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Taxifolin as a novel therapeutic agent for epileptic seizures induced by caffeine-induced oxidative stress in rats

Hasan Yasar[1,A,D–F], Durdu Altuner[2,C,E,F], Seval Bulut[2,C,E,F], Betul Cicek[3,D,F], Cebrail Gursul[3,E,F], Mehmet Kuzucu[4,B,F], Halis Suleyman[2,A,D–F]

[1] Department of Neurology, Faculty of Medicine, Erzincan Binali Yıldırım University, Turkey
[2] Department of Pharmacology, Faculty of Medicine, Erzincan Binali Yıldırım University, Turkey
[3] Department of Physiology, Faculty of Medicine, Erzincan Binali Yıldırım University, Turkey
[4] Department of Molecular Biology, Faculty of Arts and Sciences, Erzincan Binali Yıldırım University, Turkey

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Halis Suleyman
E-mail: halis.suleyman@gmail.com

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Abstract

Background. Epilepsy is a severe neurological disease that results from excessive and/or synchronized neuronal activity in the brain, and oxidative stress plays a role in its pathogenesis. Taxifolin is a flavonoid that exhibits antioxidant activity.

Objectives. To investigate the effects of taxifolin on caffeine-induced epileptic seizures in rats and reveal the role of antioxidant activity in antiepileptic therapy.

Materials and methods. Forty rats were divided into 4 groups (n = 6/group): caffeine 300 mg/kg group (CG), taxifolin 50 mg/kg + caffeine 300 mg/kg group (TCG), 2 mg/kg diazepam + 300 mg/kg caffeine group (DCG), and a healthy group (HG). Taxifolin was given to the TCG, and diazepam was given to the DCG orally. One hour later, caffeine was injected intraperitoneally into the CG, TCG and DCG rats. The time between the caffeine injection and the contractions (the latency period) was determined. Animals were euthanized 1 h after caffeine injection, and brain tissues were biochemically examined for oxidants and antioxidants.

Results. Taxifolin and diazepam prolonged the latency period to a similar extent ($p = 0.549$), while taxifolin was more successful in preventing mortality. Taxifolin suppressed the caffeine-induced increase in myeloperoxidase, total oxidant status and oxidative stress index, and decreased total glutathione, superoxide dismutase and total antioxidant status more effectively than diazepam ($p < 0.05$).

Conclusions. We showed the relationship between antioxidant activity and epilepsy treatment, and demonstrated that taxifolin may be useful for treating epilepsy.

Key words: antioxidants, diazepam, epilepsy, taxifolin, oxidative stress

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Background

The International League Against Epilepsy (ILAE) defines epileptic seizures as abnormal excessive and/or synchronized neuronal activity that occurs transiently in the brain.[1] Epilepsy is a severe neurological disorder with a 1% global prevalence[2] and is categorized by the ILAE as focal onset, generalized onset, unknown onset, and unclassified onset.[3] Experimental seizure models preferred today are generalized tonic-clonic seizures, generalized absence seizures and status epilepticus,[4] which are created using various chemicals, with systemically administered substances demonstrated to cause generalized tonic-clonic seizures.[5] The systemic administration of caffeine, as a methylxanthine derivative, has been used in a generalized tonic-clonic seizure model.[6]

The pathogenesis of epilepsy has yet to be fully clarified. However, an increase in reactive oxygen species (ROS) in epilepsy is thought to cause nerve cell mitochondrial dysfunction and oxidative damage.[7] Mitochondria are organelles that play vital roles in the maintenance and regulation of all brain functions, including neuroinflammation, neuroplasticity, oxidative stress, and apoptosis.[8] Most ROS, mainly superoxide anions, are products of mitochondrial respiration produced during electron flow in the mitochondrial electron transfer chain. The superoxide anion concentration in the mitochondrial matrix is 5–10 times higher than in the cytosol and the nucleus.[9] Therefore, mitochondria are vulnerable to oxidative damage.[10]

Mitochondrial dysfunction can cause many health issues, including psychiatric problems such as epileptic seizures, neurodegenerative diseases, anxiety, and depression.[11,12] In a study on rats, anxiety-like behaviors were linked to mitochondrial dysfunction in the nucleus accumbens. Mitochondrial function in the nucleus accumbens is crucial for social hierarchy establishment, and high anxiety results in low social competition.[11,12] Currently, there is no cure for mitochondrial diseases. Among the treatment strategies under investigation is oxidative stress modulation.[11]

Under normal conditions, the body produces ROS during aerobic metabolism, and a balance exists between ROS production and elimination.[13] Oxidative stress occurs when the amount of ROS exceeds the antioxidant capacity. The increase in ROS causes oxidative damage to macromolecules such as proteins, membrane lipids and deoxyribonucleic acid (DNA).[11,14] Reactive oxygen species maintained at low levels play a physiological role in intracellular signaling pathways; however, when overproduced, they cause cell and tissue damage.[9] The brain has more mitochondria, highly oxidizable lipids, a higher energy requirement, and less antioxidant capacity than other tissues; therefore, it is vulnerable to oxidative stress, which contributes to the formation of epileptic seizures.[10] On the other hand, there is a consensus that seizure activity induces ROS production, and this contributes to seizure-induced cell death, with the literature indicating that there is an affinity between ROS levels and seizure frequency.[15]

Malondialdehyde (MDA), a product of ROS-induced lipid peroxidation (LPO), has been shown to increase in chronic epilepsy.[16] Supporting this information, Turan et al. revealed an increase in MDA and myeloperoxidase (MPO) levels and a decrease in total glutathione (tGSH) and superoxide dismutase (SOD) activities. Such measurements represent the total endogenous antioxidants in the brain tissue of animals used to model epileptic seizures through the intraperitoneal administration of caffeine.[6] These findings suggest that epileptic seizures may be related to oxidative stress.

