

• Research Article

Effects of rutin on oxidative stress in mice with kainic acid-induced seizure

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OBJECTIVE: Flavonoids are present in foods such as fruits and vegetables. Several studies have demonstrated a relationship between the consumption of flavonoid-rich foods and prevention of human disease, including neurodegenerative disorders. We assessed the effect of rutin (quercetin-3-O-rutinoside) on oxidative stress in kainic acid (KA)-induced seizure.

METHODS: Thirty-six BALB/c mice were randomly divided into three groups. In the control group, saline (intra-peritoneal, i.p.) was administered for 7 d, and on the last day, KA (10 mg/kg, i.p.) was injected 30 min after administration of saline. In rutin groups, mice were pretreated with rutin (100 and 200 mg/kg, i.p.) for 7 d, and on the last day, KA (10 mg/kg, i.p.) was injected 30 min after administration of rutin. Subsequently, behavioural changes were observed in mice. Lipid peroxidation and oxidative stress were measured respectively in the early and late phases after KA-induced seizures.

RESULTS: Seizure scores in the rutin groups were significantly lower than those in the control group ($P < 0.01$). Furthermore, rutin dose-dependently inhibited the number of wet-dog shakes (WDS) ($P < 0.05$). Malondialdehyde level in the hippocampus of the rutin groups was significantly lower than that in the hippocampus of the control group on days 1 and 21 after KA administration. In the rutin groups, the thiol levels observed on day 1 after KA administration were higher than that in the control group ($P < 0.01$).

CONCLUSION: These results indicate that rutin has potential anticonvulsant and antioxidative activities against oxidative stress in KA-induced seizure in mice.

KEYWORDS: plant extracts; rutin; kainic acid; oxidative stress; epilepsy; seizure; mice

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1 Introduction

Epilepsy is a major focus of research on neurological

disorders. The incidence of epilepsy in the general population is about 45/100 000, its prevalence rate is 0.5% and lifetime probability of a seizure is approximately 3%^[1]. Administration of kainic acid (KA), an excitotoxic

substance, could stimulate glutamate receptors, thus increasing the levels of reactive oxygen species (ROS) and glutaminergic activity^[2]. Oxidative stress has been reported to be a possible molecular mechanism of KA-induced neurotoxicity and it is associated with hippocampal cell death^[3,4]. Both systemic and intracerebral injection of KA have been shown to trigger epileptiform seizures by damaging the CA3 region of the hippocampus; these KA-induced seizures have been shown to propagate to other limbic structures and demonstrate a pattern of cell death similar to that of temporal lobe epilepsy^[5]. Furthermore, increases in mitochondrial superoxide production and hippocampal neuronal loss caused by KA have been shown to be attenuated in transgenic mice overexpressing mitochondrial superoxide dismutase-2^[6]. KA induces activation of nuclear factor-kappa B (NF- κ B) in degenerating neurons within 24 h of treatment^[7]. Similarly, the role of antioxidants has been established in inhibiting seizures caused by excitotoxic agents^[2,8].

Rutin (quercetin-3-O-rutinoside), a flavonoid, is found in many plants such as buckwheat, passion flower, apple, tea and *Ginkgo biloba*^[9-11]. Rutin has been found to have neuroprotective effects in several different memory impairment models^[12-14], and in cerebral ischemia^[15,16]. In this study, we investigated the possible effects of rutin on oxidative stress in KA-induced seizure in mice. Moreover, malondialdehyde (MDA) and thiol were measured as indicators of lipid peroxidation and oxidative stress, respectively, in the early and late phases after KA-induced seizures.

2 Materials and methods

2.1 Animals

A total of 36 male BALB/c mice (body weight 20 to 25 g) were obtained from the Razi Institute (Karaj, Iran) and housed in groups of four per cage under standard laboratory conditions. They were maintained at constant room temperature (21 °C \pm 2 °C) under a 12:12 h light-dark cycle with free access to food and water. All animal experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) so as to minimize the number of animals used and their suffering.

2.2 Drugs

Rutin and KA were purchased from Sigma (St Louis, MO, USA). Other drugs used in this study included xylazine (Loughrea, Co. Galway, Ireland) and ketamine (Rotexmedica, GmbH, Germany). Rutin (8.3 mg/mL) and KA (0.5 mg/mL) were dissolved in saline. Other reagents included 2,2'-dinitro-5,5'-dithiodibenzoic acid (DTNB), 2-thiobarbituric acid (TBA), Tris (Trizma[®] base), sodium ethylenediaminetetraacetic acid (NA EDTA), methanol,

and trichloroacetic acid (TCA).

