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Comparison between the effects of quercetin on seizure threshold in acute and chronic seizure models

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Abstract

Flavonoids are important constituents of food and beverages, and several studies have shown that they have neuroactive properties. Many of these compounds are ligands for γ-aminobutyric acid type A receptors in the central nervous system. This study aimed to investigate the anticonvulsant effects of quercetin (3,3′,4′,5,7-pentahydroxyflavone), which is a flavonoid found in plants, in rats treated with pentylenetetrazole in acute and chronic seizure models. Single intraperitoneal administration of quercetin did not show anticonvulsive effects against acute seizure. Similarly, multiple oral pretreatment with quercetin did not have protective effects against acute seizure. However, multiple intraperitoneal administration of quercetin (25 and 50 mg/kg) significantly increased time to death compared with the control ($p < 0.001$). However, quercetin pretreatment had no significant effects on the pattern of convulsion development during all periods of kindling. But on the test day, quercetin (100 mg/kg) could significantly increase generalized tonic–clonic seizure onset (GTCS) and decrease GTCS duration compared with the control ($p < 0.01$, $p < 0.05$). We conclude that quercetin has a narrow therapeutic dose range for anticonvulsant activities *in vivo*, and it has different effects on the seizure threshold. The different effects of quercetin on seizure threshold may occur through several mechanisms.

Keywords

Flavonoids, quercetin, pentylenetetrazole, acute seizure model, kindling

Introduction

Epilepsy is a major focus in the study of neurological disorders. The incidence of epilepsy in the general population is 45/100,000, its prevalence is 0.5%, and the lifetime probability of a seizure is approximately 3% (Simon et al., 2007).

Pentylenetetrazole (PTZ), a selective blocker of the chloride channel coupled to the γ-aminobutyric acid type A ($GABA_A$) receptor complex, is the most popular chemoconvulsant used for evaluating antiepileptic drugs (Hansen et al., 2004). Also, kindling induced by the convulsant, PTZ, represents a model of primary generalized epilepsy (Rossi, 1996). PTZ kindling provides a useful model of post-seizure dysfunction, which can serve as a screen for potential treatments for those cognitive and emotional deficits that are often observed in human epilepsy (Mortazavi et al., 2005).

Quercetin is a flavonoid (3,3′,4′,5,7-pentahydroxy flavone; Figure 1) (Amália et al., 2007), which is found in a variety of fruits and vegetables, including apples, citrus fruits, berries, bulbs, cereal grains, onions, legumes, and tea (Harwood et al., 2007). The amount of daily absorption of flavonoids is about 20–1000 mg. Quercetin, which constitutes the important part of absorptive flavonoids (especially in onions, apples, and tea), has a long plasmatic half-life (11–28 h), and if its dosage is repeated, it can accumulate in plasma (Chen et al., 2006). A high dosage of quercetin is needed to

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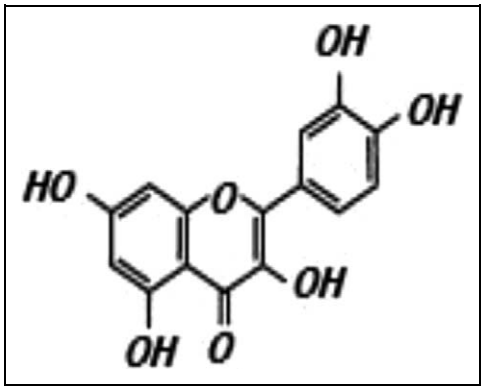

Figure 1. Chemical structure of quercetin.

achieve good results. Since, quercetin undergoes first-pass metabolism (Bhattaram et al., 2002), it has the ability to penetrate the blood–brain barrier (BBB), and in some studies, the proportion of its passage is estimated to be as high as 65.54% (Ren et al., 2010). Quercetin metabolites and glycosides seem to be less neuroprotective and penetrate BBB less efficiently than aglycone (Ossola et al., 2009).

Quercetin has several pharmacological properties, including antioxidant, anti-inflammatory, hepatoprotective, and anticancer effects (Amália et al., 2007; Borska et al., 2012; Choi et al., 2003; Huang et al., 2012; Kim et al., 2012; Liu et al., 2012; Patir et al., 2012). In addition, quercetin has shown free-radical scavenging activity in ultraviolet B-irradiated cultured human dermal fibroblasts (Chondrogianni et al., 2010). Chronic oral administration of quercetin (20–50 mg/kg) following ethanol withdrawal showed protective effects against PTZ convulsions (Joshi et al., 2005). However, it was found that quercetin could not protect seizures and movement disorders induced by *N*-methyl D-aspartate (NMDA) in rats, and it was suggested that quercetin has proconvulsant effect (Nitsinskaia et al., 2010). Furthermore, it was shown that protective and toxic effects of quercetin depend on the timing and its dose (Ossola et al., 2008).

