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Anticonvulsive and behavior modulating effects of sophoretin and rutoside

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Introduction: Seizures are the hallmarks of most types of epilepsies. Behavioral and cognitive impairments coincide with interictal periods even though it is not clear whether these impairments spring out of the seizure itself or accompanying sociopsychological burden of the disease. *Materials and methods:* In this study, we investigated behavioral and cognitive consequences of a single GABA receptor-related seizure in mice, and examined the potential anticonvulsive and behavior-modulating properties of sophoretin (quercetin) and rutoside (rutin). *Results:* The study demonstrated that sophoretin and rutoside, common flavonoids of the human diet, delay the seizure onset and reduce the seizure stage. Moreover, they exerted an antidepressant-like effect, which was independent of the seizure. Neither treatments nor seizure altered recognition and spatial memory performances of the mice. *Conclusions:* Behavioral or cognitive disturbances that are evident in epileptic patients did not appear following a single seizure. In addition, we suggest that both sophoretin and rutoside successfully alleviate the seizure severity without interfering in the behavioral stability and cognitive performance. Hence, these flavonoids may be of use as adjuncts to the current treatment options.

Original Article

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INTRODUCTION

Epilepsy is a common debilitating neurological disorder that manifests mainly with seizures and affects approximately 50 million people worldwide (Ngugi et al., 2010). Up to a quarter of the epilepsy patients display pharmacoresistance, which is to be treated with conventional antiepileptic drugs (Alexopoulos, 2013). Phytomedicines can be used for discovering novel anticonvulsant/antiepileptic medications or may be combined with current treatment options as adjuvants to boost efficiency without compromising the safety. Flavonoids, a large group of natural phytonutrients attract interest with their health benefits including those that against cancer (George et al., 2017), infections (Yildirim et al., 2016), inflammation (González et al., 2011; Li et al., 2016), atherosclerosis (Bhaskar et al., 2013; Luo et al., 2015; Shen et al., 2013), ischemia (Ahmad et al., 2011) as well as epilepsy (Choudhary et al., 2011; Quintans Júnior et al., 2008; Zhu et al., 2014), and neuropsychiatric disorders (Grosso et al., 2013; Hurley et al., 2014). Sophoretin (quercetin; 3,3′,4′,5,7-pentahydroxyflavone), which is abundant in the human diet, is one of the most potent flavonoids. The sophoretin glycoside with a higher bioavailability, rutoside (rutin; quercetin-3-rutinoside), is structurally similar to sophoretin (Makino et al., 2013). Even though ample studies have demonstrated that sophoretin and rutoside bear a potential to alleviate behavioral and cognitive impairments, few of them have particularly focused on the effectiveness of these flavonoids in seizure-induced neuropsychiatric alterations. Nevertheless, neuropsychiatric disorders are not rare in the patients suffering from seizures, and the prevalence of these disorders can reach up to 35.5%, which is about 75% higher than general population (Tellez-Zenteno et al., 2007). The clinical data suggest that behavioral and cognitive disturbances appear mainly in the epileptic patients with hippocampal sclerosis (Quiske et al., 2000), and experimental studies in which an animal model for temporal lobe epilepsy has been established support this observation (Kandratavicius et al., 2014; Reddy et al., 2013). However, these reports

denote behavioral and cognitive outcomes of recurrent seizures where there are numerous intervening events and hence consequences of a single seizure are still disputable. Moreover, clinical examination of chronic epileptics is not much explanatory since the psychosocial burden of epilepsy and central neuropathological alterations confound with outcomes of the seizure alone.

Therefore, we aimed to investigate (a) the behavioral and cognitive status following a single limbic seizure induced by the $GABA_A$-antagonist picrotoxin; (b) anticonvulsant features of the chronic pretreatment with sophoretin and rutoside; and (c) impacts of sophoretin and rutoside on depression- and anxiety-like behaviors, spatial and recognition memory, and nociception in the postictal period.

MATERIALS AND METHODS

Subjects

A total of 40 adult male Balb/c mice were equally assigned into four groups ($n = 10$ in each): Group I (CON; no seizure was provoked, received vehicle), Group II (PTX; seizure was provoked, received vehicle), Group III (QuePTX; seizure was provoked, received 50 mg/kg/day, i.p. sophoretin for 21 days), and Group IV (RutPTX; seizure was provoked, received 50 mg/kg/day, i.p. rutoside for 21 days). Sophoretin and rutoside were dissolved in a mixture of propane-1,2,3-triol (2.7 mM) and methylsulfinylmethane (2.8 mM). The mice were housed in standard cages in climate-controlled conditions (22 ± 2 °C temperature, $55\% \pm 10\%$ relative humidity, 12-h light–dark cycle). The water and chow were provided *ad libitum*. The ethical consent was acquired from the Local Ethics and Animal Care Committee (#2018/9-11), which examines experimental procedures in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 86-23, revised 1996).

