Research Article / Araştırma Makalesi

Exploring the Potentials of Flavonoids on Brain Function: Effects of Three Phenolic Compounds on Brain Electrical Activity

Flavonoidlerin Beyin Fonksiyonu Üzerindeki Potansiyellerini Keşfetmek: Üç Fenolik Bileşiğin Beyin Elektriksel Aktivitesi Üzerindeki Etkileri

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Abstract

Background: The most common polyphenolic compounds taken up by the organism through the food chain are flavonoids. Known to cross the blood-brain barrier, 7,8-DHF, L-theanine and Fisetin are reported to have neuronal effects as well as therapeutic potential for neurodegenerative diseases. However, little is known on their acute effects of neuronal function. This study aims to describe the effects of the mentioned flavonoids on the total ECoG activities and band analyzes of healthy mice.
Materials and Methods: For this purpose, 3 different groups consisting of 7 subjects were created for each flavonoid administration. After the baseline electrophysiological recordings, flavonoid administration was performed and acute effects were determined.
Results: 7.8-DHF increased the theta, alpha and beta band activities while decreasing the total ECoG power. L-theanine and Fisetin did not significantly alter the total ECoG activity. However, L-theanine statistically increased theta, alpha and beta band activities.
Conclusions: In conclusion, our data showed that flavonoids could acutely modulate the ECoG responses in a band specific manner. They can be considered as candidate molecules for drug discovery studies for central nervous system disorders.

Key Words: Flavonoids, Electrocorticography, Band activities

Öz

Amaç: Organizma tarafından besin zinciri yoluyla alınan en yaygın polifenolik bileşikler flavonoidlerdir. Kan-beyin bariyerini geçtiği bilinen 7,8-DHF, L-theanine ve Fisetin'in nörodejeneratif hastalıklar için terapötik potansiyellerinin yanı sıra nöronal etkilerinin de olduğu bildirilmiştir. Bununla birlikte, nöronal fonksiyon üzerindeki akut etkileri hakkında çok az şey bilinmektedir. Bu çalışma, söz konusu flavonoidlerin sağlıklı farelerin toplam ECoG aktiviteleri ve bant analizleri üzerindeki etkilerini açıklamayı amaçlamaktadır.
Materyal ve Metod: Bu amaçla her bir flavonoid uygulaması için 7 hayvandan oluşan 3 farklı grup oluşturulmuştur. Bazal elektrofizyolojik kayıtların ardından flavonoid uygulaması yapılmış ve akut etkiler belirlenmiştir.
Bulgular: 7.8-DHF, toplam ECoG gücünü düşürürken teta, alfa ve beta bant aktivitelerini artırdı. L-theanine ve Fisetin, toplam ECoG aktivitesini önemli ölçüde değiştirmedi. Bununla birlikte, L-theanine teta, alfa ve beta bant aktivitelerini istatistiksel olarak arttırdı.
Sonuç: Sonuç olarak, verilerimiz flavonoidlerin akut ECoG yanıtlarını banda özgü bir şekilde modüle edebildiğini gösterdi. Merkezi sinir sistemi bozuklukları için ilaç keşif çalışmalarında aday moleküller olarak kabul edilebilirler.

Anahtar Kelimeler: Flavonoidler, Elektrokortikografi, Bant aktiviteleri

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Introduction

Plants produce polyphenols that contain single or multiple hydroxyl (OH) groups attached to them as well as one or more aromatic ring structures. Polyphenol molecules, classified as secondary metabolic byproducts, considered highly essential functional foods in our diet. The polyphenolic compound most commonly taken up by the organism through the food chain is flavonoids (1). Based on a large body of the findings obtained from in vivo and in vitro experimental studies, flavonoids have a broad spectrum of biological activities. Many of these activities bear therapeutic properties such as antimicrobial, antioxidant, anti-inflammatory, antihypertensive, anti-diabetic effects and immune modulator (2-5). In this context, they have been studied to reduce chronic disease factors and as antioxidant agents in certain specific disease states (e.g., cancer, diabetes) (6-8). The forementioned studies have shown that phenolic compounds have poor absorption in the small intestine, pass 5-10% into the bloodstream and can be transported to other tissues. However, there is a lack of experimental evidence as to whether flavonoids can cross the BBB (9). Studies on some flavonoids, which are thought to be able to show their therapeutic effects especially against neurodegenerative diseases and to be used in the fight against neuronal disorders, shed light on this issue.

