnal melatonin secretion in perimenopausal women: correlation with endogenous estrogen concentrations. J Pineal Res 2000; 28: 111–118.
26. Abd-Allah ARA, El-Sayed ESM, Abdel-Wahab MH, Hamada FMA. Effect of melatonin on estrogen and progesterone receptors in relation to uterine contraction in rats. Pharmacol Res 2003; 47: 349–354.
27. Alaei H, Hosseini M. Angiotensin converting enzyme inhibitor captopril modifies conditioned place preference induced by morphine and morphine withdrawal signs in rats. Pathophysiology 2007; 14: 55–60.

28. Hosseini M, Alaei HA, Havakhah S, Neemati Karimooy HA, Gholamnezhad Z. Effects of microinjection of angiotensin II and captopril to VTA on morphine self-administration in rats. Acta Biol Hung 2009; 60: 241–252.

29. Hosseini M, Alaei HA, Headari R, Eslamizadeh MJ. Effects of microinjection of angiotensin II and captopril into nucleus accumbens on morphine self-administration in rats. Indian J Exp Biol 2009; 47: 361–367.

30. Hosseini M, Sharifi MR, Alaei H, Shafei MN, Karimooy HA. Effects of angiotensin II and captopril on rewarding properties of morphine. Indian J Exp Biol 2007; 45: 770–777.

31. Saffarzadeh F, Eslamizade MJ, Nemati Karimooy HA, Hadjzadeh MA, Khazaei M, Hosseini M. The effect of L-arginine on Morris water maze tasks of ovariectomized rats. Acta Physiol Hung 2010; 97: 216–223.

32. Azizi-Malekabadi H, Hosseini M, Saffarzadeh F, Karami R, Khodabandehloo F. Chronic treatment with the nitric oxide synthase inhibitor, L-NAME, attenuates estradiol-mediated improvement of learning and memory in ovariectomized rats. Clinics (Sao Paulo) 2011; 66: 673–679.

33. Azizi-Malekabadi H, Hosseini M, Soukhtanloo M, Sadeghian R, Fereidoni M, Khodabandehloo F. Different effects of scopolamine on learning, memory, and nitric oxide metabolite levels in hippocampal tissues of ovariectomized and sham-operated rats. Arq Neuropsiquiatr 2012; 70: 447–452.

34. Ebrahimzadeh Bideskan AR, Hosseini M, Mohammadpour T, Karami R, Khodamoradi M, Nemati Karimooy H, Alavi H. Effects of soy extract on pentylenetetrazol-induced seizures in ovariectomized rats. Zhong Xi Yi Jie He Xue Bao 2011; 9: 611–618.

35. Hosseini M, Ghasemzadeh RM, Sadeghnia H, Rakhshandeh H. Effects of different extracts of *Rosa damascena* on pentylenetetrazol-induced seizures in mice. Zhong Xi Yi Jie He Xue Bao 2011; 9: 1118–1124.

36. Hosseini M, Sadeghnia HR, Salehabadi S, Alavi H, Gorji A. The effect of L-arginine and L-NAME on pentylenetetrazole induced seizures in ovariectomized rats, an in vivo study. Seizure 2009; 18: 695–698.

37. Hosseini M, Harandizadeh F, Niazamand S, Soukhtanloo M, Mahmoudabady M. Antioxidant effect of *Achillea wilhelmsii* extract on pentylenetetrazole (seizure model)-induced oxidative brain damage in Wistar rats. Indian J Physiol Pharmacol 2013; 57: 418–424.

38. Hosseinzadeh H, Sadeghnia HR. Safranal, a constituent of *Crocus sativus* (saffron), attenuated cerebral ischemia induced oxidative damage in rat hippocampus. J Pharm Pharm Sci 2005; 8: 394–399.

39. Khodabandehloo F, Hosseini M, Rajaei Z, Soukhtanloo M, Farrokhi E, Rezaeipour M. Brain tissue oxidative damage as a possible mechanism for the deleterious effect of a chronic high dose of estradiol on learning and memory in ovariectomized rats. Arq Neuropsiquiatr 2013; 71: 313–319.

40. Pourganji M, Hosseini M, Soukhtanloo M, Zabihi H, Hadjzadeh MA. Protective role of endogenous ovarian hormones against learning and memory impairments and brain tissues oxidative damage induced by lipopolysaccharide. Iran Red Crescent Med J 2014; 16: e13954.

41. Sudha K, Rao AV, Rao A. Oxidative stress and antioxidants in epilepsy. Clin Chim Acta 2001; 303: 19–24.

42. Rodrigues AD, Scheffel TB, Scola G, Santos MTD, Fank B, de Freitas SCV, Dani C, Vanderlinde R, Henriques JA, Coitinho AS et al. Neuroprotective and anticonvulsant effects of organic and conventional purple grape juices on seizures in Wistar rats induced by pentylenetetrazole. Neurochem Int 2012; 60: 799–805.

43. Costello DJ, Delanty N. Oxidative injury in epilepsy: potential for antioxidant therapy? Expert Rev Neurother 2004; 4: 541–553.

44. Reilly C, Agnew R, Neville BG. Depression and anxiety in childhood epilepsy: a review. Seizure 2011; 20: 589–597.

45. Maldonado A, Ramos W, Pérez J, Huamán L, Gutiérrez E. Convulsive status epilepticus: clinico-epidemiologic characteristics and risk factors in Peru. Neurología 2010; 25: 478–484 (in Spanish with English abstract).

46. Liang L, Beaudoin M, Fritz M, Fulton R, Patel M. Kainate-induced seizures, oxidative stress and neuronal loss in aging rats. Neuroscience 2007; 147: 1114–1118.