Epileptic seizures can cause cognitive and psychiatric problems, with reports that frequent seizures originating from the bilateral ventromedial prefrontal cortex (vmPFC) cause prefrontal dysfunction due to tissue damage, and antisocial personality disorder may develop in affected patients.[17] In addition, studies show that vmPFC lesions can cause cognitive and behavioral disorders.[18] Furthermore, temporal lobe epilepsy causes depression and anxiety, which is associated with cell loss.[15] Both antisocial personality disorder and depression are alleviated in patients who have undergone resective epilepsy surgery.[17,19] Based on the literature, it can be stated that epilepsy is not only a neurological problem but also a social issue. Therefore, studies on epilepsy pathogenesis and new strategies for epilepsy prevention and treatment are vital.

Taxifolin (dihydroquercetin) is a flavonoid approved by the U.S. Food and Drug Administration (FDA) and is abundant in various plants, such as grapes, citrus fruits and green tea.[20,21] Studies have shown that the oral bioavailability of taxifolin is low compared to the intravenous use.[22,23] The literature also shows that gastric taxifolin absorption is better than small intestine uptake. In addition, taxifolin undergoes biotransformation by the intestinal microflora, and while some of the formed metabolites are absorbed, some are excreted in the feces.[24] Absorbed taxifolin is primarily metabolized in enterocytes and hepatocytes, generally through phase II reactions. The metabolites are then transported to the organs through the bloodstream,[25] and taxifolin is eliminated from the body through urine and feces.[25] Almost 200 taxifolin metabolites have been identified, and the drug and its metabolites have been detected in many tissues.[25,26] After reviewing the literature, no information was found on the transfer mechanism of taxifolin to the brain. However, studies have shown that taxifolin can cross the blood–brain barrier and has been found in brain tissue, albeit at relatively low concentrations.[25]

Many studies have demonstrated the neuroprotective properties of taxifolin,[23] with a recent double-blind placebo-controlled clinical trial finding that consuming taxifolin-rich foods improved brain activity and mental fatigue in healthy young adults.[27] Furthermore, taxifolin can affect gene expression that regulates the balance between cell survival and cell death and exhibits rapid neuroprotection by suppressing ROS production in inhibitory gamma-aminobutyric acid

(GABA) neurons.[21] Dok-Go et al. correlated the neuroprotective effects of taxifolin in rat cortical cells with its antioxidant activity via radical scavenging and LPO inhibition.[28] Taxifolin has also been shown to attenuate ROS production, tGSH depletion and cell death.[29] The literature on taxifolin indicates that it may be useful for preventing caffeine-related oxidative stress-induced epileptic seizures.

Objectives

No studies investigating the effects of taxifolin in caffeine-induced epileptic seizures were found when reviewing the literature. As such, this study was designed to assess the impact of taxifolin on caffeine-induced epileptic seizures in rats. Our primary aim was to determine the relationship between epileptic activity and antioxidant activity by evaluating oxidative stress, which is involved in the pathogenesis of epilepsy, by measuring oxidant and antioxidant parameters in brain tissue.

Materials and methods

The current study created a caffeine-induced epilepsy model in Albino Wistar rats to assess the effects of taxifolin on epileptic seizures and compared the effect of taxifolin to diazepam. To investigate taxifolin effects, oxidant and antioxidant levels were measured in the brain tissues of the animals, and the latent period between epilepsy induction and seizure development was recorded. To determine whether or not taxifolin was effective, results were compared to a seizure group and a diazepam group.

Animals

The experiments used 40 male Albino Wistar rats (210–220 g) procured from the Erzincan Binali Yıldırım University Experimental Animals Application and Research Center (Erzincan, Turkey). Before the experiment, the animals were housed and fed at standard room temperature (22°C) for 1 week in a laboratory environment. The Animal Experiments Local Ethics Committee Experimental approved the procedures (approval No. 2022-11/56).

Chemicals

Thiopental sodium was procured from IE Ulagay (Istanbul, Turkey), diazepam from Deva (Istanbul, Turkey), caffeine from Sigma-Aldrich (Darmstadt, Germany), and taxifolin from Evalar (Moscow, Russia).

Animal groups

The animals were split into 4 groups, including a 300 mg/kg caffeine group (CG), 50 mg/kg taxifolin + 300 mg/kg caffeine group (TCG), 2 mg/kg diazepam + 300 mg/kg caffeine group (DCG), and healthy control group (HG).

Experimental procedure

Taxifolin (25 mg/tablet, CAS No. 480-18-2) and diazepam (2 mg/tablet, CAS No. 439-14-5) were crushed into a powder and dissolved in distilled water. Solutions of 2.5 mg/mL of taxifolin and 0.1 mg/mL of diazepam were prepared. Caffeine (100 g bottle, powder, CAS No. 58-08-2) was dissolved in distilled water to obtain a 30 mg/mL solution. In the experimental application, 50 mg/kg taxifolin[30] was given to the TCG (n = 10) and 2 mg/kg diazepam to the DCG (n = 10) via oral gavage. Distilled water was administered orally at the same volume to the CG (n = 10) and HG (n = 10). One hour after administering taxifolin, diazepam, distilled water or 300 mg/kg caffeine[6] was injected intraperitoneally (ip.) into the CG, TCG and DCG. The same volume of distilled water was also injected ip. into the HG. Immediately after the caffeine injection, the animals were placed in groups in a plexiglass box (30 × 30 × 40 cm), and the caffeine injection time was recorded. The onset of tonic-clonic contractions was recorded as seizure onset. After the caffeine injection, the time until the onset of tonic-clonic contractions was measured with a stopwatch, and latency was recorded in minutes.[6] One hour after the caffeine injection, the animals were euthanized with 50 mg/kg thiopental sodium (0.5 g/20 mL vial, CAS No. 76-75-5), and the levels of MDA, MPO, tGSH, SOD, total oxidant status (TOS), and total antioxidant status (TAS), were measured in excised brain tissue.