Efficiency of quercetin in neurodegenerative disorders is doubtful. Moreover, the association between quercetin administration and neuroprotective effects is of scientific interest. However, studies on the anticonvulsive effects of quercetin in animals are controversial owing to the use of different methods. In the present study, we examined the anticonvulsant effects of quercetin using different methods of PTZ-induced seizure and different routes of administration of quercetin in rats.

Material and methods

Animals

Male Wistar rats (200–250 g) were obtained from the Razi Institute (Karaj, Islamic Republic of Iran) and housed in groups of four per cage under standard laboratory conditions. They were kept at constant room temperature (21 $\pm$ 2°C) under a normal 12-h light:12-h dark cycle with free access to food and water. All the animal experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) in such a way as to minimize the number of rats used and their suffering.

Drugs

Quercetin and PTZ were purchased from Sigma (Sigma, St Louis, Missouri, USA). PTZ and quercetin were dissolved in physiological saline before the experiments.

PTZ-induced seizures

Single administration of quercetin. The rats were divided into 3 groups with 10 animals in each group. The first group was administered with PTZ (90 mg/ kg, intraperitoneally (i.p.)) 30 min after the intraperitoneal administration of normal saline as control. In the next two groups, quercetin (50 and 100 mg/kg, i.p.) was administered 30 min before the administration of PTZ.

Multiple intraperitoneal or oral administration of quercetin. The rats were divided into 3 groups with 10 animals in each group. The first group was administered saline (i.p. or gavage) for 7 days and on the last day PTZ (90 mg/kg, i.p.) was administered 30 min after the administration of normal saline. In the next two groups, quercetin (25 and 50 mg/kg, i.p. or gavage) was administered for 7 days and on the last day PTZ (90 mg/kg, i.p.) was administered 30 min after the administration of quercetin. During both ways of administration of quercetin, the rats were individually placed in plastic boxes and observed immediately after the administration of PTZ for a 60-min period (Nassiri-Asl et al., 2008).

Latency to the first generalized tonic–clonic seizure (GTCS; the full tonic extension of both forelimbs and hindlimbs), GTCS duration, and time to death were evaluated during the administration of PTZ (Loscher et al., 1991).

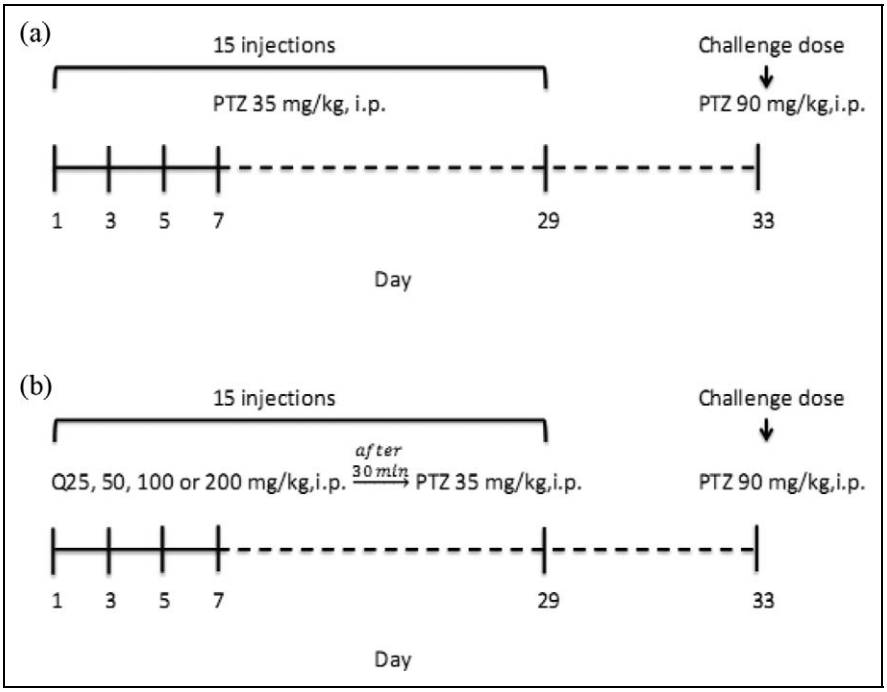

Figure 2. Protocol of PTZ kindling in control (a) and quercetin treatment groups. (b) PTZ: pentylenetetrazole.