Procedure

On the day after the last treatment, a single seizure was provoked through the intraperitoneal injection of 3 mg/kg of picrotoxin in relevant mice, as shown in Fig. 1. The seizures were staged using a modified Racine's scale as defined by Lüttjohann et al. (2009). Accordingly, the event scores were as follows: (a) behavioral arrest; (b) facial jerks; (c) neck jerks; (d) clonic seizures while sitting; (e) tonic, clonic, or tonic–clonic seizures in a prone position; and (f) wild jumping, clonic, or tonic–clonic seizures in a lateral position.

Porsolt's forced swim test

A behavioral despair model, Porsolt's forced swim test, was performed to evaluate depression-like behaviors. The test consisted of a training session, in which the mice were left to swim freely for 10 min, and a probe session that was conducted 24 h after the training. The mice were placed into cylindrical tanks (25 cm height × 20 cm diameter), which were filled with warm water (24 °C) to the height of 15 cm. All mice were video-recorded in the probe session and events of immobility, swimming, climbing, and diving were evaluated from the recorded videos using an open-source computer software (BORIS v.2.96, Life Sciences and Systems Biology Via dell'Accademia Albertina, Torino, Italy; Friard & Gamba, 2016). After each mouse, the tanks were emptied and cleaned with 70% ethanol, and the mice that completed the test were gently dried.

Open-field test

The open-field test was performed to evaluate anxiety-like behaviors as well as the locomotion. The mice were placed onto the center of a black opaque-walled, cube-shaped apparatus ($40 \times 40 \times 50$ cm), and were allowed to move freely for 5 min. The total distance moved, velocity, and time spent in the center area were video-recorded and quantified using a commercial computer software (EthoVision XT v.8.5, Noldus Information Tech., Wageningen, The Netherlands). Defecation, rearing, and grooming counts were noted down with naked eyes by a researcher during the test. The test apparatus was cleaned with 70% ethanol between trials to eliminate odor cues.

Object recognition and spatial memory

The novel object recognition and Morris' water maze tests were employed to evaluate recognition and spatial memory performances. The open-field test was also the habituation session of the novel object recognition test. On the next day, two identical objects (A_1 and A_2) were placed into the apparatus, which was same with the one used in the open-field test, and the mice were released to explore the objects

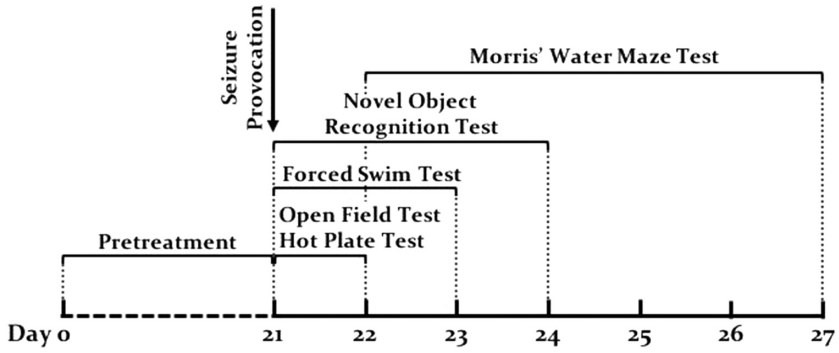

Fig. 1. The study plan

for 5 min. One and a half hours after this familiarization session, one of the familiar objects (A_2) was changed with a novel object (B), which was similar in size and color, but the shape. Then, the mice were released again into the apparatus for 5 min to evaluate the short-term memory. Twenty-four hours after the familiarization, the familiar object (A_2) was replaced, and another novel object (C), which was different only in shape, was put onto the location of the previous familiar object (A_1). Afterward, for the evaluation of the long-term memory, the mice were delivered into the apparatus and allowed to explore the objects for 5 min. The sessions were video-recorded, and events of directing the snout within 2 cm distance to the object, sniffing, and touching with the snout were considered as exploratory behavior. The preference index [$t_{\text{Familiar}-2}/(t_{\text{Familiar}-2} + t_{\text{Familiar}-1})$], recognition index [$t_{\text{Novel}}/(t_{\text{Novel}} + t_{\text{Familiar}})$], and total exploration duration were assessed using the BORIS v.2.96 computer software.