Sample preparation

To determine MDA levels, brain tissue was homogenized in a 1.15% potassium chloride solution in an icy environment and topped up to 2 mL using phosphate-buffered saline (PBS; pH 7.5) for other measurements. The solution was then centrifuged (10,000 rpm for 15 min at 4°C) and the supernatant was collected for analysis.

Malondialdehyde analysis

The method of Ohkawa et al. was adopted for MDA measurement.[31] The method is based on the spectrophotometric measurement of the absorbance (532 nm) of a pink complex created by thiobarbituric acid (TBA) and MDA at high temperature (95°C). Homogenates were centrifuged at 5000 g for 20 min, and the supernatants used to determine the amount of MDA by preparing a solution containing 250 µL of homogenate, 100 µL of 8% sodium dodecyl sulfate (SDS), 750 µL of 20% acetic acid, 750 µL of 0.08% TBA, and 150 µL of distilled water, which was pipetted into capped test tubes and vortexed. After the mixture was incubated for 60 min at 100°C, 2.5 mL

of n-butanol was added and measured spectrophotometrically. The amount of red color formed was read using 3-mL cuvettes at 532 nm, and the amount of MDA in the samples was determined using the standard curve created using the previously prepared MDA stock solution, with consideration of dilution coefficients.

Myeloperoxidase analysis

For determining the activity of MPO, an MPO-mediated oxidation reaction with hydrogen peroxide (H_2O_2) containing a 4-amino antipyrine/phenol solution as the substrate was used.[32]

tGSH analysis

The amount of GSH in brain tissue homogenates was determined using the method described by Sedlak and Lindsay.[33] Samples were weighed and homogenized in 2 mL of 50 mmol/L Tris–HCl buffer containing 20 mmol/L ethylenediaminetetraacetic acid (EDTA) and 0.2 mmol/L sucrose at pH 7.5. Homogenates were immediately precipitated with 0.1 mL of 25% trichloroacetic acid, the precipitate was removed after centrifugation at 4200 rpm for 40 min at 4°C, and the supernatant was used to determine tGSH. A total of 1500 µL measurement buffer (200 mmol/L Tris–HCl buffer containing 0.2 mmol/L EDTA at pH 7.5), 500 µL supernatant, 100 µL 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) (10 mmol/L), and 7900 µL methanol were added to the tube and vortexed and incubated for 30 min at 37°C. The DTNB was used as the chromogen, and it created a yellow-colored complex with sulfhydryl groups. The absorbance was measured at 412 nm using a Beckman DU 500 spectrophotometer (Beckman Coulter, Gaithersburg, USA). The standard curve was obtained using reduced GSH.

SOD analysis

The measurements were performed using the method of Sun et al.,[34] with SOD formed when xanthine was converted into uric acid with xanthine oxidase. When a nitro blue tetrazolium (NBT) dye is added to this reaction, it reacts with SOD to form a purple-colored formazan dye. Samples were weighed and homogenized in 2 mL of 20 mmol/L PBS with 10 mmol/L EDTA (pH 7).[8] Samples were then centrifuged at 6000 rpm for 10 min, and the supernatant was used as the assay sample. The measurement mixture, containing 2450 µL of measurement mixture (0.3 mmol/L xanthine, 0.6 mmol/L EDTA, 150 µmol/L NBT, 0.4 mol/L Na_2CO_3, and 1 g/L bovine serum albumin), 500 µL supernatant, and 50 µL xanthine oxidase (167 U/L), was vortexed and incubated for 10 min. Formazan developed at the end of the reaction. The absorbance of the purple-colored formazan was measured at 560 nm. Less of the superoxide radical reacted with NBT when more of the enzyme was present.

TOS and TAS status measurement

The TOS and TAS levels of the samples were determined using an automated measurement method created by Erel and commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey).[35,36] The TAS method was based on bleaching the characteristic color of the more stable 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation by antioxidants, and the measurements were taken at 660 nm. The results were given as nmol of H_2O_2 equivalent/L. For the TOS method, the oxidants in the sample oxidized the iron ion-o-dianisidine complex to a ferric ion. The iron ion produced a colored complex with xylenol orange in an acidic environment. The color intensity was measured spectrophotometrically at 530 nm and was associated with the total amount of oxidant molecules in the sample. The results were indicated as µmol trolox equivalent/L. The percentage of TOS to TAS was used as the oxidative stress index (OSI) and calculated according to the formula: OSI = TOS/TAS*0.1.[37]

Statistical analyses

Statistical analyses employed IBM SPSS Statistics 22.0 (IBM Corp., Armonk, USA). A Shapiro–Wilk test determined if the data were normally distributed, and Levene's test assessed homogeneity of variance. Data with a normal distribution were analyzed using one-way analysis of variance (ANOVA) with Bonferroni's correction and are presented as mean ± standard deviation (M ±SD) and 95% confidence intervals (95% CIs). Meanwhile, data with a non-normal distribution were analyzed using the Kruskal–Wallis (K–W) test followed by Dunn's test, with the results expressed as median (1^{st}–3^{rd} quartile (Q1–Q3)). Performing all analyses on the same data set increased the probability of type 1 error. To prevent this situation, the level of significance was determined as 0.00625. This value was obtained by dividing 0.05 by the comparison number of eight.

