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Selected flavonoids and their role in the treatment of epilepsy – a review of the latest reports from experimental studies

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Epilepsy is a chronic neurological disease characterized by recurrent seizures that affects about 70 million people worldwide. Antiepileptic drugs are the most commonly used medications in the treatment of epilepsy. They help control seizures in about 60-70% of people. The remaining percentage of patients suffer from drug-resistant epilepsy, prompting scientists to look for natural substances that would prevent seizures or support the effects of drugs in add-on therapy while reducing side effects. Currently, there is a lot of emphasis on natural product. Flavonoids are included in this group, and their use in the treatment of epilepsy could support the effect of other drugs. Due to very good results of preclinical studies, flavonoids are a promising candidate for epilepsy related clinical trials related. The article is an overview of literature reports from the past 10 years including mainly *in vivo* preclinical research on various models of experimental epilepsy with the use of selected flavonoids.

Key words: flavonoids, antiepileptic drugs, experimental epilepsy

INTRODUCTION

Epilepsy is a serious neurological disorder affecting around 70 million people around the world. Antiepileptic drug (AED) therapy is effective in 60–70% of patients, however in 30-40% of patients seizure control is not achieved (Kalilani et al., 2018). Drug resistance in epilepsy is defined by International League Against Epilepsy as the failure of treatment with two properly used and well-tolerated AEDs (both, monotherapy and combination) used for a sufficiently long time in controlling seizures (Kwan et al., 2010). The mechanism of drug resistance is not fully known or fully explained. It is probably influenced by both genetic and environmental factors (Tang et al., 2017). For those suffering from drug-resistant epilepsy (DRE) numerous drug side effects, memory disorders, stigmatization become a social problem, and the lack of expected treatment effects contribute to the occurrence of depression in even half of the patients (Kozera-Kępniak et al., 2013). Sudden unexpected death in epilepsy (SUDEP) is the main cause of death in patients with DRE and accounts for up to 50% documented causes of death in this group of patients (Opeskin and Berkovic 2003; Tomson et al., 2008; Surges et al., 2009; Shorvon and Tomson, 2011). Therefore, the main goal of DRE therapy is to extend the seizure control period, minimize side effects and improve quality of life (Boon et al., 2015). Currently, a number of scientific studies include the use of natural substances with antiepileptic properties as adjuncts to standard antiepileptic drugs in the DRE treatment and flavonoids and may be a potential group of natural drugs that increase the effects of commonly used anticonvulsants.

Flavonoids, a class of secondary plant metabolites, have been shown to demonstrate a wide spectrum of biological activity, among others antioxidant (Santos et al., 1999), anti-inflammatory (Pintho et al., 2016), anti-

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allergic (Kawai et al., 2006), antiatherosclerotic (Goldwasser et al., 2011), antidiabetic (Rayidi et al., 2011) as well as hepatoprotective (Renugadevi et al., 2009). Corsale et al. (2018) used a mixture of flavonoids such as diosmin, troxerutin, rutin, hesperidin, and quercetin to reduce bleeding due to hemorrhoidal disease. Cicero et al. (2019) evaluated the effectiveness of bergamot flavonoid extract in people with dyslipidemia and those overweight. Obtained results showed an improvement in lipid and glucose metabolism, adipokine pattern and systemic inflammation in overweight and dyslipidemic individuals. Kirienko et al. (2018) investigated the clinical efficacy of 1000 mg micronized purified flavonoid fraction (MPFF) administered once daily in patients with chronic venous disease (CVD), which turned out to be associated with a rapid reduction in leg pain throughout the 8-week treatment period.

Flavonoids are derivatives of 2-phenyl-benzo-γ-pyrone. A common part in the chemical structure of all flavonoids is the carbon skeleton based on the flavane system (C6-C3-C6), formed from two benzene rings (A and B) connected by a heterocyclic ring of pyran or pyrone (C) (Fig. 1, Pietta, 2000). The biosynthesis of A and B rings occurs in two pathways – acetate (A) and shikimate (B). Ring A is formed from 3 malonyl-CoA molecules obtained from glucose conversions. Ring B is formed from 4-coumaroyl-CoA, created in the shikimate pathway from phenylalanine. Condensation of the A and B ring leads to the formation of chalcone, which is cyclized with the participation of isomerase and forms flavanone - the initial compound for the synthesis of the remaining flavonoid groups (Bravo et al., 1998, Wang et al., 2018). They may exist in free form of aglycone or betaglycosides (Panche et al., 2016). Due to differences in the structure, flavonoid compounds are divided into several subclasses: flavanones, flavonols, flavones, isoflavones, chalcones and anthocyanins (Table I.) The article focuses on several compounds from the flavonoid group that have been shown to possess antiepileptic properties in various experimental models of epilepsy (Table II).