Fisetin (3,3',4',7-tetrahydroxyflavone), whose chemical formula was explained by Josef Herzig in 1891, is a flavonoid that has been used for a long time as a phytomedical compound (10). With an average daily intake of 0.4 mg, fisetin is found in the highest concentration in strawberry (160 µg/g) (11). While fisetin is partially soluble in aqueous buffer solution, it is noticeably soluble in DMSO (dimethyl sulfoxide) at 25°C at approximately 30 mg/ml and emits a yellow color (10). After oral or intraperitoneal administration, fisetin can rapidly pass through blood vessels and be distributed to the brain parenchyma. In addition, fisetin has chemo-preventive, anti-metastatic, antioxidant and anti-inflammatory effects.

7,8-Dihydroxyflavone (7,8-DHF) is a natural flavonoid derivative found in the leaves of Godmania aesculifolia, Tridax procumbens and Primula halleri (12). The compound has also been identified in the whole plant Lepisorus ussuriensis, a traditional Chinese medicine for antipyretic and detoxification (13). 7,8-DHF crosses the blood-brain barrier (BBB). Due to its potential to activate TrkB (Tropomyosin receptor kinase B), it can be used effectively to combat neuropsychiatric disorders. This compound has been shown to be neuroprotective in various disease conditions such as Alzheimer's disease, Parkinson's disease, Rett syndrome, and Huntington's disease (14).

Theanine, (γ-glutamethylamide), is a non-protein amino acid found in Camellia species and the edible laurel boletes mushroom Xerocomus badius, but otherwise rare in nature. It is the main amino acid in tea and is thought to be a flavoring component of tea leaves (15). It makes up 1% to 2% of the dry weight of tea, which corresponds to 25-60mg per 200ml serving. The dominant form of theanine in tea is the L isomer. It is thought that L-theanine crosses the blood-brain barrier and shows its effects directly on the brain within 30 minutes (16).

Studies have shown that a number of flavonoids exhibit direct effects on neurological function considering the neurodegenerative disease. However, most of existing studies have used in vitro models, ignoring the metabolism and matrix axis. Most in vivo studies consist of epidemiological studies and some clinical applications. In vivo animal experiments with the continuity of physiological and metabolic functions are important for understanding the contribution of flavonoids to neurological function in pathological neuroconditions.

In this study, it is aimed to determine the short time effects of the three flavonoids, 7,8-DHF, L-theanine and fisetin, on brain function in mice through in vivo ElectroCorticoGraphy (ECoG) recordings. Results revealed that two of the molecules have a strong modulatory effect of the power of signals and this effect was band specific.

Materials and Methods

Subjects

3 month old male C57BL/6 wild type (WT) mice were used. Mice were obtained from the KTU Medical Faculty Surgical Research Center, where they were housed and bred. Mice weighing 20-25 grams were maintained on a 12:12 hour light-dark cycle (light on at 7:00 am) and room temperature 20 ± 2 °C. Standard feed pellets and tap water were available ad libitum throughout the experiment. No protocol was applied to the animals before the experiments. All maintenance and experimental procedures were approved by the Animal Ethics Committee of Karadeniz Technical University. (Approval No: 2023/26).

Experimental Design and Application

Three different groups of 7 animals were formed for the administration of fisetin, 7,8-DHF and L-theanine. The animals in each group were primarily administered urethane anesthesia. For this, urethane was dissolved in sterile isotonic saline solution as a 25 % solution and injected intraperitoneally a dose of 1.75 gr/kg. Sleep status of all anesthetized animals was controlled by finger pinch method. The right cerebral cortex was carefully exposed by craniotomy, than the head of the animal was fixed by using standart stereotaxic methods as shown in Figure 1. Body temperature was maintained at 37°C with a homeothermic blanket system (Harvard Homeothermic Blanket, USA). Ag/AgCl ball electrodes were placed in the cortical region of the right hemisphere (electrodes coordinates: first electrode, 1.5 mm lateral to sagittal suture and 1 mm anterior to bregma; second electrode, 1.5 mm lateral to sagittal suture 3 mm posterior to bregma). The grounding electrode was placed on the tail.

