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ORIGINAL ARTICLE

Daidzein Inhibits Pentylenetetrazol-Induced Seizures in Mice

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Abstract

Background: Phytomolecules, through their antioxidant and anti-inflammatory effects, can improve neurodegenerative diseases. The aim of this study was to evaluate the effect of daidzein, a phytoestrogenic agent, on seizure severity and oxidative stress in a pentylenetetrazol (PTZ)-induced seizure model.

Methods: Male mice were injected intraperitoneally (i.p.) with daidzein (1 and 5 mg/kg) and, 30 minutes later, a single convulsive dose of PTZ was administered. The groups were as follows: PTZ Control, PTZ+Diazepam, PTZ+Daidzein 1 mg/kg, PTZ+Daidzein 5 mg/kg, and PTZ+DMSO. Seizure onset and seizure stages were scored over a 30-minute observation period according to the Racine classification. Serum samples were collected to evaluate oxidative stress parameters using an automated colorimetric method.

Results: Administration of daidzein at a dose of 1 mg/kg (first seizure presentation and modified stage p=0.001), but not 5 mg/kg, inhibited seizure formation similarly to diazepam. Neither dose of daidzein (1 and 5 mg/kg) increased serum antioxidant parameters (total thiol (p=0.425), native thiol(p=0.350)).

Conclusion: Low-dose daidzein can prevent seizure while causing a smaller increase in oxidative stress parameters compared to diazepam suggests that it may have potential as an anticonvulsant agent.

Keywords: Daidzein; epileptic seizure; oxidative stress.

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INTRODUCTION

Epilepsy is a chronic neurological disorder that leads to recurrent spontaneous seizures due to an imbalance between excitatory and inhibitory systems in the relevant areas of the brain (1). The pathological changes that epileptic seizures cause in the brain affect neuropsychological functions such as attention, memory, learning, and mental flexibility (2). While symptomatic treatment is partly achievable with current antiepileptic drugs, in some patients the seizures remain uncontrolled, and resistance and toxicity are observed in 20–30% of cases against well-known antiepileptic drugs. Moreover, due to the numerous side effects and low tolerability of current antiepileptic drugs, uncontrolled discontinuation of medication by patients is frequently encountered (3). Therefore, alternative treatment methods are being investigated in light of the biochemical, pharmacological, and pathophysiological data obtained from experimental studies (4).

Oxidative stress, which results from an imbalance between reactive oxygen species (ROS) and antioxidant defense mechanisms that can lead to irreversible cell damage, plays a role not only in the onset and progression of epilepsy but also in the pathogenesis of many neurodegenerative disorders (5). Brain tissue, containing a large amount of lipids and consuming oxygen at a high rate, is very susceptible to lipid peroxidation (6). When the balance between free radical production and the antioxidant system that compensates for the increase in free oxygen radicals is disrupted in favor of free radical production, oxidative damage occurs. Free radicals lead to degeneration of cells in the central nervous system (7). Thiols, which are organic sulfhydryl compounds with active –SH groups, form reversibly oxidized disulfide structures due to their strong reducing capacities. These disulfides are converted back to thiols by the enzymes thioredoxin reductase and glutathione reductase (8). In thiol-disulfide homeostasis, which represents the balance between antioxidants and oxidants, oxidative stress occurs when the balance shifts toward disulfides (9,10).

It has been demonstrated that oxidative stress plays a role in the emergence and progression of various neurodegenerative disorders (11). Another well-known fact is that reducing oxidative stress by activating antioxidant mechanisms can slow down and treat the symptoms of neurodegenerative diseases (12). Numerous studies in the literature have shown that phytomolecules, through their antioxidant, anti-inflammatory, and estrogenic effects, can improve neurodegenerative diseases and reduce neuroinflammation (13). Daidzein, one of the phytoestrogens, is a type of isoflavone with neuroprotective activities in various neurobiological mechanisms in the central nervous system, such as behavior, cognition, growth, development, and reproduction (14). The neuroprotective effect of daidzein, which reduces free oxygen radicals, has been demonstrated in focal cerebral ischemia (15) and in an Alzheimer's disease model (16). However, although it is known that the neuroprotective properties of soy proteins change in a dose-dependent manner (17), their acute effects on epilepsy and epileptic seizures remain unknown.

