Naringin Protects against Kainic Acid-Induced Status Epilepticus in Rats: Evidence for an Antioxidant, Anti-inflammatory and Neuroprotective Intervention

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The effect of naringin, a bioflavanoid, with potent antioxidant activity was studied on kainic acid (KA)-induced seizures, cognitive deficit and oxidative stress. Rats were administered KA (10 mg/kg intraperitoneally (i.p.)) and observed for behavioral changes and incidence and latency of convulsions over 4 h. The rats were thereafter sacrificed and oxidative stress parameters like malondialdehyde (MDA) and glutathione (GSH) were estimated in the brain. The level of proinflammatory cytokine, tumor necrosis factor (TNF)-α was also determined in the rat brain. It was observed that pretreatment with naringin (20, 40, 80 mg/kg, i.p.) significantly ($p<0.001$) increased the latency of seizures as compared to the vehicle treated-KA group. Naringin (40, 80 mg/kg) also significantly prevented the increase in MDA and fall in GSH levels due to KA. In addition, naringin dose-dependently attenuated the KA-induced increase in the TNF-α levels of brain. The pretreatment with naringin also significantly increased retention latency in the passive avoidance task. This shows that naringin reduced the cognitive deficit induced by KA. The results of our study suggest that naringin has therapeutic potential since it suppresses KA-induced seizures, cognitive impairment and oxidative stress in the brain. These neuroprotective effects are a result of its antioxidant and anti-inflammatory activity.

Key words kainic acid; naringin; oxidative stress; cognitive deficit

Epilepsy is one of the most common neurological disorder. In adults with temporal lobe epilepsy (TLE), complex partial seizures have the poorest prognosis of all types of seizures, with about 60—70% of all patients having intractable seizures.[1)] In addition, memory problems are more common in human TLE.[2)] The underlying mechanism is however not clear. Kainic acid (KA) is a structural analog of glutamate, an excitatory amino acid neurotransmitter.[3)] A single systemic injection of a convulsive dose of KA results in limbic status epilepticus, which is followed by long-term spontaneous recurrent seizures (SRS), as well as spatial learning impairments.[4)] KA-induced epilepsy model has been used widely to study human TLE.[5)] It has been proposed that activation of excitatory amino acid receptor can trigger the formation of reactive oxygen species (ROS).[6)] The increased ROS in turn may further release more glutamate and thereby forming a loop. This 'vicious' cycle not only causes a long lasting seizure formation, but if not arrested may also lead to the neuronal death.[7,8)] Status epilepticus (SE) is an emergency condition where seizures last for a long time and if not controlled neuronal injury occurs.[9,10)] Kainic acid induced SE following systemic or intracerebroventricular injection of the drug initiates neuronal injury and death in different parts of the limbic systems.[11)] The mechanism involved in the pathogenesis appears to be linked to oxidative stress.[12)] In various experimental studies, it has been demonstrated that antioxidants can prevent the excitotoxicity induced by agents like glutamate and kainic acid.[13—15)] Thus, antioxidants may have a potential role in preventing excitotoxicity induced seizure genesis. In this respect scavenging of free radicals by non-enzymatic/exogenous antioxidants seems to be the most practical approach. The anticonvulsant effect of several agents having antioxidant property such as curcumin, vineatrol, *trans*-resveratrol, melatonin, adenosine and alpha lipoic acid has been demonstrated in our laboratory.[6,16)]

Recent findings in experimental models and in the clinical setting suggest the possible role of inflammatory processes in the etiopathogenesis of seizures.[17)] Proinflammatory cytokines such as interleukin (IL)-1 beta, tumor necrosis factor (TNF)-α and IL-6 have been shown to be overexpressed in experimental models of seizures in brain areas of seizure generation and propagation.[18)] Therefore, possible strategies that involve the pharmacological manipulation of inflammation can lead to the development of novel approaches for the treatment of epilepsy.

