INTRODUCTION

Epilepsy, a serious neurological disease, causes unprovoked, recurrent seizures [1]. Temporal lobe epilepsy (TLE) is the most common form of epilepsy and is characterized by neuronal cell loss and granule cell dispersion (GCD), a structural abnormality of the dentate gyrus (DG) present in approximately 50% of patients with TLE [2]. GCD involves the enlargement of the granule cell layer (GCL) and loss of a clear outer boundary between the molecular and granular layers in the DG [3-5]. Recent studies have also shown morphological changes in granule cells and mossy fiber sprouting in the DG. Additionally, GCD-associated activation of mTORC1 was observed in the hippocampus of TLE patients.

Morin Prevents Granule Cell Dispersion and Neurotoxicity via Suppression of mTORC1 in a Kainic Acid-induced Seizure Model

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An abnormal reorganization of the dentate gyrus and neurotoxic events are important phenotypes in the hippocampus of patients with temporal lobe epilepsy (TLE). The effects of morin, a bioflavonoid constituent of many herbs and fruits, on epileptic seizures have not yet been elucidated, though its beneficial effects, such as its anti-inflammatory and neuroprotective properties, are well-described in various neurodegenerative diseases. In the present study, we investigated whether treatment with morin hydrate (MH) can reduce the susceptibility to seizures, granule cell dispersion (GCD), mammalian target of rapamycin complex 1 (mTORC1) activity, and the increases in the levels of apoptotic molecules and inflammatory cytokines in the kainic acid (KA)-induced seizure mouse model. Our results showed that oral administration of MH could reduce susceptibility to seizures and lead to the inhibition of GCD and mTORC1 activity in the KA-treated hippocampus. Moreover, treatment with MH significantly reduced the increased levels of apoptotic signaling molecules and pro-inflammatory mediators in the KA-treated hippocampus compared with control mice, suggesting a neuroprotective role. Therefore, these results suggest that morin has a therapeutic potential against epilepsy through its abilities to inhibit GCD and neurotoxic events in the in vivo hippocampus.

Key words: Morin, Seizure, Granule cell dispersion, Kainic acid, Neuroprotection

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and animal models [6-9].

Neuronal death and injury are observed in various models of epilepsy and are considered a prerequisite to seizure-induced epilepsy [10]. Recently, many studies demonstrated that neurotoxic inflammation in the brain could increase the predisposition to seizures and be associated with alterations in seizure-induced neuropathology and neuronal excitability [11, 12]. In addition, apoptosis may be associated with seizures, resulting in the loss of hippocampal neurons [13, 14]. The administration of kainic acid (KA) is widely used as a model of TLE and induces behavioral symptoms and pathological changes similar to human TLE [7, 15-17].

There has been a growing interest in exploiting natural, pure compounds due to their affordability, minimal side effects and beneficial effects as alternatives to traditional medicine. Their neuroprotective effects against excitotoxicity may be of particular therapeutic value for brain diseases [18, 19]. Morin is a bioflavonoid isolated as a yellowish pigment from Maclura pomifera (Osage orange), M. tinctoria (old fustic), and leaves of Psidium guajava (common guava). Administration of morin has led to various pharmacological effects, including anti-inflammatory and anti-oxidative properties, as well as induction of neurotrophic factors [20-24]. Moreover, morin has neuroprotective effects against excitotoxicity [25] and Huntington's disease [26]. However, it remains largely unknown whether morin can treat the cytoarchitectural abnormalities associated with the progression of epilepsy and induce neuroprotective effects against KA-induced neurotoxicity in vivo. In the present study, we have examined the effects of morin hydrate (MH) on GCD in a KA-induced seizure model, as well as its protective effects against KA-induced neurotoxicity.

