Original Research

Effects of *Leontice leontopetalum* and *Bongardia chrysogonum* on oxidative stress and neuroprotection in PTZ kindling epilepsy in rats

Ozlem Ergul Erkec[1*], Okan Arihan[1], Mehmet Kara[1], Ersin Karatas[2], Remzi Erten[3], Halit Demir[4], Ismail Meral[5], Muzaffer Mukemre[6], Fevzi Ozgokce[6]

[1] Department of Physiology, Faculty of Medicine, Van Yuzuncu Yil University, Van, Turkey
[2] Department of Medical Biochemistry, Faculty of Medicine, Bezmialem Vakif University, İstanbul, Turkey
[3] Department of Pathology, Faculty of Medicine, Van Yuzuncu Yil University, Van, Turkey
[4] Department of Chemistry, Faculty of Science, Van Yuzuncu Yil University, Van, Turkey
[5] Department of Physiology, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey
[6] Department of Biology, Faculty of Science, Van Yuzuncu Yil University, Van, Turkey

Abstract: We investigated the effects of *Leontice leontopetalum* and *Bongardia chrysogonum* on apoptosis, gamma-aminobutyric acid (GABA$_A$) receptor positive cell number, cyclin-B1 and bcl-2 levels and oxidative stress in pentylenetetrazol (PTZ) kindling in rats. Kindling was produced by subconvulsant doses of PTZ treatments in rats. Wistar albino rats were divided into 4 groups; Control, PTZ treated (PTZ), PTZ+*L. leontopetalum* extract treated (PTZ+LLE) and PTZ+*B. chrysogonum* extract treated (PTZ+BCE) groups. Extracts were given a dose (200 mg/kg) 2h before each PTZ injection. PTZ treatment significantly decreased the glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) activities and bcl-2 levels and increased the total oxidant status (TOS), malondialdehyde (MDA), cyclin B1, oxidative stress index (OSI) and number of neurons that expressed GABA$_A$ receptors when compared to the control. LLE and BCE possessed antioxidant activity in the brain and ameliorated PTZ induced oxidative stress, decreased cyclin-B1, increased bcl-2 levels, and kept the GABA$_A$ receptor number similar to that of the control despite the PTZ application.

Key words: Bcl-2; Brain; *Bongardia* chrysogonum; Cyclin-B1; GABA$_A$; kindling model; *Leontice leontopetalum*; Oxidative stress; Pentylenetetrazol.

Introduction

Epilepsy is the second most prevalent neurological problem worldwide (1). Despite current antiepileptic drug treatment, one-third of epileptic patients have drug resistant epilepsy (1). Temporal lobe epilepsy (TLE) is an epileptic disorder that has one of the worst prognosis (2). Kindling is an experimental model of TLE (3). Repeated application of sub-convulsive electrical or chemical stimuli, such as pentylenetetrazole (PTZ), causes progressive intensification of seizures (4). Due to chronic convulsive activity, neuronal degeneration and death occurs, which are reported both in clinical situations and experimental animal studies (5).

There is increasing evidence regarding the link between the cell cycle and neuronal death (4). Immunohistochemistry studies have shown cyclin B expression in the hippocampus of TLE patients (6) and PTZ-kindled rats (cyclin-B1)(4).

Oxidative stress is another factor contributing to seizure related neuronal death (7). Oxidative stress causes cellular injury and may cause cell death through the oxidation of biomolecules (2). The current literature research has focused on the possible interrelationship among oxidative stress, mitochondrial dysfunction, cell injury, and epilepsy (8). Hence, a compound that would decrease the reactive oxidative injury in the brain due to the seizures may be functional for treatment of epilepsy (9).

Medicinal herbs provide molecules that may be candidates for epilepsy medication (10). The roots of *Leontice leontopetalum* have been used as a cure for epilepsy in Turkish folk medicine (11). The plant has antioxidant and anti-cholinesterase activities (12). Bongardia chrysogonum has also been used in Turkish folk medicine for epilepsy (13). Although *L. leontopetalum* and *B. chrysogonum* have been used as medicinal plants for treatment of epilepsy in humans, there is no available research regarding the effect of *L. leontopetalum* aqueous root extract on any chronic models of epilepsy in experimental models. In a very recently study, in vitro antioxidant and free-radical scavenging properties and anticonvulsant activity (in mice) of *B. chrysogonum* root extract was reported (14). Therefore, the aim of this study was to determine the anticonvulsant, antioxidant, and neuroprotective effects of *L. leontopetalum* and *B. chrysogonum*, which belong to the same family, extracts and associated seizure-induced neuronal loss and alterations in the gamma-aminobutyric acid (GABA$_A$) receptor number in a PTZ kindling model of epilepsy in rats.

Materials and Methods

Plant collection

L. leontopetalum plant were collected from Tuşba, in the province of Van, Turkey, a voucher specimen was

prepared in the Van YYU VANF herbarium under number 114872. *B. chrysogonum* was collected from the Van YYU campus and a voucher specimen was prepared under number 165062 at the same herbarium.