In the Morris' water maze test, a circular tank (60-cm height and 120-cm diameter) was filled with warm water (24 °C) to the depth of 45 cm. The tank was virtually divided into four quadrants as southwest, southeast, northwest, and northeast. A circular platform (10 cm diameter) was placed onto the northeast quadrant. The platform was submerged 1 cm below the water surface. All mice were subjected to four learning trials in each day for four consecutive days, and the release point was changed from one quadrant to another in each trial. During the learning trials, mice were left to swim freely and allowed to locate the submerged platform for 60 s. The mice that could not find the platform were gently placed on the platform and allowed to inspect visual cues during a 30-s intertrial interval. In the probe trial, the platform was removed and mice were introduced to a task of locating the former platform quadrant for 60 s. The latency to reach to the platform, time spent in the platform quadrant, total distance swam, and velocity were estimated from the recorded videos using the EthoVision XT v.8.5 computer software.

Hot plate test

The hot plate test was performed to evaluate nociception. A hot plate device (Ugo Basile, Milan, Italy) was set to 54 ± 0.5 °C. The mice were placed onto the plate, and the latency to licking paws or flicking hind paws was noted down. A cut-off time of 60 s was determined to prevent the thermal injury.

Statistical analyses

The parametric data were analyzed using one-way ANOVA test (repeated measures ANOVA test for repeated variables) and *post-hoc* Tukey's test, whereas non-parametric data were analyzed using Kruskal–Wallis test and post-hoc Dunn's test. The normality and homogeneity of the data were assessed by D'Agostino-Pearson omnibus test and Bartlett's test, respectively. The analyses were performed using GraphPad Prism computer software (California, USA). The statistical significance was set to $p < .05$. The results were presented as means ± standard errors of the means except for the non-parametric data, which were expressed as medians and interpercentile ranges (25%–75%).

RESULTS

Body weight, mortality, and exclusions

At the beginning of the study, the mean animal weight was 25.9 ± 2.04 g with a minimum of 21.5 g and a maximum of 29.7 g. There was no statistically significant difference among the experimental groups for initial and final body weights, respectively [$F_{(3,\ 36)} = 1.631$, $p = .199$ and $F_{(3,\ 35)} = 2.215$, $p = .104$; data not shown]. Throughout the study, none of the mice displayed a body condition score less than 3 (Ullman-Culleré & Foltz, 1999). However, one mouse from the CON group died unexpectedly in the second week of the study. Furthermore, one mouse from the PTX group rapidly died following the injection of picrotoxin, and the seizure could not be provoked in one mouse from the QuePTX group and hence this mouse was excluded from the study. Therefore, the behavioral tests were conducted on a total of 37 mice.

Seizure onset, severity, and duration

The one-way ANOVA test for the seizure onset time indicated a significant difference among the experimental groups [$F_{(2,\ 25)} = 6.564$, $p = .005$] and the *post-hoc* analysis showed that the seizure onset time was significantly delayed as compared to the PTX group in the mice treated with sophoretin and rutoside ($p = .006$ and $p = .044$, respectively; Fig. 2A). Furthermore, the seizure stage was significantly lower in QuePTX and RutPTX groups than the PTX mice ($p < 0.001$ for both; see Fig. 2B). The groups did not differ in seizure duration [$F_{(2,\ 25)} = 2.19$, $p = 0.132$; data not shown].

Locomotor behaviors

The data for locomotor behaviors, such as total distance moved and velocity, were acquired from both open-field and Morris' water maze tests. The statistical analysis revealed a difference among the experimental groups for both variables in the open-field test, which was conducted on the first day of the 6-day-long behavioral test battery [Distance moved: $F_{(3,\ 33)} = 6.283$, $p = .002$; Velocity: $F_{(3,\ 33)} = 5.844$, $p = .003$; see Fig. 1 for the study plan]. As depicted in Fig. 3A, PTX and RutPTX groups traveled over a longer distance than the controls ($p = .022$ and $p = .001$, respectively). Similarly, velocity was higher in PTX and RutPTX mice as compared to the controls ($p = .007$ and $p = .009$, respectively; Fig. 3B). There was no difference between CON and QuePTX groups for either total distance moved or velocity ($p > .05$), indicating a reduced locomotor hyperactivity with the sophoretin treatment. Conversely, we found no significant difference in locomotion-related variables in the probe trial of the Morris' water maze test, which was performed on the last day of the behavioral test battery [Distance moved: $F_{(3,\ 33)} = 0.790$, $p = .508$; Velocity: $F_{(3,\ 33)} = 0.889$, $p = .457$; data not shown].

