SAKARYA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ DERGİSİ

Sakarya University Journal of Science
SAUJS

ISSN 1301-4048 | e-ISSN 2147-835X | Period Bimonthly | Founded: 1997 | Publisher Sakarya University | http://www.saujs.sakarya.edu.tr/

Title: Evaluation of the Antigenotoxic Effect of Quercetin Against Antiepileptic Drug Genotoxicity by Comet Analysis

Authors: Fadime CANBOLAT, Nihan AKINCI KENANOGLU, Tugba Nurcan YUKSEL, Ahmet Ali BERBER

Recieved: 7.08.2023

Accepted: 3.10.2023

Article Type: Research Article

Volume: 27
Issue: 6
Month: December
Year: 2023
Pages: 1185-1196

How to cite
Fadime CANBOLAT, Nihan AKINCI KENANOGLU, Tugba Nurcan YUKSEL, Ahmet Ali BERBER; (2023), Evaluation of the Antigenotoxic Effect of Quercetin Against Antiepileptic Drug Genotoxicity by Comet Analysis. Sakarya University Journal of Science, 27(6), 1185-1196, DOI: 10.16984/saufenbilder.1339199
Access link
https://dergipark.org.tr/en/pub/saufenbilder/issue/80994/1339199

New submission to SAUJS
http://dergipark.gov.tr/journal/1115/submission/start

Sakarya University Journal of Science 27(6), 1185-1196, 2023

Evaluation of the Antigenotoxic Effect of Quercetin Against Antiepileptic Drug Genotoxicity by Comet Analysis

Fadime CANBOLAT[1*], Nihan AKINCI KENANOGLU[2], Tugba Nurcan YUKSEL[3], Ahmet Ali BERBER[4]

Abstract

Valproic acid (VPA) is among the most commonly used antiepileptic drugs in childhood and adult epilepsy. Although VPA is well tolerated, it can cause life-threatening side effects. VPA has toxic and genotoxic effects. Antioxidants can reverse drugs' toxic and genotoxic effects. Therefore, our study aimed to evaluate the antigenotoxic protective effect of quercetin (QUE) against VPA genotoxicity by in vitro comet assay analysis method. Comet assay analysis was performed in five different groups. Group I; negative control (Sterile H_2O), Group II; positive control (H_2O_2), Group III; VPA was applied in four different dose ranges, Group IV; QUE was applied in four different dose ranges, Group V; For the simultaneous combined administration of VPA and QUE, three different doses of VPA + four different doses of QUE were administered. Low-dose administration of QUE was more effective in ameliorating the damage caused by low-dose VPA (62.5 µg/ml) administration. It is seen that the genotoxic damage caused by the application of 125 µg/ml VPA can be eliminated by QUE at all doses. It was determined that different doses of QUE exhibited a significant antigenotoxic effect against damage caused by 125 µg/mL VPA ($P<0.05$). In our study, the curative effect of QUE on DNA damage was determined by in vitro comet analysis. Our analysis results showed that QUE ameliorates VPA-induced genetic damage.

Keywords: Valproic acid, comet assay, in vitro, quercetin

1. INTRODUCTION

Epileptic seizures have been known since the early ages of humanity. Epilepsy is defined in the 2005 International League Against Epilepsy (ILAE) classification as “two or more unprovoked epileptic seizures more than 24 hours apart” [1]. Epilepsy treatment includes drug therapy, surgical applications, or a ketogenic diet. The purpose of drug therapy is to control neuronal increased stimulation. It is vital that the treatment to be

* Corresponding author: fadime.canbolat@comu.edu.tr (F.CANBOLAT)
[1]Department of Pharmacy Services, Vocational School of Health Services, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye
[2] Faculty of Science, Department of Biology, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye
[3] Department of Pharmacology, Faculty of Medicine, Namik Kemal University, Tekirdag, Türkiye
[4]Çanakkale Onsekiz Mart University, Vocational School of Health Services, Çanakkale, Türkiye
E-mail: nakinci@comu.edu.tr, tnyuksel@nku.edu.tr, aberber@comu.edu.tr
ORCID: https://orcid.org/0000-0001-6759-7735, https://orcid.org/0000-0002-3917-6412, https://orcid.org/0000-0001-5092-1674, https://orcid.org/0000-0002-2036-6929

Content of this journal is licensed under a Creative Commons Attribution-Non Commercial No Derivatives 4.0 International License.

chosen is with the drug that causes the most minor damage to the body and best prevents the development of seizures. It is preferred that an ideal antiepileptic drug can affect many types of seizures, its absorption and distribution are rapid, drug interaction is low, tolerance development is low, and its half-life is long. Antiepileptic drugs are classified as sodium channel inhibitors, calcium channel inhibitors, gamma-aminobutyric acid (GABA) potentiators, carbonic anhydrase inhibitors, and agents acting through other mechanisms according to their mechanism of action at the cellular level.

Valproic acid (VPA) is among the most commonly used antiepileptic drugs in childhood and adult epilepsy [2]. When its mechanism of action is evaluated, it is among the mechanisms explained that it increases GABA synthesis, strengthens GABA conductivity, slows down the GABA cycle, and inhibits GABA destruction [3]. In addition, VPA decreases the amount of aspartate, an excitatory amino acid, while increasing the amount of glycine, an inhibitory neurotransmitter. VPA is highly bound to plasma protein. The therapeutic dose of total VPA (free and plasma protein bound) in the blood is 50-120 μg/mL [4-6].