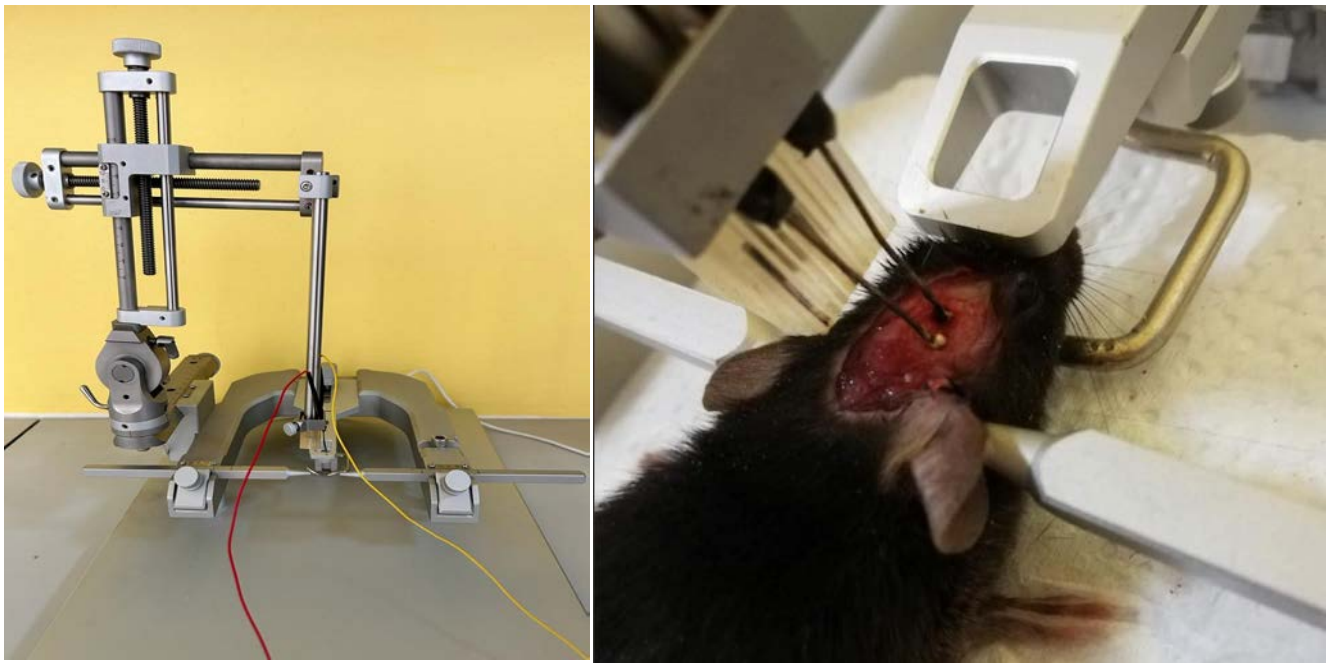


Figure 1. Stereotaxic frame was used to fix the head of animal and Ag/AgCl ball electrodes were placed in the cortex as shown.

First of all, basal ECoG recordings were taken for 10 minutes from all animals in this way, and then drug administration was performed depending on the group they belonged to. The groups are as described below;

Group 1; It consists of 7 animals. After basal records 7.8-DHF was injected intraperitoneally at a dose of 10 mg/kg. 7.8-DHF (7.8-DHF; Sigma Aldrich,Saint Louis, MO, USA) was dissolved in 17% DMSO/PBS. After waiting for the physiological distribution of the drug, ECoG recording was taken for 20 minutes.

Group 2; It consists of 7 animals. Following baseline recordings, 100 mg/kg L-theanine (L-theanine; Sigma Aldrich, Saint Louis, MO, USA) was injected intraperitoneally and ECoG recording was taken for 20 minutes after drug distribution.

Group 3; It consists of 7 animals. After baseline recordings, 250 mg/kg fisetin (Fisetin; MedChem Express) was injected intraperitoneally. Fisetin was dissolved in 0.1% DMSO/PBS. After waiting for the distribution of fisetin, 20 min ECoG recording was taken in the same procedure.