Porsolt's forced swim test

In Porsolt's forced swim test, there was a difference among groups for the total immobile time and climbing duration

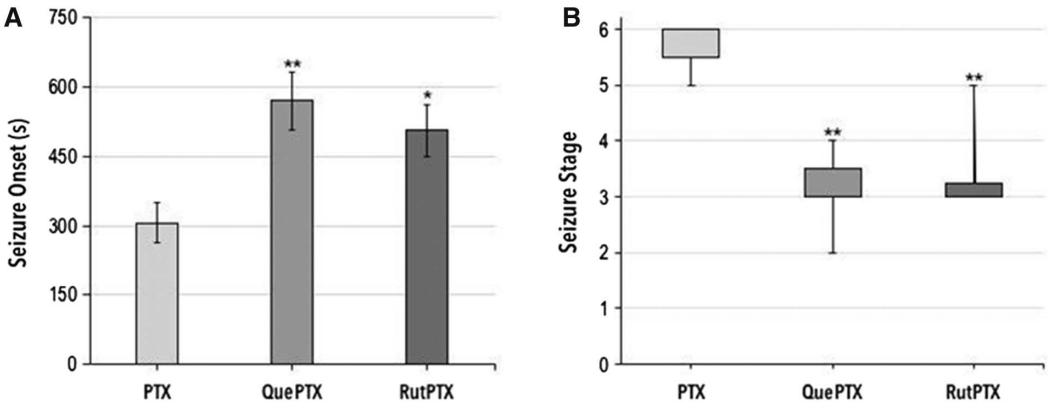

Fig. 2. (A) Seizure onset time and (B) seizure stage. Sophoretin (QuePTX) and rutoside (RutPTX) delayed the seizure onset. Also, both treatments resulted in lower seizure stages. Asterisks (* and **) indicate statistical significance versus the picrotoxin group (PTX) ($p < .05$ and $p < .01$, respectively)

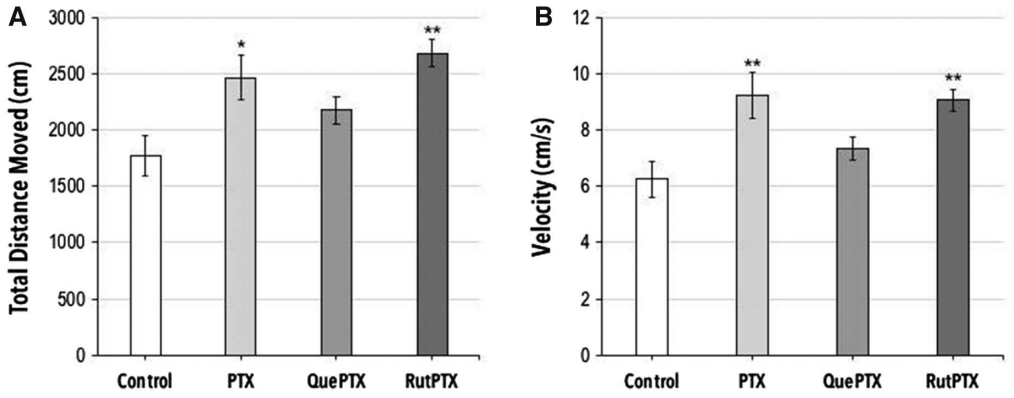

Fig. 3. (A) Total distance moved and (B) velocity in the open-field test. Picrotoxin (PTX) led to an increase in locomotion, which was hindered by sophoretin (QuePTX), but not by rutoside (RutPTX). Asterisks (* and **) indicate statistical significance versus controls ($p < .05$ and $p < .01$, respectively)

[$F_{(3,\ 33)} = 7.895$, $p < .001$ and $F_{(3,\ 33)} = 14.58$, $p < .001$, respectively]. As shown in Fig. 4A, B, a single seizure did not generate a significant effect for either immobility or climbing behavior ($p > .05$ for both); however, in both sophoretin- and rutoside-treated mice, the time passed in an immobile posture decreased ($p = .002$ and $p = .005$, respectively), whereas climbing duration increased as compared to the controls ($p = .004$ and $p < .001$, respectively). In comparison to the CON group, relative mobilizing effect of the treatment was 35% for sophoretin and 32.9% for rutoside (Fig. 4C).

Open-field test and hot-plate test

There was no statistically significance among experimental groups the open-field test in regard to anxiety-related variables including the time spent in the center zone [$F_{(3,\ 33)} = 0.357$, $p = .785$; Fig. 5A–C), defecation count ($p = .105$), and grooming [$F_{(3,\ 33)} = 0.179$, $p = .910$]. In the hot plate test, latency to the first discomfort did not differ among the groups [$F_{(3,\ 33)} = 2.424$, $p = .083$; data not shown].