Although VPA is well tolerated, it can cause life-threatening side effects [7]. The effects of antiepileptic drugs on the antioxidant system are known. Previous studies have shown that long-term use of antiepileptics increases the formation of free radicals and causes oxidative damage in neurons. Increasing free radicals cause disruption of vital structures such as DNA, protein, and lipids and cause cell damage. DNA is one of the main targets of free radicals, and mutant components are formed on DNA by oxidation of nucleotides [8]. In particular, the use of VPA causes a decrease in carnitine levels. It can also trigger oxygen-dependent tissue damage and increase free radicals in the body [9]. Free radical attacks also cause the activation of the polysynthetase enzyme (ADP-ribose), leading to programmed cell death and DNA fragmentation [10].

Antioxidants can reverse drugs' toxic and genotoxic effects by restoring the antioxidant balance. Antioxidants protect cells directly and indirectly against the undesirable effects of xenobiotics and free radicals [2]. In recent years, there has been a great interest in plant-derived foods and their active components, which have antioxidant activity on oxidative stress. Polyphenols are the primary antioxidants in the plant-derived foods. They are classified according to their aromatic rings and other components [11].

Flavonoids are the largest polyphenols group, subdivided into flavonols, isoflavones, anthocyanins, flavanols, flavones, and flavanones. These compounds have two benzene rings in their structure and a three-carbon chain connecting them. Quercetin (QUE) is one of the flavonoids found in high amounts in plants, especially vegetables and fruits. There are three important functional groups in the structure of QUE. It has been reported that hydroxyl, catechol, and oxo groups in the structure of Que are effective in antioxidant activity. Que exerts its antioxidant effect by scavenging reactive oxygen species (ROS) and chelating metal cations. This feature stops the lipid peroxidation mechanism [12, 13].

The fact that QUE has many different biological activities, including antioxidant, anti-inflammatory, antiproliferative, proapoptotic, and antiangiogenic, supports its evaluation as a natural anti-cancer agent and suggests that it may have antigenotoxic effects [11- 13]. It is necessary to elucidate the restorative effects of antioxidants on the toxicity of drugs used in chronic diseases that can trigger each other and to conduct further research. It has been established that the protective effects of antioxidant substances against VPA-induced genotoxicity have not been sufficiently explored in the existing literature. Hence, our study seeks to assess the antigenotoxic protective potential of QUE

against VPA genotoxicity through the in vitro comet assay analysis method.

2. MATERIALS AND METHODS

2.1. Chemicals

Valproic acid (VPA) was purchased from Toronto Research Chemicals (North York, Canada). Quercetin dihydrate and all other chemicals in this study were purchased from Sigma-Aldrich (USA).

2.2. Collection Of Blood Samples

Peripheral venous blood was drawn from four healthy donors, two male and two female, aged 20 – 25 years, for all genotoxicity tests. These donors had no history of chromosome fragility, recent viral infection, or exposure to known mutagenic agents or drug therapy within the previous two years, nor had they received ionizing radiation within six months.

2.3. Study Groups

Comet assay analysis was performed in five different groups. *i)* Group I; Sterile H_2O was used for negative control. *ii)* Group II; H_2O_2 (3.4 µg/mL) was used for positive control. *iii)* Group III; For VPA administration, VPA was applied in four different dose ranges (31.2, 62.5, 125, 250 µg/mL), including subtherapeutic, therapeutic, and toxic doses. *iv)* Group IV; For QUE administration, four different doses of QUE (1.6, 3.2, 6.5, 13 µg/mL) were applied, including lower dose intervals than in the literature [14]. *v)* Group V; For the simultaneous combined administration of VPA and QUE, three different doses of VPA (62.5, 125, 250 µg/mL), including therapeutic and toxic doses + four different doses of QUE (1.6, 3.2, 6.5, 13 µg/mL), were administered

2.4. Comet Assay In Isolated Human Lymphocytes

From the work of Singh et al., 2015, the Comet assay (SCGE=Single Cell Gel Electrophoresis) has been modified [15]. Before the test, a Heparinised Peripheral Blood Sample had been taken. The Biocoll Separating solution was used for the isolation of lymphocytes. To detect cell viability, the trypan blue exclusion test has been used. The viability of the cell was more than 99%.

The obtained lymphocytes were distributed to Eppendorf as 100 µL. One hundred microliters each of negative control (sterile H_2O), positive control (H_2O_2), and test substances (III., IV., and V. group) were added. It was incubated at 37^0C for 1 hour. After these treatments, lymphocytes were centrifuged at 1060 rpm for 5 min, and the supernatant was removed. Cells were resuspended with PBS and mixed with the low-melting agarose (0.65%). After that, 100 µL of suspension was dropped onto slides and covered by lamellas (Slides were coated with normal-melting agarose (0.65%) one day ago). The slides were placed at 5 ^{o}C for 20-25 minutes.

After this process, lamellas were removed, and slides were put into a lysing solution (2.5 M NaCl, 10 mM Tris, 100 mM EDTA) at 4^{o}C for 1 hour. Then, the slides were placed in the gel electrophoresis tank with electrophoresis buffer and waited for 20 minutes. After 20 minutes, electrophoresis (1v/cm, 300mA) was carried out for 20 minutes. After electrophoresis, a neutralization buffer (0.4 M Tris, pH=7.5) was used to wash the slides. Each slide received 50 µl of EtBr (Applichem, cat. no. 1239-45-8) following the protocols, and lamellas were placed on top of the slides.

The preparations evaluated three parameters. Three characteristics of DNA's tail—its length, moment, and intensity (%) were assessed. A fluorescent microscope and image analysis systems were used to analyze 100 cells at each concentration for this evaluation.

2.5. Statistical Evaluation

The possibility of DNA damage caused by test substances was evaluated using the student t-test. The regression coefficients for the mean comet tail length, tail moment, and tail intensity were used to determine dose-response relationships.