In this study, based on the hypothesis that daidzein would prevent epileptic seizures, the anticonvulsant efficacy of daidzein was evaluated in an experimental seizure model induced by pentylenetetrazol (PTZ).

MATERIALS AND METHODS

Animals

The experimental animals were obtained from the BAIBU Experimental Animals Center. Male Swiss albino mice weighing 25–30 g were randomly divided into groups and maintained under a 12-hour dark/12-hour light cycle at a temperature of 20–21°C and 55–60% relative humidity, with ad libitum access to water and pellet feed. The methods used for animal experiments were organized in accordance with the protocols of the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Daidzein was administered at doses of 1 and 5 mg/kg 30 minutes prior to the PTZ injection. The mice were monitored with a video camera system for 30 minutes after the PTZ injection (18). In the video recordings, the time of the first seizure presentation (minute) was determined, and seizure scoring was performed according to Racine classification (Table 2) (19).

After video seizure recordings were obtained from all mice, under general anesthesia with ketamine and xylazine (100 mg/kg and 20 mg/kg i.m.), 1 ml of blood was collected via intracardiac puncture. The samples collected in biochemical tubes were centrifuged at 1500 G for 15 minutes at room temperature, then transferred into at least two separate Eppendorf tubes within 1 hour and frozen at -80°C (Figure 1).

Table 1. Groups and drug names, drug doses and applications		
PTZ Control	n=8	60 mg/kg PTZ (solvent: 0.9% salin, 0.1 ml), i.p.
PTZ + Diazepam	n=7	5 mg/kg (0,1 ml), i.m.
PTZ + Daidzein 1 mg/kg	n=7	1 mg/kg (solvent: %5 dimethyl sulphoxide (DMSO)), s.c., (20) (ChemScene cat no:CS-2332)
PTZ + Daidzein 5 mg/kg	n=7	5 mg/kg (solvent: %5 dimethyl sulphoxide (DMSO)), s.c., (21)
PTZ + DMSO	n=7	%5 DMSO, s.c., (18)

Table 2. Racine classification (21)	
Score 1	• Immobilization, with no convulsive activity.
Score 2	• Head nodding with twitching of the ear and facial muscles.
Score 3	• Myoclonic jerks.
Score 4	• Rearing with forelimb clonus.
Score 5	• Jumping, generalized clonic seizures, and falling.
Score 6	• Tonic extension of the hind limbs, leading to tonic-clonic convulsions.

