Jundishapur J Nat Pharm Prod. 2017 February; 12(1):e31384. doi: 10.5812/jjnpp.31384.

Published online 2016 October 2. **Research Article**

Evaluation of the Effect of Naringenin on Pentylenetetrazole and Maximal Electroshock-Induced Convulsions in Mice

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Received 2015 August 02; **Revised** 2016 July 07; **Accepted** 2016 September 13.

Abstract

Background: Naringenin is a flavonoid with several different biological effects in central nervous system. As mentioned, naringenin has neuroprotective, memory enhancing, anti-inflammatory and antioxidant effects.
Objectives: In this study, we investigated effects of naringenin on pentylenetetrazole and maximal electroshock-induced seizures in mice.
Methods: Naringenin was administered at doses of 50, 100 and 200 mg/kg intraperitoneally in two models of seizure. Thirty minutes after different doses of naringenin, phenytoin or diazepam and vehicle, the animal received pentylenetetrazole or current stimulus by an electroconvulsiometer.
Results: In maximal electroshock model, naringenin 200 mg/kg reduced the duration of hind limb tonic extension. In pentylenetetrazole seizure model all doses of naringenin increased the latency for convulsion and latency for Straub's tail but only naringenin 200 mg/kg showed significant reduction in duration of myoclonic seizure.
Conclusions: According to the results, naringenin showed significant anticonvulsant and neuroprotection activity in two pentylenetetrazole and electroshock models of convulsion in mice and these effects may be mediated by antioxidant properties, agonist activity on $GABA_A$ receptors and weakening of glutamate transmission.

Keywords: Naringenin, Pentylenetetrazole, Maximal Electroshock, Convulsion, Mice

1. Background

Epilepsy is a chronic neurological disorder by recurrent, spontaneous seizures (1). Epilepsy is described as a complex neurological dysfunction and it affects 50 million people around the world (2). The cause of epilepsy is often unknown, although it may progress after brain attack, such as trauma, tumor growth, infection or other types of neurological disorders (3). Current antiepileptic drugs are effective in controlling seizures in 70% of cases. Side effects such as chronic toxicity and teratogenicity have limited the use of anticonvulsant drugs (4, 5). Thus, recently herbal medicines have played an important role in human health and preclinical evaluation for seizures or recognized mechanisms of action that are related to epilepsy (6). Flavonoids are a class of naturally polyphenolic compounds present in a broad variety of human nutriment and possess a wide range of biological effects including a variety of neuroprotective activities (7). The biological effects of flavonoids, including those on the brain, have been recognized to be related to their antioxidant actions (8). Naringenin (4',5,7-trihydroxyflavanone), a dietary flavonoid in fruits and vegetables such as grapefruit, tomato and orange has been reported to have a number of biological effects, such as neuroprotective (9) and mono amine oxidase inhibitory (10), anti-inflammatory as well as antioxidant (11) and memory enhancing activity (12). It has been suggested that neuroprotective effects of naringenin in experimental stroke is mediated by suppression of NF-κB-mediated neuroinflammation (13). Furthermore, naringenin was found to be a neuroprotective agent in Parkinson's disease, amnesia, Alzheimer's disease and prevents oxidative injury in numerous pathophysiological disorders due to their ability to cross into the brain (14-16).

2. Objectives

According to previous researches, this study was designed to investigate the effects of naringenin on

Pentylenetetrazole (PTZ) and Maximal Electroshock (MES)-induced seizures in mice.

3. Methods

3.1. Experimental Animals

Male C57 albino mice, born and reared in the research center and experimental animal house of Ahvaz Jundishapur University of Medical Sciences, were used in the present study. Young healthy male mice (21 - 25 g) were housed and maintained at 23 ± 2°C under laboratory conditions with alternating food and water except for a short time when they were removed from their cages for testing. The animals were fasted two hours before and during the test. Animal care was carried out in accordance with institutional guidelines for animal care and use, and all possible measures were taken to minimize the number of animals used and their suffering, including immediate euthanasia after acute experiments. Each mouse was used only once. Animals were adapted to the new environment for at least one hour before testing.