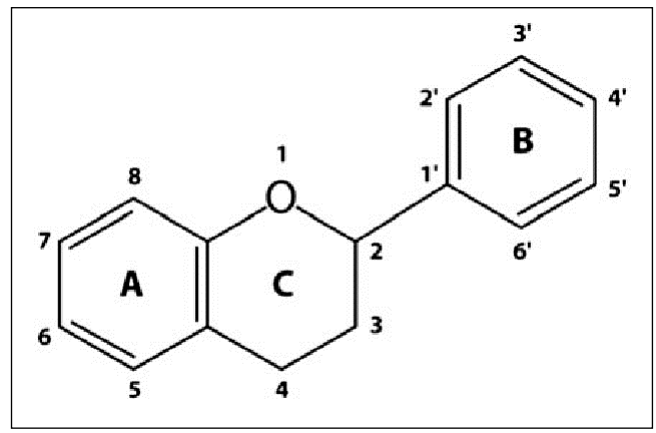

Fig. 1. The main structure of flavonoids with rings and numbered positions.

Anticonvulsant properties of flavonoids

Naringenin/naringin

Naringenin (2,3-dihydro-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, Fig. 2) is a natural phenolic compound found in citrus species and Chinese plants such as: *Drynaria fortunei*, *Citrus aurantium* and *Citrus medica* (Jadeja et al., 2014; Chen et al., 2016). Shakeel et al. (2017) indicated anticonvulsant properties for naringenin in the mouse pilocarpine-induced seizures (PILO). Results they obtained showed that 15-day treatment naringine (20 mg/kg and 40 mg/kg), followed by the administration of pilocarpine on the last day of treatment, seizures reduced in comparison with the control group. Naringenin has been shown to inhibit the onset and duration of seizures in a dose-dependent manner. Naringin in a dose of 20 mg shortened the seizure duration by about 1 min, and delayed seizures by 12 min, whereas the

Table I. Flavonoids – subclasses division.

Flavonoids				
Flavonols	Flavanones	Flavones	Isoflavones	Chalcones
quercetin routine	naringenin	apigenin	daidzein	phloretin
kemferol	naringin	diosmetin	genistein	arbutin
mirecitin	hesperidin	luteolin		phloridzin
fistein	hesperedine	baicalin		chalconaringenin
morina				

Table II. Antiepileptic properties of selected flavonoids in animal models of epilepsy – a summary of scientific reports.

Flavonoids	Animal model	Results	Author
Naringenine	PILO-model (mice)	• reduction of seizures	Shakeel et al. (2017)
	KA-model (mice/rats)	• reduction of seizures, undisturbed cognitive functions	Park et al. (2016) Golechha et al. (2011).
	PTZ-model (rats)	• protection against seizures, reduction of cognitive impairment	Golechha et al. (2014)
Apigenin	picrotoxin-induced seizures (mice)	• delayed the onset of seizures	Avallone et al. (2000)
	KA-model (mice)	• reduction of the number of seizures	Han et al. (2012)
Luteolin	PTZ-model (mice/rats)	• delayed the onset of myoclonic jerks, onset of clonic seizures and onset of hind limb extension • protection against mortality • neuroprotective effect	Tambe et al. (2015) Zhen et al. (2016)
	PILO-model (mice)	• delayed the onset of seizures and shortened the duration of clonic seizures • improvement of cognitive functions	Smillin et al. (2020)
Epigallocatechin	PTZ- kindling model (rats)	• reduced cognitive deficits and oxidative damage	Xie et al. (2012)
	cocaine-induced seizures (rats)	• reduction of seizures	Park et al. (2001)
	lithium-PILO model of TLE (rats)	• reduction of the frequency of spontaneous recurring seizures • neuroprotective effect	Qu et al. (2019)
Hesperidin	PTZ-model (mice/zebrafish)	• increased anticonvulsant effect alone and in combination with diazepam • neuroprotective effect	Kumar et al. (2014) Rosa-Falero et al. (2015)
Quercetin/routine	PTZ-kindling model (rats/mice)	• attenuation of seizures • memory improvement	Nassiri-Asl et al. (2013) Singh et al. (2017)
	PTZ model (rats)	• reduced generalized seizure	Sefil et al. (2014)
	MEST (mice)	• reduced seizures	Nieoczym et al. (2014)
	6 Hz model (mice)	• reduced seizures • no side effects in behavioral tests in combination with LEV and VPA	Nieoczym et al. (2014)
Baicalin	PILO-model (rats)	• significant delay in seizures • reduced neuronal death	Liu et al. (2012)
	KA-model (mice)	• neuroprotective effect	Liao et al. (2016)

higher dose (40 mg/kg) shortened seizure duration by 2 min and delayed seizures by 27 min. Moreover, naringenin treatment restored the antioxidant status and reduced lipid peroxidation in hippocampus of epileptic mice.