2.3 KA administration and experimental design

The pH of KA solution was adjusted to 7.2 to 7.4 with NaOH^[17]. Mice were divided into three groups of 12 animals each. The control group was given an intraperitoneal (i.p.) injection of saline (10 mL/kg) daily for 7 d, and on the last day, KA (10 mg/kg, i.p.) was injected 30 min after administration of saline. In the two treatment groups, rutin (100 and 200 mg/kg, i.p.) was administered daily for 7 d, and on the last day, KA (10 mg/kg, i.p.) was injected 30 min after administration of rutin^[18]. Following administration of KA, mice were observed for behavioural changes over a period of 2 h. The behavioural scores were as follows: 0, no response; 1, immobility; 2, rigid posture; 3, scratching/circling/head bobbing; 4, forelimb clonus/rearing/falling; 5, repetitive pattern of 4; and 6, severe tonic-clonic seizures^[19]. Also, onset and number of wet-dog shake (WDS), which is characteristic behavioural response^[20,21], was counted for 2 h after i.p. injection of KA^[22].

Finally, 1 and 21 d after KA administration, mice were anaesthetized with i.p. injection of ketamine (100 mg/mL, 60 mg/kg)/xylazine (20 mg/mL, 6 mg/kg) and sacrificed. The hippocampi of animals were immediately removed, cleaned with chilled saline (0.9%) and used for biochemical analysis.

2.4 Measurement of lipid peroxidation

MDA level of hippocampi was measured spectrophotometrically. MDA reacts with TBA as a thiobarbituric acid reactive substance (TBARS) to produce a red coloured complex that has a peak absorbance at 535 nm^[23]. Hippocampus was homogenized with cold 1.5% KCl (15 mg/mL) to make a 10% homogenate. To 1.0 mL of the hippocampus homogenate, 2.0 mL of TCA-TBA-HCl was added and mixed thoroughly. The solution was heated for 60 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1 000 \times g for 10 min. The absorbance at 535 nm was measured by Genesys 10 UV-Spectrophotometer (Spectronic Unicam, Model, Rochester, NY, USA) against a blank (TBA, 1 mL) that contained all the reagents except the sample. The amount of MDA equivalents formed was calculated using a molar extinction coefficient of 1.56 \times 10⁵/mol \cdot cm and expressed as nmol MDA equivalents/mg protein.

2.5 Measurement of total thiol

Total thiol was measured using DTNB. This reagent reacts with the thiol to produce a yellow-coloured complex that has a peak absorbance at 412 nm^[24]. Briefly, 1 mL Tris-EDTA buffer (0.25 mol/L Tris; 2 mmol/L EDTA; pH 8.6) was added to 50 μ L hippocampus homogenate in the 2-mL cuvettes. Sample absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Next, 20 μ L of DTNB

(10 mmol/L in methanol, purity $\geq 99.5\%$) was added to the mixture, and after 15 min (stored at laboratory temperature), the sample absorbance was read again (A₂). The absorbance of the DTNB reagent was read as a blank (B)^[25]. Total thiol concentration (mmol/L) was calculated from the following equation: total thiol concentration (mmol/L) = (A₂ - A₁ - B) × (1.07/0.05) × 13.6 and expressed as nmol MDA equivalents/mg protein.

2.6 Data analysis

Seizure score, time of onset, number of WDS, MDA level and thiol concentration were expressed as mean ± standard error of mean. Differences among experimental groups were analysed by one-way analysis of variance (ANOVA), and the post-hoc Tukey's test was used for multiple comparisons (Prism software ver 3.0). Also, we used two-way ANOVA to investigate the interaction between the effects of treatment and test day. $P < 0.05$ was considered to be statistically significant.

3 Results

3.1 Effects of KA on seizure

Seizure scores in the rutin-pretreated groups were significantly lower than those in the control group ($F(2,33) = 31.9$, $P < 0.01$; Figure 1A). The delay to onset of WDS increased significantly in the rutin groups compared to that in the control group ($F(2,33) = 13.6$, $P < 0.01$; Figure 1B). During the 2-hour observation period after KA administration, the number of WDS in the rutin groups was significantly lower than that in the control group ($F(2,33) = 5.8$, $P < 0.05$; Figure 1C).

3.2 TBARS measurement

Pretreatment with rutin significantly decreased MDA levels in the hippocampus at doses of 100 and 200 mg/kg compared to the control group on day 1 ($F(2,15) = 7$, $P <$

0.05, $P < 0.01$, respectively; Figure 2). Furthermore, MDA level in the rutin groups was significantly lower than that in the control group on day 21 ($F(2,15) = 5.3$, $P < 0.05$; Figure 2).

