Original Article

Effects of *Ginsenosides* on Pentylenetetrazol-Induced Convulsions during Estrus Cycle in Rat

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ABSTRACT

Ginseng is known as the king of all herbs in terms of antioxidant and anti-inflammatory activities and recently has become more involved in the treatment of neurological diseases. In this regard, this study aimed to determine the effects of *Ginsenosides* on pentylenetetrazol-induced epilepsy during the estrus cycle. For this purpose, 30 rats were randomly divided into five groups, namely control (saline), valproic acid (VPA, 75 mg/kg), *Ginsenosides* (50 mg/kg), *Ginsenosides* (100 mg/kg), and *Ginsenosides* (150 mg/kg) with four subgroups (proestrus, estrus, metestrus, and diestrus). Subsequently, the initiation time of myoclonic seizures (ITMS), initiation time of tonic-clonic seizures **(**ITTS), and seizure duration (SD) were determined. According to the results, ITMS and ITTS significantly increased in the VPA-treated group (P<0.05). *Ginsenosides* (100 and 150 mg/kg) administration significantly increased ITMS and ITTS (P<0.05). Moreover, the ITMS and ITTS in *Ginsenosides*-treated rats were significantly higher in luteal phases, compared to the follicular phase (P<0.05). In addition, pretreatment with VPA significantly decreased SD, compared to the control group (P<0.05). A significant decrease in SD was observed in the rats pretreated with *ginsenosides* (100 and 150 mg/kg) (P<0.05). Seizure duration significantly decreased in animals that received *Ginsenosides* in luteal phases, compared to the follicular phase (P<0.05). These results suggested that *Ginsenosides* have anticonvulsant effects that are more prominent during the luteal phase than the follicular phase.

Keywords: Estrus cycle, *Ginsenosides*, PTZ, Rat, Seizure

1. Introduction

Ginseng is known as the king of all herbs in folk medicine in East Asian countries for the treatment of diseases. Due to its reputation, it has been recently noticed and has become one of the most popular herbs worldwide (1). The main pharmacological and therapeutic properties of ginseng are related to triterpene saponins, known as *Ginsenosides* (2). To date, more than 150 *Ginsenosides* have been reported; however, their basic structure is the same as they contain 30 carbon atoms with steroidal structure (3). *Ginsenosides* have antioxidant and anti-inflammatory activities and have recently become more involved in the treatment of neurological diseases, such as Alzheimer's disease, Parkinson's disease, epilepsy, and depression (4).

Catamenial epilepsy is one of the central brain-related disorders which occurs as a result of hormonal imbalances during the menstrual cycle (5). Fluctuations in ovarian hormones during the menstrual cycle and electrolyte imbalance are the main factors for this type of epilepsy (6). Estradiol has convulsant effects, while progesterone and its metabolites (mainly allopregnanolone) play an anticonvulsant role. Therefore, hormonal changes during the menstrual cycle can affect seizure incidence. It is well known that estrogen increases the incidence of epilepsy, while progesterone inhibits it via excitatory glutamate receptors allopregnanolone that acts via gamma-aminobutyric type A ($GABA_A$) receptors (7).

Antiepileptic drugs, such as carbamazepine, phenytoin, phenobarbital, oxcarbazepine, and valproic acid (VPA) are routinely prescribed to control epilepsy; however, they decrease libido and imbalance sex hormone levels are their side effects (8). Due to the adverse effects of the available antiepileptic medications, there is a growing interest in natural resources for the development of antiepileptic medications (9). Due to the antiepileptic and anti-neuroinflammatory effects of red ginseng, Kim, Kim (10) administered red ginseng (50 mg/kg) for 4 weeks to mice and found that its anticonvulsant effects are similar to VPA without serious side effects.

Ginseng extract (60 mg/kg) decreased pentylenetetrazol (PTZ)-induced seizures, increased seizure latency, and decreased seizure score in rats (11). Despite the research that has been performed on the antiepileptic activity of the ginseng extract, there is no report on its role in catamenial epilepsy during different phases of the estrous cycle. Therefore, the present study aimed to determine the effects of *Ginsenosides* on PTZ-induced convulsions during the estrus cycle in rats.