3. RESULTS

Tail length, tail moment, and tail intensity changes in the results of the comet analysis to determine the genotoxic effect of VPA and QUE were evaluated between the groups (groups III and IV) compared to the negative control group. The in vitro genotoxic evaluation of VPA application at different doses is given in Table 1. When Table 1 was examined, it was observed that the DNA tail length increased at four different VPA doses (31.2, 62.5, 125, 250 μg/mL) compared to the negative control group. This increase was found to be statistically significant (P<0.001). When the increase in tail length was evaluated according to VPA doses, it was seen that the increase was dose-dependent (r: 0.96) (Figure 1). Similarly, it was observed that the tail moment increased at four different VPA doses (31.2, 62.5, 125, 250 μg/mL) compared to the negative control group. This increase was found to be statistically significant (P<0.005). When the increase in the tail moment was evaluated according to VPA doses, it was seen that the increase was dose-dependent (r: 0.83) (Figure 1). When the tail intensity between the groups was examined, the tail intensity was statistically significant in three VPA-treated groups (31.2, 125, 250 μg/mL) compared to the negative control group (P<0.05) (Table 1). When the increase in tail intensity was evaluated according to VPA doses, it was seen that the increase was dose-dependent (r: 0.83) (Figure 1). As a result of these results, it was determined that VPA showed genotoxic properties at application doses.

Table 1 In vitro genotoxic evaluation of valproic acid (VPA) and quercetin (QUE) in human lymphocytes

Dose (μg/mL)	Tail Length (μm)	Tail Moment	Tail Intensity (%)
Valproic Acid (VPA)			
Negative Control H_2O; 0	2.085±0.13	1.142± 0.03	171.501±12.59
31.2	3.995±0.23**	1.296±0.04*	537.694±116.83*
62.5	5.795±0.85**	1.371±0.08*	234.560± 39.29
125	19.235±3.35**	4.289±0.85**	369.951± 38.45*
250	Toxic	Toxic	Toxic
Positive Control H_2O_2; 3.4	33.480 ± 4.43	6.913 ± 0.85	308,570± 69.73
Quercetin (Que)			
Negative Control H_2O; 0	2.085 ± 0.13	1.146 ± 0.03	277.001± 47.16
1.6	1.540 ± 0.10	0.946 ± 0.03	327.625± 159.52
3.2	2.590 ± 0.37	1.087 ± 0.05	491.952± 50.91*
6.5	2.935 ± 0.75	1.243 ± 0.08	373.504± 35.82
13	3.040 ± 0.34*	1.256 ± 0.04*	234.581± 16.70
Positive Control H_2O_2;3.4	33.48 ± 4.43	5.127 ± 0.86	762.920± 123.71

* statistically significant compared to control (P<0.05), ** statistically significant compared to control (P<0.001)

In vitro comet assay genotoxic evaluation of QUE doses is given in Table 1. When Table 1 is examined, the tail length of the lowest dose of QUE (1.6 μg/mL) was determined to be

lower than the negative control. It was determined that tail length increases at medium doses (3.2 and 6.5 µg/mL) were not statistically significant (P> 0.05). A statistically significant increase in tail length was detected by applying high-dose QUE (P<0.05). When the increase in tail length was evaluated according to the QUE dose, a strong positive regression was detected (r: 0.78) (Figure 1). When the tail moment results were analyzed, it was found that the tail moment decreased at two low doses of QUE (1.6 and 3.2 µg/mL) compared to the negative control group. A significant increase in tail moment was observed for the high two doses compared to the negative control group (6.5 and 13 µg/mL). Only the highest dose (13 µg/mL) of these increases was statistically significant (P<0.05). When the increase in the tail moment was evaluated according to the QUE dose, a significant positive regression was detected in the increase (r: 0.67) (Figure 1). When the tail intensity between the groups was examined, a decrease in tail intensity was determined with 13 µg/mL QUE application compared to the negative control group. In other dose applications, an increase in tail intensity was observed compared to the negative control group (Table 1). A negative regression was detected when the increase in tail intensity was evaluated according to the QUE dose (r: -0.34) (Figure 1).