Park et al. (2016) using a kainic acid (KA) mouse model of temporal lobe epilepsy (TLE) showed that treatment with naringenin (100 mg) significantly delayed the occurrence of KA-induced seizures and impaired granular cell dispersion (GCD). Moreover, the effects of naringenin treatment were similar to those of rapamycin (RA) treatment. RA is not a universal antiepileptic drug and it's use is limited to tuberous sclerosis. In these studies, it was used as a positive control for changes in GCD mediated by mTORC1 inactivation. Nissel staining showed that naringenin significantly reduced changes in GCD, suggesting that it may act by inhibiting mTORC1 pathway activation. Many studies have indicated that hyperactivation of the mTORC1 pathway leads to epilepsy (Laplante et al., 2012; Sha et al., 2012; Lasarge et al., 2014; Shima et al., 2015). Immunofluorescence results and Western blot analysis showed that naringenin treatment attenuated the increase in TNFα and IL-1β expression and significantly reduced their levels within the dentate gyrus (DG) of the hippocampus of KA-treated mice. These results

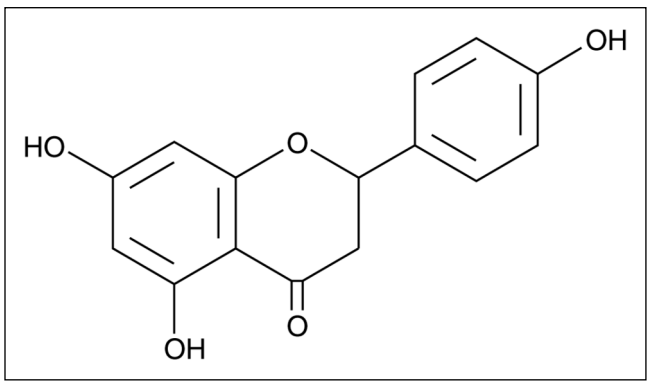

Fig. 2. Structure of naringenin.

suggest that naringenin may have anti-inflammatory effects in the KA-induced TLE model.

Interestingly, a flavanone glycoside naringin was tested in the KA induced epilepsy model in rats (Golechha et al., 2011). Results they obtained indicated, that initial treatment with naringin at doses 20, 40, 80 mg/kg significantly increased seizure delay compared to the KA control group, which suggests that naringin suppresses KA-induced seizures. Behavioral studies in the passive avoidance test showed cognitive impairment in the KA control group, while pretreatment with naringin reversed the KA-induced cognitive deficit (Golechha et al., 2011). Additionally, in the rat pentylenetetrazol (PTZ) model naringin (20, 40 and 80 mg/kg) administered for 7 days significantly delayed the occurrence of myoclonic seizures depending on the dose (Golechha et al., 2014). Obtained results indicated that treatment with naringin (80 mg/kg) protected all rats against PTZ seizures, and reduced brain malondialdehyde and TNF-α levels and conserved glutathione, which highlighted the antioxidant, anti-inflammatory and anticonvulsant potential of naringin. Additional studies were conducted in order to check whether the anticonvulsant effect of naringin is GABA dependent. For this purpose, the GABA-A receptor was blocked by flumazenil, an antagonist of GABA-A. Obtained results showed, that flumazenil pretreatment decreased the prolongation of convulsant latency induced by naringin, suggesting a possible modulation of the benzodiazepine site of the GABA-A receptor by naringin to produce its anticonvulsant effect (Golechha et al., 2014).