MATERIALS AND METHODS

Animals and the KA-induced seizure model

Male C57BL/6 mice (age: 8 weeks, body weight: 22–23 g) were purchased from Daehan Biolink (Eumseong, Korea) and maintained for 1 week before experiments. The mice were housed in a controlled environment and provided with food and water ad libitum. All procedures and experiments involving animals were performed in accordance with approved animal protocols and guidelines established by the Animal Care Committee of Kyungpook National University (No. KNU 2016-42). In the present study, mice were anesthetized with 115 mg/kg of ketamine (Yuhan, Korea) and 23 mg/kg of rompun (Bayer Korea Ltd., Korea).

Seizures were induced by a unilateral injection of KA in the hippocampus of mice brain as previously described with minor modifications [7]. Briefly, mice were positioned on a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) after induction of anesthesia. Each mouse received an unilateral injection of KA [0.2 μg in 2 μl phosphate-buffered saline (PBS); Sigma] using a 10 μl Hamilton syringe (30-S needle) attached to a syringe pump (KD Scientific, New Hope, PA, USA) into the hippocampus (AP: -2.0 mm; ML: -1.2 mm; DV: -1.5 mm, relative to the bregma). Mice injected with 2 μl of PBS alone were used as controls. After the injection, the needle was left in place for an additional 5 minutes to limit reflux along the injection track.

Drug administration

To investigate the effects of MH [2′,3,4′,5,7-pentahydroxy-flavone] (Fig. 1A) in preventing epileptic seizures, the drug was freshly prepared in suspension of 0.01 M PBS. Experiments were performed according to the scheme presented in Fig. 1B. Mice were administered orally with MH in various doses (20, 40, or 80 mg/10 ml/kg per day; Sigma) 1 hour and 1 day before the KA injection to measure seizure onset. To analyse the occurrence of spontaneous recurrent seizures (SRS) and the population of mossy fiber sprouting, MH was orally administered to the mice, one times daily from 2 days before the KA injection to 35 days. To study the effects of MH on properties such as the inhibition of GCD and neurotoxicity, the drug was initiated 1 day before the KA injection, then continued for 7 days. For experiments assessing the protein levels of mammalian target of rapamycin complex 1 (mTORC1), neuroinflammation and apoptosis, mice were treated with MH for 3 days and sacrificed 2 days after KA treatment.

Monitoring of KA-induced seizures

After KA treatment, mice were monitored for up to 3 hours in order to evaluate the time of seizure onset. The severity of seizures was classified into five stages according to a modified scale as previously described [8, 27, 28]. The stages were: stage 1, characterized by facial movements; stage 2, characterized by head nodding and myoclonic twitching; stage 3, characterized by forelimb clonus with lordotic posture; stage 4, characterized by forelimb clonus with reared posture; and stage 5, characterized by tonic-clonic seizures without postural control. In the present study, mice exhibiting seizures with at least stage 3 severity were considered positive for seizure onset. In addition, 3 weeks after KA treatment, the frequency of SRS was measured over a period of two weeks [34 h/week (9 h/day; 6 days/week)] using video recording, as previously described, with some modification [29].

Nissl staining and GCD measurement

Seven days after KA treatment, mice were sacrificed and subjected to transcardial perfusion with 4% paraformaldehyde in...
0.1 M PBS. The brains were obtained and post-fixed overnight at 4°C and then cut in 30-μm-thick coronal sections on a HM525 NX cryostat (Thermo Scientific, Germany). For Nissl staining, the sections were mounted on gelatin-coated slides and stained with 0.5% cresyl violet (Sigma). The presence of GCD was determined by measuring the average width of the GCL in the mid and medial quarter portions of the upper blade of the DG [29]. GCD was expressed as a percentage of the GCL width relative to the contra-lateral side.