Results

Latent period results

The period elapsed until seizure onset, after the caffeine-driven induction of an epileptic attack, was calculated in minutes as the latent period. There was a difference in the variable latent period between groups with respect to the variable group ($H [2] = 20.066$, $p < 0.001$). As shown in Fig. 1, Table 1 and Table 2, the latent period for the CG was higher than for the TCG ($p = 0.004$) and DCG ($p < 0.001$), while latency was similar in the taxifolin and diazepam groups ($p = 1.000$). All animals in the CG, TCG and DCG had epileptic seizures. All animals that had a seizure in the CG died (100%), while the number of animals that died in TCG and DCG was 3 (30%) and 9 (90%), respectively.

Table 1. Analysis results of the data obtained from the study

Biochemical parameters	Contents	Shapiro–Wilk				Levene's test	One-way ANOVA				
		CG	TCG	DCG	HG		sum of squares	df	mean square	f	sig.
MDA	statistic	0.977	0.908	0.949	0.929	8.070	8135.814	3	2711.938	865.927	0.000
	df	10	10	10	10	3/36	112.746	36	3.132	–	–
	sig.	0.945	0.270	0.662	0.439	0.000	8248.560	39	–	–	–
MPO	statistic	0.965	0.969	0.908	0.910	12.697	2196.455	3	732.152	253.522	0.000
	df	10	10	10	10	3/36	103.965	36	2.888	–	–
	sig.	0.837	0.877	0.268	0.283	0.000	2300.420	39	–	–	–
tGSH	statistic	0.932	0.964	0.849	0.952	0.999	405.690	3	135.230	1837.087	0.000
	df	10	10	10	10	3/36	2.650	36	0.074	–	–
	sig.	0.473	0.826	0.057	0.693	0.404	408.340	39	–	–	–
SOD	statistic	0.960	0.991	0.973	0.976	6.937	2109.204	3	703.068	210.255	0.000
	df	10	10	10	10	3/36	120.380	36	3.344	–	–
	sig.	0.783	0.998	0.915	0.941	0.001	2229.584	39	–	–	–
TOS	statistic	0.988	0.914	0.945	0.949	15.237	2362.577	3	787.526	429.338	0.000
	df	10	10	10	10	3/36	66,034	36	1.834	–	–
	sig.	0.994	0.313	0.605	0.653	0.000	2428.611	39	–	–	–
TAS	statistic	0.982	0.869	0.859	0.918	9.926	343.047	3	114.349	251,577	0.000
	df	10	10	10	10	3/36	16.363	36	0.455	–	–
	sig.	0.974	0.098	0.075	0.342	0.000	359.410	39	–	–	–
OSI	statistic	0.895	0.967	0.789	0.945	Kruskal–Wallis test	34.077	–	–	–	–
	df	10	10	10	10		3	–	–	–	–
	sig.	0.192	0.862	0.011	0.613		0.000	–	–	–	–
Latent period	statistic	0.889	0.935	0.805	–		20.066	–	–	–	–
	df	10	10	10	–		2	–	–	–	–
	sig.	0.166	0.499	0.017	–		0.000	–	–	–	–

CG – 300 mg/kg caffeine-administered group; TCG – 50 mg/kg taxifolin + 300 mg/kg caffeine-administered group; DCG – 2 mg/kg diazepam + 300 mg/kg caffeine-administered group; HG – healthy group; MDA – malondialdehyde; MPO – myeloperoxidase; tGSH – total glutathione; SOD – superoxide dismutase; TOS – total oxidant status; TAS – total antioxidant status; OSI – oxidative stress index; df – degrees of freedom; df1 – number of groups: 1; df2 – total number of samples: number of groups; sig. – significance; f – (largest variance)^2/(smallest variance)^2.

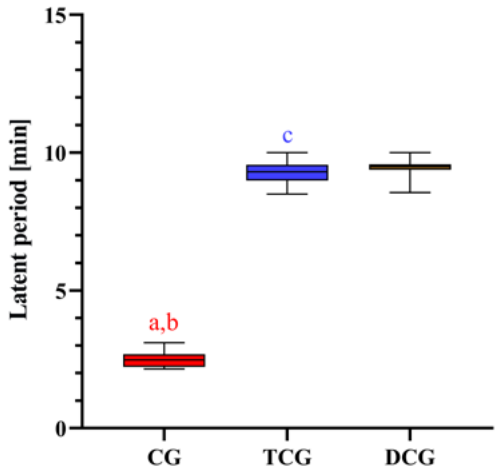


Fig. 1. Comparison of latency period data of the CG, TCG and DCG. Data are presented as median and Q1–Q3 percentile (horizontal line = median; bottom line of the box = Q1 (25th); top line of the box = Q3 (75th))

CG – 300 mg/kg caffeine-administered group; TCG – 50 mg/kg taxifolin + 300 mg/kg caffeine-administered group; DCG – 2 mg/kg diazepam + 300 mg/kg caffeine-administered group; (a). p = 0.04 compared to the TCG; (b). p < 0.001 compared to the DCG; (c). p = 1.000 compared to the DCG.