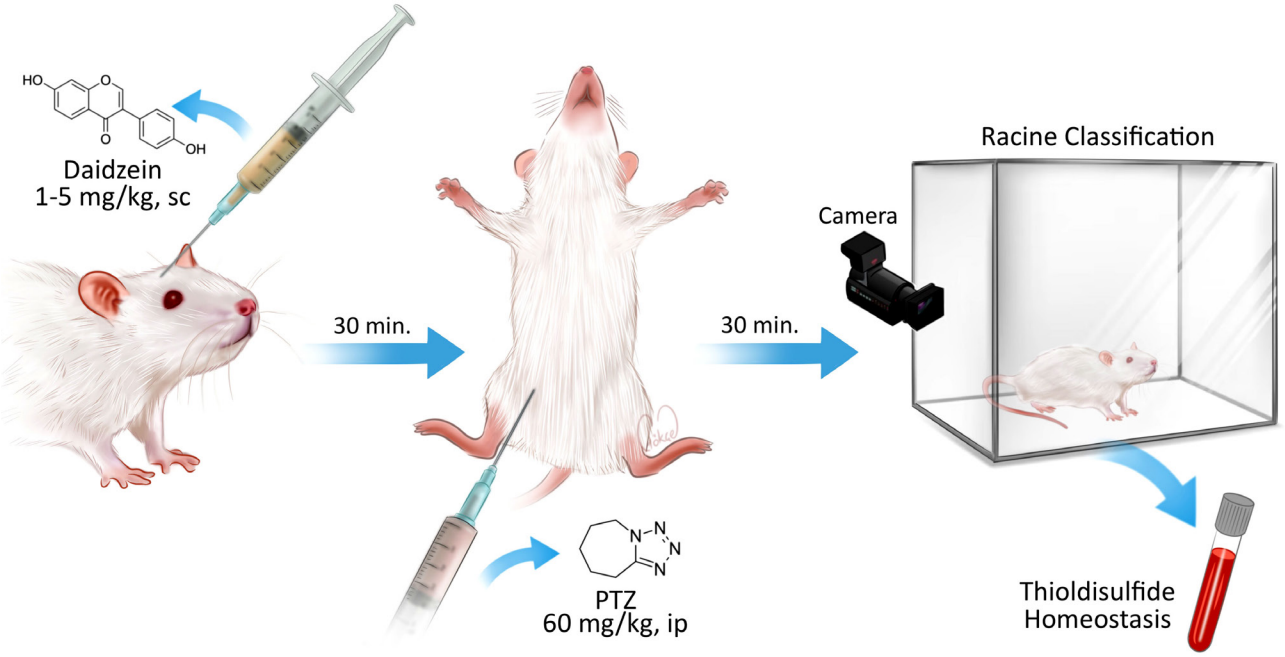

Figure 1: Experimental flowchart

Measurement of Serum Thiol-Disulfide Homeostasis Parameters

Serum thiol-disulfide homeostasis parameters were determined using the automated colorimetric method developed by Erel and Neselioglu. This method consists of two steps. In the first step, the native thiol levels present in the sample are detected by measuring the absorbance at 412 nm of the yellow color formed by the reaction of thiol groups with DTNB [5,5'-dithiobis-(2-nitrobenzoic) acid]. In the second step, the dynamic and reducible disulfide bonds (-S-S) are reduced to free functional (reactive) thiol groups (-SH) in the presence of sodium borohydride 'NaBH4'. The unused reducing agent, NaBH4, is eliminated by reacting with formaldehyde. Subsequently, the total thiol groups, which include both the NaBH4-reduced and native thiols, are detected following reaction with DTNB. After determining the concentrations of native thiol (-SH) and total thiol (-SH + -S-S), half of the difference between these two values gives the amount of dynamic disulfide. Additionally, the percentage ratios of disulfide (-S-S) to native thiol (-SH), disulfide (-S-S) to total thiol (-SH + -S-S), and native thiol (-SH) to total thiol (-SH + -S-S) are calculated (8).

Statistical Analysis

The data were evaluated in the IBM SPSS Statistics 25.0 (IBM Corp., Armonk, New York, USA) statistical package program. The normal distribution of the data of numerical variables was evaluated with the Shapiro Wilk test of normality. Levene's test was used to assess the homogeneity of group variances. Descriptive statistics of the data are presented as n (%) and mean±standard deviation (x±SD) if the variable is normally distributed, otherwise as median (minimum-maximum) or median (1st quartile -3rd quartile). Comparisons between groups were made with one-way analysis of variance for normally distributed variables, and Kruskal-Wallis analysis for non-normally distributed variables. Tukey HSD was used for normally distributed variables and Mann-Whitney U test with Bonferronni correction was used for non-normally distributed variables as a multiple comparison test. A p value of <0.05 was considered statistically significant.

RESULTS

Comparing the five groups in terms of the first seizure presentation, significant differences were observed between PTZ Control group and PTZ + Diazepam and PTZ + Daidzein 1 mg/kg groups ($p = 0.001$). However, no significant differences were found between PTZ Control group and the PTZ+Daidzein 5 mg/kg and PTZ+DMSO. The mean value of PTZ+ DMSO group was significantly lower than that of PTZ + Diazepam, PTZ + Daidzein 1 mg/kg, and PTZ + Daidzein 5 mg/kg, except for PTZ Control group (for each, $p< 0.01$). Additionally, the mean of the PTZ + Daidzein 5 mg/kg group was significantly lower than those of PTZ + Diazepam and PTZ + Daidzein 1 mg/kg groups. Based on this result, seizures in the PTZ + Diazepam and PTZ + Daidzein 1 mg/kg groups had a delayed onset (Figure 1).