Object recognition and spatial memory

In learning trials of the Morris' water maze test, there was no difference between mice for the platform latency ($p > .05$; Fig. 6A). Furthermore, the time passed in the target quadrant [$F_{(3,\ 33)} = 0.559$, $p = .646$] was not significantly different (Fig. 6B). In regard to the novel object recognition test, the results from the familiarization session showed that no mice displayed a place preference [$F_{(3,\ 33)} = 0.080$, $p = .970$; data not shown). In the short-term memory session, there was no statistical significance for the exploration time [$F_{(3,\ 33)} = 0.437$, $p = .728$] and recognition index [$F_{(3,\ 33)} = 0.448$, $p = .721$], as depicted in Fig. 7A, B. In addition, the time consumed for exploring the objects and recognition indices of the groups were similar in the long-term memory session [$F_{(3,\ 33)} = 0.656$, $p = .585$ and $F_{(3,\ 33)} = 0.399$, $p = .755$, respectively; Fig. 7C, D].

DISCUSSION

In sum, we found that (a) both sophoretin and rutoside delayed the seizure onset and alleviated the seizure severity, (b) a single seizure resulted in the increase of locomotion in an acute course and the treatment with sophoretin decreased the locomotor hyperactivity; however, this effect was temporary and not observed on the long-term, (c) sophoretin and rutoside possessed an antidepressant-like effect, but this was independent of the seizure since a single seizure did not

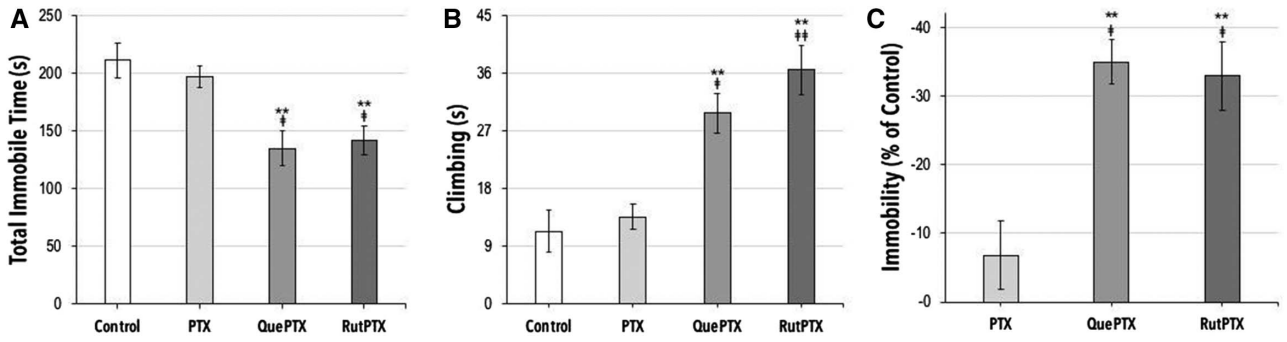

Fig. 4. (A) Total immobile time, (B) duration of the climbing behavior, and (C) immobilization relative to the controls in the Porsolt's forced swim test. In comparison to the controls, picrotoxin (PTX) did not alter the mobility in the forced swim test. However, both sophoretin (QuePTX) and rutoside (RutPTX) decreased immobility and increased climbing. Double asterisks (**) indicate statistical significance versus controls ($p < .01$) and palatal clicks (ǂ and ǂ ǂ) versus the PTX group ($p < .05$ and $p < .01$, respectively)

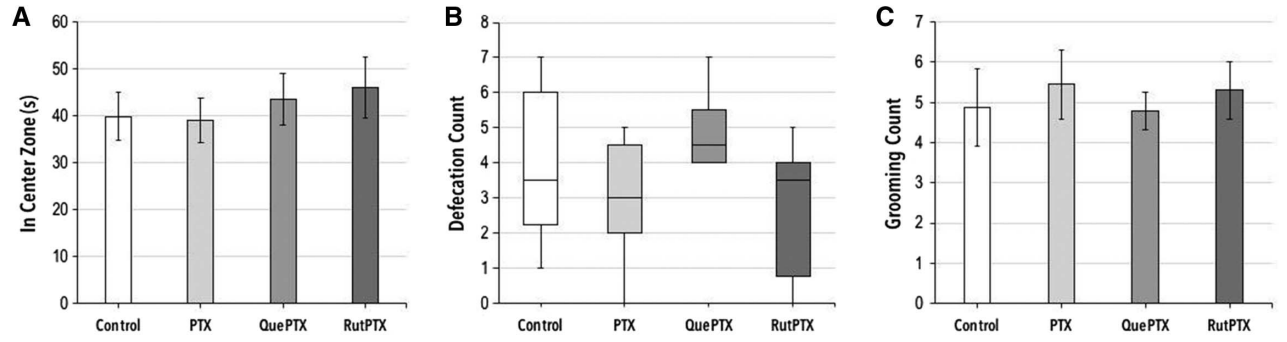

Fig. 5. (A) The time passed in the central zone, and (B) defecation, and (C) grooming counts in the open-field test. There was no significance between groups for either main (in-center time) or adjunct (defecation and grooming) indicators of anxiety ($p > .05$)

generate a depressive status, (d) neither a single seizure nor the treatments altered anxiety-like behaviors and nociception, and (e) spatial and recognition memories were not influenced by either a single seizure or the treatments (see Supplementary Fig. S1 for a graphical abstract).