Table 2. Analysis of experimental groups in terms of latent period

Parameters	Contents	Latent period [min]
Median (Q1–Q3)	CG (n = 10)	2.48 (2.23–2.69)
	TCG (n = 10)	9.30 (8.98–9.56)
	DCG (n = 10)	9.51 (9.37–9.57)
Kruskal–Wallis test	H	20.066
	p-value	<0.001
Post hoc test p-values	CG vs. TCG	0.004
	CG vs. DCG	<0.001
	TCG vs. DCG	1.000

CG – 300 mg/kg caffeine-administered group; TCG – 50 mg/kg taxifolin + 300 mg/kg caffeine-administered group; DCG – 2 mg/kg diazepam + 300 mg/kg caffeine-administered group. Statistical analysis was performed with Kruskal–Wallis test, followed by Dunn's test.

MDA and MPO analysis results

There was a difference in MDA ($F[3.36] = 865.927$, $p < 0.001$) and MPO ($F[3.36] = 253.522$, $p < 0.001$) with respect to the variable group. As seen in Fig. 2, Table 1 and Table 3, MDA and MPO levels in the brain tissues of rats in the CG were higher than those in the TCG, DCG and HG ($p < 0.001$). While taxifolin and diazepam inhibited the increase in MDA at the same level ($p = 1.000$), taxifolin suppressed the MPO increase better than diazepam ($p < 0.001$).

tGSH and SOD analysis results

There was a group difference in tGSH ($F[3,36] = 1837.087$, $p < 0.001$) and SOD ($F[3,36] = 210.255$, $p < 0.001$) with respect to the variable group. The tGSH and SOD levels in the CG were lower than those in the TCG, DCG and HG ($p < 0.001$). Taxifolin inhibited this decrease in tGSH and SOD levels better than diazepam ($p < 0.001$) (Fig. 3, Table 1 and Table 3).

TOS, TAS and OSI analysis results

There were group differences in TOS ($F[3,36] = 429.338$, $p < 0.001$) and OSI ($H[3] = 34.077$, $p < 0.001$) with respect to the variable group. As shown in Fig. 4, Table 1 and Table 3, the CG had the highest TOS and OSI values, and there was a statistically significant difference between the other groups ($p < 0.001$). While TOS values were similar in the TCG and DCG, OSI values were lower ($p = 1.000$ and $p < 0.001$, respectively). The OSI values in the TCG were similar to the HG ($p = 1.000$). Furthermore, there was a group difference in TAS with respect to the variable group ($F[3,36] = 251.577$, $p < 0.001$). The TAS levels were lower in the CG than in the other groups ($p < 0.001$). Taxifolin inhibited the decrease in TAS levels better than diazepam ($p < 0.001$). There was no statistically significant difference in TAS levels between the TCG and HG ($p = 1.000$).

The latent period data for the CG were higher than for the TCG and DCG. All animals in the CG, TCG and DCG had epileptic seizures. All animals who had a seizure in the CG died, while the number of animals that died in the TCG and DCG was 3 and 9, respectively. The MDA and MPO were higher in the CG than in the TCG, DCG and HG. While taxifolin and diazepam inhibited the MDA increase to the same extent, taxifolin suppressed the MPO increase better than diazepam. The SOD and tGSH were lower in the CG than in the TCG, DCG and HG. Taxifolin inhibited this decrease in tGSH and SOD better than diazepam. While TOS values were similar in the TCG compared to the DCG, OSI scores were lower. Oxidative stress index was similar for the TCG and HG. Meanwhile, TAS was lower in the CG than in the other groups. Taxifolin inhibited the decrease in TAS better than diazepam. There was no difference in TAS levels between the TCG and the HG.

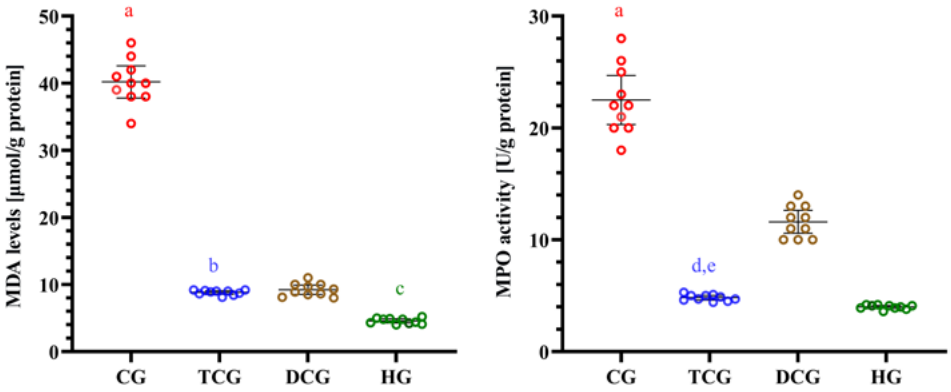


Fig. 2. Comparative analysis of MDA and MPO levels obtained from experimental groups. Data were presented as mean with 95% confidence intervals

(a). $p < 0.001$ compared to the TCG, DCG and HG; (b). $p = 1.000$ compared to the DCG; (c). $p < 0.001$ compared to the CG, TCG and DCG; (d). $p < 0.001$ compared to the DCG; (e). $p = 1.000$ compared to the HG; MDA – malondialdehyde; MPO – myeloperoxidase; CG – 300 mg/kg caffeine-administered group; TCG – 50 mg/kg taxifolin + 300 mg/kg caffeine-administered group; DCG – 2 mg/kg diazepam + 300 mg/kg caffeine-administered group; HG – healthy group.

