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Effects of epigallocatechin-3-gallate on pentylenetetrazole-induced kindling, cognitive impairment and oxidative stress in rats

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ABSTRACT

Cognitive dysfunction is commonly observed in epileptic patients. It has been shown that not only epilepsy but also antiepileptic drugs could induce cognitive impairment. Thus, there is an urgent need for drugs that can suppress seizures without causing cognitive deficit. Recent studies have shown that oxidative stress is involved in the pathophysiology of epilepsy, and many antioxidants have an antiepileptic property. Epigallocatechin-3-gallate (EGCG), a catechin polyphenols component, is found to be an effective antioxidant. The purpose of this study was to assess the effect of EGCG against seizures, seizure-induced oxidative stress and cognitive impairment in pentylenetetrazole-induced kindling. Male Sprague-Dawley rats were injected intraperitoneally with a dose of 35 mg/kg of pentylenetetrazole (PTZ) once every alternate day for 13 injections. EGCG was administered daily in two doses (25 mg/kg and 50 mg/kg) intraperitoneally along with alternate-day PTZ. Morris water maze test was carried out 24 h after the last injection of PTZ, and the oxidative stress parameters (malondialdehyde and glutathione) were assessed after the completion of the behavioral test. The results showed that EGCG dose-dependently suppressed the progression of kindling. EGCG also ameliorated the cognitive impairment and oxidative stress induced by PTZ kindling. These observations suggest that EGCG may be a potential agent for the treatment of epilepsy as well as a preventive agent against cognitive impairment induced by seizure.

Epilepsy, one of the most common neurological disorders, affects 1% of the world population [7]. Clinical investigations have shown that over half of the epileptic patients suffer from cognitive impairment [18]. A variety of factors can adversely affect the cognition in patients with epilepsy, including the etiology of the seizures, seizure type, and psychosocial problems [14]. Another factor that may affect cognition is antiepileptic drugs (AEDs), though AEDs have been clinically available for several decades, however the side effects of AEDs cannot be ignored, especially the negative effect on cognitive function. Therefore, there is an urgent need to find new drugs that can suppress seizures effectively and prevent cognitive decline in the meantime.

Oxidative stress, which has been indicated to play an important role in the pathogenesis of seizures [19], may account for the underlying mechanisms for the deficits in cognitive function [8]. As a result, medicinal plants recently have been given particular attention as a protective agent against epilepsy and oxidative stress [5,12,3]. Epigallocatechin-3-gallate (EGCG), the main polyphenol of green tea, has been characterized as having antioxidant, anti-inflammatory, and anti-apoptotic properties [4,20,10]. Furthermore, EGCG could enhance spatial cognitive ability in normal animals [6]. Recent researches demonstrate that EGCG could attenuate oxidative damage in diabetic rats [1] and in brain ischemia model [11]. These properties raise the possibility of EGCG as a substance used in preventing epileptic seizures. Thus, the present study was aimed to examine the effects of EGCG on seizures, oxidative stress, and cognitive impairment in pentylenetetrazole (PTZ) kindled rats.

Adult male Sprague-Dawley rats weighing 180–220 g, obtained from Hebei Medial University, were housed in groups of four to five per cage in a room that was maintained at a constant temperature ($25 \pm 1\,^{\circ}C$) and humidity (40–60%). The rats were kept on a 12 h light/dark cycle, with lights on at 08:00 AM and with free access to food and water. Animal experiments were performed according to the regulations of laboratory animal management promulgated by the Ministry of Science and Technology of the People's Republic of China [1988] No. 134, which conforms to the internationally recognized NIH guidance for care and use of laboratory animals.

EGCG and PTZ were purchased form Sigma (St. Louis, MO, USA). Glutathione (GSH) detection kit and malondialdehyde (MDA) detection kit were obtained from Nanjing Jiancheng Bioengineering Institute (China). Prior to the experiments, EGCG and PTZ were

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dissolved in physiological saline. Then, PTZ was injected intraperitoneally (i.p.) on alternate day in a dose of 35 mg/kg (13 injections total), while EGCG was injected intraperitoneally (i.p.) daily. The administration work was conducted between 08:00 and 09:00 AM.

The animals were randomly divided into five groups with ten in each group. Group I (control group) received 0.9% saline i.p. every other day (3.5 ml/kg, 13 injections total). Group II (PTZ group) received saline pretreatment along with PTZ every other day. Groups III and IV (PTZ + EGCG group) received EGCG pretreatment in doses of 25 and 50 mg/kg, respectively, in addition to alternate-day treatment of PTZ for 13 injections. In these groups, EGCG was given 30 min before PTZ. Group V (EGCG group), received 50 mg/kg of EGCG alone to study any effect of EGCG on the cognitive functions and the biochemical parameters.

The animals were observed for 30 min after each PTZ administration. The latency to myoclonic jerks and the generalized tonic clonic seizures (GTCS), as well as the duration of GTCS were recorded. Seizure stage was evaluated using the following scale [17]: Stage 0: no response; Stage 1: hyperactivity and vibrissae twitching; Stage 2: head nodding, head clonus and myoclonic jerk; Stage 3: unilateral forelimb clonus; Stage 4: rearing with bilateral forelimb clonus; Stage 5: generalized tonic-clonic seizure (GTCS) with loss of postural control.