There is no consensus in the medical literature regarding antiepileptic and/or anticonvulsant features of sophoretin and rutoside, probably due to the methodological diversities. For example, Nieoczym et al. (2014) have reported that a single injection of sophoretin or rutoside to Swiss mice in the doses between 100 and 800 mg/kg did not exert an anticonvulsant effect in pentylenetetrazole-induced seizures, but both flavonoids were effective in the doses of 10–200 mg/kg in 6 Hz-induced seizures. Akin to these divergent results, Guo et al. (2011) showed that the ethanol extract of *Abelmoschus manihot,* a sophoretin- and rutoside-containing plant, dose-dependently exerted anticonvulsant effects in a single pentylenetetrazole-induced seizure. In a series of studies by Nassiri et al. (2008, 2010, 2013), sophoretin and rutoside reported to have antiepileptic and/or anticonvulsant actions in a variety of experimental rodent models. Our results of a delayed seizure onset and reduced severity of the seizures with sophoretin and rutoside in the picrotoxin-induced model support the hypothesis of an anticonvulsant feature of these flavonoids. The exact mechanism for how these flavonoids and also others bring about an anticonvulsant effect is not clear yet; however, it likely appears to be a benefit that emerged by their antioxidant properties and interactions with both excitatory and inhibitory neurotransmitters (predominantly with inhibitory $GABA_A$-benzodiazepine receptor complex) (Nassiri-Asl et al., 2008; Nieoczym et al., 2014).

Picrotoxin-related locomotor hyperactivity has been linked to the disinhibition of mesolimbic dopaminergic neurons (Chang et al., 2004; Mogenson et al., 1980). In this study, we observed exaggerated locomotor behaviors on the day after the induction of the seizure; however, the hyperactivity was impermanent and seen to be reversed in the probe trial of the Morris water maze test, which was performed 5 days after the first locomotion-dependent test, the open-field test. The present finding indicates that the dopaminergic alterations generated by a single seizure do not extend to neuroplastic changes. This hypothesis is supported by the studies in which a sustained locomotor hyperactivity has been reported in kindled mice (Caldecott-Hazard, 1988; Watanabe et al., 2004). Moreover, we found that sophoretin attenuated the locomotor hyperactivity. Considering the inhibitory feature of sophoretin on dopamine-degrading enzymes, namely catechol-O-methyl transferase and monoamine oxidase (Bandaruk et al., 2012; Singh et al., 2003), our aforementioned finding with sophoretin is surprising and requires to be examined comprehensively to define probable transient modifications that resulted in increased dopamine sensitivity, and to demonstrate possible pharmacodynamic/pharmacokinetic interactions of picrotoxin and sophoretin.

Depression is the most common neuropsychiatric disorder in epileptic patients (Tellez-Zenteno et al., 2007). Even though it occurs more frequently in epilepsy sufferers than