Naringin is a flavanoid present in grapefruit and other citrus fruits. Naringin is hydrolyzed to a major metabolite, naringenin (4′5,7-trihydroxyflavanone) which readily crosses the blood brain barrier.[19,20)] It has been reported to possess antioxidant, antihypertensive, hypocholesterolemic, hepatoprotective and neuroprotective effects.[21—24)] Naringin has potent antioxidant activity which has been observed in various *in-vitro* and *in-vivo* animal models.[25,26)] Like most flavonoids, naringin also has metal chelating, antioxidant and free radical scavenging properties[27—29)] and offers some protection against mutagenesis and lipid peroxidation.[30)] The antioxidant effects of naringin have been shown to be similar to glutathione (GSH) and furthermore, it is reported to inhibit hydrogen peroxide-induced lipid peroxidation.[31)] Recent studies have shown that naringin plays an important role in regulating antioxidative capacity by increasing superoxide dismutase (SOD) and catalase (CAT) activities and by up regulating the gene expression of SOD, CAT, and GSH-Px.[32)]

Since, naringin has very high antioxidant activity and the primary underlying pathology of status epilepticus is attributable to increased oxidative stress, it was considered worth-

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while to evaluate the anticonvulsant effect of naringin in the KA-model of epilepsy. Thus, the aim of the present study was, to investigate the effect of naringin in kainic-acid induced status epilepticus, oxidative stress and cognitive impairment in rats. Furthermore, we have also evaluated the effect of naringin on TNF-α, a proinflammatory cytokine.

MATERIALS AND METHODS

Animals Study was carried out using male Wistar rats weighing 150—200 g. They were obtained from the central animal house facility of Dr. B. R. Ambedkar Center for Biomedical Research, 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 *ad libitum*. 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.

Drugs and Chemicals Naringin, kainic acid, diazepam, radio-immunoprecipitation assay (RIPA) lysis buffer, and dimethylsulfoxide (DMSO) were purchased from Sigma Chemical Co. (Sigma, St. Louis, MO, U.S.A.). The protease inhibitors 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.

Kainic Acid Induced Status Epilepticus Rats were administered kainic acid (KA) at a dose 10 mg/kg intraperitoneally (i.p.). The pH of kainic acid solution was adjusted to 7.2±0.1. Following administration of KA rats were observed for behavioral changes (grooming, rearing, hind limb scratching, urination, defecation, wet dog shakes, jaw movements, salivation, head nodding), incidence and latency of convulsions and mortality over a total period of 4 h.[16)]

Experimental Design Rats were divided into seven groups and each group consisted of a minimum of six animals. Separate animals were used for each experiment.

Group I: The control group. These rats were injected with vehicle (saline 10 ml/kg, i.p.) for 7 d. This group was used in biochemical and behavioral studies.

Group II: The naringin *per se* group. Naringin (80 mg/kg, i.p.) was administered for 7 d. This group was used in biochemical studies.

Group III: The vehicle treated-KA group. The vehicle (saline 10 ml/kg, i.p.) was administered for seven consecutive days. Kainic acid (10 mg/kg, i.p.) was administered on the seventh day 30 min after the administration of vehicle and animals were observed over a period of 4 h for any change in behavioral parameters, incidence and latency of convulsions.

Group IV: The positive control group. Diazepam (5 mg/kg, i.p.) was administered on the seventh day followed by Kainic acid (10 mg/kg, i.p.) 30 min after the administration of diazepam. The rats thereafter were observed over a period of 4 h for any change in behavioral parameters, incidence and latency of convulsions.

Group V, VI and VII: Naringin (20, 40, 80 mg/kg respectively per group) was administered i.p. for 7 d. After 30 min of administration of last dose of naringin on the seventh day, KA (10 mg/kg, i.p.) was administered and rats were observed over a period of 4 h for any change in behavioral parameters, incidence and latency of convulsions.

