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Antiepileptic effect of nobiletin in PTZ-induced catamenial seizures in the rat

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Abstract

Background and Objective: Catamenial epilepsy is a seizure pattern associated with the menstrual cycle that happens in specific women with epilepsy while menstruating. Nobiletin is a type of polymethoxy flavonoid and with many beneficial physiological effects. This study aimed to determine antiepileptic effect of nobiletin in PTZ-induced catamenial seizures in rats.

Materials and Methods: Thirty female Wistar rats, separated into 5 experimental groups based on their estrus cycle stage (proestrus, estrus, metestrus, diestrus). In the control group, rats received saline, while in experiment 2, the animals were given VPA (75 mg/kg). During experiments 3-5, animals received nobiletin at doses 12.5, 25, and 50 mg/kg, respectively. Then, the animals received an 80 mg per kilogram intraperitoneal injection of PTZ (80 mg/kg). Following the start of the seizure, the animals' actions were observed for a period of 30 minutes to evaluate the onset of ITMS and ITTS. Experiments were conducted between 9 a.m. and 12 p.m. to minimize the influence of circadian rhythm on vulnerability to seizures.

Results: Based on findings, nobiletin (20 and 30 mg/kg) increased the initiation time myoclonic seizures (ITMS) and initiation time of tonic-clonic seizures (ITTS) and decreased seizure duration (SD) after PTZ administration in rats during different stages of the estrous cycle ($p<0.05$). The effects of nobiletin were more pronounced during metestrus and diestrus in comparison to proestrus and estrus ($p<0.05$).

Conclusion: Findings suggested that nobiletin has antiepileptic effect in PTZ-induced catamenial seizure in rat.

Keywords: Antiepileptic, Nobiletin, PTZ, Seizure

1. Introduction

Epilepsy is considered one of the oldest neurological disorders. Catamenial epilepsy is a seizure pattern associated with the menstrual cycle that happens in specific women with epilepsy while menstruating (1). It is regarded as one of the earliest neurological problems seen in women with focal or general epilepsies (2). The main reasons for menstrual epilepsy are variations in ovarian hormones and an imbalance in electrolytes. Steroid hormones can impact the onset and propagation of seizures by affecting convulsions through their anticonvulsant and proconvulsant properties in P4 and E2. A relationship was observed in rodents between seizures happening during ovulatory cycles and the concentrations of serum estradiol and P4. The greatest number of seizures was linked to increased levels of estrogen. PTZ has been widely used in the management of catamenial epilepsy seizures. The gamma-aminobutyric acid (GABA)A receptor is antagonized by its own GABAergic inhibition. Neurotransmitters in the CNS are responsible for regulating seizures associated with sex hormones. Metabolic substances such as allopregnanolone (AP) and pregnanolone (PREG) have the potential to influence the

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GABAergic receptors in the brain (3).
Nobiletin, a type of polymethoxy flavonoid, is primarily found in *Pericarpium Citri Reticulatae*, which is a traditional Chinese herbal remedy. Nobiletin is present in the outer peel of citrus fruits and offers many beneficial physiological effects that support overall health. Consequently, nobiletin has generated curiosity in the medical sector (4). Nobiletin possesses anti-inflammatory, antioxidant, and anticancer qualities, in addition to aiding in decreasing atherosclerosis, decreasing blood sugar levels, safeguarding the liver, and supporting nerve health (5). Previous studies have demonstrated that rat hippocampal cells cultured in nobiletin can enhance the protein kinase A/extracellular signal regulated kinase/cAMP response element binding protein signaling pathway to alleviate memory impairment caused by P amyloid protein in mice models of Alzheimer's disease and genetically engineered amyloid precursor protein. Additionally, it can improve cognitive function impacted by cerebral ischemia (6). There is only one report for antiepileptic effect of nobiletin which indicates nobiletin (12.5, 25, or 50 mg/kg) via oral gavage for 6 consecutive days and prior to PTZ injection improved muscle strength and motor coordination and reduced seizure severity in mice. Also, nobiletin modulated the expression of GABAA and restored the glutamate/GABA balance (7). Based on the importance of these hormone sin incidence of the catamenial epilepsy, there is no report for role of the nobiletin in seizures during estrous cycle. Thus, this study aimed to determine antiepileptic effect of nobiletin in PTZ-induced catamenial seizures in rats.