Morris water maze (MWM) test was assessed 24 h after the last administration of PTZ. The water maze consisted of a circular water tank (180 cm in diameter, 70 cm in height) that was partially filled with 23 ± 1 °C water. The pool was divided virtually into four equal quadrants labeled N-S-E-W. A colorless escape plat (10 cm in diameter) was hidden 1.5 cm below the surface of the water in a fixed location. The maze was located in a quiet test room, surrounded by many visual cues outside of the maze which was visible from within the pool and could be used by the rats for spatial orientation. The experiments were conducted two sessions per day for 5 days, each session comprising four trials, with an intertrial interval of 60 s and an intersession interval of 2 h. In each trail, the rats were gently placed in the middle of the circular edge in a randomly selected quadrant, with the nose pointing toward the wall. If rats failed to find the escape platform within 120 s by themselves, they were placed on the platform for 10 s by the experimenter and their escape latency was accepted as 120 s. After climbing onto the platform, the animal remained there for 30 s before the commencement of the next trial. On the sixth day, a probe trial without the platform was assessed, and the time spent in the target quadrant where the platform had been located was recorded. The test was performed from 10:00 AM to 17:00 PM to exclude variations in performance resulting from circadian rhythmicity.

Shortly after completion of the behavioral tests, the animals were sacrificed and the brains were removed and cleaned with ice-cold (4 °C) saline. Brain tissue samples were thawed, and a 10% (w/v) homogenate was made with ice-cold (4 °C) 0.1 M phosphate buffer (PH 7.4). The homogenates were used to assess lipid peroxidation product and reduced glutathione. Malondialdehyde (MDA), an index of lipid peroxidation, was measured spectrophotometrically with the method described by Okhawa [15]; reduced glutathione (GSH) was estimated spectrophotometrically by the method described by Ellman [2].

All data are shown as the mean ± standard error of mean (SEM). Significance of seizure stage and escape latencies in MWM were

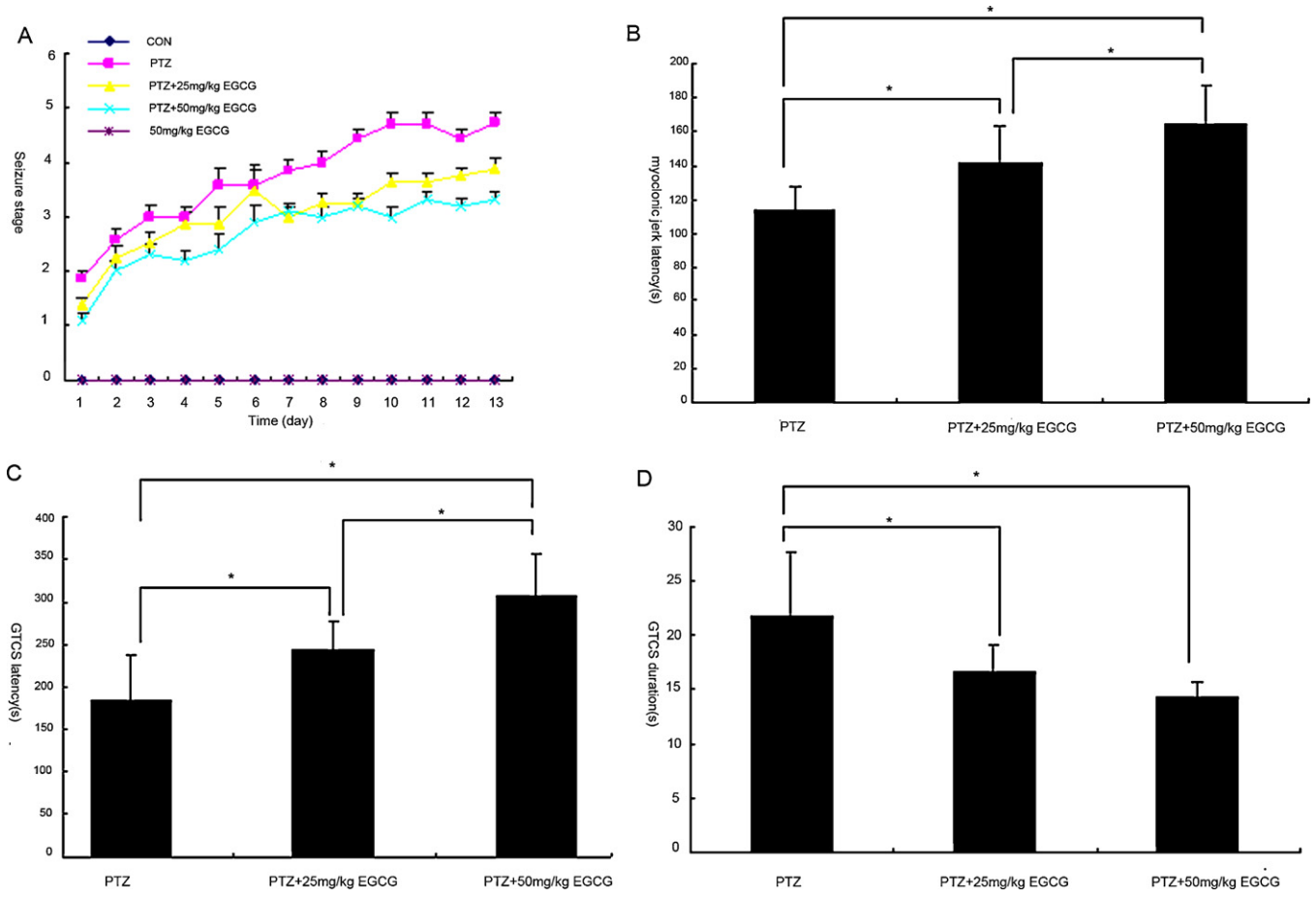

Fig. 1. Effect of EGCG treatment in PTZ-kindled seizures. (A) Mean seizure score. (B) Latency to myoclonic jerks. (C) Latency to GTCS. (D) Duration of GTCS. Values are expressed as mean ± S.E.M. $^{*}p < 0.05$.