In groups III—VII, the effect of KA on learning and memory was tested using one trial passive avoidance test. The initial latency (IL) was recorded 24 h after KA-administration and the retention latency (RL) was noted after 48 h *i.e.* on the ninth day. The rats were thereafter sacrificed for estimation of markers of oxidative stress (malondialdehyde (MDA) and glutathione) and determination of brain levels of TNF-α.

Behavioral Tests. Single-Trial Passive Avoidance Test Memory retention deficit was evaluated by step through passive avoidance apparatus. The apparatus consist 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 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 KA, 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 the 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 was moistened with water before delivering the foot shock as this is known to reduce the wide inter animal variability in paw skin resistance of the rats.[33)]

Biochemical Tests. 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 rat brain were used to determine lipid peroxidation and glutathione.

Measurement of Lipid Peroxidation MDA, a measure of lipid peroxidation, was measured as described by Ohkawa *et al.*[34)]

Measurement of Reduced Glutathione Glutathione was measured according to the method of Ellman.[35)]

TNF-α Assay in Rat Brain by Enzyme Linked Immunosorbent Assay (ELISA) The brain was homogenized in 1 ml of ice-cold lysis buffer (radio-immunoprecipitation assay, RIPA) containing 50 mM Tris–HCl (pH 8.0), 150 mM sodium chloride, 1.0% Iepal CA-630 (NP-40), 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1% phosphatase inhibitor cocktail and a protease inhibitor cocktail. The lysate was centrifuged (15000×g 4 °C) for 15 min, and the supernatant was added to 96-well ELISA plates. The TNF-α concentration was then determined by reading the ELISA plate.

Statistical Analysis of Data Data are expressed as mean±S.E.M. Statistical differences between the treatment and the control groups were evaluated by one-way ANOVA followed by Tukey–Kramer post test. The value with $p<0.05$ was considered to be significant.

RESULTS

Effect of Kainic Acid on Behavioral Symptoms and Convulsions All the rats in the vehicle treated-KA group exhibited behavioral signs like grooming, rearing, hind limb scratching, urination, defecation, wet dog shakes, jaw movements, salivation, and head nodding within 5 min after kainic acid administration (10 mg/kg, i.p.). Also, all the rats (100%) in this group exhibited convulsions with the mean latency of 36.24±3.19 min.

Effect of Naringin on Kainic Acid-Induced Status Epilepticus In the naringin+KA groups, the rats were pretreated with naringin 20, 40 or 80 mg/kg, i.p. for 7 d prior to KA (10 mg/kg, i.p.) administration. All the animals in the above groups exhibited behavioral signs as well as seizures following injection of KA However, in the naringin 40 and 80 mg/kg pretreatment groups, the latency of occurrence of behavioral signs significantly ($p<0.001$) increased to 22.12±1.80 min and 32.94±1.97 min respectively as compared to 4.48±0.33 min in the vehicle treated-KA group. In addition, the latency of convulsions increased significantly to 47.44±3.29 min ($p<0.05$), 73±2.44 min ($p<0.001$) and 98.48±2.15 min ($p<0.001$) respectively with naringin pretreatment at 20, 40 and 80 mg/kg doses respectively as compared to 36.24±3.19 min in the vehicle treated-KA group (Fig. 1). The rats pretreated with diazepam did not show any behavioral signs and convulsions.

Effect of Naringin on the Levels of MDA in Kainic Acid Induced Status Epilepticus in Rats The rat brain MDA level in the control group was 168.58±6.30 nmol/g wet tissue. This significantly ($p<0.001$) increased to 358.69±7.37 nmol/g wet tissue in the KA treatment group. The brain MDA levels of rats in the naringin+KA groups were 324.08±4.68, 246.43±5.26 and 217.44±5.78 nmol/g wet tissue in naringin 20, 40 and 80 mg/kg, i.p. pretreatment groups respectively. Thus, pretreatment with naringin dose-dependently and significantly led to a decrease in the levels of MDA as compared to the vehicle treated kainic acid group (Fig. 2). We had also determined the MDA levels in the naringin (80 mg/kg, i.p.) *per se* group. It was observed that 7 d pretreatment had no significant effect upon the MDA levels.