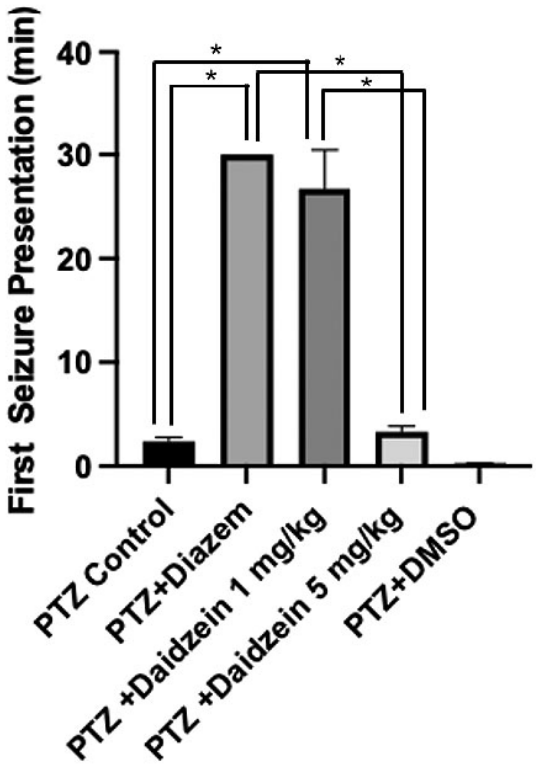

Figure 2: First seizure presentation (minute).

In terms of the modified stage, it was observed that the PTZ + Diazepam and PTZ + Daidzein 1 mg/kg groups were significantly lower than PTZ + Daidzein 5 mg/kg,

PTZ Control, PTZ+DMSO (for each, p<0.01). Additionally, the mean of PTZ+DMSO group was significantly higher than that of PTZ + Diazepam, PTZ + Daidzein 1 mg/kg, and PTZ + Daidzein 5 mg/kg, PTZ Control. No other differences were found to be significant (Figure 3).

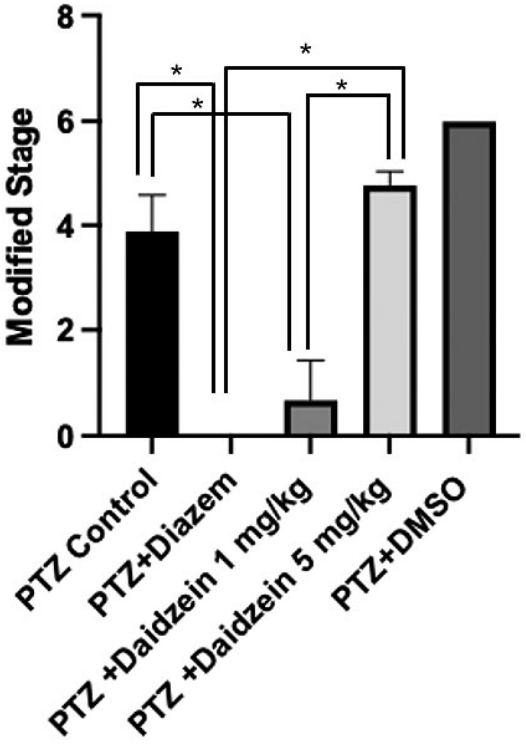

Figure 3: Modified Stage Between Groups.

No significant difference was found among the groups in terms of the average total thiol and native thiol levels. Regarding the disulfide (SS) levels, the differences between the PTZ Control and PTZ + Daidzein 5 mg/kg groups (p = 0.012), PTZ Control and PTZ + Daidzein 1 mg/kg groups (p = 0.018), PTZ Control and PTZ + Diazepam groups (p = 0.004), and DMSO and PTZ + Diazepam groups (p = 0.05) were significant. In terms of the SS/SH ratio, the differences between the PTZ Control and PTZ + Daidzein 1 mg/kg groups (p = 0.014), PTZ Control and PTZ + Daidzein 5 mg/kg groups (p = 0.008), and PTZ Control and PTZ + Diazepam groups (p = 0.003) were significant (Figure 4).

DISCUSSION

According to our study results, 1 mg/kg daidzein prevented seizure formation similar to of diazepam; however, both doses of daidzein (1 and 5 mg/kg), which did not increase antioxidant parameters, demonstrated an increased oxidative stress.