PTZ kindling

Rats were divided into 5 groups of 10 animals each. In the control group, saline was injected 30 min before the administration of PTZ every other day (35 mg/kg, i.p., 15 injections in total).

After each injection, the animals were placed individually in plastic boxes, and the convulsive behavior was recorded for 20 min and the behavioral changes were graded according to the criterion described by Racine (1972): 0: *no behavioural changes*; 1: *facial movements and ear and facial twitching*; 2: *myoclonic convulsions without rearing*; 3: *myoclonic convulsions with rearing*; 4: *clonic convulsion with loss of posture*; 5: *GTCS*. In the remaining 4 groups, 25, 50, 100, or 200 mg/kg quercetin was administered 30 min before the administration of PTZ every other day (35 mg/kg, i.p., 15 injections in total; Figure 2).

PTZ test dose. On the test day (i.e. day 33), all kindled groups received PTZ (90 mg/kg, i.p.), and the animals were tested for this challenge dose and latency to the first GTCS, GTCS duration, and the protection percentage against seizure were recorded during 2 h (Figure 2).

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Data from behavioral studies were analyzed using analysis of variance, followed by the Tukey–Kramer multiple comparison test for statistical evaluation. Fisher's exact probability test was used to analyze seizure protection. The values of $p < 0.05$ were considered significant.

Results

Effects of single administration of quercetin on acute seizure

In the control group, administration of saline 30 min before the administration of PTZ (90 mg/kg, i.p.) induced GTCS. Quercetin (50 and 100 mg/kg, i.p.) administered 30 min before the administration of PTZ prolonged the latency to GTCS compared with the control (Table 1). However, this effect was not significant. GTCS duration did not decrease (Table 1). Time to death in quercetin groups increased compared with that in the control group. However, these effects were not significant (Table 1).

Effects of multiple oral administration of quercetin on acute seizure

The first group administered with saline (gavage) for 7 days and PTZ (90 mg/ kg, i.p.) 30 min after the administration of normal saline on the last day induced GTCS. Quercetin (25 mg/kg, gavage) administered for

Table 1. Effects of acute administration of quercetin on acute seizure induced by PTZ in rats.[a]

Treatment (dose)	Onset (s)	Duration (s)	Time to death (min)
Control	50.1 ± 3.2	23.5 ± 1.4	5.8 ± 1.2
Q (50 mg/kg)	63.6 ± 51.2	23.6 ± 0.68	7.18 ± 2.7
Q (100 mg/kg)	115.6 ± 54	24.5 ± 1.7	12.4 ± 3.9

Q: quercetin; i.p.: intraperitoneally; PTZ: pentylenetetrazole.
[a]Drugs were administered (i.p.) 30 min before the injection of PTZ (90 mg/kg, i.p.). Values are the mean ± SEM for 10 rats, Tukey–Kramer test.

Table 2. Effects of multiple oral administration of quercetin on acute seizure induced by PTZ in rats.[a]

Treatment (dose)	Onset (s)	Duration (s)	Time to death (min)
Control	60.6 ± 2.2	24.5 ± 2.1	4 ± 1.1
Q (50 mg/kg)	77.2 ± 16	19.25 ± 2.9	10.57 ± 2.5
Q (100 mg/kg)	68.6 ± 8.7	19.2 ± 4.6	11.2 ± 3

Q: quercetin; i.p.: intraperitoneally; PTZ: pentylenetetrazole.
[a]Drugs were orally administered for 7 days. The last dose was given 30 min before the injection of PTZ (90 mg/kg, i.p.). Values are the mean ± SEM for 10 rats, Tukey–Kramer test.

Table 3. Effects of multiple intraperitoneal administration of quercetin on acute seizure induced by PTZ in rats.[a]

Treatment (dose)	Onset (s)	Duration (s)	Time to death (min)
Control	58.6 ± 5.2	22.5 ± 1.6	6.9 ± 1.3
Q (25 mg/kg)	76.2 ± 10.5	21.2 ± 3.3	53.38 ± 30.9[b]
Q (100 mg/kg)	137.8 ± 47.8	15.4 ± 1.9	53.39 ± 30.9[b]

Q: quercetin; i.p.: intraperitoneally; PTZ: pentylenetetrazole.
[a]Drugs were administered (i.p.) for 7 days. The last dose was given 30 min before the injection of PTZ (90 mg/kg, i.p.). Values are the mean ± SEM for 10 rats, Tukey–Kramer test,
[b]$p < 0.001$ compared with control, Tukey–Kramer test.