3.2. Drugs and Chemicals and Solutions

Naringenin (Sigma-Aldrich, USA), PTZ (Sigma-Aldrich, USA), Phenytoin (HIDANTIC, Caspian Tamin, Iran) and diazepam (ZEPADIC, Caspian Tamin, Iran) were used in the present study. All chemicals were dissolved in 0.9% saline except naringenin, which was dissolved in 0.1% Carboxy Methyl Cellulose (CMC). Drug solutions were prepared fresh.

3.3. Maximal Electroshock-Induced Seizures Test

Mice were divided to five groups each containing six animals and treated with either vehicle, naringenin (50, 100 or 200 mg/kg, i.p.) or phenytoin (25 mg/kg, i.p.). Thirty minutes later, seizures were induced by a current stimulus (50 mA, 80 Hz for 0.2 second) delivered through using ear electrodes by an electroconvulsiometer (Borjsanat Company, Iran). In order to improve electrode contact, the electrodes were moistened with normal saline before being attached to the ears of mice. The current used was predetermined before experimentation and was the current that caused Hind-Limb Extension (HLTE) in all control mice in the trials. The duration of HLTE (i.e., the hind limbs of animals outstretched at 180° to the plane of the body axis) was recorded (17, 18). In this method of MES convulsion no death occurred.

3.4. Pentylenetetrazole-Induced Seizures Test

Mice were divided to five groups each containing six animals and received either vehicle naringenin (50, 100 or 200 mg/kg, i.p.) or diazepam (1 mg/kg, i.p.). Thirty minutes later, seizures were induced by pentylenetetrazole (85 mg/kg, i.p.). The animals were observed during the first 30 minutes for latency of convulsion, latency for Straub's tail, duration of myoclonic and protection of mortality.

3.5. Statistical Analysis

All the results are expressed as mean ± Standard Deviation (SD). Latency to induce seizures, latency to straub's tail and myoclonic duration in PTZ model and HLTE in MES model were analyzed by One-way Analysis Of Variance (ANOVA) followed by post hoc Tukey's test comparison. P values of < 0.05 were considered statistically significant.

4. Results

4.1. Effect of Naringenin on Maximal Electroshock-Induced Seizures

In the MES model, 200 mg/kg of naringenin significantly reduced the duration of hind limb tonic extension compared to the untreated group. However, phenytoin completely prevented hind limb tonic extension (Table 1).

4.2. Effect of Naringenin on PTZ-Induced Seizures

In PTZ-induced seizures, administration of naringenin at doses of 50, 100 and 200 mg/kg increased the latency for convulsion and latency for straub's tail compared to the negative control group, vehicle ($P < 0.001$), yet only 200 mg/kg of naringenin decreased the duration of myoclonic in mice ($P < 0.05$). In addition, naringenin and diazepam showed significant protection against mortality as well as seizures. Whereas diazepam-treated (1 mg/kg, i.p.) animals failed to show any signs of convulsions and all mice were protected from PTZ-induced convulsions (Table 2).

5. Discussion

In present study, anticonvulsant activity of naringenin was demonstrated in two pentylenetetrazole and electroshock models of convulsion. In MES-induced seizure model, naringenin 200 mg/kg decreased HLTE duration as compared to the vehicle group. Phenytoin anticonvulsant effects on MES was mediated by blocking the voltage-gated sodium and calcium channels (19). Considering the impact of drugs on general and complex seizures induced by MES (20), naringenin can be raised for improvement of this type of seizure. In PTZ-induced seizure model, the lowest mortality and reduction in duration of myoclonic

Table 1. Effect of Naringenin on Maximal Electroshock-Induced Seizures in Mice[a,b]

Treatment	Dose (mg/kg)	N	Hind Limb Tonic Extensions (sec)
Vehicle	10 mL/kg	6	18.62 ± 1.15
Phenytoin	25	6	0 ± 0[c]
NAR 50	50	6	16.99 ± 0.49
NAR 100	100	6	17.00 ± 1.68
NAR 200	200	6	15.87 ± 1.36[d]

[a] Values are expressed as mean ± SD. Data were analyzed by one-way ANOVA followed by Tukey - HSD multiple comparison test
[b] $F(4,25) = 258.7$; $P < 0.001$.
[c] $P < 0.001$ as compared to vehicle group.
[d] $P < 0.01$.