Soybean isoflavones (genistein, daidzein, and the daidzein metabolite S-equol) are known as phytoestrogens due to their structural similarities to endogenous estrogen, 17-β-estradiol (22, 23). Experimental studies have shown that they exhibit strong neuroprotective properties against neurodegenerative diseases, modulate neurotransmitters (24), and protect against neurodegeneration by interacting with estrogen receptors (25). One of the neurodegenerative diseases, studies investigating the effects of soy-based isoflavones on epilepsy, were evaluated and in the literature, another isoflavone, genistein, has been shown to prolong seizure latency and reduce seizure intensity scores as well as the duration of generalized tonic-clonic seizures in a PTZ-induced epilepsy model (26). Another isoflavone, biochanin A, has also demonstrated antiepileptic characteristics in a PTZ-induced epilepsy model (27). In our study, it was found that 1 mg/kg daidzein prevented seizure formation with an effect similar to diazepam, whereas the 5 mg/kg dose did not exhibit anticonvulsant effects. Consistent with our study results, an another study reported that low doses of daidzein showed anxiolytic effects, but this effect was not observed at higher doses (28), and that a high-dose (750 mg/kg) daidzein diet triggered epileptic seizures (29). Similarly dose dependent, it is known that, among soy-based isoflavonoids, genistein at low doses (1–5 mg/kg) reduces aggressive behaviors, whereas at high doses (100–300 mg/kg) it triggers anxiety and aggression (30, 31). While no increase in epilepsy risk has been observed with the formula milk used for infants in the Korean population, an increased frequency of febrile convulsions has been reported with soy-based formula milk used in the American population (17). In conclusion, we can state that a soy-based diet, particularly with daidzein, exhibits dose-dependent proconvulsant-anticonvulsant effects, and that low doses fall within a safe range for epileptic seizures. In addition, the demonstration that genstein and daidzein block T-type calcium channels