7 days and on the last day administered 30 min before PTZ increased seizure onset and decreased GTCS duration. However, these effects were not significant (Table 2). Quercetin (25 and 50 mg/kg, gavage) increased time to death, and these effects were not significant (Table 2).

Effects of multiple intraperitoneal administration of quercetin on acute seizure

The first group that was administered with saline for 7 days and PTZ (90 mg/ kg, i.p.) 30 min after the administration of normal saline on the last day induced GTCS.

Quercetin (25 and 50 mg/kg, i.p.) administered for 7 days and 30 min before PTZ administration on the last day increased seizure onset and decreased GTCS duration. However, these effects were not significant (Table 3). Quercetin (25 and 50 mg/kg, i.p.) significantly increased time to death compared with that of the control ($p < 0.001$; Table 3).

Effects of quercetin pretreatment on kindling development

The effects of different doses of quercetin during kindling are shown in Figure 3. The repeated application of 35 mg/kg PTZ induced behavioral seizures with increasing severity that culminated in clonic convulsion with loss of posture of stages 4 at the end of kindling. Pretreatment with quercetin (25, 50, 100, and 200 mg/kg) before each kindling injection modified the stage of kindling development differently. Quercetin pretreatment had no significant effects on the pattern of convulsion development during all periods of kindling.

However, on the test day (day 33), 100 mg/kg quercetin significantly increased GTCS onset compared with the control ($p < 0.01$; Figure 4(a)). Also,

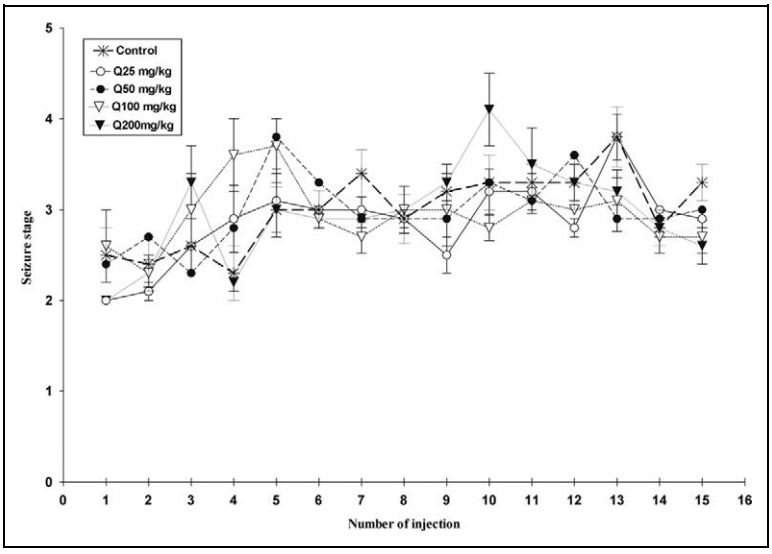

Figure 3. The effects of repeated administration of quercetin (25, 50, 100, and 200 mg/kg) on the development of PTZ induced kindling (35 mg/kg, i.p., 15 injections total) in comparison with the control. Data are mean seizure stage ± SEM, $n = 10$, Tukey–Kramer test. PTZ: pentylenetetrazole; i.p.: intraperitoneally.