**Timm’s staining**

Brain sections were mounted on gelatin-coated slides and subsequently treated as previously described [30-32], using Timm’s staining to reveal mossy fibers. Briefly, the slides were placed in the staining solution containing a 6:1:3 mixture of gum arabic (50%, w/v), 2 M citric acid-sodium citrate buffer, 0.5 M hydroquinone, and with 0.25 ml of a silver nitrate solution (17%, w/v). The developing process was monitored periodically over a period of 3 hours until the desired level of staining was attained. The sections were rinsed in water for 15 min, dehydrated with ethanol, and cleared in xylene before being cover slipped. To semi-qualitatively assess mossy fiber sprouting, sections at equivalent positions relative to bregma ipsilateral and contralateral to injury were examined and assigned Timm scores ranging from 0~3, with a score of 0 corresponding to little to no granular staining, 1 indicating moderate Timm staining through the granule cell layer, but not into the inner molecular layer, 2 indicating continuous staining through the granule cell layer with discontinuous puncta in the inner molecular layer, and 3 indicating a continuous band of staining in the inner molecular layer. The scorer was blinded to treatment [30, 31].

**Immunohistochemistry and immunofluorescence staining procedure**

Brain sections were prepared for immunohistochemistry and immunofluorescence staining as previously described [7]. Briefly, free-floating sections were washed with 0.1 M PBS and blocked with 0.5% bovine serum albumin in 0.1 M PBS. Primary antibody incubation was performed two overnight at 4°C. The following
primary antibodies were used: anti-neuronal nuclei (NeuN; 1:500; Millipore, Temecula, CA, USA) and anti-phospho-4E-BP1 [p-4E-BP1, a phosphorylated form of eukaryotic initiation factor 4E-binding protein 1 (4E-BP1); 1:1000; Cell Signaling, Beverly, MA, USA]. The sections were subsequently washed and incubated with biotin-conjugated secondary antibodies, followed by an avidin-biotin complex kit (Vector Laboratories, Burlingame, CA, USA). The signal was visualized using 3,3'-diaminobenzidine (0.5 mg/ml Sigma) with hydrogen peroxide (0.003%; Sigma).

The sections used for immunofluorescence were incubated two overnight with one pair of the following antibodies: anti-NeuN (1:500; Millipore), anti-cleaved caspase-3 (c-caspase-3; 1:400; Cell Signaling), anti-cleaved Poly [ADP-ribose] polymerase-1 (PARP-1) (c-PARP-1; 1:400; Cell Signaling), anti-ionized calcium-binding adapter molecule 1 (Iba1; 1:500; Wako Pure Chemical Industries, Japan), anti-Interleukin 1 beta (IL-1β; 1:500; Santa Cruz), antitumor necrosis factor alpha (TNF-α; 1:1000; Santa Cruz) and anti-inducible nitric oxide synthase (iNOS; 1:200; Abcam, Cambridge, MN, USA). The sections were then incubated with Texas Red-conjugated IgG (1:400; Vector Laboratories) and fluorescein isothiocyanate-conjugated IgG (1:200; Jackson ImmunoResearch, West Grove, PA, USA), and mounted with Vectashield mounting medium (Vector Laboratories). The stained sections were examined under a microscope (Axio Imager; Carl Zeiss, Gottingen, Germany).

**Counting of hippocampal CA1 neurons**

The number of hippocampal CA1 neurons was quantified as previously described with some modifications [33]. All quantitative analyses were performed in a blind manner. Alternate sections were prepared from the coronal brain slice of each animal at 1.7, 1.8, 2.0, and 2.1 mm posterior to bregma. To ensure consistency in tissue sampling, a rectangular box (1×0.05 mm) was centered over the CA1 cell layer beginning 1.0 mm lateral to the midline. Only neurons with visible nuclei were counted using a light microscope (Carl Zeiss) at a magnification of 200×. The mean number of CA1 neurons in the ipsilateral hippocampus was expressed as a percentage, compared with the contralateral control.