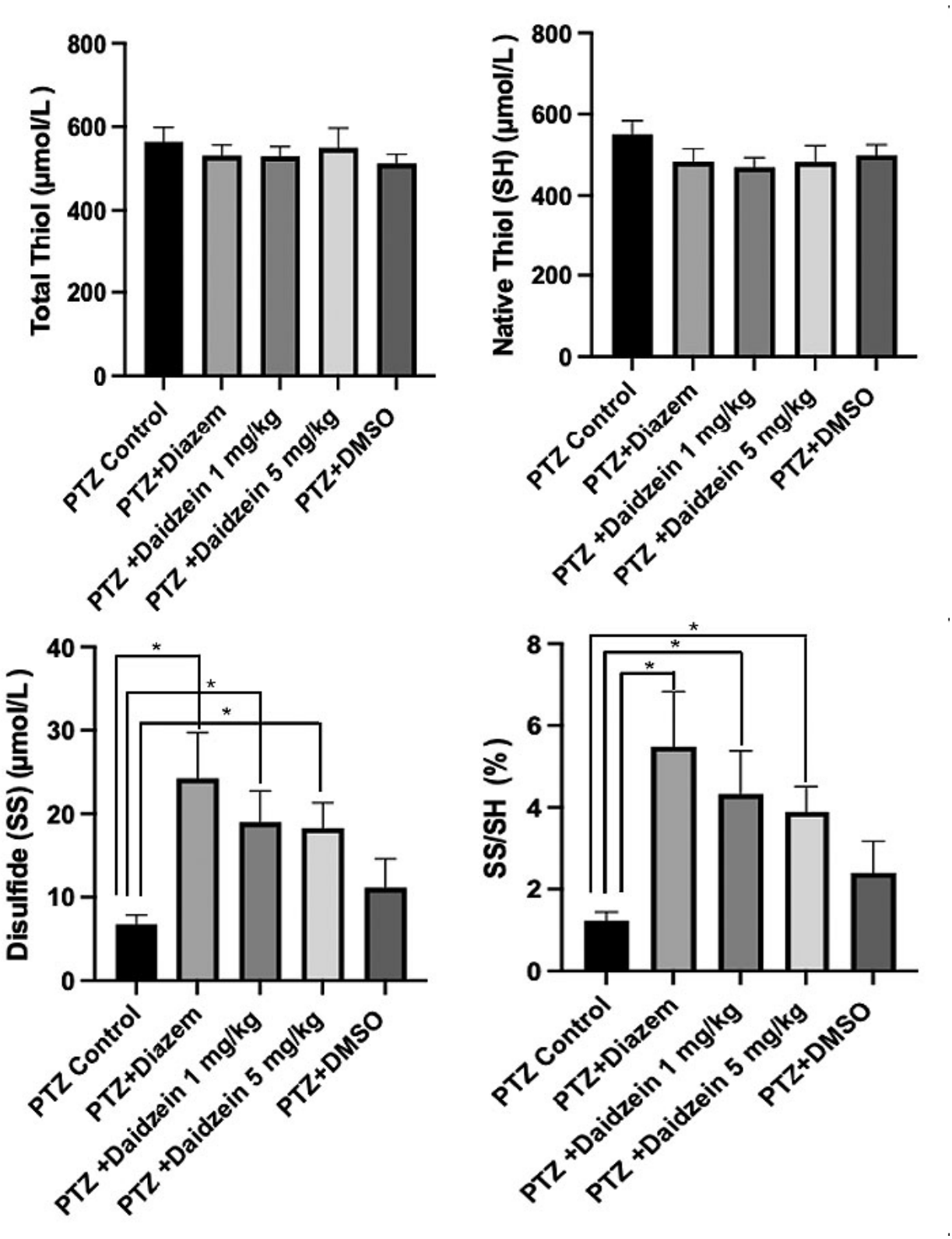

Figure 4: Comparison Results of Thiol Measurements Among Groups

in a dose-dependent manner explains our study results from a pathophysiological perspective (32).

It is also known that daidzein exerts its therapeutic effects through its potent antioxidant properties (33). Daidzein has been shown to exhibit antioxidant effects in traumatic brain injury (34), reduce hypothalamic oxidative stress (35), and decrease free radicals in focal cerebral ischemia (15). Contrary to expectations in our study, both 1 and 5 mg/kg daidzein were found to increase disulfide levels compared to the epilepsy control, indicating an oxidant effect. In a related neuron culture study, it was shown that 50 and 100 micromolar daidzein increased LDH release in neurons (36). Additionally, in a group of men and women who were fed a soy diet for one week, soy did not reduce the levels of oxidant proteins in men; the researchers attributed this finding to gender-dependent effects of soy and the short-term nature of the outcomes (37). Presumably, if the long-term effects of multiple doses of daidzein had been evaluated, its antioxidant effect might have been more clearly observed, and we could have explained our experimental results in terms of gender-dependent effects. Moreover, the fact that daidzein increased disulfide levels to a lesser extent than diazepam is also a noteworthy finding.

A limitation of our study is that we did not evaluate the mechanistic effects of daidzein at the molecular, electrophysiological, and histological levels.

In conclusion, among the soy isoflavones that exhibit neuroprotective effects in the central nervous system through their antioxidant actions, daidzein demonstrates an anticonvulsant effect on epileptic seizures at low doses. Further studies designed on a chronic and gender-based basis are needed to elucidate the antioxidant effects of daidzein in relation to its antiepileptic properties.