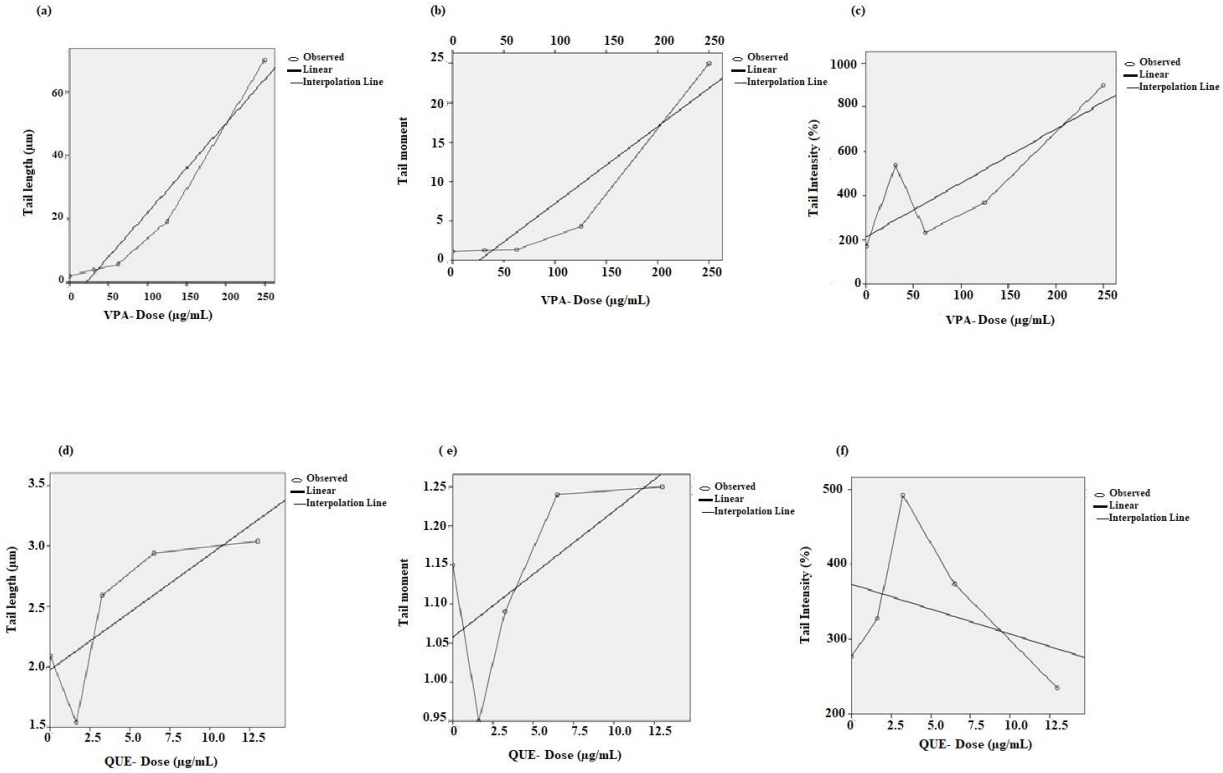

Figure 1 Dose-dependent regression graph of comet analysis results for groups III and IV. Valproic acid (VPA) dose-dependent regression graph of tail length (r: 0.96) (a), valproic acid (VPA) dose-dependent regression graph of the tail moment (r: 0.83) (b), valproic acid (VPA) dose-dependent regression graph of tail intensity (r: 0.83) (c), quercetin (Que) dose-dependent regression graph of tail length (r: 0.78) (d), quercetin (Que) dose-dependent regression graph of the tail moment (r: 0.67) (e), quercetin (Que) dose-dependent regression graph of tail intensity (r: -0.34) (f)

Tail length, tail moment, and tail intensity change in group V comet analysis to determine the antigenotoxic effect of Que were evaluated compared to the condition without Que dose. The results of the comet analysis performed with the simultaneous combination of VPA and QUE at different administration doses in human lymphocytes

are given in Table 2. Each VPA dose is combined with QUE doses (1.6, 3.2, 6.5, and 13 µg/mL). These results determined that the lowest dose (1.6 µg/mL) of QUE combined with 62.5 µg/mL VPA administration significantly reduced the tail length and tail moment. (P<0.05). When the comet tail intensity was evaluated, it was determined that the 13 µg/ml QUE combination caused a statistically significant decrease. When the changes in the tail length (r: 0.71) and tail moment (r: 0.91) depending on the QUE administration doses were examined, a positive increase was determined depending on the dose (Figure 2).

Table 2 Genotoxic profile of the combination of valproic acid (VPA) and quercetin (QUE) in human lymphocytes

VPA µg/mL	QUE µg/mL	Tail Length (µm)	Tail Moment	Tail Intensity (%)
62.5	0	5.795 ±0.85	1.371±0.08	494.560 ± 62.29
	1.6	2.015±0.18*	1.044±0.06*	989.920 ± 118.21
	3.2	4.680 ±0.80	1.233 ± 0.05	415.390 ± 55.11
	6.5	4.745 ±1.43	1.481 ± 0.23	734.643 ± 83.01
	13	8.490 ±0.70	2.883 ± 0.27	79.914 ± 4.59 *
125	0	19.235±3.35	4.289 ± 0.85	369.951± 38.45
	1.6	2.290±0.18**	1.092±0.04**	357.125 ± 29.77
	3.2	2.465±0.20**	1.164±0.06*	440.290 ± 43.69
	6.5	3.595±1.08**	1.404 ±0.18*	343.880 ± 28.73
	13	2.945±0.43**	1.163±0.05**	217.251 ± 11.16*
250	0	Extremely toxic	Extremely toxic	Extremely toxic
	1.6	NC	NC	NC
	3.2	NC	NC	NC
	6.5	NC	NC	NC
	13	NC	NC	NC
Positive Control (H_2O_2)	3.4	39.265 ± 2.47	1051.298 ± 99.28	806.160 ± 93.66

* statistically significant compared to control (P<0.05), ** statistically significant compared to control (P<0.001), NC; not comparable

On the other hand, negative regression was detected in tail intensity depending on the QUE dose (r:-0.63) (Figure 2). QUE exhibited a dose-dependent significant antigenotoxic effect on the comet tail intensity caused by 62.5 µg/mL VPA. QUE doses (1.6, 3.2, 6.5 ve 13 µg/mL) combined with 125 µg/mL VPA administration statistically reduced comet tail length occurrences (P<0.001). The tail moment showed a statistically significant decrease at all combination doses of QUE (P<0.001). When the tail intensity was evaluated, a decrease was observed in the tail intensity in 1.6, 6.5, and 13 µg/mL QUE combinations, while an increase was observed in the 3.25 µg/ml QUE combination.

The QUE combination level, which is statistically significant in the decrease in comet tail intensity, was determined as 13 µg/ml (P<0.05) (Table 2). Que doses combined with 125 µg/mL VPA administration decreased tail length (r: -0.50) (Figure 2), tail moment (r: -0.51) (Figure 2), and tail intensity (r: -0.82) (Figure 2) dose-dependently. It was determined that different doses of QUE exhibited a significant antigenotoxic effect against DNA damage caused by 125 µg/mL VPA (Figure 2). The administration of 250 µg/mL VPA could not be included in the analysis due to its highly

toxic effect on human lymphocytes; therefore, no comparison could be made (Table 2).

Figure 3 shows photomicrographs showing the varying intensities of fluorescence in the comet tail in our study groups. While DNA damage was not observed in the negative control and Que-treated groups (Figure 3), DNA damage was observed in the groups exposed to VPA (Figure 3).