While determining the acute drug doses applied in the study, an average value was chosen based on the studies in the literature (17-19). After all procedures, animals were decapitated.

Electrophysiologic Techniques

Multi-channel data acquisition system (Axon CNS digidata 1440A) was used to record the electrical activity of cortex. The signals from the electrodes were amplified (x10k) and filtered (0.1-50 Hz bandpass) using A-M Systems Differantial AC Amplifier 1700. They were digitized at a sampling rate of 1 kHz. All recordings were stored on a computer.

Data Analysis

EEG analyzes (power spectrum and EEG band analyzes) of the obtained data were performed offline using Clampfit 10 software. Fast Fourier Transform (FFTs) of signals of 15 minutes recordings were calculated as power by using Hamming windows function. The spectral resolution was 1.22 Hz. The values of 1 s windows were averaged without omitting or excluding any of the windows. 0.5-50 Hz range of all spectra was used for further analysis. EEG power was computed in the selected frequency bands; delta band (0.5-4 Hz), theta band (4-8 Hz), alpha band (8-13 Hz), beta band (13-30 Hz), and gamma band (30-50 Hz). Absolute power was calculated for each frequency band.

Statistical Analysis

Statistical intragroup comparisons were made using GraphPad Prism 6 and paired t-test (Wilcoxon test). Statistical significance was set at $p<0.05$. The results are given as the means±SEM.

Results

In this study, the effect of three flavonoids on basal EEG activity in adult mice was investigated. For this purpose, EEG recordings of animals were taken before the application and immediately after the application of 7,8-DHF, L-theanine and fisetin to separate animal group. Based on the results of the obtained data, it was found that 7.8-DHF application had a significantly reduced the total power ($p=0.0156$). L-theanine and fisetin administration caused a general decrease when compared to basal recordings, but it was not statistically significant. Examples of recordings are shown in Figure 2.

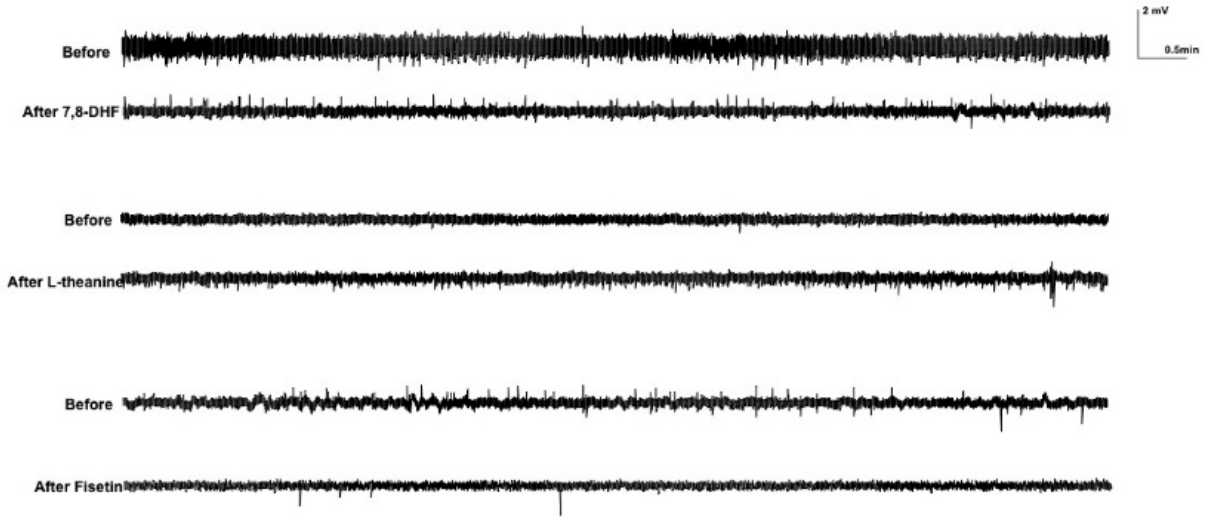


Figure 2. Sample traces of the original electrocorticography recordings before and after flavonoids administration. All recordings were obtained from cortex and presented at 10 minutes.