REFERENCES

1. Dichter MA. The epilepsies and convulsive disorders. In: Isselbacher KJ, editor. Harrison's Principles of Internal Medicine. New York: McGraw- Hill; 1994. P.2223–33.
2. Shen B, Shi Y, Fu Y, Cao Y, Wang Y, Fang J. Deep brain stimulation on cognition in epilepsy: A concentration on learning and memory. Brain Res Bull. 2024;219:111134.
3. Fisher RS. Animal models of the epilepsies. Brain Res Brain Res Rev. 1989;14(3):245-78.
4. Löscher W, Schmidt D. Strategies in antiepileptic drug development: is rational drug design superior to random screening and structural variation? Epilepsy Res. 1994;17(2):95-134.
5. Chung YH, Kim WK, Ko KH, Bach JH, Ko KH, Bach JH, et al. Role of oxidative stress in epileptic seizures. Neurochem Int. 2011;59(2):122–37.
6. Menon B, Ramalingam K, Kumar RV. Oxidative stress in patients with epilepsy is independent of antiepileptic drugs. Seizure 2012; 21(10):780–784
7. Perrone S, Negro S, Tataranno ML, Buonocore G. Oxidative stress and antioxidant strategies in newborns. J Matern Fetal Neonate 2010; 23(Suppl 3):63–65
8. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014; 47(18):326–332
9. Matteucci E, Giampietro O. Thiol signalling network with an eye to diabetes. Molecules 2010;15(12):8890–8903
10. Prabhu A, Sarcar B, Kahali S, Yuan Z, Johnson JJ, Adam KP et al. Cysteine catabolism: a novel metabolic pathway contributing to glioblastoma growth. Cancer Res 2014;74(3):787–796
11. Sumien N, Cunningham JT, Davis DL, Engelland R, Fadeyibi O, Farmer GE, et al. Neurodegenerative Disease: Roles for Sex, Hormones, and Oxidative Stress. Endocrinol. 2021;162(11):bqab185.
12. Teleanu DM, Niculescu AG, Lungu II, Radu CI, Vladâcenco O, Roza E, et al. An Overview of Oxidative Stress, Neuroinflammation, and Neurodegenerative Diseases. Int J Mol Sci. 2022;23(11):5938.
13. Sharma D, Singh V, Kumar A, Singh TG. Genistein: A promising ally in combating neurodegenerative disorders. Eur J Pharmacol. 2025;991:177273.
14. Ahmed T, Javed S, Tariq A, Budzynska B, D'Onorrio G, Daglia M, et al. Daidzein and its effects on Brain. Curr Med Chem. 2016;24(4):365-375.
15. Aras AB, Guven M, Akman T, Ozkan A, Sen HM, Duz U, et al. Neuroprotective effects of daidzein on focal cerebral ischemia injury in rats. Neural Regen Res 2015; 10(1):146- 152.
16. Wei J, Yang F, Gong C, Shi X, Wang G. Protective effect of daidzein against streptozotocin-induced Alzheimer's disease via improving cognitive dysfunction and oxidative stress in rat model. J Biochem Mol Toxicol. 2019 ;33(6):e22319.
17. Ariyani W, Koibuchi N. The effect of soy isoflavones in brain development: the emerging role of multiple signaling pathways and future perspectives. Endocr J. 2024;71(4):317-333.

18. Öğün MN, Çetinkaya A, Beyazçiçek E. The effect of vortioxetine on penicillin-induced epileptiform activity in rats. Sci ELO Arq. Neuro-Psiquiatr. 2019; 77 (6):412-417.

19. Racine RJ, Gartner JG, Burnham WM. Epileptiform activity and neural plasticity in limbic structures. Brain Res. 1972;47(1):262-8.

20. Parida S, Singh TU, Thangamalai R, Narasimha Reddy ChE, Panigrahi M, Kandasamy K, et al. Daidzein pretreatment improves survival in mouse model of sepsis. J Surg Res. 2015;197(2):363-73.

21. . Kim JW, Jin YC, Kim YM, Rhie S, Kim HJ, Seo HG, et al. Daidzein administration in vivo reduces myocardial injury in a rat ischemia/reperfusion model by inhibiting NF-kappaB activation. Life Sci. 2009;84(7-8):227-34

22. Křížová L, Dadáková K, Kašparovská J, Kašparovský T. Isoflavones. Molecules 2019; 24: 1076.

23. Zaheer K, Humayoun Akhtar M. An updated review of dietary isoflavones: Nutrition, processing, bioavailability and impacts on human health. Crit Rev Food Sci Nutr. 2017;57(6):1280-1293.

24. Mahdy HM, Mohamed MR, Emam MA, Karim AM, Abdel-Naim AB, Khalifa AE. Puerarin ameliorates 3-nitropropionic acid-induced neurotoxicity in rats: possible neuromodulation and antioxidant mechanisms. Neurochem Res. 2014;39(2):321-32.