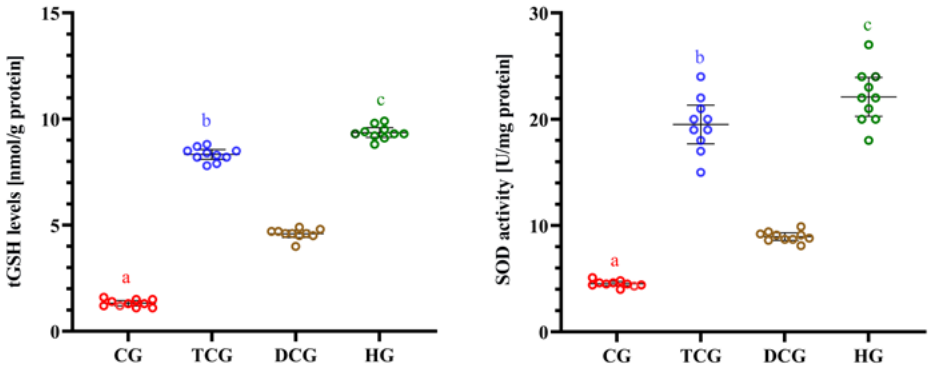


Fig. 3. Comparative analysis of tGSH and SOD levels obtained from experimental groups. Data were presented as mean with 95% confidence intervals

(a). $p < 0.001$ compared to the TCG, DCG and HG; (b). $p < 0.001$ compared to the DCG; (c). $p < 0.001$ compared to the CG, TCG and DCG; tGSH – total glutathione; SOD – superoxide dismutase; CG – 300 mg/kg caffeine-administered group; TCG – 50 mg/kg taxifolin + 300 mg/kg caffeine-administered group; DCG – 2 mg/kg diazepam + 300 mg/kg caffeine-administered group; HG – healthy group.

Table 3. Statistical analysis of biochemical data obtained from experimental groups

Parameters	Contents		MDA	MPO	tGSH	SOD	TOS	TAS	OSI
X ±SD or median (Q1–Q3)	CG (n = 10)		40.20 ±3.36	22.50 ±3.06	1.32 ±0.18	4.52 ±0.29	25.50 ±2.64	3.19 ±0.32	0.84 (0.68–0.86)
	TCG (n = 10)		8.82 ±0.37	4.82 ±0.30	8.33 ±0.32	19.50 ±0.55	7.70 ±0.34	9.97 ±0.60	0.08 (0.07–0.08)
	DCG (n = 10)		9.21 ±0.96	11.60 ±1.43	4.59 ±0.24	8.96 ±0.50	7.15 ±0.39	5.81 ±0.38	0.12 (0.12–0.13)
	HG (n = 10)		4.57 ±0.42	3.99 ±0.19	9.36 ±0.32	22.10 ±2.56	8.51 ±0.35	10.14 ±1.10	0.08 (0.08–0.09)
95% CI for the mean change	CG	lower–upper	37.97–42.60	20.31–24.69	1.20–1.45	4.31–4.73	23.62–27.39	2.96–3.42	–
	TCG	lower–upper	8.56–9.08	4.62–5.03	8.10–8.56	17.68–21.32	7.46–7.95	9.54–10.40	–
	DCG	lower–upper	8.52–9.90	10.58–12.62	4.42–4.76	8.61–9.31	6.87–7.43	5.54–6.08	–
	HG	lower–upper	4.27–4.87	3.85–4.13	9.13–9.59	20.27–23.93	8.26–8.76	9.35–10.93	–
ANOVA or K–W	F (3.36) or H		865.927	253.522	1837.087	210.255	429.338	251.577	34.077
	p-values		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Post hoc test p-values	HG vs. TCG		<0.001	1.000	<0.001	0.018	1.000	1.000	1.000
	HG vs. DCG		<0.001	<0.001	<0.001	<0.001	0.186	<0.001	0.118
	HG vs. CG		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	TCG vs. DCG		1.000	<0.001	<0.001	<0.001	1.000	<0.001	0.004
	TCG vs. CG		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	DCG vs. CG		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.335

CG – 300 mg/kg caffeine-administered group; TCG – 50 mg/kg taxifolin + 300 mg/kg caffeine-administered group; DCG – 2 mg/kg diazepam + 300 mg/kg caffeine-administered group; HG – healthy group; MDA – malondialdehyde; MPO – myeloperoxidase; tGSH – total glutathione; SOD – superoxide dismutase; TOS – total oxidant status; TAS – total antioxidant status; OSI – oxidative stress index. Statistical analysis was performed with one-way analysis of variance (ANOVA) or the Kruskal–Wallis (K–W) test. One-way ANOVA was preferred for statistical analysis of MDA, MPO, tGSH, SOD, TOS, and TAS. Bonferroni correction was then applied. Statistical analysis for OSI was done using the K–W test and post hoc Dunn's test was applied.

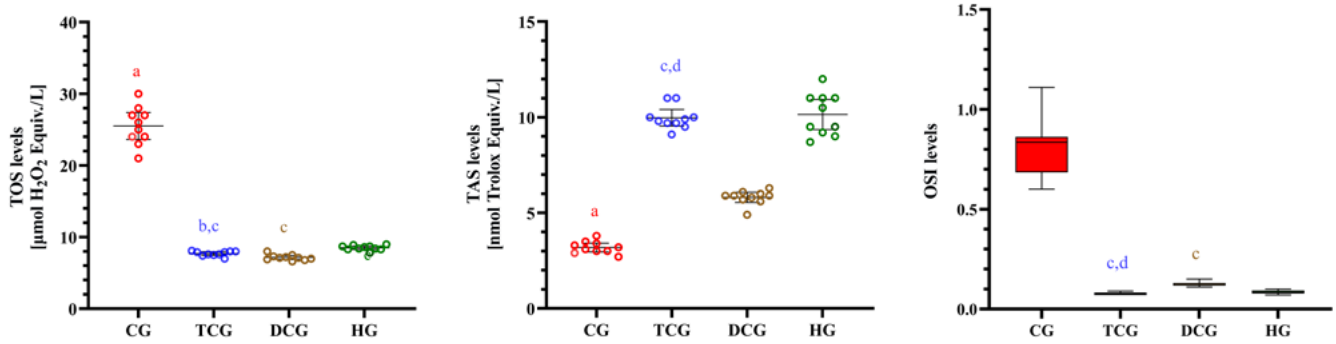


Fig. 4. Comparative analysis of TOS, TAS and OSI levels obtained from experimental groups. Data were presented as mean with 95% confidence intervals for TAS and TOS, and as median and Q1–Q3 percentile for OSI (horizontal line = median; bottom line of the box = Q1 (25th); top line of the box = Q3 (75th))