2. Materials and Methods

2.1. Animals

In total, 30 female Wistar rats (200±50 g) were randomly divided into five experimental groups. Animals were maintained under standard laboratory conditions (22±2 °C, 12 h dark/light cycle) following European community regulations for laboratory animals (12). Sexual puberty was approved using vaginal smears, two regular estrous cycles were employed to select the rats, and estrus synchronization was performed (13). Vaginal smears were obtained every day to estimate the stage of the estrus cycle based on the most common cell type, including proestrus large round nucleated cells, estrus masses of the cornfield squamous epithelial cells, metestrus round nucleated epithelial cells with the leukocyte infiltration, and diestrus consisting of a predominance of leukocytes (14).

2.2. Study Procedure

Rats were randomly allocated to 5 groups of control (saline), VPA (75 mg/kg), *Ginsenosides* (50 mg/kg), *Ginsenosides* (100 mg/kg), and *Ginsenosides* (150 mg/kg). *Ginsenosides* were provided by Sigma Chemical Co. St Louis, MO, USA, (CAS No:14197-60-5) (Table 1). Each group consisted of four subgroups, namely proestrus, estrus, metestrus, and diestrus. Subsequently, each group was subjected to the i.p. administration of the saline or vehicle i.p. administration of PTZ (80 mg/kg). After the seizure was induced, the behavior of animals was monitored for 30 min to assess seizure duration (SD), mortality rate, initiation time of myoclonic seizures (ITMS), and initiation time of tonic-clonic seizures **(**ITTS) (15). All experiments were conducted from 9 to 12 a.m. to reduce the impact of circadian rhythm on seizure susceptibility (15).

Table 1. Treatment procedure

Group Estrous Cycle	First injection	Second injection*
Control	Normal saline	PTZ (80 mg/kg)
Valproic Acid	Valproic Acid (75 mg/kg)	PTZ (80 mg/kg)
Ginsenosides (50 mg/kg)	Ginsenosides (50 mg/kg)	PTZ (80 mg/kg)
Ginsenosides (100 mg/kg)	Ginsenosides (100 mg/kg)	PTZ (80 mg/kg)
Ginsenosides (150 mg/kg)	Ginsenosides (150 mg/kg)	PTZ (80 mg/kg)

*30 min after the first injection; PTZ, Pentylenetetrazol

2.3. Statistical Analysis

The collected data were analyzed by one-way analysis of variance (ANOVA) using SPSS software (version 16.0) for Windows (SPSS, Inc., Chicago, IL, USA) and reported as mean±SD. The ANOVA was followed by Tukey–Kramer multiple comparison post-hoc tests used for the analysis of data (P<0.05).

3. Results

Based on figure 1, ITMS significantly increased in the VPA-treated group, compared to the control group (P<0.05). Administration of *Ginsenosides* (100 and 150 mg/kg) significantly increased ITMS in the recipients, compared to the control group (P<0.05). There was a significant difference regarding ITMS in different phases of the estrous cycle as ITMS of the *Ginsenosides*-treated rats was significantly higher in luteal phases, compared to the follicular phase (P<0.05).

According to figure 2, ITTS was significantly enhanced in VPA-receiving rats, compared to the control group (P<0.05). Moreover, pretreatment with *Ginsenosides* (100 and 150 mg/kg) significantly increased ITTS in the recipients, compared to the control group (P<0.05). In addition, there was a significant difference regarding ITTS in different phases of the estrous cycle as the ITTS of the *Ginsenosides*-treated rats was significantly higher in luteal phases, compared to that in the follicular phase (P<0.05).