2. Materials and Methods

The study involved thirty female Wistar rats, separated into 5 experimental groups based on their estrus cycle stage (proestrus, estrus, metestrus, diestrus). The animals were kept in accordance with European community regulations for laboratory animals, under standard conditions of 22 ± 1°C and a 12-hour cycle of darkness and light. They had unrestricted access to food and water. Mattson and Cramer (8) examined puberty by analyzing vaginal smears. Researchers used vaginal smears to identify the stage of estrous cycles by analyzing the most common cell type (9).

2.1. Study procedure

In the control group, rats received saline, while in experiment 2, the animals were given VPA (75 mg/kg, Sigma CAS Number, 1069-66-5). During experiments 3-5, animals received nobiletin at 12.5, 25, and 50 mg/kg doses, respectively, sourced from Sigma (CAS Number 1405-86-3) (7). The seizures were classified based on Rj (10) score as follows: stage 0, no response; stage I, ear and facial twitching; stage II, myoclonic jerks without upright position; stage III, myoclonic jerks, upright position with bilateral forelimb clonus; stage IV, tonic–clonic seizures; stage V, generalized tonic–clonic seizures and loss of postural control (11). Then, the animals received an 80 mg per kilogram intraperitoneal injection of PTZ. Each experiment carried out during the different stages of the estrous cycle (proestrus, estrus, metestrus, and diestrus). Following the start of the seizure, the animals' actions were observed for a period of 30 minutes to evaluate the onset of ITMS and ITTS. Experiments were conducted between 9 a.m. and 12 p.m. to minimize the influence of circadian rhythm on vulnerability to seizures.

2.2. Statistical analysis

The data was analyzed using SPSS with ANOVA followed by Tukey-Kramer post hoc tests, and the results were presented as mean ± SD ($P<0.05$).

3. Results

The ITMS was affected by VPA and different doses of nobiletin (12.5, 25, and 50 mg/kg), as shown in Figure 1. It was observed that valproic acid injection raised ITMS after PTZ administration in proestrus, estrus, metestrus, and diestrus stages ($p<0.05$). Administering nobiletin at a dosage of 12.5 mg/kg did not have a notable effect on ITMS during proestrus, estrus, metestrus, and diestrus ($p>0.05$). Nobiletin at doses of 25 and 50 mg/kg increased the ITMS after PTZ administration in rats during different stages of the estrous cycle compared to the control group ($p<0.05$). The effects of nobiletin were more pronounced during metestrus and diestrus in comparison to proestrus and estrus ($p<0.05$).