Table 2. Effect of Naringenin on PTZ-Induced Seizures in Mice[a,b,c,d]

Treatment	Dose (mg/kg)	N	Latency for Convulsion (sec)	Latency for Straub's Tail (sec)	Duration of Myoclonic (sec)	Protection of Mortality (sec)
Vehicle	10 ml/kg	6	65.52 ± 2.48	90 ± 2	11.54 ± 0.51	0/6 (0.00)
Diazepam	1	6	1280.2 ± 26.58[e]	1800 ± 0[e]	0 ± 0[e]	6/6 (100.00)
NAR 50	50	6	428.17 ± 7.67[e]	438.17 ± 3.06[e]	11.73 ± 0.49	2/6 (33.33)
NAR 100	100	6	535.83 ± 5.81[e]	568 ± 5.93[e]	10.46 ± 1.16	3/6 (50.00)
NAR 200	200	6	797.2 ± 5.80[e]	828.6 ± 4.61[e]	9.78 ± 1.30[f]	4/6 (66.67)

[a] Values are expressed as mean ± SD. Data were analyzed by one-way ANOVA followed by Tukey-HSD multiple comparison test.
[b] $F(4,25) = 6591.799$; $P < 0.001$ (Latency for convulsion).
[c] $F(4,25) = 47674.494$; $P < 0.001$ (Latency for Straub's tail).
[d] $F(4,25) = 204.779$; $P < 0.001$ (Duration of myoclonic).
[e] $P < 0.001$ as compared vehicle group.
[f] $P < 0.05$.

seizures was obtained with the 200 mg/kg dose. Thus, this dose was introduced as the most effective dose in PTZ-induced seizures. Clinically effective drugs against PTZ-induced seizure could be used in treatment of myoclonic and absence epilepsy (21-23). Accordingly, naringenin can be effective in absence epilepsy. This study is the first report of naringenin in improvement of convulsion in animal models. Thus, there are no available researches for comparison with the current study. One of the most common causes of seizures in humans and animals is decreasing the GABAergic and increasing of glutamatergic system activity. The $GABA_A$ receptors are ligand-gated ion channels, which mediate the most common inhibitory transmission in synapses. The $GABA_A$ receptor function not only prevents the development of epilepsy, but also inhibits the development of convulsive activity throughout the cerebral cortex tissues (24). Pentylenetetrazole mediates convulsive behavior through blocking of $GABA_A$ receptors, which are present in the membrane of neurons in the central nervous system. This blocking effect can trigger transmission of glutamatergic system. Therefore, the drugs which are agonists for $GABA_A$ can prevent PTZ-induced seizure. Blocking of $GABA_A$ receptor gated chloride channels and triggering glutamate transmission increase influx of calcium ions, which led to an increase in production of superoxide radicals. The evidences showed that flavonoids have anti-anxiety and anticonvulsant effects by binding to the benzodiazepine binding site on GABA receptors in the central nervous system (25, 26). In a study for evaluating naringin protection against kainic acid-induced status epilepticus, the results showed that pretreatment with naringin significantly increased the latency of seizures as compared to the vehicle treated group. Accordingly, naringin has therapeutic potential for preventing kainic acid-induced seizures (27). Naringin is hydrolyzed to a major metabolite, naringenin, which readily crosses the blood brain barrier (28, 29). Thus one of the possible mechanisms of naringenin to inhibit the effects of PTZ is weakening of glutamatergic neurotransmission. Oxidative stress and mitochondrial dysfunction can make seizure attacks on brain that lead to the production of free radicals and oxidative damage to proteins, lipids and nucleic acid (30, 31). The free radicals aggravate epilepsy through inhibition of glutamine synthase and consequently increase brain gluta-

mate level (32). Thus, oxidative stress is known as one of the leading causes of seizures. It seems that antioxidants such as flavonoids can inhibit pentylenetetrazole-induced seizures by reducing oxidative stress (33). Flavonoids such as naringenin can increase the antioxidant activity in the body and enhances the activity of antioxidant enzymes that reduce the production of oxygen free radicals, and tissue damage (34, 35). In conclusion, the results of naringenin anticonvulsant effects in both models of convulsion in mice suggest that the neuroprotective effects of naringenin may be mediated by antioxidant properties, agonist activity on $GABA_A$ receptors and weakening of glutamate transmission.

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