Compans and coworkers (2018) investigated a potent anticonvulsant properties of two methylated flavanones: naringenin 4',7-dimethyl ether (NRG-DM) and naringenin 7-O-methyl ether (NRG-M) in zebrafish pentylenetetrazole (PTZ) seizure model. Results they obtained showed, that both NRG-M NRG-DM decreased PTZ-induced seizure activity in larval zebrafish up to approximately 20-30% compared to PTZ controls. In addition, NRG-DM was shown to be active in two standard mouse models of acute epilepsy, the PTZ test and in the 6 Hz psychomotor seizure model, suggesting that methylation facilitates the absorption of the compound, which translates into higher anticonvulsant activity. NRG-DM certainly deserves further research in the treatment of generalized seizures, and especially in the treatment of drug-resistant focal seizures (Compans et al., 2018).

Apigenin

Apigenin (4',5,7-trihydroxyflavone, Fig. 3) is one of the most common flavonoids in plants and belongs to the flavone subclass. It has been shown, that the main source of the apigenin are plants belonging to *Asteraceae*, such as *Artemisia* (Ornano et al., 2016), *Achillea* (Venditti et al., 2015; 2016), *Matricaria* (Sharifi-Rad et al., 2018) and *Tanacetum* (Venditti et al., 2018). Apigenin has received particular interest because of its anxiolytic, sedative, neuroprotective, anticonvulsant and antidepressant properties (Ali et al., 2017). Interestingly, apigenin can also ameliorate memory dysfunction linked to Alzheimer's disease (Zhao et al., 2013).

Studies by Avallone et al. (2000) determining a potent anticonvulsant efficacy of apigenin in picrotoxin-induced seizures in Sprague-Dawley male rats indicated, that apigenin at doses of 25 and 50 mg/kg administered 15 min before picrotoxin (6 and 8 mg/kg) delayed the onset of convulsions.

Interestingly, apigenin administrated for five days (25-50 mg/kg) followed by a KA injection on the last day, was shown to reduce both the number and duration of seizures in mice (Han et al., 2012). Moreover, apigenin was found to block KA-induced electroencephalogram discharge activity in the cerebral cortex. Cresol violet staining of the brain sections of KA-treated mice showed that KA causes the loss of approximately 20% of neurons, whereas apigenin administration af-

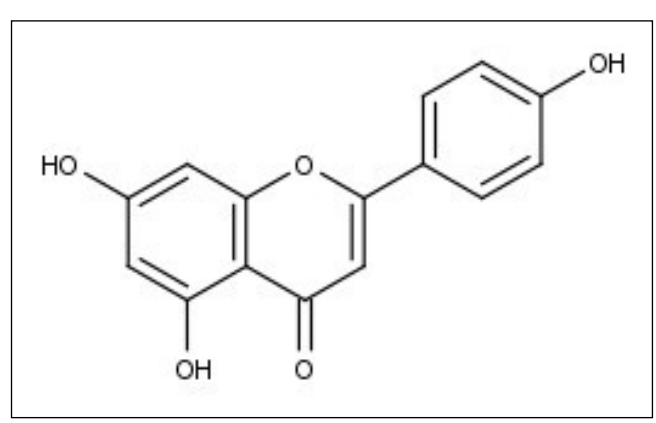

Fig. 3. Structure of apigenin.

ter KA treatment for 5 days rescued about 32-40% of the neuronal loss in the CA3 region. Additionally, apigenin (25-50 mg) in dose-dependent manner reversed the reduction of glutathione (GSH) levels induced by KA in hippocampus tissues. *In vitro* studies on primary hippocampal cell culture indicated that apigenin inhibited KA-induced excitotoxicity in a dose-dependent manner as measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. Moreover, apigenin (5, 20, 60 μM) treatment dose-dependently inhibited intracellular reactive oxygen species (ROS) elevations in cultured hippocampal neurons. Other *in vitro* studies using SH-SY5Y cells indicated apigenin's neuroprotection against oxidative stress-induced cell death by blocking caspase-3 activity and glutamate-induced neurotoxicity by reducing NMDA receptor-mediated responses, which was demonstrated in cultured cortical neurons (Losi et al., 2004). In addition, apigenin was reported to inhibit the influx of extracellular Ca2+ and release of intracellular Ca2+ in the rat thoracic aorta and NMDA or gamma-aminobutyric acid (GABA) receptors channels (Ko et al., 1991).