(a). $p < 0.001$ compared to the TCG, DCG and HG; (b). $p = 1.000$ compared to the DCG; (c). $p > 0.05$ compared to the HG; (d). $p < 0.05$ compared to the DCG; TOS – total oxidant status; TAS – total antioxidant status; OSI – oxidative stress index; CG – 300 mg/kg caffeine-administered group; TCG – 50 mg/kg taxifolin + 300 mg/kg caffeine-administered group; DCG – 2 mg/kg diazepam + 300 mg/kg caffeine-administered group; HG – healthy group.

Discussion

An epileptic seizure results from abnormal synchronized neuronal activity in the brain and causes transient clinical signs or symptoms. The incidence is increasing, especially in developing countries.[3] The literature states that approx. 30% of epilepsy patients are resistant to current treatments. As such, new treatment options are needed to successfully control seizures and improve patient quality of life.[38] Although the pathogenesis of epilepsy has not been fully elucidated, oxidative stress is thought to play a role.[7] Therefore, this study investigated the effects of taxifolin on caffeine-induced epileptiform activity in rats and analyzed its relationship with oxidative stress.

In this study, the latent period was significantly longer in the taxifolin and diazepam groups compared to the epilepsy group. Also, all animals were followed up clinically after the episode, and mortality rates were determined. While all rats in the epilepsy group died from seizures, the death rate was 30% in the taxifolin group and 90% in the diazepam group. Compared to the HG, the CG had higher MDA, MPO, TOS, and OSI values, and lower tGSH, SOD and TAS values. While taxifolin and diazepam inhibited the MDA and TOS increase at a similar level,

taxifolin was more successful than diazepam in suppressing the changes in MPO, tGSH, SOD, TAS, and OSI.

Previous studies reported that increased ROS production caused oxidative stress, disrupting the antioxidant balance and causing epilepsy-induced neuronal death.[39] Lipid peroxidation, due to increased ROS, can produce large amounts of MDA,[13] which is an important parameter used to analyze oxidative damage associated with an epileptic event.[40,41] Moreover, LPO causes impaired cell membrane permeability, decreased membrane potential and cell damage, with the latter exacerbated by MDA formation.[42] Turan et al. revealed that MDA levels in the brain tissue of rats increased significantly in a caffeine-induced epileptic seizure model.[6] Furthermore, previous studies using experimental epileptic episode models reported increased MDA levels in tissues and damaged neuronal cells.[6,40,41,43] Obtaining high MDA levels in the epilepsy group in the current study indicates that our experimental results are in line with the literature.

In the current study, TOS measurement was performed to analyze oxidative stress in more detail. The experimental results revealed that TOS and OSI levels were also high in the epilepsy group alongside high MDA levels. As is well known, there are various types of oxidant molecules, and measuring these oxidants separately increases costs. However, it is possible to determine the levels of all ROS using TOS analysis.[36] In the current literature, there were no studies analyzing the TOS level in the brain tissue of rats subjected to caffeine-induced epilepsy. Nonetheless, a study reported increased TOS and OSI values in parallel with elevated MDA levels in a rat model of pentetrazol-induced epilepsy.[44] Consistent with the literature, our findings showed high MDA, TOS and OSI levels in the epilepsy group.

The accumulation of ROS in brain regions is a cellular threat that can cause significant neuronal damage if not appropriately prevented by local and systemic antioxidants.[45] Measuring changes in antioxidant levels is one of the most frequently used methods for clarifying the mechanisms of brain damage induced by ROS in epilepsy.[46] Therefore, SOD and tGSH levels, known as the endogenous antioxidants, were measured in brain tissue. Superoxide dismutase oxidizes one superoxide radical to oxygen and catalyzes the reduction of another superoxide radical to H_2O_2, which is a less reactive molecule.[46,47] On the other hand, GSH reacts with H_2O_2 and organic peroxides catalyzed by active glutathione peroxidase, detoxifies them and protects the cells from ROS damage.[46,48,49] Our findings showed a significant decrease in SOD and tGSH levels in the brain tissue of rats as a result of caffeine treatment, which is in agreement with a previous study demonstrating that decreased SOD and tGSH levels were associated with brain damage in the caffeine-induced epileptic seizure rat model.[6] In our study, TAS level was measured to analyze the effects of caffeine on SOD, tGSH, and other antioxidants. Total antioxidant status is used to measure the cumulative antioxidative effects of all antioxidants in organisms.[35] We found that the TAS level decreased in parallel with decreased SOD and tGSH levels in brain tissue. However, no studies investigating the level of TAS in caffeine-induced epilepsy models were found in the literature. Nonetheless, studies reported that TAS levels decreased and neuronal oxidative damage developed in experimental epilepsy models.[50,51]

Clinical and experimental evidence suggests that inflammatory processes in the brain play an important role in the pathophysiology of seizures and epilepsy. There is also evidence that inflammation may be a consequence as well as a cause of epilepsy.[52] Indeed, various experimental epilepsy models have reported that the overproduction of ROS triggers an increase in inflammation in neurons.[53] Myeloperoxidase is an enzyme abundant in neutrophil granules, and its secretion increases after neutrophil activation,[54] which generates hypochlorous acid from H_2O_2 in the environment using chloride ions. Hypochlorous acid is a strong oxidant that reacts readily with biological molecules and causes neuronal damage.[54,55] Previous studies reported that increased MPO levels in experimental epilepsy models were associated with increased inflammation and oxidative stress, and this caused epileptic-induced neuronal cell death.[6,39,56] In the current study, a significant increase in MPO level was found in parallel with the increasing oxidative stress parameters and decreasing antioxidant parameters in the caffeine-induced epilepsy model, which is in line with the literature.