4. DISCUSSION

This study evaluated the genotoxic effect of the antiepileptic drug VPA on human lymphocytes and the antigenotoxic role of QUE. VPA is among the antiepileptic drugs widely and long-term used by epileptic patients in a wide age range. VPA is absorbed rapidly and easily after oral administration. The time to peak plasma concentration is approximately 2-8 hours, and the half-life is approximately 8-18 hours. It is rapidly distributed throughout the body and reaches the central nervous system within minutes. It is approximately 90% protein bound, primarily to albumin [4, 5]. It is extensively metabolized in the liver, and some metabolites are active as anticonvulsants. Effective blood levels recommended for use as antiepileptic and antimanic are approximately 50-120 µg/ml. If the blood level is 100 µg/ml, the possibility of side effects increases gradually [4-6]. When the literature studies on the genotoxic effects of VPA are examined, it is seen that VPA toxicity represents an increasing concern for toxicologists. In the study of Sardas *et al.* (1994), sister chromatid exchanges (SCE) were analyzed in epilepsy patients receiving anticonvulsant medication.

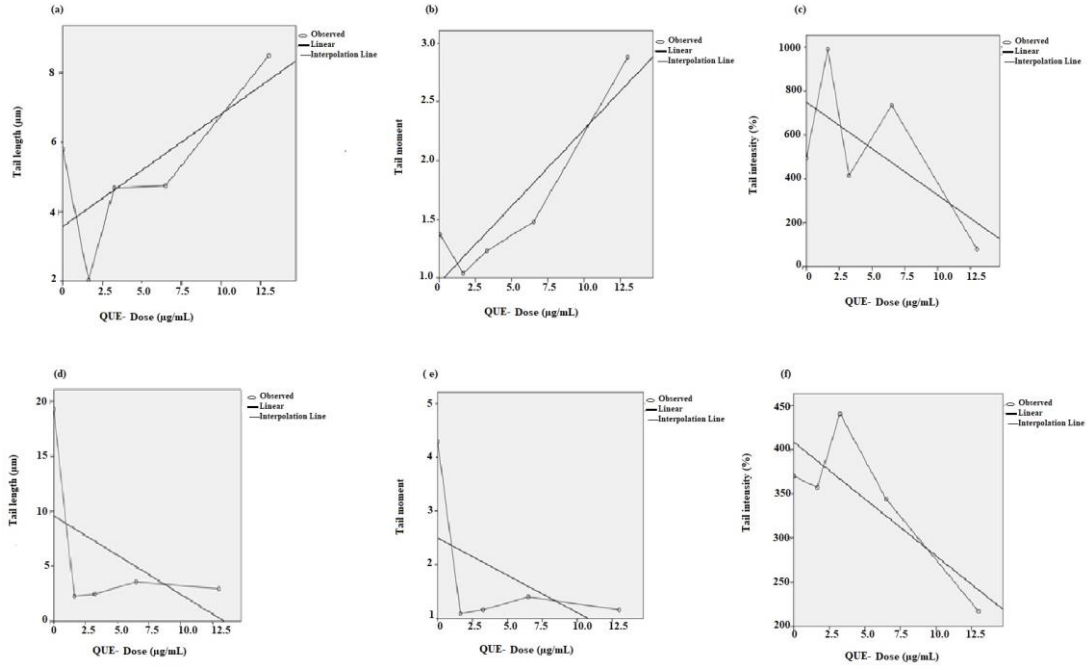

Figure 2 Dose-dependent regression graph of comet analysis results for group V. Quercetin (Que) dose-dependent regression graph of tail length (r: 0.71) in 62.5 µg/mL valproic acid (VPA) toxicity (a), quercetin (Que) dose-dependent regression graph of the tail moment (r: 0.91) in 62.5 µg/mL valproic acid (VPA) toxicity (b), quercetin (Que) dose-dependent regression graph of tail intensity (r:-0.63) in 62.5 µg/mL valproic acid (VPA) toxicity (c), quercetin (Que) dose-dependent regression graph of tail length (r: -0.50) in 125 µg/mL valproic acid (VPA) toxicity (d), quercetin (Que) dose-dependent regression graph of tail moment (r: -0.51) in 125 µg/mL valproic acid (VPA) toxicity (e), quercetin (Que) dose-dependent regression graph of tail intensity (r: -0.82) in 125 µg/mL valproic acid (VPA) toxicity (f)