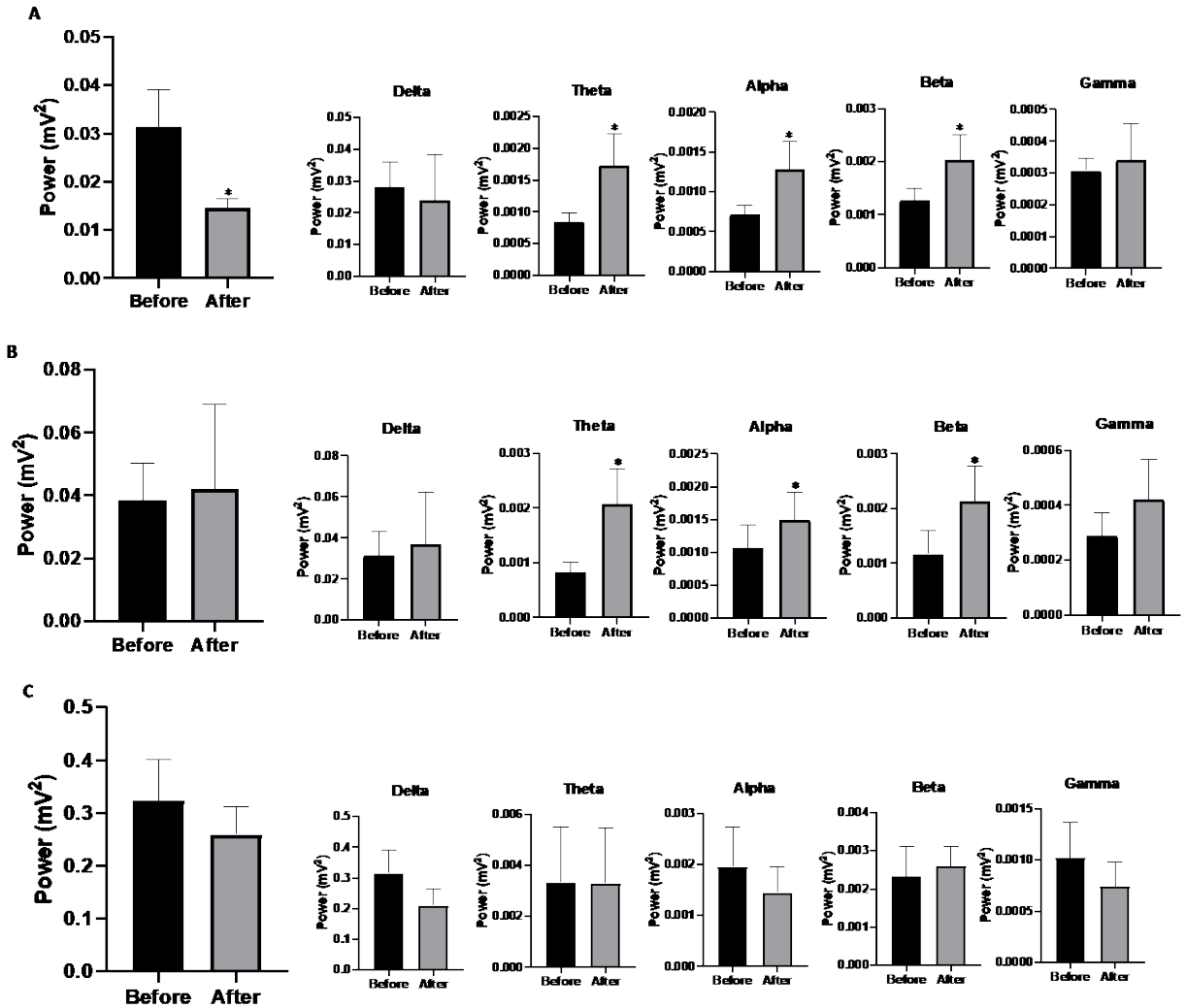