Luteolin

Luteolin (3',4',5,7-tetrahydroxy flavone, Fig. 4) also belongs to the group of flavones. This compound is present in herbs such as thyme, chamomile, celery and green peppers. It can also be found in vegetables and fruits such as parsley, celery, broccoli, apples, onion leaves (Miean et al., 2001; Mencherini et al., 2007). Plants rich in luteolin have been used in the treatment of hypertension, inflammatory diseases and cancer (Harborne and Williams, 2000). The blood-brain barrier is permeable to luteolin, which makes it useful in the treatment of diseases of the central nervous system, including brain cancer (Wruck et al., 2007). Shaikh et al. (2013) indicated no anticonvulsant effect of doses (0.3-10 mg/kg) of luteolin in the 6 Hz seizure model, PTZ-induced seizure test, maximal electroshock (MES) tests as well as in PILO model with second hit PTZ seizure model in mice. On the other hand, Tambe and colleagues (2015) evaluated the antiepileptic potential of luteolin isolated from *Eclipta alba* in PTZ-induced seizure test in mice. Obtained results showed that the administration of luteolin at a dose of 10 or 20 mg/kg delayed the occurrence of myoclonic seizures, clonic seizures and extension of the hind limbs in comparison with the PTZ control animals. In addition, the above doses showed 100% protection against PTZ-induced mortality. The efficacy was comparable to that of diazepam, suggesting that luteolin is anti-epileptic in the PTZ-induced seizure test in a dose-dependent manner.

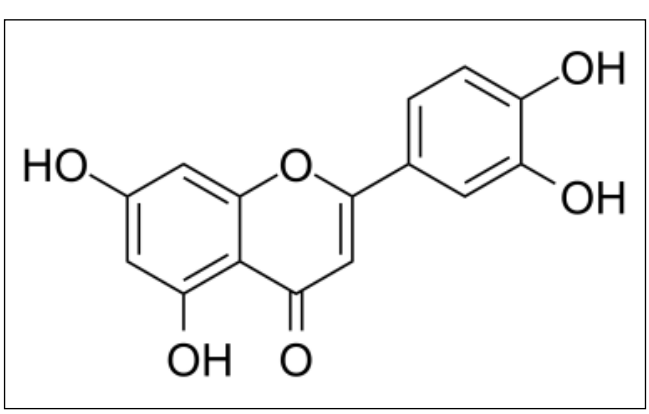

Fig. 4. Structure of luteolin.

Zhen et al. (2016) studied the anticonvulsant effect of luteolin in the PTZ test in rats. They showed that initial treatment with luteolin at a dose of 100 mg/kg increased seizure delay, reduced the mean seizure duration and seizure severity compared to the PTZ group. Mortality in the PTZ group was 33.3%, while in the PTZ+100 mg/kg luteolin rats it decreased to 8.3%. Despite the fact that luteolin did not completely inhibit seizures, the results of Nissel's hippocampus staining and the Morris water maze behavioral test showed a significant reduction in the neurological damage and cognitive deficits associated with daily seizures. Biochemical studies indicated that the neuroprotective effect of luteolin on seizure-induced cognitive dysfunction was exerted by inhibition of ROS production and increased phosphoactivation of the cyclic AMP binding protein (CREB) pathway and expression of derived neurotrophic factor (BDNF) in the hippocampus (Zhen et al., 2016).

Interesting results were shown by Smilin Bell Aseervatham (2020) studying *Passiflora caerulea* (PCAE), which contains polyphenol compounds such as ginsenoside, naringenin, chrysoeriol 8-c-glucoside, luteolin-6-C-glucoside, apigenin-6,8-di-C-β-D-glucopyranoside. PCAE (200 mg/kg) significantly delayed the onset of seizures and reduced the duration of clonic seizures compared to the PCAE group (100 mg/kg) and diazepam in the mouse PILO model of epilepsy. Furthermore, PCAE-treated mice showed an improvement in cognitive function. It is likely that the antioxidant substances contained in PCAE have a protective effect on PILO-induced seizures by neutralizing oxidative damage (Smilin Bell Aseervatham et al., 2020).

Epigallocatechin

Epigallocatechin gallate (EGCG, 2R, 3R-3',4',5,5',7-pentahydroxy flavan-3-yl gallate, Fig. 5) is classified in hydroxylated flavans. It constitutes 50% of all polyphenols

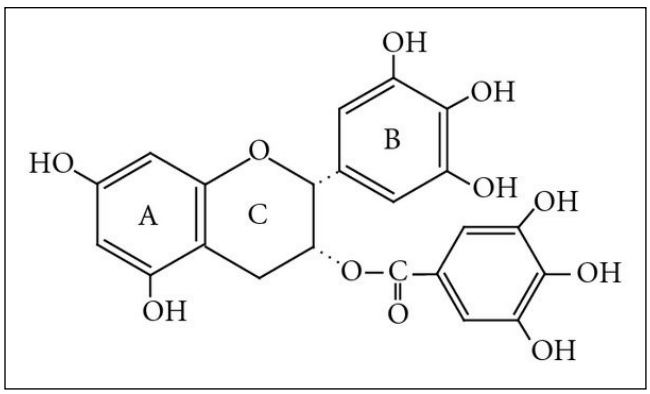

Fig. 5. Structure of epigallocatechin.