25. Gong G, Ganesan K, Wan Y, Liu Y, Huang Y, Luo Y, et al. Unveiling the neuroprotective properties of isoflavones: current evidence, molecular mechanisms and future perspectives. Crit Rev Food Sci Nutr. 2024; 24:1-37.

26. Hu QP, Yan HX, Peng F, Feng W, Chen FF, Huang XY, et al. Genistein protects epilepsy-induced brain injury through regulating the JAK2/STAT3 and Keap1/Nrf2 signaling pathways in the developing rats. Eur J Pharmacol. 2021;912:174620.

27. El-Sayed RM, Fawzy MN, Zaki HF, Abd El-Haleim EA. Neuroprotection impact of biochanin A against pentylenetetrazol-kindled mice: Targeting NLRP3 inflammasome/TXNIP pathway and autophagy modulation. Int Immunopharmacol. 2023;115:109711.

28. Kalandakanond-Thongsong S, Daendee S, Thongsong B, Srikiatkhachorn A. Daidzein, but not genistein, has anxiolytic-liked effect on intact male Wistar rats. Behav Brain Res. 2024 ;474:115172.

29. Westmark CJ, Westmark PR, Malter JS. Soy-based diet exacerbates seizures in mouse models of neurological disease. J Alzheimers Dis. 2013;33(3):797-805.

30. Rodriguez-Gomez A, Filice F, Gotti S, Panzica G. Perinatal exposure to genistein affects the normal development of anxiety and aggressive behaviors and nitric oxide system in CD1 male mice. Physiol Behav. 2014;133:107-14.

31. Wisniewski AB, Cernetich A, Gearhart JP, Klein SL. Perinatal exposure to genistein alters reproductive development and aggressive behavior in male mice. Physiol Behav. 2005;84(2):327-34.

32. Rangel-Galván M, Rangel A, Romero-Méndez C, Dávila EM, Castro ME, Caballero NA, et al. Inhibitory Mechanism of the Isoflavone Derivative Genistein in the Human CaV3.3 Channel. ACS Chem Neurosci. 2021;12(4):651-659.

33. Laddha AP, Kulkarni YA. Daidzein ameliorates peripheral neuropathy in Sprague Dawley rats. Front Pharmacol. 2024;15:1385419.

34. Haider T, Khan S, Bibi T, Zahra SA, Ali H, Din FU, et al. Daidzein ameliorates experimental traumatic brain injury-induced neurological symptoms by suppressing oxidative stress and apoptosis. J Biochem Mol Toxicol. 2024;38(11):e70019.

35. Pang D, Yang C, Luo Q, Li C, Liu W, Li L, et al. Soy isoflavones improve the oxidative stress induced hypothalamic inflammation and apoptosis in high fat diet-induced obese male mice through PGC1-alpha pathway. Aging (Albany NY). 2020 ;12(9):8710-8727.

36. Jin Y, Wu H, Cohen EM, Wei J, Jin H, Prentice H, et al. Genistein and daidzein induce neurotoxicity at high concentrations in primary rat neuronal cultures. J Biomed Sci. 2007;14(2):275-84.

37. Celec P, Hodosy J, Pálffy R, Gardlík R, Halčák L, Ostatníková D. The short-term effects of soybean intake on oxidative and carbonyl stress in men and women. Molecules. 2013;18(5):5190-200.

Abbreviations List

PTZ: pentylenetetrazol, ROS: reactive oxygen species, DMSO: dimethyl sulfoxide, LDH: lactate dehydrogenase, DTNB: 5,5'-dithiobis-(2-nitrobenzoic) acid, BAIBU: Bolu Abant Izzet Baysal University, i.p.: intraperitoneal, s.c.: subcutaneous, i.m.: intramuscular.

Ethics Approval and Consent to Participate

The ethical approval for the study was obtained from the Bolu Abant Izzet Baysal University (BAIBU) Local Ethics Committee for Experimental Animals (2022/19).

Consent for Publication

It does not contain any personal data.

Availability of Data and Materials

The data sets are available from the corresponding author on reasonable request.

Competing Interests

There is no conflict of interest.

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Author Contributions

Study Design CA; Data Acquisition: CA, HC, CKÖ; Analysis: MA, CKÖ; Writing: CA, HC, ŞNT.

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