There was no interaction between the effects of treatment and test day ($P > 0.2$, two-way ANOVA).

3.3 Total thiol assay

The content of thiol in the hippocampus of the rutin-treated (100 and 200 mg/kg) groups was significantly higher than that of the control group on day 1 ($n=6$, $F(2,15) = 9.2$, $P < 0.01$, respectively; Figure 3). However, there was no significant elevation in the total content of thiol in the hippocampus of the rutin groups compared to that of the control group on day 21 ($n=6$, $F(2,15) = 4.4$, $P > 0.05$; Figure 3). Two-way ANOVA showed that the interaction between the effects of treatment and test day was not significant ($P > 0.285$).

4 Discussion

In the present study, we investigated the possible effects of rutin on oxidative stress in KA-induced seizure in mice. Pretreatment with rutin dose-dependently reduced the seizure score relative to control animals.

Another significant finding in the present study is that rutin pretreatment was able to significantly and dose-dependently decrease the KA-evoked WDS that is characteristic of experimental convulsive seizures^[26]. The KA-induced WDS represents neuronal hyperactivity in limbic structures that spread to midbrain areas and to the motor system^[21]. Interestingly, an increased number of γ -aminobutyric acid (GABA) receptors has also been reported after limbic stimulation^[27], suggesting that the WDS is a sign of the progression of limbic seizures towards generalized seizure^[26]. The present data indicate

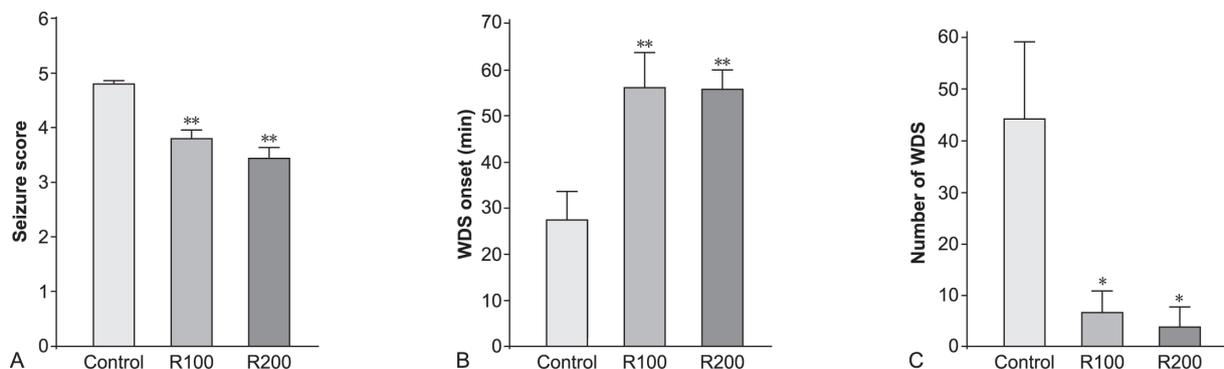


Figure 1 The effects of rutin (100 and 200 mg/kg) on seizure score (A), onset of WDS (B) and the number of WDS (C) in mice with KA-induced seizure

The control group was administered saline (i.p.) for 7 d, and on the last day, KA (10 mg/kg, i.p.) was injected 30 min after administration of saline. Rutin (100 and 200 mg/kg, i.p.) was administered for 7 d, and on the last day KA (10 mg/kg, i.p.) was injected 30 min after the administration of rutin. Data are expressed as mean ± standard error of mean. * $P < 0.05$, ** $P < 0.01$, vs control group, $n=36$, analyzed by post-hoc Tukey's test. KA: kainic acid; WDS: wet-dog shake.