**Western blot analysis**

Hippocampus were dissected from brain tissue and used for western blotting, as described previously [7, 34]. Briefly, each tissue was homogenized in lysis buffer containing a protease inhibitor cocktail (Sigma) and cleared by centrifugation. The protein concentration was determined using a BCA assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Proteins were separated by gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories). The PVDF membranes were incubated overnight at 4°C with the following antibodies: anti-c-4E-BP1 (1:1000, Cell signaling), anti-p-4E-BP1 (1:1000, Cell signaling), anti-p70S6 kinase (p70S6K, 1:1000, Cell Signaling), anti-phospho-p70S6K (p-p70S6K, 1:1000, Cell signaling), anti-β-actin (1:4000, Santa Cruz), anti-caspase-3 (1:1000, Cell Signaling), anti-c-caspase-3 (1:1000, Cell Signaling), anti-PARP-1 (1:1000, Cell signaling), anti-c-PARP-1 (1:1000, Cell signaling), anti-iNOS (1:1000, Abcam). The membranes were subsequently incubated with secondary antibodies (GE Healthcare, Piscataway, NJ, USA), and blots were developed using enhanced chemiluminescence western blotting detection reagents (GE Healthcare). For semi-quantitative analyses, band densities were measured using a computer imaging device and accompanying software (Fuji Film, Tokyo, Japan).

**Statistical analysis**

All values are expressed as mean±standard error of the mean (SEM). The Shapiro-Wilk test was used to confirm normal distribution prior to statistical analyses. Differences between the two groups were analyzed with either the Student’s t-test or Mann-Whitney U test. Multiple comparisons among groups were performed using either the one-way analysis of variance (ANOVA) or Kruskal-Wallis test, followed by Tukey’s post hoc tests. All statistical analyses were performed using SigmaPlot 12.0 (Systat Software, San Leandro, CA, USA).

**RESULTS**

**Morin treatment delays the onset of KA-induced seizures and inhibits GCD and mTORC1 activation in the hippocampus**

KA-induced excitotoxicity is established to induce seizures; behavioral changes; and neuro-pathophysiological features, such as GCD, similar to those found in patients with TLE [2, 7]. The hyperactivation of mTORC1 was also observed in dispersed granule cells in a mouse model of TLE and in patients with a sclerotic hippocampus [6]. To investigate the effects of MH on KA-induced seizures, various doses of MH (20, 40, or 80 mg/10 ml/kg per day) were orally administrated 1 hour and 1 day before the KA injection. The effect of MH in delaying seizures was dose-dependent. 80 mg/kg of MH significantly delayed the onset time of seizures compared to KA alone within 3 hours after KA treatment (Fig. 1C; *p<0.01 vs. KA-treated mice). Furthermore, we investigated whether oral administration of MH could repress the occurrence of SRS after KA treatment. To measure SRS, mice received 80 mg/kg of MH 1 hour and 1 day before KA injection. This continued
for 5 weeks. The frequency of SRS tended to decrease in the MH-treated mice compared to mice which received KA alone (Fig. 1D, \( p=0.163 \) vs. KA-treated mice).

To evaluate whether the administration of MH could diminish KA-induced morphological changes in GCD and mossy fiber sprouting in the DG, we further measured GCD and mossy fiber sprouting using Nissl staining and Timm’s staining, respectively, after a latency period of 7 days. The results of Nissl staining revealed that a unilateral injection of KA into the hippocampus induced GCD in the DG, but not in the DG of single MH-treated mice (Fig. 2A). However, treatment with MH resulted in a dose-dependent reduction of GCD in KA-treated hippocampus (Fig. 2A and B). The alterations in GCD were quantified by comparing the experimental sides with the contralateral sides. KA treatment led to a significant increase in GCD by 143%, compared to controls (Fig. 2B; **\( p<0.01 \)). Notably, administration of 40 and 80 mg/kg of MH significantly inhibited the formation of GCD caused by KA treatment, compared with KA alone (Fig. 2B; ‘\( p<0.05 \) and ‘\( p<0.05 \), respectively, vs. KA alone). Furthermore, Timm-positive staining showed that the aberrant mossy fiber sprouting was significantly higher in the GCL and inner molecular layer, where mossy fibers project in the KA-treated mice than in controls (Fig. 2C and D; \( p<0.001 \)). In contrast, MH-treated mice for 5 weeks showed a decreasing tendency of mossy fiber sprouting in the DG of KA-treated hippocampus (Fig. 2C and D; \( p=0.088 \)).