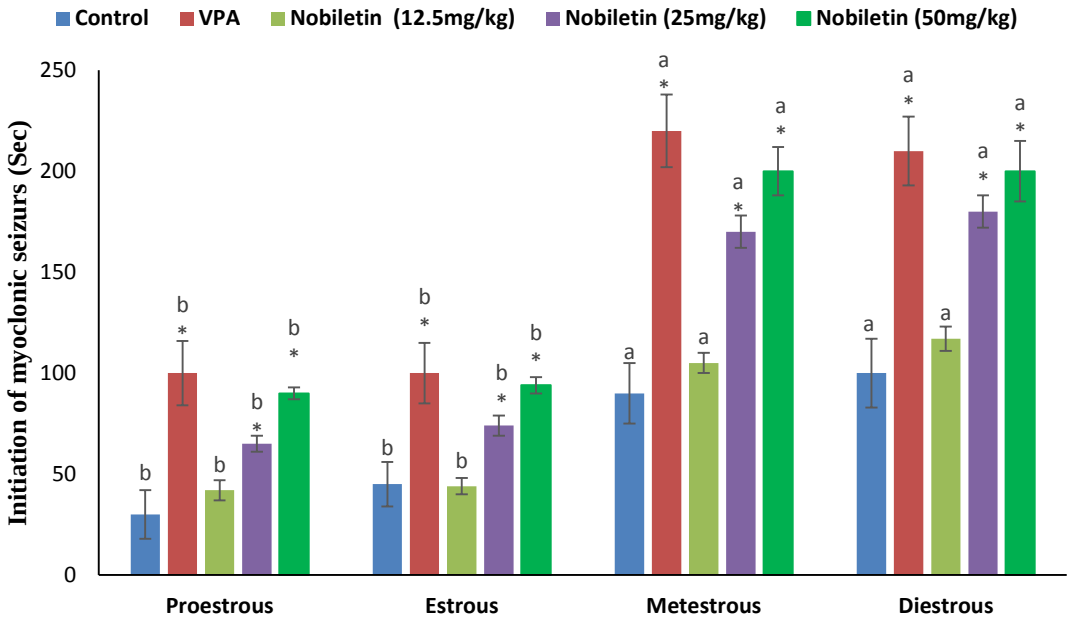

Figure 1. Effects of nobiletin on the initiation time myoclonic seizures (ITMS) in rats induced with Pentylentetrazole kindling during their estrous cycle. *Significant differences were observed in each phase of the estrous cycle compared to the control group ($P < 0.05$). Distinct letters (a, b and c) represent notable variances for each category during every stage of the estrous cycle ($P < 0.05$). Data are displayed as average plus or minus standard error.

During proestrus, estrus, metestrus, and diestrus phases, VPA injection resulted in a rise in ITTS after PTZ administration, as shown in figure 2 ($p<0.05$). No significant effect was observed on ITTS after PTZ administration during all stages of the estrous cycle with 12.5 mg/kg dose of nobiletin ($p>0.05$). The occurrence of ITTS was decreased by 25 and 50 mg/kg of nobiletin in the stages of proestrus, estrus, metestrus, and diestrus ($p<0.05$).

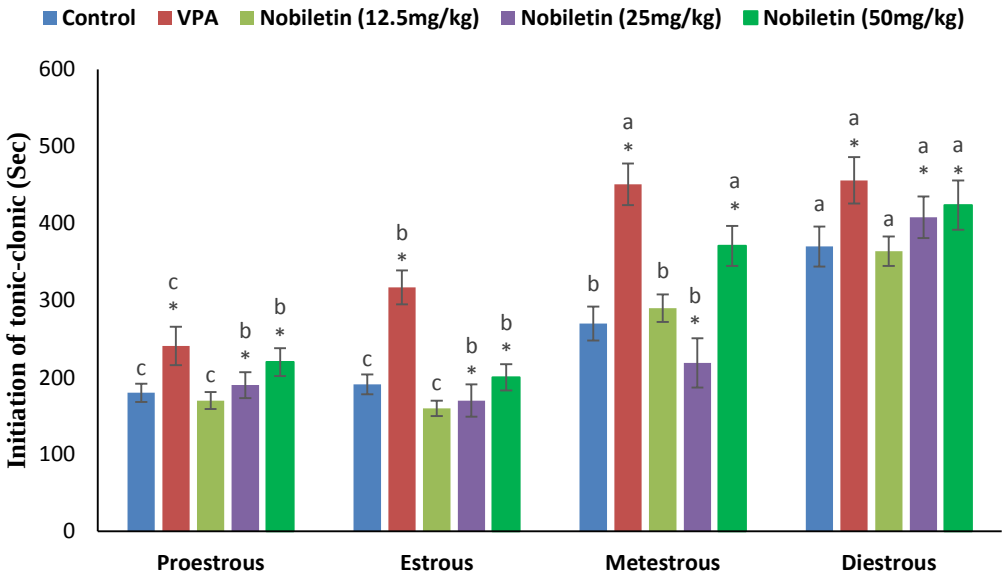

Figure 2. Effects of nobiletin on the initiation time of tonic-clonic seizures (ITTS) in rats induced with Pentylentetrazole kindling during their estrous cycle. *Significant differences were observed in each phase of the estrous cycle compared to the control group ($P < 0.05$). Distinct letters (a, b and c) represent notable variances for each category during every stage of the estrous cycle ($P < 0.05$). Data are displayed as average plus or minus standard error.