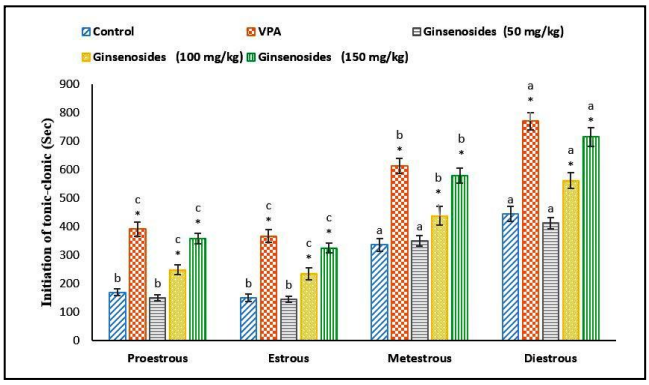

Figure 1. Antiepileptic effects of Ginsenosides (50, 100, and 150 mg/kg) on the initiation time of myoclonic seizures (ITMS) (sec) during various phases of the estrous cycle. *Asterisks indicate a significant difference in each estrous cycle phase compared with the control group (P < 0.05). Different letters (a and b) indicate significant differences for each group in each estrous cycle phase (P < 0.05). Data are presented as mean ± SEM

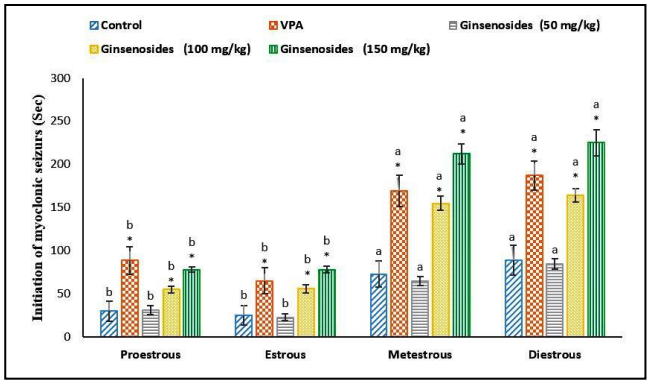

Figure 2. Antiepileptic effects of Ginsenosides (50, 100, and 150 mg/kg) on the initiation time of tonic-clonic seizures (ITTS) (sec) during various phases of the estrous cycle. *Asterisks indicate a significant difference in each estrous cycle phase compared with the control group (P < 0.05). Different letters (a, b, and c) indicate significant differences for each group in each estrous cycle phase (P < 0.05). Data are presented as mean ± SEM

As illustrated in figure 3, pretreatment with VPA significantly decreased SD in comparison to the control group (P<0.05). A significant decrease in SD was observed in the group that was pretreated with *Ginsenosides* (100 and 150 mg/kg) in comparison to the control group (P<0.05). Seizure duration significantly decreased in animals that received *Ginsenosides* during metestrus and diestrus phases, compared to proestrus and estrus phases (P<0.05). Moreover, there was a significant difference regarding SD in different phases of the estrous cycle; accordingly, the SD of the *Ginsenosides*-treated rats was significantly lower in luteal phases, compared to that in the follicular phase (P<0.05).

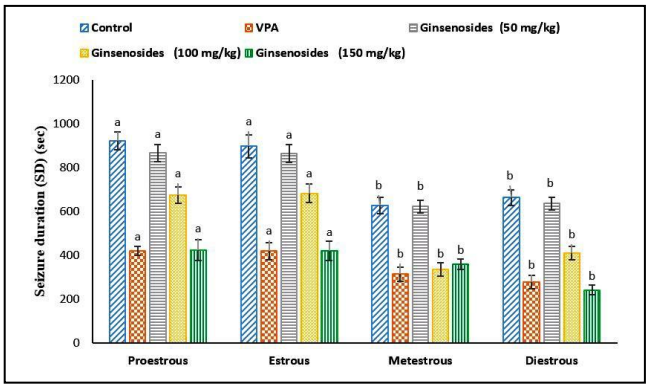

Figure 3. Antiepileptic effects of Ginsenosides (50, 100, and 150 mg/kg) on seizure duration (sec) during various estrous cycle phases. *Asterisks indicate a significant difference in each estrous cycle phase compared with the control group (P < 0.05). Different letters (a, b, and c) indicate significant differences for each group in each estrous cycle phase (P < 0.05). Data are presented as mean ± SEM