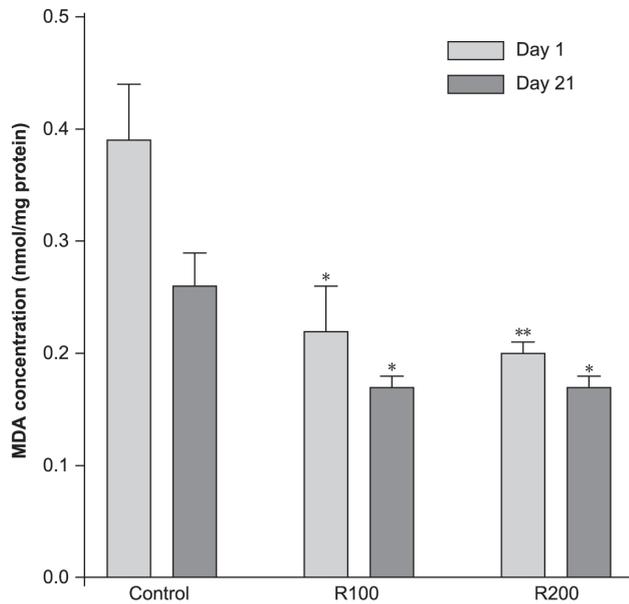


Figure 2 Effect of rutin on MDA in the hippocampus on days 1 and 21 after kainic acid administration

Values are expressed as mean \pm standard error of mean. * $P < 0.05$, ** $P < 0.01$, vs control group, $n=6$, analyzed by post-hoc Tukey's test. MDA: malondialdehyde.

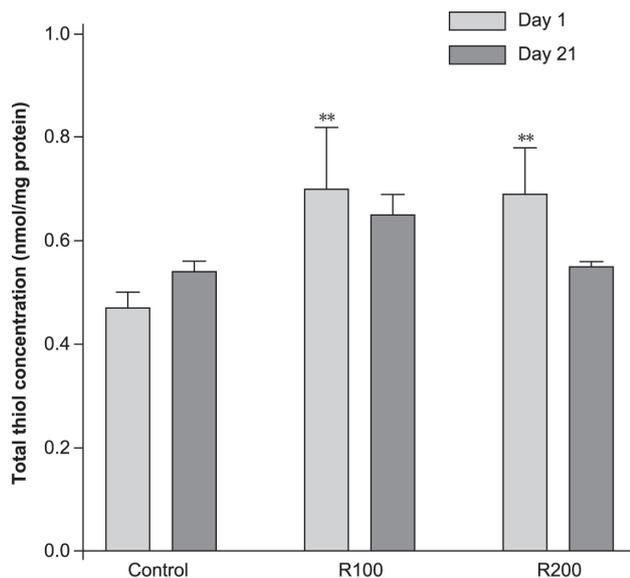


Figure 3 Effect of rutin on total thiol concentrations in the hippocampus on days 1 and 21 after kainic acid administration

Values are expressed as mean \pm standard error of mean. ** $P < 0.01$, compared to control group, $n=6$, analyzed by post-hoc Tukey's test.

that rutin, activated by the GABAergic system, could prevent neuronal hyperactivity in specific regions of the brain.

In our previous studies, rutin demonstrated potential anticonvulsant effects in pentylenetetrazol (PTZ)-induced

acute and chronic seizures in rats^[28,29]. It has also been reported that many flavonoids are ligands for GABA_A receptors in the central nervous system^[30,31]. Thus, the same GABAergic system may have contributed to the anticonvulsant effects of rutin in the present work.

Monitored in the hippocampus, MDA levels of both rutin groups were significantly lower than those of the control group on days 1 and 21 of the present study. It seems that the effect of rutin on day 1 was dose-dependent. However, this effect was not dose-dependent on day 21. In addition, the total content of thiol in both rutin groups on day 1 (but not day 21) was significantly higher than that of the control group. However, this effect was not dose-dependent. Elevation in lipid peroxidation in the hippocampus has been reported at an early time-point (e.g., 4 and 24 h) following the administration of KA^[32,33]. This initial increase of TBARS in the hippocampus has been shown to decrease 7 d after status epilepticus, and can return to the basal level, but not below it^[34]. Further, KA administration has been shown to elevate MDA and extracellular glutamate, while, decreasing glutathione (GSH) levels in the brain^[18,22,35]. GSH can act directly as an antioxidant^[36], and may modulate neurotransmission by reacting with neurotransmitters and their binding sites^[37]. Flavonoids are excellent at scavenging free radicals. Their antioxidant activity can be greater than those of vitamins C and E^[38], and has been attributed to several factors, including their physical structure. It is possible that rutin, by virtue of its antioxidant properties, is able to scavenge free radicals and attenuate oxidative stress. Furthermore, the protective effect of rutin on MDA level in the brain may have led to the less intense seizures observed in rutin-treated mice, relative to the control group.

In this study, we observed that rutin has anticonvulsant effects and attenuated oxidative stress in KA-induced seizure. Modulatory effects of rutin on GABA receptors complex and antioxidant activity are suggested to be two possible mechanisms for explaining these results. Further studies are needed to find other possible mechanisms.

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6 Competing interests

The authors declare that they have no competing interests.

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