In accordance with figure 3, VPA (75 mg/kg) reduced SD after PTZ injection in the proestrus, estrus, metestrus, and diestrus phases ($p<0.05$). There was no significant effect on sexual maturation when nobiletin was given at a dose of 12.5 mg/kg during the proestrus, estrus, metestrus, and diestrus stages ($p>0.05$). Nobiletin reduced SD at doses of 25 and 50 mg/kg ($p<0.05$). Nobiletin had a greater effect during metestrus and diestrus than during proestrus and estrus ($p<0.05$).

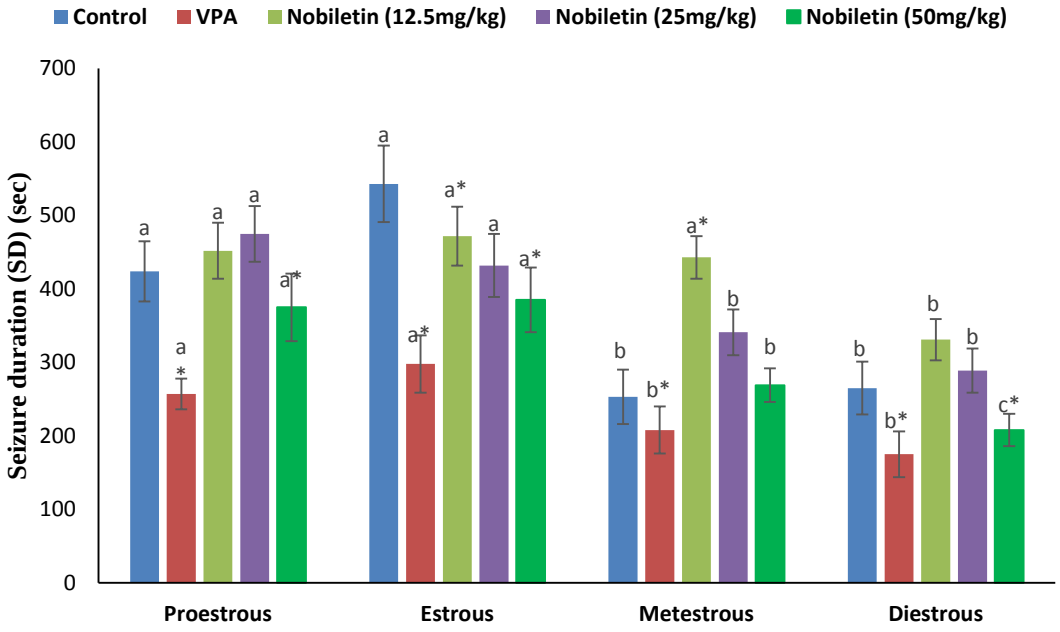

Figure 3. Effects of nobiletin on seizure duration in rats induced with pentylentetrazole kindling during their estrous cycle. *Significant differences were observed in each phase of the estrous cycle compared to the control group ($P < 0.05$). Distinct letters (a and b) represent notable variances for each category during every stage of the estrous cycle ($P < 0.05$). Data are displayed as average plus or minus standard error.