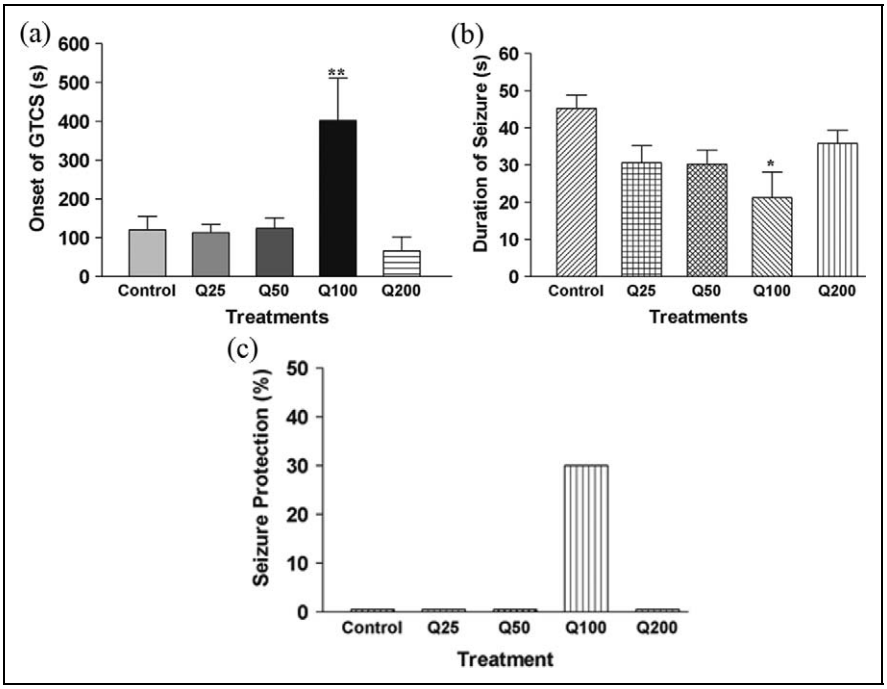

Figure 4. Response to challenge dose of PTZ in all kindled groups. On the test day (day 33), all kindled groups received PTZ (90 mg/kg, i.p.). Values are the mean ± SEM for 10 rats. *n*: number of animals; *$p < 0.05$, **$p < 0.01$: compared with the control, Tukey–Kramer test. PTZ: pentylenetetrazole; i.p.: intraperitoneally.

100 mg/kg quercetin significantly reduced GTCS duration compared with the control ($p < 0.05$; Figure 4(b)). Furthermore, 100 mg/kg quercetin could increase the protection percentage against seizure compared with the control. However, this effect was not significant ($p > 0.05$, Fisher's exact test; Figure 4(c)). Similar to kindling, 200 mg/kg quercetin did not show an anticonvulsive effect, and the duration of GTCS was not significantly reduced. Furthermore, there was no protection against seizure with increasing dose ($p > 0.05$, Fisher's exact test; Figure 4(c)).

Discussion

In the present study, the anticonvulsant effects of single and multiple administration of quercetin were investigated in acute and chronic models of seizure, which was induced by PTZ in rats.

Our results demonstrated that acute intraperitoneal administration of quercetin did not affect GTCS induced by PTZ. Also, multiple oral administration of quercetin did not significantly affect GTCS induced by PTZ. At the same dose, multiple intraperitoneal administration of quercetin significantly increased only time to death compared with that of the control.

Quercetin pretreatment had no significant effects on the pattern of convulsion development during all periods of kindling. On the test day (after kindling), 100 mg/kg quercetin significantly increased GTCS onset and reduced GTCS duration. Furthermore, 100 mg/kg quercetin increased the protection percentage against seizures but this effect was not significant. Similar to kindling, 200 mg/kg quercetin did not show an anticonvulsive effect, and GTCS duration was not significantly reduced. Moreover, there was no protection against seizure when the dose was increased to 200 mg/kg.

Flavonoids are a group of structurally related phenyl benzopyrones. They are subdivided into the following main classes: flavones, flavanones, flavonols, isoflavones, flavans, and flavan-3-ols. They are found in all plants and therefore form part of the human diet (Hall et al., 2005).

It was established that in the PTZ kindling model, the susceptibility to seizures following injection of initially subconvulsive doses of PTZ is increased and culminated in GTCS. After repeated or single dose administration of PTZ, the GABAergic function is decreased, and the stimulation and modification of sensitivity of different glutamate receptor subtypes in many brain regions occur (Corda et al., 1992; Rauca et al., 1999). With regard to the anticonvulsant effects of quercetin on the test day, it was reported that quercetin, at concentrations of about 0.34 pM, could inhibit $\alpha_1\beta_1\gamma_{2\ s}$ $GABA_A$, and ρ_1 $GABA_c$ receptors, and it was suggested that quercetin acts as a modulator of α_1GABA receptors (Goutman et al., 2003). Thus, these protective effects of quercetin on the test day may relate to its modulatory effect on GABA receptors in our study.