Several previous reports indicated that KA-induced status epilepticus results in increases in mTOR activity in adult animals [35-39]. The use of mTOR inhibitors, such as rapamycin, were also suggested as a rational and efficacious strategy for preventing epilepsy [9]. To examine whether MH treatment can prevent KA-induced activation of mTORC1 in the DG, we measured the levels of p-4E-BP1, an mTORC1 substrate, in the DG of mice brains at early time points. Mice were pretreated with 80 mg/kg of MH 1 hour and 1 day prior to KA treatment. Subsequently, one group of mice was sacrificed 30 min and 90 min after KA injection. The activation of mTORC1 was assessed by measuring phosphoryla-

![Fig. 2. Morin diminishes GCD and mossy fiber sprouting in the KA-treated DG. (A, B) Morin (20, 40, and 80 mg/kg per day) was orally administered to the mice 1 day before the KA injection, and then administered for 7 more days. (A) The representative sections of the ipsilateral DG following Nissl staining by cresyl violet. CON, contralateral side. Scale bar, 200 μm. (B) The quantification of GCD normalized to the contralateral side for each sample. **\( p<0.01 \) vs. CON, ‘\( p<0.05 \) vs. KA alone (one-way ANOVA and Tukey’s post-hoc analysis; \( n=4 \), each experimental group). (C, D) Morin (80 mg/kg per day) was orally administered to the mice 1 day before the KA injection, and then administered for 35 more days. (C) The representative sections of the ipsilateral DG following Timm’s staining. (D) The semi-quantification of Timm’s staining results. Data was presented by mean Timm scores for each sample. ***\( p<0.001 \) vs. CON (one-way ANOVA and Tukey’s post-hoc analysis; \( n=4 \), each experimental group).](https://doi.org/10.5607/en.2018.27.3.226)
tion of p70S6K in the DG using western blot analysis (Fig. 3A). Our results showed that phosphorylation of p70S6K significantly increased in the DG at early time points (both 30 min and 90 min) after KA injection compared to controls (Fig. 3B, **p<0.01, ***p<0.001 vs. CON, respectively), and MH-treated mice showed a decreasing trend in the levels of p-p70S6K at 30 min after KA-treated hippocampus (Fig. 3B; p=0.144). Moreover, after 90 min KA-treatment, we found a significant reduction of p-p70S6K in MH-treated mice compared to KA alone-treated mice (Fig. 3B; ’p<0.05 vs. KA alone). In addition, to confirm whether continuous administration of MH can inhibit the activation of mTORC1 following KA-treatment, mice received MH for 2 more days after KA-treatment. Our immunohistochemical results showed that KA induced phosphorylation of 4E-BP1 in the DG, and MH-treatment effectively inhibited the p-4E-BP1 in the KA-treated hippocampus (Fig. 3C). Similar to these results, KA treatment induced a

![Figure 3](https://doi.org/10.5607/en.2018.27.3.226)
significant increase in the levels of p-4E-BP1 and p-p70S6K 2 days after seizure induction (Fig. 3D, E; ***p<0.001 vs. CON and 80 mg/kg MH-treated mice, respectively). However, treatment with MH following KA administration led to a significant reduction in the levels of p-4E-BP1 and p-p70S6K compared to KA alone (Fig. 3D, E; #p<0.05).