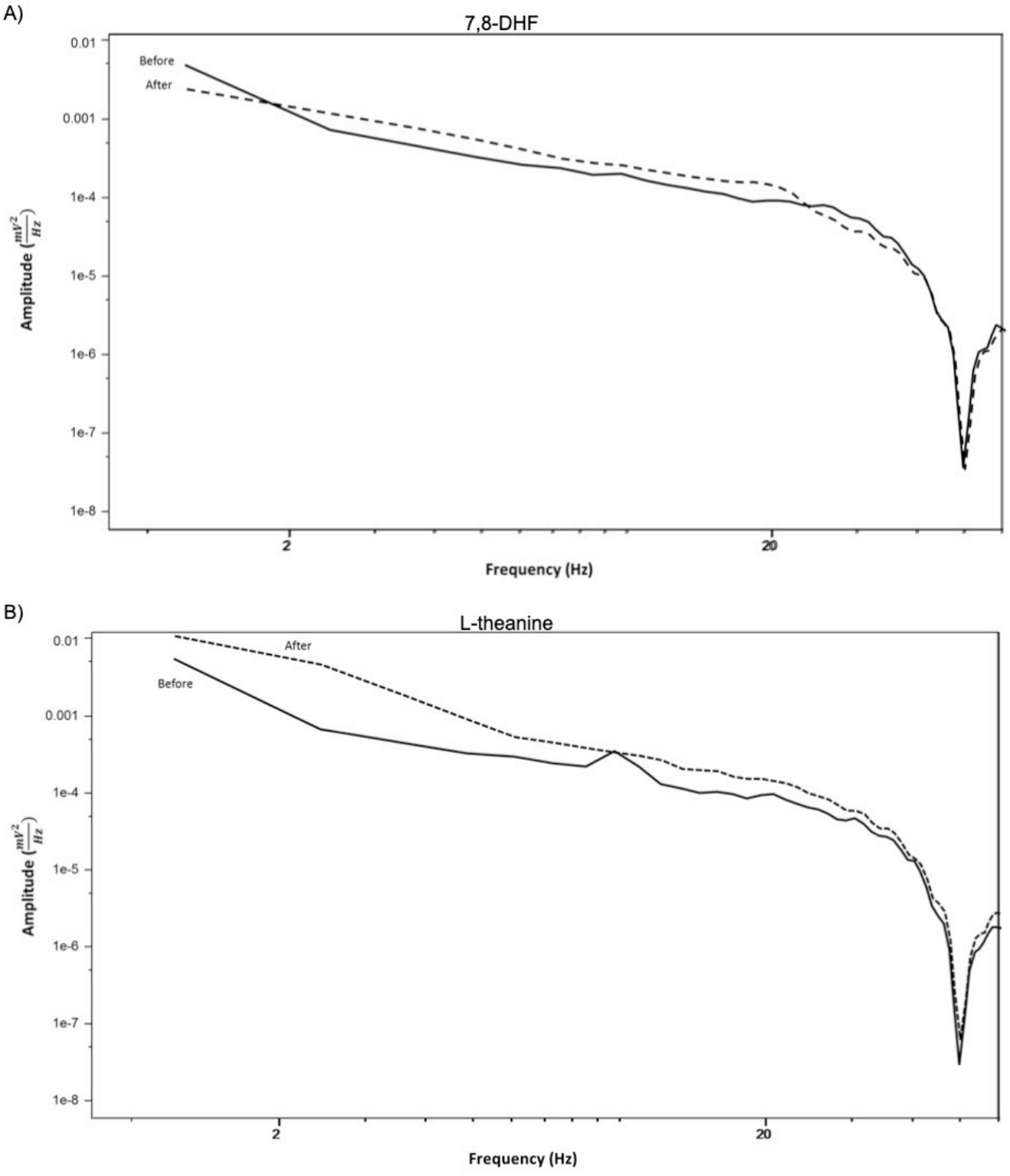