Effect of Naringin on the Levels of GSH in Kainic Acid Induced Status Epilepticus in Rats The rat brain glutathione level in the control group was 102.91±5.08 μg/g wet tissue. KA administration produced a significant ($p<0.001$) decrease (46.75±3.92 μg/g wet tissue) in the glutathione level as compared to the control group. The brain glutathione levels of rats in the naringin+KA group were 54.64±3.81, 71.88±3.72 and 90.17±3.93 μg/g wet tissue in naringin 20, 40 and 80 mg/kg, i.p. pretreatment groups respectively. The glutathione levels were significantly higher in the naringin 40 and 80 mg/kg, i.p. pretreatment groups as compared to the vehicle treated-KA group (Fig. 3). In the naringin (80 mg/kg, i.p.) *per se* group, it was observed that 7 d pretreatment had no significant effect upon the GSH levels.

Effect of Naringin on the Levels of TNF-α in Kainic Acid Induced Status Epilepticus in Rats The brain level of TNF-α was significantly raised after KA-administration (794.16±25.98 pg/ml) as compared to the control group rats

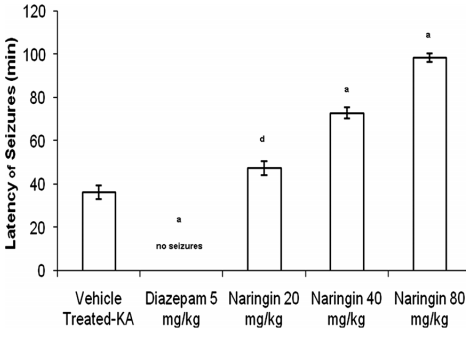

Fig. 1. Effect of 7 d Pretreatment with Naringin on Kainic Acid Induced Seizures in Rats

Each value represents the mean±S.E.M. for 6 rats. [a]$p<0.001$, [d]$p<0.05$ compared to vehicle treated-KA (ANOVA followed by Tukey–Kramer post test). 'KA' represents 'kainic acid.'

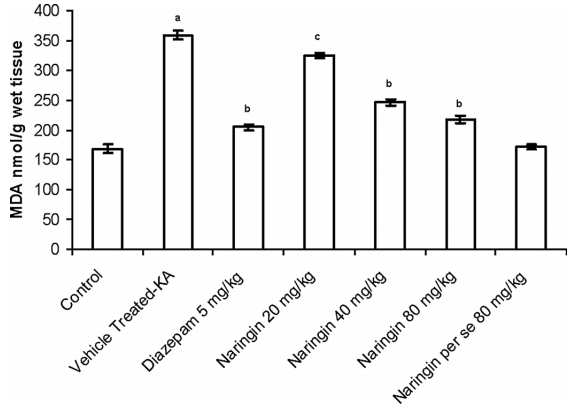

Fig. 2. Effect of 7 d Pretreatment with Naringin on Levels of MDA in Kainic Acid Induced Seizures in Rats

Each value represents the mean±S.E.M. for 6 rats. [a]$p<0.001$ compared to control, [b]$p<0.001$, [c]$p<0.01$ compared to vehicle treated-KA (ANOVA followed by Tukey–Kramer post test). 'KA' represents 'kainic acid.'