4. Discussion

Epilepsy is characterized by seizures and antiepileptic drugs are used for its treatment; however, these drugs can also lead to neurological disorders in many epileptic patients. Therefore, it is necessary to find new antiepileptic drugs for the treatment of this disease (3). Based on the main findings of the current study, pretreatment with *Ginsenosides* increased ITMS and ITTS and decreased SD in the model of PTZ. There are previous reports on the role of the *Ginsenosides*, especially the panaxadiol groups (Rb1, Rb2, Rb3, Rc, Rd, Re, Rg1, Rg2, and Rg3) in neurological disorders, such as memory, anxiety, depression, and epilepsy (1). Ginsenoside decreases depression by increasing noradrenaline and upregulating 5-$HT2_A$ receptor levels (16).

Previous studies have reported the antiepileptic effects of *Ginsenosides*. Lian, Zhang (11) have studied the anticonvulsant activities of the panaxadiol group of ginseng using kainic acid, pilocarpine, and PTZ model of the seizures and reported obvious anticonvulsant effects. It is reported that *Ginsenosides* have anticonvulsant effects, while some *Ginsenosides* do not have such effects on all types of seizures. Therefore, it is helpful to investigate the efficacy of Ginsenosides on all kinds of epilepsy (17).

The involvement of steroid hormones and their metabolites in seizures is well documented (18). A positive correlation exists between seizure incidence and plasma estradiol levels. In epileptic females, seizure frequency decreases during the luteal phase and increases in the follicular phase; therefore, increasing the estradiol levels can promote epileptogenesis, whereas progesterone may be used for preservation (19). Hence, sex hormones can stimulate or suppress seizures. Therefore, it is useful to increase the consumption of the medication during the follicular phase and decrease its dosage during the luteal phase.

As observed in this study, pretreatment with *Ginsenosides* was more effective in PTZ-induced epilepsy during the luteal phase, compared to the follicular phase. Progesterone and allopregnanolone act through their allosteric effect on $GABA_A$ receptors. Allopregnanolone induces Cl^- influx in $GABA_A$ receptors, inhibiting the firing of new neural action (20). Intracellular calcium concentration increases in epilepsy. *Ginsenosides* inhibit the increase of Ca^{2+} induced by Mg^{2+} and inhibition of the NMDA glutamate receptor (21). GABA is an important inhibitory neurotransmitter and low GABAergic function plays a key role in the pathophysiology of catamenial epilepsy. Numerous triterpene saponins exert their antidepressant and anticonvulsant effects via

the regulation of the GABAergic system. The Zn or Mg^{2+} deficiency can induce depression or epilepsy via glutamatergic and GABAergic receptors (22). Moreover, *ginsenoside* Rb1 plays a neuroprotective role against PTZ-induced brain damage and Mg^{2+} free-induced neuron injury. *Ginsenoside* Rb1 has anticonvulsant activities against glutamate-induced excitotoxicity (11).

It is reported that *ginsenoside* Rb1 improves cognitive impairment via the GABAergic system in the prefrontal cortex (23). *Ginsenoside* Rb1 exerts anticonvulsant effects by enhancing the $GABA_A$ receptor-mediated inhibitory synaptic transmission in the hippocampus (24). Based on the finding of the present study, it is assumed that *Ginsenosides* exert their effect via the GABAergic system in P, 25TZ-induced convulsions during the estrus cycle in rats and this effect is more prominent during the luteal phase (25, 26).

Authors' Contribution

A. A.: data collect, draft of paper
Sh. H.: advisor, study design, revise of paper
Z. Gh.: data collect, draft of paper

Ethics

All experimental procedure approved by ethic committee of the Science and Research Branch, Islamic Azad University, Tehran, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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