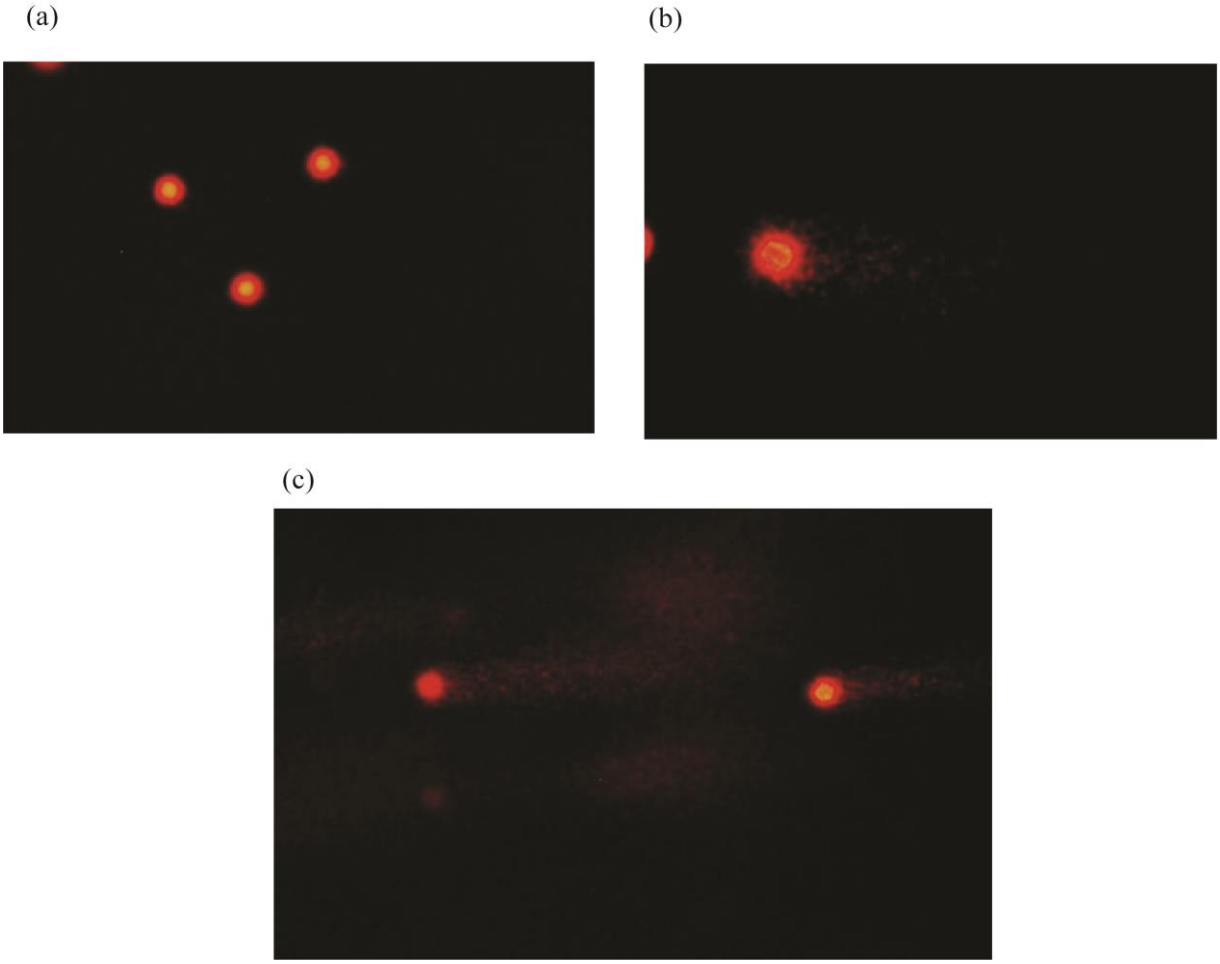

Figure 3 Photomicrographs showing the varying intensities of fluorescence in the comet tail in our study groups. Undamaged (a) in the negative control and Que-treated groups, slightly damaged (b), maximally damaged (c) in the groups exposed to valproic acid (VPA)

At the end of the study, it was observed that the frequency of SCE per metaphase increased significantly in all patients compared to the control group, and it was reported that anticonvulsant drugs may have genotoxic effects [16]. Hu *et al.* (1990) reported that the frequency of SCE in peripheral lymphocytes of epileptic children treated with VPA monotherapy was significantly higher compared to the control group [17].

Another in vitro study reported a significant increase in SCE frequency and decreased proliferation rate index (PRI) values of lymphocyte cultures treated with VPA monotherapy [18]. Mahmoud *et al.*, in their 2019 study, studied the DNA damage that may develop due to the use of VPA in epileptic children. The study concluded that patients treated with VPA were at risk of developing significant DNA damage effects compared to those who did not receive treatment [19]. In a 2011 study by Khan *et al.*, germ cell damage and genotoxic effects of VPA use were investigated in male mice. At the end of the study, it was observed that VPA treatment significantly decreased sperm count, increased sperm head abnormality, sperm DNA damage, and oxidative stress in the testes of mice [20].

Denli *et al.* (2000) found that the frequency of comet scores in epileptic patients treated with VPA was significantly higher than in the control group [21]. In the animal study of Sakr *et al.* 2014, cytogenetic and testicular damage caused by VPA was investigated in rats. In the study, it was reported that VPA causes

chromosomal aberrations and DNA damage [22].

In our study, when the genotoxic effect of VPA on human lymphocytes was examined, including the therapeutic plasma concentration range, it was observed that the tail length and tail moment increased depending on the VPA dose (Table 1). When the tail intensity was evaluated, the tail intensity formed at 31.2, 125, and 250 µg/mL VPA exposure was higher than the tail intensity formed with the H_2O_2 exposure used as a positive control. The highest dose of the working range, 250 µg/mL of VPA, caused highly toxic effects (Table 1). As a result of these results, it was determined that VPA showed genotoxic properties at application concentrations (Figure 1). Considering the literature data and the in vitro comet assay analysis data in our study (Figure 3), it is thought that long-term use of VPA at the upper limits of the therapeutic range or alarm level may cause adverse effects on human lymphocytes.

Natural-origin products with known medicinal effects are often used alongside drug treatments to eliminate the side effects that may occur or increase the drug's expected effect. QUE is found in many pharmaceutical and dietary supplements taken by patients suffering from epilepsy and being treated with antiepileptic drugs. No significant adverse effects of QUE were reported in the 50 to 500 mg/kg/day dose range [23]. The effect of QUE on the central nervous system has been demonstrated in both experimental and clinical studies [24]. In the study of Chaudhary *et al*. (2014), it was determined that QUE supplementation with VPA significantly restored the levels of antioxidant enzymes and non-enzymatic antioxidants in rats [25].

In addition to its potent antioxidant properties, QUE's antigenotoxic effects are also being investigated. The ability of QUE to protect against ionizing radiation (IR)-induced genotoxicity in human lymphocytes was investigated by Patil *et al.* [14]. The study observed that QUE (10-25 µg/ml) gradually reduced comet formation in irradiated human lymphocytes [14]. When the genotoxic effect of QUE (1.6, 3.2, 6.5, 13 µg/ml) used at low dose ranges on human lymphocyte cells is examined in our study, it is observed that the tail length and tail moment are close to the negative control group. It is observed that these values increase depending on the dose (Table 1).