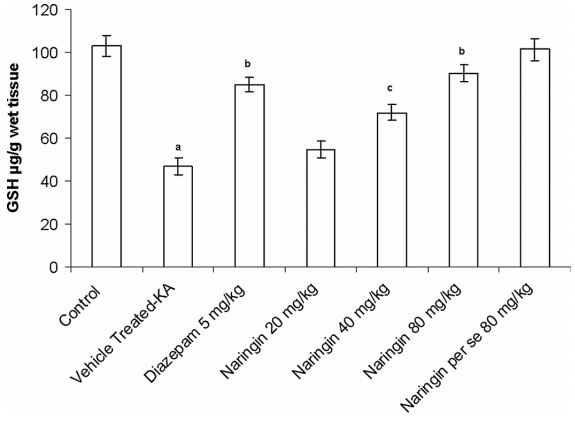

Fig. 3. Effect of 7 d Pretreatment with Naringin on Levels of GSH in Kainic Acid Induced Seizures in Rats

Each value represents the mean±S.E.M. for 6 rats. [a]$p<0.001$ compared to control, [b]$p<0.001$, [c]$p<0.01$ compared to vehicle treated-KA (ANOVA followed by Tukey–Kramer post test). 'KA' represents 'kainic acid.'

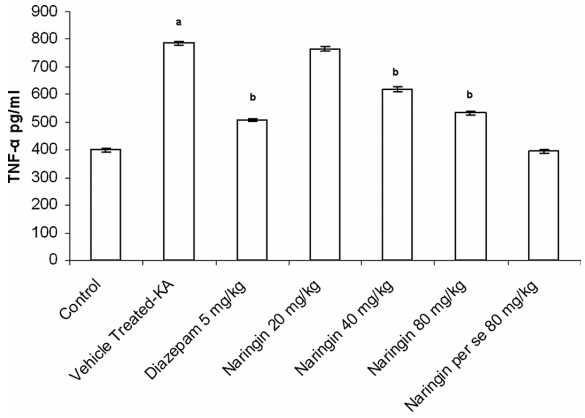

Fig. 4. Effect of 7 d Pretreatment with Naringin on Levels of TNF-α in Kainic Acid Induced Seizures in Rats

Each value represents the mean±S.E.M. for 6 rats. [a]$p<0.001$ compared to control, [b]$p<0.001$, [c]$p<0.01$ compared to vehicle treated-KA (ANOVA followed by Tukey–Kramer post test). 'KA' represents 'kainic acid.'

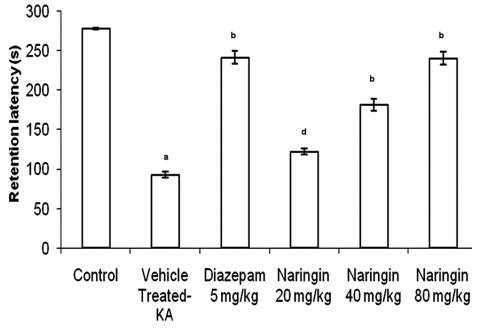

Fig. 5. Effect of 7 d Pretreatment with Naringin on Kainic Acid Induced Cognitive Impairment in Rats

Each value represents the mean±S.E.M. for 6 rats. [a]$p<0.001$ compared to control, [b]$p<0.001$, [c]$p<0.01$, [d]$p<0.05$ compared to vehicle treated-KA. (ANOVA followed by Tukey–Kramer post test). 'KA' represents 'kainic acid.'

(476.29±21.53 pg/ml) ($p<0.001$). In the naringin+KA groups, naringin pretreatment dose dependently attenuated the KA induced rise in brain levels of TNF-α (Fig. 4). The maximum inhibition was observed with naringin at the dose of 80 mg/kg in the naringin+KA group ($p<0.001$). Further, in the naringin (80 mg/kg, i.p.) *per se* group, the 7 d pretreatment with naringin had no significant effect upon the brain TNF-α level.

Effect of Naringin on Cognitive Impairment Induced by Kainic Acid Induced Status Epilepticus in Rats The retention latency 48 h after the administration of kainic acid in the vehicle-treated KA group was significantly ($p<0.001$) less (92.70±3.94 s) as compared with the control group rats (277.95±0.91 s). This indicates significant cognitive impairment due to KA. The mean retention latencies in the naringin (20, 40, 80 mg/kg, i.p.)+KA groups were 122.12±3.45, 181.48±7.84 and 240.36±8.07 s respectively. Naringin pretreatment produced a dose-dependent and significant reversal of KA-induced cognitive deficit (Fig. 5).