Acute administration of quercetin (50 mg/kg) to animals withdrawn from ethanol did not prevent withdrawal-induced anxiety. However, single and multiple oral administration of quercetin (20–50 mg/kg) following ethanol withdrawal showed protective effects against a nonconvulsive dose (40–60 mg/kg) of PTZ (Joshi et al., 2005). In our study, acute intraperitoneal administration of quercetin (50 mg/kg) could not show protection against PTZ-induced seizure; furthermore, multiple oral administration of quercetin (20–50 mg/kg) did not protect against PTZ-induced seizure. Since our applied dose of PTZ was a convulsive dose. However, in our study, multiple intraperitoneal administration of quercetin (50 mg/kg) shows some protective effects and increased time to death of the animals.

It was reported that low concentrations of the flavonoid could stimulate cell proliferation and increase total antioxidant capacity (TAC) of the cells. On the other hand, higher concentrations of the flavonoid decreased cell survival and viability, thiol content, TAC, and activities of superoxide dismutase, catalase, and glutathione *S*-transferase. Furthermore, quercetin decreased the production of reactive oxygen species in the cells but produced peroxides in the medium. Subsequently, it has been suggested that quercetin has complex oxidative and antioxidative effects (Robaszkiewicz et al., 2007). Quercetin causes no significant toxicity in several animal studies and has beneficial effects on various *in vivo* models of neural disorders, particularly in cerebrovascular insults. However, it has narrow therapeutic dose range in *in vitro* studies. Thus, the risk for neurotoxicity is not negligible (Ossola et al., 2009). For this reason, it is possible that quercetin at dose of 200 mg/kg did not show any anticonvulsant effects during kindling, and quercetin (100 mg/kg) did not protect against acute administration of PTZ.

Pretreatment with quercetin had no effects on tyrosine hydroxylase positive cells in the substantia nigra or dopamine levels (Zbarsky et al., 2005). Furthermore, it was found that a single dose of quercetin did not have protective effect against 6-hydroxydopamine toxicity in Parkinson's disease (Ossola et al., 2008). Similarly, in our study, a single dose of quercetin did not have a protective effect against PTZ-induced acute seizure.

In agreement with our study, quercetin did not protect seizures and movement disorders induced by NMDA in rats, and it was discussed that the proconvulsant effect of quercetin was associated with inhibition of heat shock protein 70 (Hsp 70) expression (Nitsinskaia et al., 2010). It was shown that quercetin

inhibited the expression of Hsps in *in vivo* and *in vitro* studies. It is suggested that quercetin may induce cell toxicity via changes in protein expression (Nitsinskaia et al., 2010; Sasaki et al., 2007). However, it was shown that intracellular metabolism of quercetin in glial cells can decrease quercetin neuronal toxicity potential (Vafeiadou et al., 2008).

On the other hand, oxidative stress causes phosphorylation of connexin 43 (Cx43), a major part of gap junction channels, causes conformational changes in gap junctions and then inhibits gap junction intercellular communication. It was shown that antioxidant properties of quercetin may be responsible for the suppression of hydrogen peroxide-induced phosphorylation of extracellular signal-regulated protein kinase 1/2 and Cx43 and prevention of gap junction intercellular communication (Lee et al., 2010). It was shown that changes in the expression of Cx43 may be involved in seizure activity in human epileptic tissue (Garbelli et al., 2011). Gap junction blocker could attenuate hippocampal seizure activity (Mylvaganam et al., 2010). Thus, it may be another possibility that explains why quercetin did not increase the seizure threshold with some doses in the experiments.

Quercetin is an oral metabolite of rutin (Nakayama et al., 1993). It was shown that, biochemically, the 4-oxo group and the 2,3 double bond in the C ring common to both rutin and quercetin are related to their neuroprotective action. Nevertheless, it was also demonstrated that rutin has a more significant effect on spatial memory impairment than quercetin (Pu et al., 2007).

In agreement with the above studies, in our previous studies, rutin has potential anticonvulsant effects in PTZ-induced acute and chronic seizure in rats (Nassiri-Asl et al., 2008, 2010).

In our study, it seems that the efficacy of intraperitoneal administration of quercetin is greater than that of oral administration. This may be related to its oral first-pass metabolism that leads to a reduction in the efficacy of quercetin at same doses compared with intraperitoneal administration (Bhattaram et al., 2002).

Conclusion

In this study, we conclude that quercetin has a narrow therapeutic dose range for anticonvulsant activities and that it has different effects on the seizure threshold. It might therefore be assumed that the effects of quercetin on seizure threshold occur through different mechanisms. Also, the route of administration of quercetin is another important issue that may change the effect of quercetin on seizure threshold. Further study is necessary to evaluate the molecular mechanisms underlying the different effects of quercetin.

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