47. Czapinski P, Blaszczyk B, Czuczwar SJ. Mechanisms of action of antiepileptic drugs. Curr Top Med Chem 2005; 5: 3–14.

48. Xie T, Wang WP, Mao ZF, Qu ZZ, Luan SQ, Jia LJ, Kan MC. Effects of epigallocatechin-3-gallate on pentylenetetrazole-induced kindling, cognitive impairment and oxidative stress in rats. Neurosci Lett 2012; 516: 237–241.

49. Liu SH, Chang CD, Chen PH, Su JR, Chen CC, Chaung HC. Docosahexaenoic acid and phosphatidylserine supplementations improve antioxidant activities and cognitive functions of the developing brain on pentylenetetrazol-induced seizure model. Brain Res 2012; 1451: 19–26.

50. Bikjdaouene L, Escames G, Leon J, Ferrer JM, Khaldy H, Vives F, Acuña-Castroviejo D. Changes in brain amino acids and nitric oxide after melatonin administration in rats with pentylenetetrazole-induced seizures. J Pineal Res 2003; 35: 54–60.

51. Yokozawa T, Dong E. Influence of green tea and its three major components upon low-density lipoprotein oxidation. Exp Toxicol Pathol 1997; 49: 329–335.

52. Reiter RJ. Melatonin: lowering the high price of free radicals. Physiology 2000; 15: 246–250.

53. Mohanan P, Yamamoto HA. Preventive effect of melatonin against brain mitochondria DNA damage, lipid peroxidation and seizures induced by kainic acid. Toxicol Lett 2002; 129: 99–105.

54. Borowicz KK, Kamiński R, Gasior M, Kleinrok Z, Czuczwar SJ. Influence of melatonin upon the protective action of conventional anti-epileptic drugs against maximal electroshock in mice. Eur Neuropsychopharmacol 1999; 9: 185–190.

55. Yildirim M, Marangoz C. Anticonvulsant effects of melatonin on penicillin-induced epileptiform activity in rats. Brain Res 2006; 1099: 183–188.

56. Abbasi F, Krumholz A, Kittner SJ, Langenberg P. Effects of menopause on seizures in women with epilepsy. Epilepsia 1999; 40: 205–210.

57. Verrotti A, Latini G, Manco R, De Simone M, Chiarelli F. Influence of sex hormones on brain excitability and epilepsy. J Endocrinol Invest 2007; 30: 797–803.

58. Penovich PE, Helmers S. Catamenial epilepsy. Int Rev Neurobiol 2008; 83: 79–90.

59. Galanopoulou AS, Alm EM, Velíšková J. Estradiol reduces seizure-induced hippocampal injury in ovariectomized female but not in male rats. Neurosci Lett 2003; 342: 201–205.

60. Hoffman G, Moore N, Fiskum G, Murphy A. Ovarian steroid modulation of seizure severity and hippocampal cell death after kainic acid treatment. Exp Neurol 2003; 182: 124–134.

61. Pereira M Jr, Soares JM Jr, Valente SG, Oliveira PB, Cavalheiro EA, Amado D, Baracat EC. Estrogen effects on pilocarpine-induced temporal lobe epilepsy in rats. Maturitas 2009; 62: 190–196.

62. Frye CA. Role of androgens in epilepsy. Expert Rev Neurother 2006; 6: 1061–1075.

63. Reddy DS. Role of neurosteroids in catamenial epilepsy. Epilepsy Res 2004; 62: 99–118.

64. Riazi K, Honar H, Homayoun H, Rashidi N, Dehghani M, Sadeghipour H, Gaskari SA, Dehpour AR. Sex and estrus cycle differences in the modulatory effects of morphine on seizure susceptibility in mice. Epilepsia 2004; 45: 1035–1042.

65. Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle–linked changes in $GABA_A$ receptors mediating tonic inhibition alter seizure susceptibility and anxiety. Nat Neurosci 2005; 8: 797–804.

66. Kokate T, Cohen A, Karp E, Rogawski M. Neuroactive steroids protect against pilocarpine-and kainic acid-induced limbic seizures and status epilepticus in mice. Neuropharmacology 1996; 35: 1049–1056.

67. Członkowska AI, Krząścik P, Sienkiewicz-Jarosz H, Siemiątkowski M, Szyndler J, Bidziński A, Płaźnik A. The effects of neurosteroids on picrotoxin-, bicuculline-and NMDA-induced seizures, and a hypnotic effect of ethanol. Pharmacol Biochem Behav 2000; 67: 345–353.

68. Jahnke G, Marr M, Myers C, Wilson R, Travlos G, Price C. Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague-Dawley rats. Toxicol Sci 1999; 50: 271–279.

69. Karasek M, Kowalski AJ, Zylinska K. Serum melatonin circadian profile in women suffering from the genital tract cancers. Neuro Endocrinol Lett 1999; 21: 109–113.

70. Costa-Lotufo LV, Fonteles MMF, Lima ISP, de Oliveira AA, Nascimento VS, de Bruin VMS, Viana GSB. Attenuating effects of melatonin on pilocarpine-induced seizures in rats. Comp Biochem Physiol C 2002; 131: 521–529.

71. Acufla-Castroviejo D, Escames G, Macks M, Hoyos AM, Carballo AM, Arauzo M, Montes R, Vives F. Minireview: Cell protective role of melatonin in the brain. J Pineal Res 1995; 19: 57–63.

72. Yahyavi-Firouz-Abadi N, Tahsili-Fahadan P, Riazi K, Ghahremani MH, Dehpour AR. Involvement of nitric oxide pathway in the acute anticonvulsant effect of melatonin in mice. Epilepsy Res 2006; 68: 103–113.