DISCUSSION

Epilepsy is the commonest neurological disorder encountered in clinical practice. Approximately 2 million persons in the United States have epilepsy, and 3% of persons in the general population will have epilepsy at some point in their lives.[36] In recent years, important advances have been made in the diagnosis and treatment of seizure disorders. The exact mechanism for the genesis of seizures is not clear. However, it has been proposed that there may be over excitation of excitatory amino acid receptor and or inhibition of GABAergic system.[37,38] It has been well demonstrated that excessive activation of excitatory amino acid receptors can cause prolonged seizures.[39] The free radicals are generated as a result of over excitation of excitatory amino acid receptors and thus they play a critical role in seizure genesis.[40] Thus, intervention by antioxidants can be a potential beneficial approach in the treatment of epilepsy.

In the present study, KA produced behavioral changes as well as convulsions in all the animals. Naringin pretreatment dose dependently increased the latency of onset of seizures as compared to the vehicle treated-KA group. Moreover, naringin (40, 80 mg/kg, i.p.) pretreatment prevented KA induced oxidative stress. In the KA *per se* group, there was a significant increase in the levels of MDA and a significant decrease in the levels of GSH suggesting thereby production of oxidative stress. It was observed that naringin (80 mg/kg i.p.) pretreatment *per se* had no significant effect upon the MDA and GSH levels. MDA is an end product of lipid peroxidation, a measure of free radical generation. The significantly less increase in MDA levels in the groups treated with naringin as compared to the vehicle treated KA group indicates attenuation of lipid peroxidation. Also, there was a simultaneous significant increase in the glutathione levels in the naringin (40, 80 mg/kg, i.p.) group as compared to vehicle treated KA group. Glutathione is the most abundant intracellular thiol and low molecular weight tripeptide found in living cells.[41] It reacts with the free radicals and can protect cells from singlet oxygen, hydroxyl radical and superoxide radical damage. The attenuation of KA-induced reduction in glutathione levels by naringin indicates that naringin enhanced the endogenous defensive capacity of the brain to combat oxidative stress induced by KA. A number of experimental studies have reported the potent antioxidant property of naringin.[22,42,43] Naringin has been reported to prevent alterations in mitochondrial lipid peroxides and mitochondrial dysfunction following isoproterenol induced myocardial infarction in rats.[25,44] This suggests that naringin helps to combat oxidative stress at the level of the mitochondria. It is our contention that the animals will exhibit seizures when a threshold value of excitation is reached after administration of kainic acid. The kainic acid administration stimulates glutamate receptors, which enhances ROS level, which in turn will enhance glutaminergic activity.[45] Naringin has been reported to modulate glutamatergic activity *in vitro*.[46] It is difficult to predict at what level naringin acts. However, it is evident that it breaks the vicious chain, thus decreasing the excitotoxicity and thereby showing the protective effect. Hence, a dual effect of naringin acting both as an antioxidant and an anti-excitotoxic agent cannot be ruled out.

Inflammation is known to participate in the mediation of a growing number of acute and chronic neurological disorders. However, the role of inflammatory responses in the pathogenesis of epilepsy and seizure-induced brain damage has been appreciated only recently. Inflammatory processes, in-