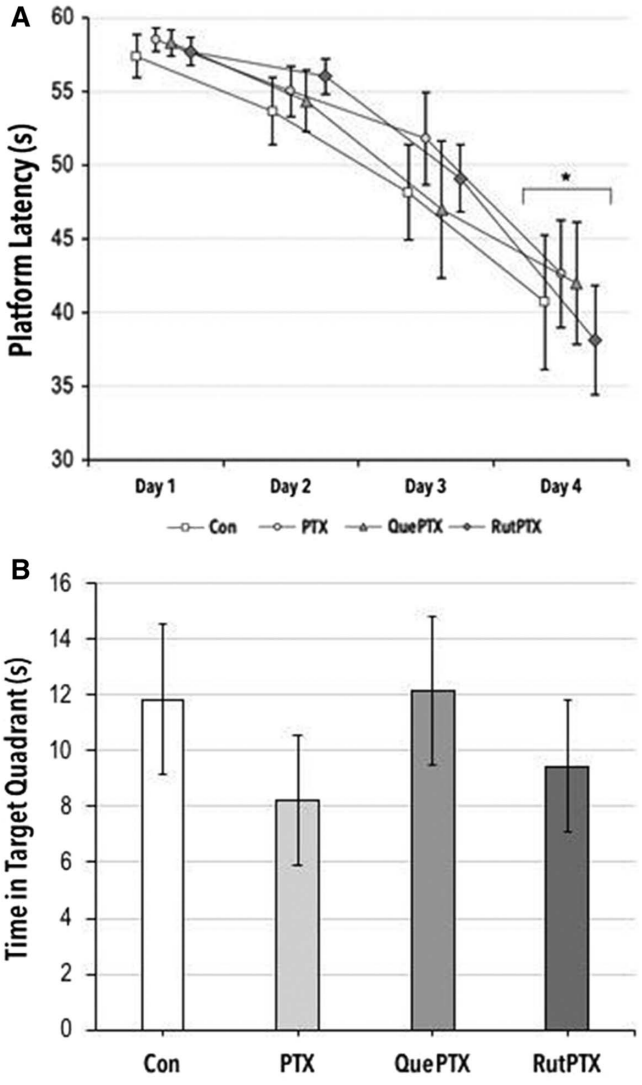

Fig. 6. (A) Platform latency in the learning trials and (B) time passed in the target quadrant in the probe session of the Morris' water maze. The latency in learning trials was similar in all groups ($p > .05$). The temporal comparison of the latency revealed that all animals had learned the location of the platform. Asterisk (*) indicates statistical significance versus Day 1 ($p < .05$). There was no significance for the time passed in the target quadrant ($p > .05$)

the general population, the incidence of depression is akin to that is seen in the patients with a chronic disease (Stefansson et al., 1998). Despite the epidemiological data, experimental studies suggest a connection between epilepsies and depression particularly in genetic and kindling models. The genetic rat models of absence epilepsy have been shown to be prone to anhedonic and depressive behaviors (Jones et al., 2008; Sarkisova & van Luijtelaar, 2011; Sarkisova et al., 2003), whereas the kindling in wild-type mice has been reported to result in depression-like status (Chen et al., 2016; Godlevsky et al., 2014; Medel-Matus et al., 2017). Mazarati et al. (2007) associated depressive behaviors in animal models with the hippocampal injury. Indeed, this association is favored by experimental and clinical studies. Müller et al. (2009) reported that the mice without any hippocampal injury did not display depression- or anxiety-like behaviors, whereas these affective disturbances were observed in the mice with a hippocampal damage. Furthermore, Hecimovic et al. (2014) demonstrated that depressive symptoms are significant in the patients with a decreased hippocampal volume unless the hippocampal atrophy becomes severe. The results of this study, in which a single seizure has been provoked without causing a hippocampal injury as well as any depressive behavior, also support the role of hippocampus in affective disorders in seizures. Remarkably, similar to the study by Guo et al. (2011), anti-depressant-like effects of sophoretin and rutoside were not influenced by the seizure.

Cognitive decline in epilepsy can be originated in seizures, epileptiform discharges, antiepileptic drugs, and social disabilities (Loughman et al., 2016). The design of this study allowed us to examine cognitive effects of a single seizure isolated from other confounding cognition-deteriorating events, and to investigate influences of sophoretin and rutoside. As for the neurobehavioral disturbances, the most prominent neurological insult that leads to a cognitive disruption is hippocampal sclerosis (Kandratavicius et al., 2014). In contrast, acute seizures generate a milder deficit, which does not bring about hippocampal sclerosis (Müller et al., 2009; Norwood et al., 2010). Consistently, our results for both spatial and recognition tests indicate that a single GABA receptor-related seizure does not create a cognitive impairment. In their comprehensive review, Huberfeld et al. (2015) stated that altered chloride channel expressions are responsible for the interictal cognitive deficits. Hence, with regard to this study, it can be assumed that a single seizure did not disturb the chloride homeostasis. Contrarily to the scarcity of previous studies regarding rutoside, sophoretin is known to interact with chloride homeostasis through increasing the expression of sodium-potassium-chloride cotransporter (NKCC), and to promote the neurite outgrowth. Notably, its effect on the expression level of NKCC is dependent on the number of available cotransporters. Therefore, the absence of a cognitive effect of sophoretin in this study is conceivable, since a single seizure did not result in learning and memory impairments. As a sophoretin glucoside, the case with rutoside may be same as sophoretin. The supporting evidence of this hypothesis comes from the study of Nassiri-Asl et al. (2010) in which they have demonstrated that rutoside bolstered the cognition in kindled mice, which had a broader neurological insult.

Overall, the investigated flavonoids, such as sophoretin and rutoside, that are common in the daily human diet, were demonstrated to exhibit an anticonvulsive activity. This feature may be attributed to their antioxidant properties (Geronzi et al., 2018). It is important to note that both flavonoids did not alter behavioral and cognitive parameters, which show their safety with regard to neuropsychiatric adverse effects. Moreover, these flavonoids possessed an antidepressant-like effect and this may be of use against depression in epileptic patients while also contributing their existing anticonvulsive treatment. A single GABA receptor-related seizure was found not to bring about behavioral or cognitive deficits. This result indicates that the neuropsychiatric disruptions in epileptic patients are a consequence of a cumulative insult to affective and cognitive brain regions. The sociopsychological burden of the disease should also be considered in the medical evaluation of the patients with epilepsy.