Morin treatment prevents the loss of hippocampal neurons in the KA-induced seizure model

To investigate the protective effect of morin against KA-induced neurotoxicity in the hippocampus, 80 mg/kg of MH was orally administered 1 day before the KA injection, then continued for 7 days. As shown in Fig. 4A, an intra-hippocampal injection of KA resulted in considerable neuronal loss in the hippocampal CA1 regions as assessed by immunohistochemical staining for NeuN. Administration of 80 mg/kg of MH reduced neuronal cell loss in the KA-treated hippocampus. When quantified and expressed as a percentage of preserved neurons in the area of interest of the ipsilateral compared with the contralateral CA1 controls, KA treatment significantly reduced the number of hippocampal neurons by 64% (Fig. 4B; ***p<0.001 vs. CON). However, treatment with MH (80 mg/kg) significantly protected the hippocampal neurons against KA-induced neurotoxicity (Fig. 4B; #p<0.01 vs. KA-treated mice).

Morin inhibits KA-induced apoptotic molecules

Extensive neuronal cell loss in the epileptic brain could be caused by excitotoxicity involved in apoptosis-mediated signaling pathways [40]. To examine whether morin treatment inhibits KA-induced apoptosis, the levels of c-caspase-3 and c-PARP-1, pro-apoptotic molecules, were measured by double-immunofluorescence staining and western blotting. As shown in Fig. 5A and B, double-immunofluorescence staining demonstrated that KA treatment significantly enhanced the expression of c-caspase-3 and c-PARP-1 in the hippocampal CA1 region, compared with controls, while the upregulation was diminished in mice treated with MH. Consistent with the immunofluorescence results, western blot analysis demonstrated that the increased levels of c-caspase-3 and c-PARP-1 in the KA-treated hippocampus (Fig. 5C and D; **p<0.01 and *p<0.05, respectively, vs. control mice) were significantly attenuated by treatment with MH, compared with KA alone (Fig. 5C and D; #p<0.05, respectively, vs. KA-treated mice).

Morin leads to downregulation of pro-inflammatory mediators in the hippocampus of KA-treated mice

The inflammatory events in a KA-induced rodent epilepsy model and in the hippocampus of patients with TLE are important to understand as they may relate to the pathological mechanisms involved in seizure-related neuronal cell death [7, 41]. Thus, we ex-

Fig. 4. Morin prevents the loss of hippocampal neurons in a KA-induced seizure model. Mice were treated with a unilateral intra-hippocampal injection of KA and killed 7 days later. Morin (80 mg/kg) was administered orally 1 day prior to the KA injection, then continued once daily for 7 days. (A) Representative coronal sections of the hippocampus following NeuN immunostaining. Scale bar, 500 μm and 50 μm. (B) The histogram quantitatively shows the percentage of NeuN-positive neurons in the counting area of the hippocampal CA1 region compared with contralateral controls. ***p<0.001 vs. CON, **p<0.01 vs. CON, and #p<0.01 vs. KA alone (one-way ANOVA and Tukey’s post-hoc analysis; n=4, each experimental group). All values are expressed as the mean±SEM.
examined whether morin treatment effects the expression of inflammatory mediators, such as TNF-α, IL-1β and iNOS, which may be produced by activated microglia [7], in the hippocampus of KA-treated mice. As shown in Fig. 6A, B, and C, immunofluorescence staining demonstrated that KA treatment resulted in microglial activation and an increase in TNF-α, IL-1β, and iNOS, in the hippocampus, while these increases in inflammatory mediators were inhibited by MH treatment. Consistent with these results, western blots also showed that MH treatment inhibited the increase in expression of TNF-α, IL-1β, and iNOS following the KA injection (Fig. 6D and E; *p<0.05 and **p<0.01, respectively, vs. KA alone). Thus, these results suggest that morin protects hippocampal neurons by inhibiting the production of apoptotic signaling molecules and pro-inflammatory mediators.