cluding activation of microglia and astrocytes and production of proinflammatory cytokines like TNF-α, IL-1β, IL-6, and related molecules have been described in human epilepsy patients as well as in experimental models of epilepsy.[17,47] It is well known that the microglial cells are the resident macrophages of the brain and spinal cord. They are primarily responsible for providing the active immune defence in the central nervous system (CNS). Flavanoids, like luteolin and resveratrol, are polyphenols present in plants. Luteolin is rich in leafy vegetables like parsely and celery and resveratrol is present in the skin of red grapes. These flavanoids have been shown to affect the function of microglia. Luteolin has been reported to enhance spatial working memory by reducing microglia associated inflammation in the hippocampus.[48] In another study, resveratrol has been reported to exert neuroprotection against lipopolysaccharide (LPS) induced dopaminergic neurodegeneration via attenuation of the activation of mitogen activated protein kinase and nuclear factor kappa B signaling pathways in microglia.[49] Thus, it is possible that naringin may also affect the activity of the microglial cells. TNF-α, interleukin-1 (IL-1) beta and IL-1ra are highly inducible under different forms of stress, such as excitatory neurotransmitter excess occurring during seizures, in infection and inflammation and during neurotrauma.[50] For these reasons, KA has been adapted for studies on various neurological disorders involving excitotoxicity and inflammation, such as epilepsy, Huntington's chorea and Alzheimer's disease.[51] In the present study we have observed that KA causes induction of brain levels of TNF-α. The naringin (80 mg/kg i.p.) *per se* group had no effect upon the TNF-α. In the naringin+kainic acid groups, naringin (40, 80 mg/kg, i.p.) significantly attenuated the KA-induced rise in the brain level of TNF-α ($p<0.001$). In earlier studies also, naringin has been reported to attenuate TNF-α levels.[52] It has been reported to inhibit expression of inducible nitric oxide synthase (iNOS) and suppress apoptosis *via* its anti-inflammatory activity.[53] It has also shown inhibitory effects on LPS-induced iNOS expression and nitric oxide production in macrophages and has also been reported as the most potent flavanoid which suppresses the TNF-α secretion. The latter effect might explain, at least partly, its favourable activity in diseases involving inflammation.[54,55] In another *in vitro* study, naringin has been reported to suppress LPS-induced iNOS, TNF-α, inducible cyclooxygenase (COX)-2 and IL-6. These effects were mediated *via* inhibiton of the activation of NF-kappa B.[56]

COX-2 is the inducible isoform expressed at the injury/inflammation sites and constitutively at the central nervous system, and plays a role in neurodegenerative diseases associated with increased excitatory activity.[57] The excitatory neurotransmitter glutamate by activating *N*-methyl-D-aspartate (NMDA) receptor leads to increased expression of COX-2 resulting in increased synthesis of prostaglandins.[58] COX-2 over-expression has been associated with neurotoxiticy in acute conditions, such as hypoxia/ischemia and seizures.[59] Moreover, recently in our laboratory we found that naringin possesses potent anti-inflammatory activity in both acute and chronic model of inflammation (unpublished observations). This finding is supported by the study of Vanamala *et al.* and other workers in which they have reported that naringin reduced the iNOS and COX-2 expression levels by inhibiting NF-kappa B activation.[53,60] In light of the above mentioned observations, it may be reasonable to assume that the potent antioxidant and anti-inflammatory effects of naringin contributes to its anti-convulsant activity.

In the present study, it was observed that the administration of kainic acid resulted in seizures that were associated with cognitive impairment as evidenced by reduction of retention latency in passive avoidance behavior. These results are in conformity with findings of other workers who also demonstrated cognitive impairment after administration of chemoconvulsants.[61—63] The administration of naringin for seven consecutive days dose dependently prevented cognitive deficit associated with kainic acid induced seizures as indicated by increased retention latency in passive avoidance behavior. All major antiepileptic drugs (AEDs) have been reported to be associated with cognitive side effects, though uncertainty exists regarding the degree of cognitive deficits.[64]

The present study has demonstrated the beneficial effect of naringin in attenuating the kainic acid induced seizures as well as cognitive impairment. Naringin, thus, has a potential to be used in the clinical management of status epilepticus. In addition, the present study also demonstrates that naringin can prevent cognitive impairment and may act as a useful adjuvant to the conventional antiepileptic therapy.

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