4. Discussion

Epilepsy is a prevalent neurological disorder characterized by recurrent unprovoked seizures (12). Although the exact hormonal mechanisms related to catamenial epilepsy are not entirely understood, sex hormones have neuroactive effects. The proconvulsant effects of estradiol and the anticonvulsant effects of progesterone. In the follicular phase, heightened estradiol levels lead to a greater chance of having a seizure. During the luteal phase, the increase in progesterone levels leads to a reduction in the frequency of seizures (13). The effect of progesterone on the frequency of seizures is especially significant for focal seizures that progress to bilateral tonic-clonic seizures. A greater daily average seizure in this seizure type was observed in women with an anovulatory menstrual cycle when compared to those with an ovulatory cycle; this observation was linked to variations in the estradiol/progesterone ratio (14). In ovulatory menstrual cycles, high progesterone phases lead to a reduction in seizure frequency, while in anovulatory cycles, high estradiol phases result in an increase in seizure frequency (15).

A more profound comprehension of the underlying pathology associated with the initiation and progression of seizures, as well as related factors, may assist in pinpointing molecular targets for novel antiepileptic drugs. The induced onset of seizures with PTZ, a chloride channel inhibitor that impacts GABAA receptors, is a frequently used experimental model for epilepsy (13). Based on our findings, PTZ injection decreased the ITMS and ITTS and increased SD in rats. GABA, the main inhibitory neurotransmitter, along with its receptors, regulates inhibitory neurotransmission and stops excessive neuronal excitation. The effect of flavonoids on GABA receptors is concentration-dependent and biphasic, in low concentrations they strengthen the effect of the receptor and in high concentrations they inhibit them (16). Flavonoids have an agonistic effect on GABA receptors, and in the absence of GABA, they directly interact with the receptor and cause its valves to open, so it is said that some flavonoids have an active binding site on the GABA receptor (17). Based on main findings, nobiletin increased the ITMS and ITTS and decreased SD after PTZ administration in rats during different stages of the estrous cycle. The effects of nobiletin were more pronounced during metestrus and diestrus in comparison to proestrus and estrus. In a sole and previous report on anti-nociceptive activity of the nobiletin, it is reported

nobiletin considerably reduced the severity of seizures induced by PTZ. Moreover, nobiletin pretreatment enhanced the effectiveness of clonazepam, and the combination treatment demonstrated greater efficacy than using each agent alone. Moreover, the pairing of nobiletin led to a notable increase in muscle strength and motor coordination, evaluated through the chimney and grip tests, exceeding the outcomes achieved from using clonazepam or nobiletin alone (7). Based on sole previous report on antiepileptic activity of the nobiletin, it is reported nobiletin administration following PTZ increased GABA and decreased glutamate levels in brain (7). Nobiletin increased key enzyme for GABA production and GABAA (18). Nobiletin reduced the production of reactive oxygen species (ROS) and the expression of nuclear NF-κB p65 in a rat model of carotid artery damage. Nobiletin displayed strong anti-neuroinflammatory properties, inhibited activation of signaling pathways associated with microglial activation like the PI3K/Akt, ERK, JNK, p38MAPKs pathways. Nobiletin improved scratching behavior by blocking the activation of NF-κB, activator protein-1, and p38 in mice (19). Nobiletin might reduce cisplatin-induced acute kidney injury by enhancing Bax expression and demonstrating antioxidant, anti-inflammatory, and anti-apoptotic properties. BDNF is a crucial member of the glial cell line-derived neurotrophic factor family, mainly found in the central nervous system, especially in the hippocampal region. BDNF influences differentiation, proliferation, and nourishment of various neuron types (20). Nobiletin has beneficial influences on the production of neurotransmitters and neurotrophic factors, and is strongly linked to learning, memory, and cognitive functions (21). The PI3K/Akt signaling pathway is a crucial regulatory pathway that plays a role in the proliferation, growth, and survival of neuronal cells. Activation of the pathway has been demonstrated to provide neuroprotective benefits against seizures (21).

Conclusion

In conclusion, findings suggested that nobiletin has antiepileptic effect in PTZ-induced catamenial seizure in rat. Based on literature, there is limited report on interaction of the nobiletin with GABA receptors. However, because of its small molecular size, it can cross the blood-brain barrier to function as a therapeutic agent on brain neurons. Further researches needed to determine more information regarding antiepileptic activity of the nobiletin.

Conflicts of interest

The authors declare that they have no competing interests.

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