The tail length and tail moment at the lowest dose of QUE (1.6 µg/ml) were also lower than the negative control group. The tail intensity between groups varied independently of dose. The tail intensity at the highest dose of QUE (13 µg/ml) was lower than that of the negative control group. Tail length, moment, and intensity values in human lymphocytes exposed to QUE at all levels significantly differed from positive control (H_2O_2). Since the effects of all levels of QUE used in the study were close to the negative control group, these levels were found to be reliable in terms of genotoxicity (Table 1).

Genotoxicity-safe doses of QUE used in the study were applied to human lymphocytes in combination with the therapeutic and toxic doses of VPA (62.5, 125, 250 µg/ml) in plasma (Table 2). Our study's data shows that QUE can correct DNA damage depending on the dose of VPA. QUE exhibited a dose-dependent significant antigenotoxic effect on the comet tail intensity caused by 62.5 µg/mL VPA (r: -0.63) (Figure 2). Low-dose administration of QUE was more effective in ameliorating the damage caused by low-dose VPA (62.5 µg/ml) administration (Table 2). It is seen that the genotoxic damage caused by the application of 125 µg/ml VPA can be eliminated by QUE at all doses. (Table 2). It was determined that different doses of QUE exhibited a significant antigenotoxic effect against damage caused by 125 µg/mL VPA (Figure 2). Since the genotoxic effect of the highest dose of VPA was found to be extremely toxic, a comparison with QUE doses could not be made. In a 2008 study by Ramos *et al*., the chemoprotective effect of

QUE on tert-butyl hydroperoxide (t-BHP)-induced DNA damage was investigated by human hepatoma cell line (HepG2) comet assay. At the end of the research, it was determined that quercetin has an effect on removing DNA damage [26]. In our study, the curative effect of QUE on DNA damage was determined by in vitro comet analysis. Our analysis results showed that QUE ameliorates VPA-induced genetic changes.

5. CONCLUSION

It is known that long-term drugs used in chronic diseases can adversely affect many mechanisms in our body, especially oxidative stress. Bioactive molecules with high antioxidant activity, which are widely accepted as safe, are used to naturally reduce these adverse effects of drugs. Our study has also shown that QUE, one of these molecules, can reverse any damage that may occur in the body thanks to its potent antioxidant activity. Several previous literature studies have reported the curative effect of QUE against genotoxicity. However, since the studies on the curative effect of QUE against genotoxicity that may occur with the use of VPA are limited, the data of our study contributed to the elimination of this deficiency in the literature. In our study data, QUE has been shown to ameliorate the genetic damage caused by VPA. In light of these data, it is thought that VPA toxicity can be reduced by incorporating QUE as a dietary supplement or in new drug formulations.

Funding
The author (s) has not received any financial support for the research, authorship, or publication of this study.

Authors' Contribution
F.C is the first author of the study. Concept: F.C, Design: F.C, Data Collection and Processing: F.C, A.A.B, Analysis and Interpretation F.C., N.A.K., T.N.Y., A.A.B., Literature Search: F.C., Writing: F.C., T.N.Y., A.A.B.

The Declaration of Conflict of Interest/ Common Interest
The authors have declared no conflict of interest or common interest.

The Declaration of Ethics Committee Approval
The study was approved by Çanakkale Onsekiz Mart University Clinical Research Ethics Committee on 26.07.2023 (Decision no; 2023/10-07).

The Declaration of Research and Publication Ethics
The article's authors declare to respect SAUJS scientific, ethical, and citation rules in all processes of the paper and that they did not falsify the collected data. Furthermore, they state that the Sakarya University Journal of Science and its editorial board are not responsible for any ethical violations that may be encountered and that this study has not been evaluated in any environment. scholarly publications other than the Sakarya University Journal of Science.

Acknowledgments
Thank you to the management of Uskudar University Clinical Pharmacogenetics Laboratory and Istanbul University SANKARA Brain and Technology Research Center for providing reference standards in our study.

REFERENCES

[1] P. Haznedar, “Çocukluk çağı epilepsilerinde levetirasetam ve valproik asit tedavisinin karaciğer fonksiyonları, plazma serbest karnitin ve lipid peroksidasyonu ile oksidatif DNA hasarı üzerine etkileri,” Çocuk Sağlığı ve Hastalıkları Anabilim Dalı Tıpta Uzmanlık Tezi, Ankara üniversitesi, 2017.

[2] D. A. Colak, C. Ersöz, “Sisplatin ve valproik asitin indüklediği toksisiteye karşı kudret narının Drosophila melanogaster’in yaşama yüzdesi ve

ömür uzunluğu üzerine etkisi," Eurasian Journal of Biological and Chemical Sciences, vol.2, no.2, pp.73-78, 2018.

[3] T. Tomson, D. Battino, E. Perucca, "Valproic acid after five decades of use in epilepsy: time to reconsider the indications of a time-honored drug," Lancet Neurology, vol.15, no.2, pp.210-218, 2016.

[4] M. D. Sztajnkrycer, "Valproic acid toxicity: overview and management," Journal of Toxicology: Clinical Toxicology, vol. 40, no. 6, pp.789-801, 2002.

[5] I. T. Okay, C. Kısa, N. Dilbaz, "Psikiyatrik bozukluklarda valproat kullanımı", Klinik Psikiyatri, vol. 5, 33-41, 2002.

[6] C. Hiemke, P. Baumann, N. Bergemann, A. Conca, O. Dietmaier, K. Egberts, M. Fric, M. Gerlach, C. Greiner, G. Grunder, E. Halen, U. Havemann-Reinecke, E. Jaquenoud Sirot, H. Kircherr, G. Laux, U.C. Lutz, T. Messer, M.J. Müller, B. Pfuhlmann, B. Rambeck, P. Riederer, B. Schoppek, J. Stingl, M. Uhr, S. Ulrich, R. Waschgler, G. Zernig, "AGNP consensus guidelines for therapeutic drug monitoring in psychiatry: update 2011", Pharmacopsychiatry, vol. 44, no. 06, pp.195-235, 2011.