Figure 3. Spectral power analysis of ECoG recorded experimental groups. Astrerix indicate significant differences between bars (*p< .05). A) The absolute power of each EEG band. The acute application of 7,8-DHF altered the absolute power and bands of tetha, alpha, beta significantly. B) The absolute power of each EEG band. The acute application of L-theanine altered power of tetha, alpha and beta bands significantly. C) The absolute power of each EEG band. The acute application of fisetin did not alter power of any EEG band significantly

In addition, delta, theta, alpha, beta and gamma band analyzes were performed in EEG recordings. Especially after 7,8-DHF and L-theanine applications, a significant increase was detected in the total power analysis in theta, alpha and beta bands. However, there was no significant change in band analysis after fisetin application.

Bar graphs are presented in Figure 3.

The graphic representation of the EEG recordings of each flavonoid application, especially the band frequencies, clearly shows the changes in the total power averages of the recordings in Figure 4.



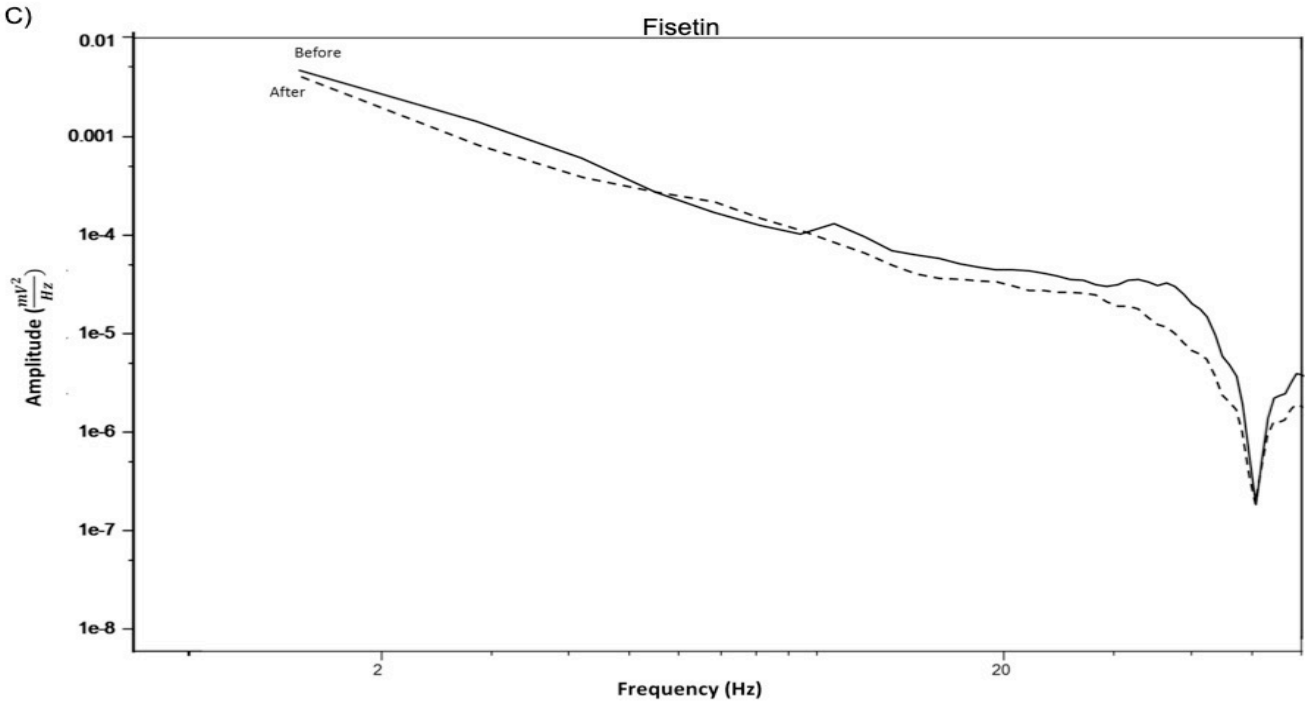


Figure 4. Basal and A) 7,8-DHF (10 mg/kg, i.p.) B) L-theanine (100 mg/kg, i.p.) and C) Fisetin (250 mg/kg, i.p.) administrated average power spectrum.

Discussion

Phenolic compounds that cross the blood-brain barrier may interact with neurological targets. Therefore, they have been extensively studied and some of them have been proposed as therapeutic agents in various neurological disorders. To be able to provide a better insight to the acute modulation of neuronal functions by phenolic compounds, current study investigated changes in cortical ECoG spectral power (alpha, beta, etc. oscillations) in healthy mice, in response to 7,8-DHF, L-theanine, and fisetin administration. Although there was no significant change in fisetin administration, exposure to 7,8-DHF and L-theanine significantly increased the powers of the theta, alpha, and beta bands in particular.