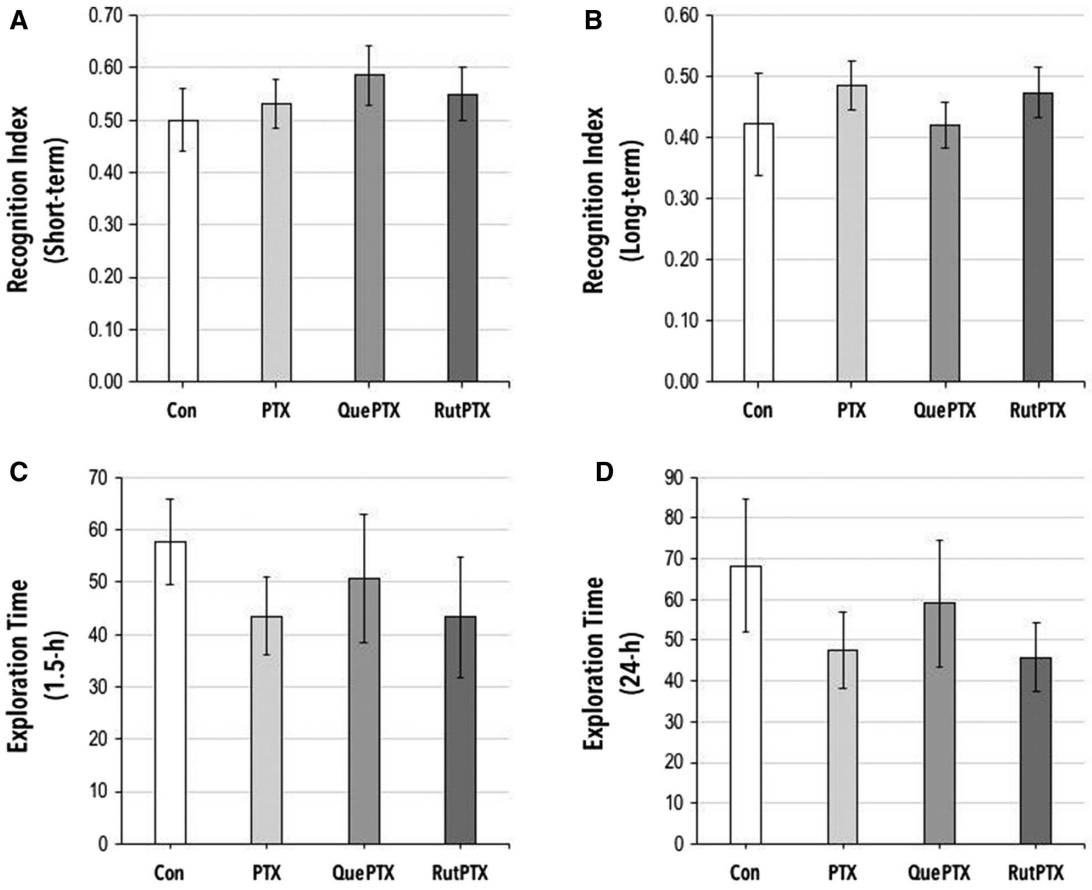

Fig. 7. (A) Short-term and (B) long-term recognition indices, and the duration of exploratory behaviors following (C) 1.5-h and (D) 24-h intertrial intervals in the novel object recognition test. Neither recognition indices nor the time spent for exploration were statistically significant ($p > .05$)

CONCLUSION FOR FUTURE BIOLOGY

In this study, we provoked a single GABA receptor-related seizure and investigated anticonvulsive effects of sophoretin (quercetin) and rutoside (rutin) as well as behavioral and cognitive consequences of a single seizure and the treatments. Thus, there were no spontaneously occurring paroxysmal neural discharges. In this experimental arrangement, our results demonstrated that a single seizure does not bring about behavioral or cognitive impairments, and the chronic treatment with either sophoretin or rutoside successfully alleviates the seizure severity without interfering in the behavioral stability and cognitive performance. Conclusively, these two flavonoids may be acknowledged as experimentally effective anticonvulsant agents.

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Ethical Statement: The ethical consent was acquired from the Local Ethics and Animal Care Committee (no. 2018/9-11).

Funding Statement: No funding has been received for this study.

Data Accessibility: The authors prefer not to provide research data publicly. The data can be provided on demand only for reviewing purposes.

Competing Interests: The authors declare no conflict of interest.

Authors' Contributions: EAD conceived the hypothesis, performed behavioral/cognitive tests, and drafted the manuscript. AO, OT, and HD carried out the experimental protocol. CT supervised and critically reviewed the manuscript.

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