[7] F. E. Dreifuss, N. Santilli, D. H. Langer, K. P. Sweeney, K. A. Moline, K. B. Menander, "Valproic acid hepatic fatalities: a retrospective review," Neurology, vol. 37, no. 3, pp. 379-85, 1987.

[8] F. J. Romero, F. Bosch-Morell, M. J. Romero, E. J. Jareño, B. Romero, N. Marín, J. Romá, "Lipid peroxidation products and antioxidants in human disease," Environmental Health Perspectives, vol. 106, no. 5, pp. 1229, 1998.

[9] D. Coulter, "Carnitine deficiency: a possible mechanism for valproate hepatotoxicity," The Lancet, vol. 323, no. 8378, pp.689, 1984.

[10] T. Devasagayam, J. Tilak, K. Boloor, K. S. Sane, S. S. Ghaskadbi, R. Lele, "Free radicals and antioxidants in human health: current status and future prospects," Journal of the Association of Physicians of India, vol. 52, no.10, pp.794-804, 2004.

[11] A. S. Yalçın, A. M. Yılmaz, E. M. Altundag, S. Koçtürk, "Kurkumin, kuersetin ve çay kateşinlerinin anti-kanser etkileri," Marmara Pharmaceutical Journal, vol. 21, no.1, pp. 19-29, 2017.

[12] Y. J. Moon, I. Wang, R. DiCenzo, M. E. Morris, "Quercetin pharmacokinetics in humans," Biopharmaceutics and Drug Disposition, vol. 29, pp. 205-17, 2008.

[13] W. Bors, W. Heller, C. Michel, M. Saran, "Flavonoids as antioxidants: determination of radical-scavenging efficiencies," Methods in Enzymology, vol. 186, pp. 343–55, 1990.

[14] S. L. Patil, K. Swaroop, N. Kakde, H. M. Somashekarappa, "In vitro protective effect of rutin and quercetin against radiation-induced genetic damage in human lymphocytes," Indian Journal of Nuclear Medicine, vol. 32, no. 4, pp. 289, 2017.

[15] N. P. Singh, M. T. McCoy, R. R. Tice, E. L. Schneider, "Simple technique for quantitation of low levels of DNA damage in individual cells," Experimental Cell Research, vol 175, no. 1, pp.184-191, 1988.

[16] S. Şardaş, M. Ada, A.E. Karakaya, N. Aydin, "Sister-chromatid exchanges in epileptic patients on anticonvulsant therapy," Mutation Research/Environmental Mutagenesis and Related Subjects, vol. 313, no. 1, pp. 21-24, 1994.

[17] L. Hu, X. Lu, B. Lu, Y. Huang, "The effect of valproic acid on SCE and chromosome aberrations in epileptic children," Mutation Research Letters, vol. 243, no. 1, pp. 63-66, 1990.

[18] I. Karapidaki, M. T. Ekonomopoulou, K. Akritopoulou, D. Anestakis, Z. Iakovidou-Kritsi, "Cytogenetic effects of valproic acid and ziprasidone in human lymphocyte cultures," Neuropsychobiology, vol. 64, no. 4, pp. 219-223, 2011.

[19] A. T. Mahmoud, M. A. Tawfik, S. A. Abd-El-Naby, D. A. E. A. Hammad, "A study of DNA damage in epileptic children treated with valproic acid or carbamazepine," Menoufia Medical Journal, vol. 32, no. 3, pp. 1078, 2019.

[20] S. Khan, T. Ahmad, C. V. Parekh, P. P. Trivedi, S. Kushwaha, G. Jena, "Investigation on sodium valproate induced germ cell damage, oxidative stress and genotoxicity in male Swiss mice," Reproductive Toxicology, vol. 32, no. 4, pp. 385-394, 2011.

[21] M. Denli, H. I. Aydin, R. Döndaröz, T. Özişik, E. Erdem, V. Baltaci, "Genotoxicity evaluation in female patients on valproic acid monotherapy using alkaline single cell gel electrophoresis (comet assay)," Eastern Journal of Medicine, vol. 5, no. 2, pp.61-65, 2000.

[22] S. A. Sakr, M. E. Zowail, A. M. Marzouk, "Effect of saffron (Crocus sativus L.) on sodium valproate induced cytogenetic and testicular alterations in albino rats," Anatomy & cell biology, vol. 47, no. 3, pp. 171-179, 2014.

[23] M. Russo, C. Spagnuolo, I. Tedesco, S. Bilotto, G. L. Russo, "The flavonoid quercetin in disease prevention and therapy: facts and fancies," Biochemical pharmacology, vol. 83, no. 1, pp. 6-15, 2012.

[24] D. Nieoczym, K. Socała, G. Raszewski, P. Wlaź, "Effect of quercetin and rutin in some acute seizure models in mice," Progress in Neuro-Psychopharmacology and Biological Psychiatry, vol. 54, pp. 50-58, 2014.

[25] S. Chaudhary, P. Ganjoo, S. Raiusddin, S. Parvez, "Nephroprotective activities of quercetin with potential relevance to oxidative stress induced by valproic acid," Protoplasma, vol. 252, pp. 209-217, 2015.

[26] A. A. Ramos, C. F. Lima, M. L. Pereira, M. Fernandes-Ferreira, C. Pereira-Wilson, "Antigenotoxic effects of quercetin, rutin and ursolic acid on HepG2 cells: evaluation by the comet assay," Toxicology Letters, vol. 177, no. 1, pp. 66-73, 2008.