contained in green tea, but also in chocolate and wine (Lin et al., 2003; Del Rio et al., 2004; Voung et al., 2010). It has many beneficial properties, such as antioxidant, anti-inflammatory and anti-apoptotic effects (Khalatbary et al., 2018; He et al., 2018). A neuroprotective effect of EGCG was found in Alzheimer's disease, Parkinson's disease, ischemic stroke and spinal cord injury (Cano et al., 2019; Zhou et al., 2019).

Xie et al. (2012) assessed the effect of EGCG (25, 50 mg) on PTZ-induced kindling in rats. The study showed that EGCG dose-dependently inhibited the kindling progress. In addition, EGCG reduced cognitive deficits and oxidative damage caused by PTZ kindling. Therefore, EGCG may be a potential antiepileptic agent. Results obtained by Xie et al. (2012) are consistent with previous Park and coworkers studies (2001), where EGCG (10 mg) administered for 7 days has prevented cocaine-induced seizures and protected against death as well as with earlier experimental models of epilepsy induced by intracortical injection of Fe-ions, where EGCG prevented or slowed the onset of epilepsy (Yokoi et al., 1989).

Qu et al. (2019) examined the effects of EGCG after status epilepticus (SE) on behavior in the rat lithium-pilocarpine model of TLE. Obtained results showed that EGCG treatment after SE tended to reduce the frequency of spontaneous recurrent seizures and the duration of the seizure, partially attenuating the hippocampal late-phase long-term potentiation recording impairment caused by epilepsy. Importantly, histopathological staining of the rat brain showed that after EGCG treatment, the number of surviving pyramidal neurons significantly increased in the CA1 and CA3 regions of the hippocampus, indicating that EGCG has a neuroprotective effect. EGCG was shown to reduce spontaneous chronic seizures by reducing the expression of inflammatory factors such as TLR4, NF-κB and IL-1β expression, which shows that the anti-inflammatory effect can prevent epilepsy from progressing.

Hesperidin

Hesperidin ((*S*)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-[[(2*R*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy-2,3-dihydrochromen-4-one, Fig. 6) belongs to flavanones and is mainly found in lemons and oranges. Hesperidin has been shown to have neuroprotective, anti-inflammatory, analgesic, anti-bacterial, anti-fungal, anti-viral, anti-hypercholesterolemic and anti-cancer properties (Sudha et al., 2001; Cho et al., 2006). Kumar et al. (2014) assessed the effects of hesperidin alone and in combination with gabapentin and diazepam in the mouse PTZ-induced epilepsy. Obtained results indicated that administration of hesperidin (200 mg/kg) 30 min before PTZ administration significantly delayed the onset of seizures. A lower dose of hesperidin (100 mg/kg) in combination with diazepam (0.2 mg/kg) or gabapentin (10 mg/kg) has also been shown to increase the protective effect compared to diazepam and gabapentin used alone. These results suggest that neuroprotective effects of hesperidin against PTZ-induced convulsions may be related to its antioxidant properties and the synergy with the ligands at the GABAA-benzodiazepines receptors. Results obtained from other studies using an aqueous extract made of *Citrus aurantium* tree leaves containing hesperidin, neohesperidin and neohesperidindihydochalcone confirmed anticonvulsant properties of hesperidin against PTZ seizures in adult zebrafish (Rosa-Falero et al., 2015). *C. aurantium* extract at a dose of 28 mg/ml, gave maximum protective effect with very limited toxic effects. Moreover, *in vitro* results using ligand binding assay showed a significant reduction of [(3)H] Glu binding

