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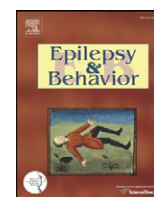

Naringin ameliorates pentylenetetrazol-induced seizures and associated oxidative stress, inflammation, and cognitive impairment in rats: Possible mechanisms of neuroprotection

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ABSTRACT

Oxidative stress and cognitive impairment are associated with PTZ-induced convulsions. Naringin is a bioflavonoid present in the grapefruit. It is a potent antioxidant, and we evaluated its effect on PTZ-induced convulsions. Rats were pretreated with normal saline, naringin (20, 40, and 80 mg/kg, i.p.), or diazepam (5 mg/kg, i.p.) 30 min prior to the administration of PTZ. The administration of PTZ induced myoclonic jerks and generalized tonic–clonic seizures (GTSs). We observed that naringin significantly prolonged the induction of myoclonic jerks dose-dependently. Naringin (80 mg/kg, i.p.) pretreatment protected all rats, and this protective effect was annulled by the $GABA_A$ receptor antagonist, flumazenil. In addition, naringin reduced brain MDA and TNF-α levels and conserved GSH. The pretreatment also enhanced the performance of rats in the passive avoidance task. Our observations highlight the antioxidant, antiinflammatory, and anticonvulsant potential of naringin. Also, naringin modulates the $GABA_A$ receptor to produce anticonvulsant effects and to ameliorate cognitive impairment.
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1. Introduction

Epilepsy is one of the most common neurological disorders affecting approximately more than 50 million people globally [1]. Antiepileptic drugs possess a wide range of side effects, and some require continuous monitoring to prevent toxicity [2]. Thus, there is a need for developing safer and more efficacious drugs. Nowadays, many medicinal plants are being screened for their possible antiepileptic effects [3]. Much scientific exploration is underway to identify a new plant-based drug or adjunctive agent to either enhance the efficacy or reduce the adverse effects of currently available antiepileptic drugs.

A convulsive episode is usually triggered whenever there is an imbalance between the levels of inhibitory and excitatory neurotransmitters in the central nervous system (CNS) [4]. A decline in the levels of γ-aminobutyric acid (GABA) or an increase in glutamate concentration in the CNS may cause convulsions. One of the animal models used to screen drugs for their potential anticonvulsant properties is the pentylenetetrazole (PTZ) model [5,6]. Pentylenetetrazol triggers seizures in animals by blocking GABA transmission. Pentylenetetrazol also increases reactive oxygen species (ROS) levels in the rat brain which further enhances the neurotoxic and convulsant effects of PTZ [7]. In contrast, antioxidants not only minimize convulsions but also limit ROS-induced damage [8].

Vezzani and Granata [9] studied the areas of the brain which were involved in the epileptic activity and exhibited rise in inflammatory mediators. In particular, a rapid onset of inflammatory response occurred in the glia as a result of seizures induced by chemoconvulsants or by electrical stimulation [9,10]. Also, molecular and pharmacological studies of *in vivo* models showed that cytokines like IL-1β and TNF-α play a significant role and are amongst the first inflammatory mediators to rise following seizures [11]. Thus, the role of cytokines in epilepsy needs to be elucidated and this understanding can potentially be applied to new drug development in epilepsy.

Naringin (4′,5,7-trihydroxyflavanone 7-rhamnoglucoside) is a flavanone glycoside that is ubiquitous in citrus herbs and grapefruit. It possesses potent antioxidant, superoxide scavenging, antiapoptotic, antiatherogenic, and metal chelating activities [12–14]. Naringin is hydrolyzed by intestinal microflora to yield naringenin (4′,5,7-trihydroxyflavanone). The latter is readily absorbed and also has good penetration across the blood–brain barrier [15]. A recent study has reported naringin's neuroprotective potential against D-galactose-induced cognitive impairment and oxidative stress in mice [16]. Epilepsy is usually associated with cognitive decline, and use of some antiepileptic drugs is also associated with cognitive impairment.

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Further, our previous work on naringin has demonstrated the antiepileptic potential of naringin against kainic acid-induced status epilepticus in rats [17]. Therefore, it is worthwhile to assess the neuroprotective property of naringin against PTZ-induced seizures in rats in view of its multiple favorable pharmacological activities.

2. Materials and methods

2.1. Animals

The study was carried out using male Wistar rats weighing 150–200 g obtained from the central animal house facility of Dr. B. R. Ambedkar Center for Biomedical Research (ACBR), University of Delhi, India. The rats were group housed in polyacrylic cages (38 × 23 × 10 cm) with not more than four animals per cage and were maintained under standard laboratory conditions with natural dark and light cycles (approximately 14 h light–10 h dark cycle) and a room temperature of 25 ± 1 °C. They were allowed free access to standard dry diet (Golden Feeds, India) and tap water. All the behavioral procedures were carried out between 0900 and 1300 h. All procedures described were reviewed and approved by the Institutional Committee for Ethical Use of Animals, and care of animals was taken as per guidelines of CPCSEA, Ministry of Forests and Environment, Government of India.

2.2. Drugs and chemicals

Naringin, pentylenetetrazole (PTZ), diazepam, flumazenil, radioimmunoprecipitation assay (RIPA) lysis buffer, and dimethylsulfoxide (DMSO) were purchased from Sigma Chemical Co. (Sigma, St. Louis, MO, U.S.A.). The protease inhibitor cocktail was purchased from Roche Applied Science (Mannheim, Germany). Enzyme-linked immunosorbent assay (ELISA) kits were from Pierce Biotechnology, Inc. (Rockford, IL, U.S.A.). All other materials were of the highest grade available.