ECoG band power analyzes consist of 5 bands, each of which is in a different frequency range, representing specific brain activity. Delta wave activity in the 0.5-4 Hz frequency range is an important component for deep sleep. In its most general definition, it gives information about sleep quality (20-22). Theta wave activity, which is in the 4-8 Hz frequency range, is a band observed in both sleep (REM) and wakefulness states, and its relationship with learning and memory has been defined in the literature (23-25). The alpha band, defined as the 8-13 Hz frequency range, is associated with quiet waking. Finally, beta (13-30 Hz) and gamma (30-50 Hz) band activities with higher frequency range were matched with attention and other cognitive activities (26-28).

Studies have found that fisetin, which is known to bind to HCN2 channels with high affinity, significantly reduces ictal discharge duration, especially in mice with epilepsy models (29). In addition, there are studies providing evidence that fisetin is neuroprotective in animal models of different disease models and cell culture studies. The therapeutic effects of fisetin lie in its antioxidant properties as well as its interaction with many pathways associated with neurological disease (30). According to a study on the cognitive and behavioral effects of fisetin depending on age, the relative spectral power of α and the relative spectral power of β was higher in aged fisetin supplemented rats than aged controls. This study demonstrates that fisetin's role in improving electrical communication is regulatory (31). However, this study conducted in healthy adult mice, no significant change was found due to fisetin administration. Therefore, in addition to studies proving the neuroprotective and neurotherapeutic effect of fisetin, especially in pathological conditions, we can say that it does not have a significant effect on EEG bands under normal conditions.

7,8-DHF, a TrkB agonist that can mimic BDNF, has been extensively investigated in various neurologic disease models. It exhibits physiological functions such as promoting neuronal survival, enhancing synaptogenesis, learning and memory. In addition, its therapeutic effect in neurological diseases has been studied and demonstrated for many models. However, in a fresh brain slice study, it was determined that epileptic discharges caused a significant increase and showed that excessive activation of the TrkB pathway could lead to devastating results (32). In addition to studies showing that BDNF microinjections increase slow wave activity, it has been determined that 7,8-DHF application significantly

increases the powers of alpha and sigma bands, especially in EEG bands (33, 34). This is consistent with the results of our study. In healthy mice, dose-dependent 7,8-DHF treatment significantly increased slow wave activity. This may be due to the active role of the TrkB pathway in excitability.

There are studies suggesting that L-theanine exhibits neuroprotective effects against neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. In addition, opinions about the need for further investigation of its neuroeffective properties have also been reported (35). Besides, there are studies reported the relation between L-theanine and electrophysiological alterations. A human study conducted to examine the effect of GABA (gamma-aminobutyric acid) administration showed that L-theanine increased alpha band power compared to basal activity (36). A different study supports the higher alpha band activity of administered L-theanine versus placebo (37). Similarly, an EEG study showed that acute administration of L-theanine increased alpha activity (38). In line with those findings, our results showed that alpha activity is increased by L-theanine administration. The obtained results verified the effect in an animal model.

Conclusion

This study, in which electrophysiological band analyzes and total power activities are presented, contains new information in terms of the effect of phenolic compounds 7,8-DHF, L-theanine and fisetin on the basal recordings of healthy mice. It fills the gap in the literature in terms of determining its contributions not only to disease states but also to healthy individuals. We would like to draw attention to the fact that flavonoids, which are known to exist in abundant types and amounts in nature, may easily cross blood-brain barrier and interact with neuronal targets. Hence, they are precious molecules with a potential to be utilized in drug discovery research and will contribute to the treatment of various neurological diseases.

Ethical Approval: *Ethical committee approval of this study was obtained from "Animal Ethics Committee of Karadeniz Technical University, Trabzon, Türkiye" (Approval No: 2023/26).*

Author Contributions:
Concept: H.Ö., İ.A.
Literature Review: H.Ö., H.B.
Design : H.Ö., İ.A., S.A.
Data acquisition: H.Ö., H.B.
Analysis and interpretation: H.Ö., S.A.
Writing manuscript: H.Ö.
Critical revision of manuscript: İ.A., S.A., H.B.

Conflict of Interest: *The authors have no conflicts of interest to declare.*

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