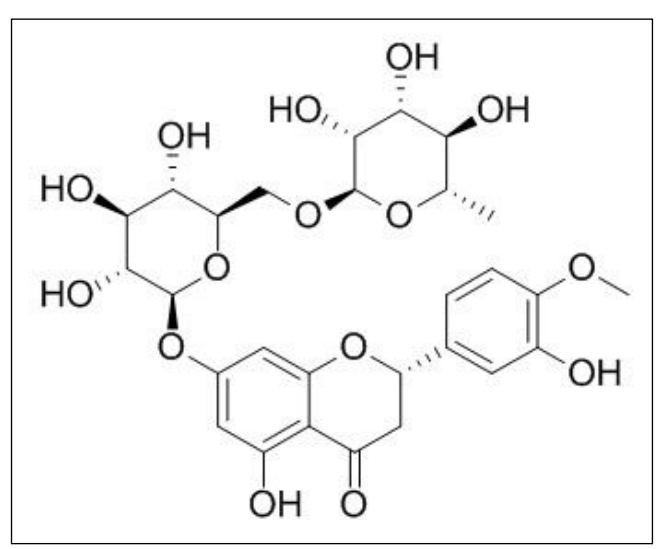

Fig. 6. Structure of hesperidin.

indicating an interaction with glutamate receptors, in particular with NMDA receptors and mGluR II, which suggests that the extract may be an antagonist at the glutamate binding site (Rosa-Falero et al., 2015).

Quercetin

Quercetin (3,3,4,4,7-pentahydroxy flavone, Fig. 7), a natural flavonoid classified as flavonols. Quercetin is found in the apples, blueberries, nuts, grapes, onions or chamomile (Srivastava et al., 2010). Nassiri-Asl et al. (2013) investigated the possible effects of quercetin on oxidative stress and memory retrieval in rat PTZ kindling model. Obtained results indicated that pretreatment with 50 mg/kg of quercetin attenuated seizure severity from the beginning of the kindling procedure by lowering the mean seizure stages compared to the control group. Moreover, quercetin at dose of 50 mg/kg significantly increased the step-through latency of the passive avoidance response compared to the control rats.

Nieoczym et al. (2014) investigated the effects of routine and quercetin in some selected mouse epilepsy models, (6 Hz seizure, maximal electroshock seizure threshold MEST and intravenous (iv) PTZ threshold seizure tests). Rutine and quercetin (400 mg/kg) showed statistically significant anticonvulsant effect in the 6 Hz seizure test. There was no effect on thresholds in MEST and iv PTZ tests in mice. Moreover, they evaluated the effect of quercetin on the anticonvulsant effect of two AEDs, i.e., valproic acid (VPA) and levetiracetam (LEV) in a 6 Hz mouse model. Obtained results showed no significant impact of quercetin on the AEDs anticonvulsant efficacy. Similarly, no side effects in behavior (long-term memory, muscle strength or motor coordination) were observed.

Sefil et al. (2014) showed that quercetin administration (10 and 20 mg/kg i.p.) reduced generalized seizure duration in PTZ (45 mg/kg i.p.) induced seizures in rats, whereas in picrotoxin induced seizures was ineffective. Quercetin also significantly decreased the seizure severity score and prolonged the onset of seizure at 10 mg/kg dose in PTZ induced seizure.

Furthermore, quercetin combined with LEV dose dependently attenuated depressive behavior in PTZ kindling mice (Singh et al., 2017). Long-term treatment with LEV without quercetin significantly reduced seizure severity, but exacerbated depressive behavior in mice with epilepsy and comorbid depression. This is due to the facts that LEV treatment of PTZ mice alters the kynureuin (KYN) and tryprophan (TRP) ratio in both cortical and hippocampal areas of the mouse brain, while LEV + quercetin treatment restores KYN: TRP levels to normal (Singh et al., 2017).

Baicalin

Baicalin ((2S,3S,4S,5R,6S)-6-(5,6-dihydroxy-4-oxo-2-phenyl-chromen-7-yl)oxy-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid, Fig. 8), is a flavone glycoside found in the plant species *Scutellaria*. It has the following pharmacological properties: anti-tumor, antioxidant, antibacterial (Huang et al., 2019). Research by Liu et al. (2012), indicated anticonvulsant and neuroprotective effects of this compound on pilocarpine-induced epileptic model in rats. They showed that pretreatment baicalin (100 mg) delayed PILO-induced seizures in rats as well as decreased animal mortality. Additionally, baicalin reversed the changes in oxidative stress levels in the hippocampus (significantly reduced nitrite, glutathione and lipid peroxidation) compared to the PILO group. Immunihistochemical studies (Nis-