2.2.1. Pentylenetetrazole (PTZ)-induced seizures

Pentylenetetrazole was dissolved in saline and administered intraperitoneally (i.p.) at the dose of 60 mg/kg. This dose has been standardized previously in our laboratory and is associated with the least mortality [18]. With this dose of PTZ, 99.0% of the animals exhibit a generalized tonic seizure (GTS). In GTSs, there is symmetric forelimb and hindlimb tonus followed by hindlimb clonus and flipping activity.

2.2.2. Experimental design

The rats were divided into six groups, and each group consisted of a minimum of six animals. Separate animals were used for each experiment. The naringin dose used in this experiment was determined from previous studies [16,17]. The visual scoring for seizure onset was done unblinded.

Group I (control group): The rats were injected with saline, i.p., for 7 days.

Group II (vehicle + PTZ group): Vehicle (saline) was administered, i.p., for 7 successive days before PTZ (60 mg/kg, i.p.) administration.

Group III (positive control group): Diazepam was administered on the seventh day followed by PTZ (60 mg/kg, i.p.) 30 min after the administration of diazepam.

Groups IV, V, and VI (naringin + PTZ group): Naringin in the doses of 20, 40, and 80 mg/kg/day, i.p., was administered for 7 days respectively to rats in different groups prior to PTZ.

On the seventh day, 30 min after the drug treatment, 60 mg/kg, i.p., PTZ was administered and the animals were observed for 30 min for latencies to myoclonic jerks and GTSs as well as duration of GTSs.

In groups II–VI, 24 h after PTZ administration, initial latency (IL) was noted and 48 h later, *i.e.*, on the ninth day, retention latency (RL) was noted using one trial of the passive avoidance task. This was done to assess the effect of PTZ on learning and memory. The rats were thereafter sacrificed for the estimation of oxidative stress markers and determination of brain levels of TNF-α.

2.3. GABAergic modulation by naringin in PTZ-induced seizures

Flumazenil (a benzodiazepine receptor ($GABA_A$) antagonist) was used to assess whether it blocks or reverses naringin ameliorating effects. The rats were pretreated with flumazenil (10 mg/kg, i.p.) and then after 15 min, the animals received an injection of naringin (80 mg/kg, i.p.), vehicle (10 ml/kg, i.p.), or diazepam (1 mg/kg, i.p.). Finally, PTZ (60 mg/kg, i.p.) was administered 30 min after naringin injection to induce seizures. The rats were observed for 30 min for latencies to myoclonic jerks and GTSs as well as duration of GTSs.

2.4. Behavioral test: single-trial passive avoidance test

Memory retention deficit was evaluated by a step-through passive avoidance apparatus. The apparatus consisted of equal-sized light and dark compartments (30 × 20 × 30 cm). A 40-W lamp was fixed 30 cm above its floor in the center of the light compartment. The floor consisted of a metal grid connected to a shock scrambler. The two compartments were separated by a trap door that could be raised to 10 cm. Twenty-four hours after the administration of PTZ, the rats were placed in the light compartment and the time lapse before each animal entered the dark compartment and had all four paws inside it was measured in seconds and termed as initial latency (IL). Immediately after the rat entered the dark chamber with all four paws inside the dark chamber, the trap door was closed and an electric foot shock (50 V a.c.) was delivered for 3 s. Five seconds later, the rat was removed from the dark chamber and returned to its home cage. Twenty-four hours after the IL, the latency time was again measured in the same way as in the acquisition trial and was termed as the retention latency (RL). However, during the retention trial, no foot shock was delivered, and the latency time was recorded to a maximum of 600 s. To improve the reliability and validity of the foot shock avoidance test, the grid as well as the rat paw were moistened with water before delivering the foot shock as this is known to reduce the wide interanimal variability in paw skin resistance of the rats [18].

2.5. Biochemical tests

2.5.1. Tissue preparation

Brain tissue samples were thawed and homogenized with 10 times (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4). Aliquots of homogenates from the rat brain were used to determine lipid peroxidation and glutathione.

2.5.2. Estimation of lipid peroxidation

Malondialdehyde (MDA), which is a measure of lipid peroxidation, was measured spectrophotometrically [19]. Briefly, brain tissues were homogenized with 10 times (w/v) 0.1 M sodium phosphate buffer (pH 7.4). The reagents 1.5 ml acetic acid (20%), pH 3.5, 1.5 ml thiobarbituric acid (0.8%), and 0.2 ml sodium dodecyl sulfate (8.1%) were added to 0.1 ml of the processed tissue sample. The mixture was then heated at 100 °C for 60 min. The mixture was cooled with tap water, and 5 ml of n-butanol:pyridine (15:1% v/v) and 1 ml of distilled water were added. The mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was withdrawn and absorbance was measured at 532 nm using a spectrophotometer.

2.5.3. Estimation of reduced glutathione

Glutathione (GSH) was measured spectrophotometrically [20]. Briefly, brain tissues were homogenized with 10 times (w/v) 0.1 M sodium phosphate buffer (pH 7.4). This homogenate was then centrifuged with 5% trichloroacetic acid to centrifuge out the proteins. To

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