DISCUSSION

Despite many new anti-epileptic drugs developed over the last two decades, anti-convulsant therapies are still limited and unable to control seizures in all patients [42]. To overcome these limitations, many studies have focused on the neuroprotective actions of flavonoids against brain injury induced by neurotoxins. The beneficial neuroprotective effects are thought to include the ability to suppress neuroinflammation and excitotoxicity with minimal toxic side effects compared with synthetic compounds [19, 43]. Moreover, recent studies have reported anti-epileptic effects of some flavonoids in the KA-induced models of seizures [7, 15, 44], suggesting various natural flavonoids as alternative medicines for patients with epileptic seizures.

The formation of GCD, an abnormal widening of the GCL in the
DG, was found in the surgical hippocampal specimens of patients with TLE [45]. The development of GCD in the hippocampus was influenced by abnormal activation of mTORC1 in rodent models of TLE.

Notably, inhibition of mTORC1 with rapamycin treatment markedly reduced the KA-induced GCD in the hippocampus [5, 7, 46], suggesting that activation of mTORC1 is an important risk factor in the progression of epilepsy.

Morin, a bioflavonoid constituent of many herbs and fruits, has been reported to prevent neurodegeneration and motor disability by reducing oxidative stress in a rat model of Huntington’s disease [26]. Moreover, its beneficial effects, such as its anti-excitotoxicity, anti-apoptotic, and anti-inflammatory properties and its induction of neurotrophic factors were well described in previous studies [20-26, 47]. However, it was largely unknown whether morin treatment affects the cytoarchitectural abnormalities associated with the progression of epilepsy and have neuroprotective effects against KA-induced neurotoxicity in vivo. In the present study, our results showed that MH treatment could delay the onset of KA-induced seizures (Fig. 1C) and reduce the frequency of SRS.
Against KA-induced epileptic seizures in vivo. Our results demonstrated that morin is a useful natural compound to prevent GCD via the inhibition of mTORC1, resulting in anti-seizure activities.

Seizures induced by chemical convulsants, such as KA, can cause hippocampal neuronal death [48]. This neuronal death during chronic epilepsy may be due to apoptosis triggered by the activation of several signal transduction factors including caspase-3, PARP-1, Bcl-2, and Bax [49, 50]. This evidence suggests that controlling apoptosis may be important in protecting hippocampal neurons following epileptic seizures. Our results showed that MH treatment protected the hippocampal CA1 neuronal fibers and cell bodies against KA-induced excitotoxicity 2 days and 7 days post-lesion (Fig. 4). Moreover, morin treatment prevented the cleavage of caspase-3 and PARP-1 following the KA injection (Fig. 5). Thus, these results suggested that MH treatment attenuated the apoptotic signal induced by KA treatment at early time points, resulting in protection from neuronal fiber degeneration. MH treatment also protected the hippocampal CA1 neurons against KA-induced neurotoxicity.

In addition to the increases in apoptotic signaling molecules, the increased levels of inflammatory mediators, including IL-1β, TNF-α, cyclooxygenase-2, and iNOS, may be involved in the neurotoxic events in the brain following epileptic seizures. The control of these inflammatory pathways may be one of the many potential strategies for the prevention and treatment of seizures [7, 51-53]. In the present study, our results further demonstrated that morin treatment could inhibit microglial activation in the KA-treated hippocampus, resulting in a decrease in the levels of IL-1β, TNF-α, and iNOS (Fig. 6). These results suggest that the anti-apoptotic and anti-inflammatory effects of morin are associated with the protection of hippocampal neurons from KA-induced neurotoxicity.

In conclusion, we evaluated the beneficial effects of morin against KA-induced epileptic seizures in vivo. Our results demonstrate that morin induces anti-seizure effects through the inhibition of GCD, SRS, and mossy fiber sprouting via the suppression of mTORC1 activation. Moreover, we found that the protective properties of morin against KA-induced excitotoxicity may be mediated by the inhibition of microglial activation and apoptotic signaling molecules in the hippocampus. Our findings suggest that morin is an effective natural compound for the prevention and treatment of epileptic seizure.

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