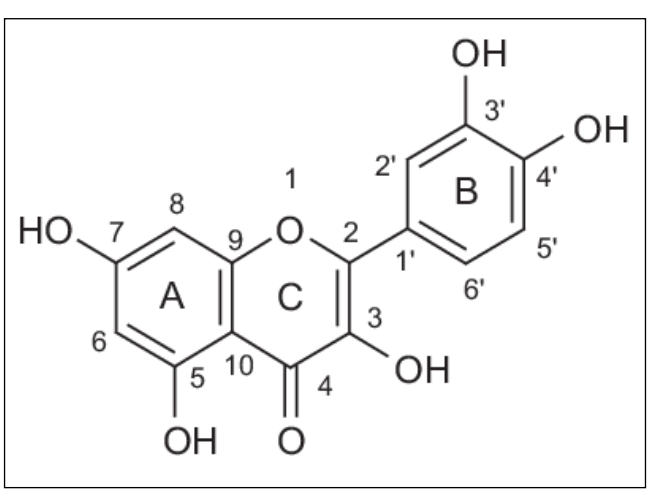

Fig. 7. Structure of quercetin.

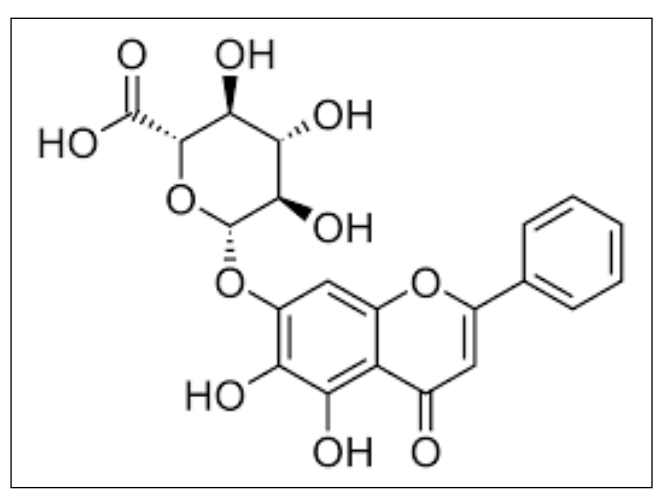

Fig. 8. Structure of baicalin.

sel, Fluoro-Jade B and TUNEL staining) showed that baicalin protected the hippocampus against neuronal loss, apoptosis and pilocarpine-induced degeneration. Liao et al. (2016) demonstrated anti-apoptotic properties of baicalin and proved that it protects against damage to the hippocampus in mice after KA-induced SE. Research indicates a decrease in the expression of miR-49 responsible for neuronal death and an increase in the level of Bcl-2 (an anti-apoptotic protein). It can be concluded that baicalin has a protective effect in SE and could become a potential supplement in brain damage prevention and, with further research, the treatment of epilepsy.

Toxicity studies

Since flavonoids are of great interest due to their broad spectrum of activity, they have also been tested for toxicity. Liang et al. (2017) assessed the toxicity of the flavonoid fraction from *Lithocarpus polystachyus* in rodents. In the case of acute toxicity, doses of 5000 mg/kg were used, and in the case of sub-chronic toxicity, doses were established at 0, 70, 140, 560 mg/kg for 26 weeks. The dose of 5000 mg/kg caused no mortality or any significant toxicity symptoms. In the sub-chronic toxicity experiment in rats no behavioral changes, convulsions, coma, or eating problems were observed. Analysis of blood coagulation parameters and biochemical parameters (creatinine, alkaline phosphatase, glucose, total protein, alanine aminotransferase, aspartate aminotransferase, bilirubin, total cholesterol, triglycerides) showed no significant changes in comparison to the control. Hematology only showed an increase in white blood cell (WBC) levels compared to the control. WBC levels were still normal. Increasing the level of WBC can help to strengthen the immune function of the body. Histopathological examinations did not reveal any pathological changes within organs. Peng and coworkers (2016) studied the toxicity of corn silk flavonoids in mice. General clinical signs, mortality, hematological, biochemical and histopathological parameters were examined. The analysis of the results did not show any significant changes compared to the control group. Research shows that the use of corn silk as functional food is safe.

CONCLUSIONS

Despite high availability of several generations of AEDs, patients resistant to a one drug treatment only require an additional anticonvulsant, which carries the risk of increasing side effects. Flavonoids were shown to have a strong pharmacological effect and a wide spectrum of activity. In the light of the results of numerous *in vivo* studies, there is no doubt that due to the prevalence of flavonoids in the plant world and the presence of vegetables and fruits in the diet, these compounds are of great importance in the prevention of many civilization diseases and could prove effective as an add-on therapy for epilepsy. Thus, flavonoids can be useful for developing new therapeutic strategies for treating epilepsy. Although antiepileptic effects of flavonoids have been verified in preclinical studies, there is a great need for including flavonoids as antiepileptic agents in clinical trials.

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