Taxifolin, analyzed in terms of its effect against caffeine-induced epileptic activity, significantly decreased the caffeine-induced increase in MDA and TOS levels in brain tissue. There was no information indicating the possible effects of taxifolin against the oxidative damage caused by epilepsy in the brain in the literature. However, Okkay et al. reported that increased MDA levels in the brain tissue of rats with hepatic encephalopathy were suppressed by taxifolin and revealed a neuroprotective effect.[57] Another study by Akagunduz et al. demonstrated that increased MDA and TOS levels in a rat model of pazopanib-induced oxidative liver injury were significantly decreased by taxifolin treatment.[30] Our experimental results and information obtained from the literature suggest that taxifolin has an antioxidant effect on ROS. Indeed, we demonstrated an oxidant/antioxidant balance change in favor of oxidants in the epilepsy group, whereas this balance was maintained in the taxifolin group, with the predominance of antioxidants. Turovskaya et al. reported that taxifolin protected neurons against ischemic damage by activating antioxidant systems.[58] Furthermore, other studies show that taxifolin prevented thioacetamide-associated GSH reduction in the hippocampus and protected the central nervous system from oxidative damage.[57]

It was determined that increased MPO levels due to epileptic activity-induced brain damage decreased significantly as a result of taxifolin treatment. Low MPO levels

in the taxifolin group rather than in the epilepsy group indicate that it antagonized caffeine-induced neuroinflammation. No previous studies have investigated the effects of taxifolin on MPO levels in caffeine-induced epileptic activity. However, Unver et al. reported that taxifolin significantly reduced the increase in MPO in extra-brain tissues and protected against inflammation-induced damage.[59]

The positive effects of taxifolin on epileptic seizures may be associated with oxidative stress, which is involved in the pathogenesis of epilepsy. Sun et al. determined that, in an epilepsy model induced by lithium chloride-pilocarpine in rats, ROS and MDA levels increased in the epilepsy group, while SOD and mitochondrial membrane potential decreased.[60] They also reported that GSH decreased with increasing MDA and ROS levels in brain tissues after epileptic seizures induced by pentylenetetrazole in rats.[61] Previous studies show that epileptic seizures occur with similar pathology, although they are induced in different ways, which suggests that taxifolin may also be effective in epileptic seizures induced by various means.

Numerous clinical studies in adults have revealed that melatonin and cannabidiol added to antiepileptic treatment reduce the frequency and severity of seizures and increase the quality of life, and these results were associated with the antioxidant and anti-inflammatory properties of the agents used.[62,63] Therefore, it is not surprising that taxifolin, which has shown antioxidative[57] and anti-inflammatory properties,[59] has a neuroprotective effect in caffeine-induced epileptic seizures.

Limitations

Objective measurement methods that could assess the effect of taxifolin on the epileptic seizure model, such as electroencephalography and histopathological examination, were excluded due to technical constraints. Also, brain tissue, cerebrospinal fluid and blood concentrations of caffeine and taxifolin could not be measured for technical reasons. Furthermore, additional groups could have been added to assess the effects of using taxifolin in combination with existing antiepileptics.

Conclusions

The current study investigated the effects of taxifolin on caffeine-induced epileptiform activity in rats and analyzed its relationship with oxidative stress. Taxifolin prolonged the latency period of caffeine-induced epilepsy-like convulsions and reduced the severity of tonic-clonic seizures. In addition, taxifolin prevented a decrease in antioxidant capacity and an increase in oxidant and inflammatory markers in brain tissue after caffeine administration. Our experimental results showed that taxifolin may be useful for treating epilepsy and epilepsy-like convulsions. Based on our findings, using taxifolin, which has antioxidant and anti-inflammatory effects, either alone or in combination with existing antiepileptics or a reduced dose of antiepileptic drugs, could be a new treatment strategy. Since no side effects have been found for taxifolin in human studies,[23] it could be used as a food supplement or to treat various diseases. In addition, this study may lead to further studies on the effects of other antioxidant and anti-inflammatory agents for epilepsy treatment.

Supplementary data

The Supplementary materials are available at https://doi.org/10.5281/zenodo.8354869. The package consists of the following files:

Supplementary Table 1. Normality assumption test results for latent period.

Supplementary Table 2.Normality assumption test results for biochemical data.

Supplementary Table 3. Homogeneity of variances assumption test results for MDA, MPO, tGSH, SOD, TOS and TAS.

ORCID iDs

Hasan Yasar https://orcid.org/0000-0003-1115-8359
Durdu Altuner https://orcid.org/0000-0002-5756-3459
Seval Bulut https://orcid.org/0000-0003-4992-1241
Betul Cicek https://orcid.org/0000-0003-1395-1326
Cebrail Gursul https://orcid.org/0000-0001-6521-6169
Mehmet Kuzucu https://orcid.org/0000-0002-7786-7687
Halis Suleyman https://orcid.org/0000-0002-9239-4099

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