Preparation of the extract

The roots of *L. leontopetalum* and *B. chrysogonum* were separately dried for 1 month, and following pounding, they were sieved through a 1 mm mesh. The powder obtained was dissolved in distilled water and macerated in a water bath for 50 min at 70 °C with continuous stirring. Following 10 min of cooling, it was filtered through a filter paper and the root aqueous extract was prepared.

Animals and treatments

Male Wistar albino rats, aged 2 months, were obtained from the Van Yuzuncu Yil University, Experimental Animals Facility. The animals were kept in a light/dark photoperiod of 12:12 and under stable heating conditions (21 °C). Standard rat pellet and tap water was given ad libitum. The study was approved by the Ethics Committee of the Van Yuzuncu Yil University.

The animals were divided into 4 groups (n = 6): (1) the control group: saline (SF) (0.5 ml, orally (po), (2) the pentylenetetrazol (PTZ) group: SF (0.5 ml, po) + PTZ (35 mg/kg, intraperitoneally (ip), 3) PTZ + L. leontopetalum extract (LLE), (PTZ+LLE) group: LLE (200 mg/kg, po) + PTZ (35 mg/kg, ip), and 4) PTZ + B. chrysogonum extract (BCE) (PTZ+BCE) group: BCE (200 mg/kg, po) + PTZ (35 mg/kg, ip). SF, LLE, or BCE were given to rats orally via a gauge every other day (Monday, Wednesday, and Friday) 2h before each PTZ injection. Rats in the control group treated only saline. PTZ kindling was produced by injecting a subconvulsant dose of PTZ, 35 mg/kg (a total of 18 injections, every other day). PTZ was administered 75 mg/kg, as a challenge dose in 19th injection day. Seizure intensity was scored after each PTZ injection for 30 min according to the Racine scale (15) . Blood and brain samples were obtained under anaesthesia. Brain tissue samples were kept in -80°C until analysis.

Antioxidative Parameters

Tissue preparation

The frozen brain tissue samples were thawed and homogenized using an ultrasonic homogenizer in glass porcelain for 5 min in ice-cold media. Next, they were centrifuged at 10000g, 30 min at 4 C°. Supernatant fractions were used for the determination of antioxidant defense enzymes and oxidative stress parameters.

Analysis of the oxidative stress

Serum total antioxidant status (TAS) (16) and total oxidant status (TOS) (17) were determined spectrophotometrically using the Rel assay diagnostics kit (Mega Tıp, Gaziantep, Turkey). Using those findings, the oxidative stress index (OSI) was calculated (18). Brain lipid peroxidation was determined by measuring the malondialdehyde (MDA) levels (19). Superoxide dismutase (SOD) activity was determined by using the method of Spitz and Oberley (20). Glutathione peroxidase (GSH-Px) enzyme activity was determined using the method

by Paglia and Valentine (21). Measurement of the nitric oxide (NO) was performed using the colorimetric nitric oxide assay kit (Relassay®, Gaziantep, TR), which uses Griess reagents to convert nitrite into a deep purple azo compound.

Western blot analysis

Tissues obtained from the brain and hippocampus were homogenized using steel beads in a homogenization buffer (2 mM Tris-HCl pH 7.2, 150 mM NaCl, 10% glycerol, 0.2% NP-40 additionally proteases inhibitors which inhibits cysteine, serine, aspartic, and metalloproteases.). Following centrifugation (Centric 200R) occurred at 4 °C for 10 min at 14000 RPM. The final supernatant was used as the total protein. Protein quantities were measured using the method described by Bradford (22). All the samples equated to the same total protein amount using the homogenization buffer as a diluent. 30 µg of total protein from each sample was loaded onto a 4-12% SDS-PAGE gel for separation. The separated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA). After incubation in 5% skim milk powder containing TBS-T (20 mM Tris HCl, 137 mM NaCl and 0.1% Tween-20, pH 7.6) for 2 h at room temperature to block nonspecific binding, the PVDF membrane was reacted for 16 h at 4 ° C with primary antibodies against Bcl-2 (ProteinTech, 1:1000), ß -Tubilin (ProteinTech, 1:1000), and Cyclin ß1 (ProteinTech 1:1000), washed in TBS-T, and incubated for 1 h at room temperature with corresponding horseradish peroxidase labelled anti-rabbit secondary antibody (Cell Signaling, 1:5000). As the final step after three times rinses with TBS-T, the ECL developer solution was applied to the membranes to visualize by using an imaging system (Chemidoc MP, Biorad). The Image J analysis software was used to determine the integrated intensity of individual bands (23).

Sample preparation and histological evaluations

Specimens were fixed in 10% neutral buffered formalin (NBF) and embedded in paraffin. Paraffin sections of 4 µm were stained using $GABA_A$ receptor-alpha immunohistochemistry and the TUNEL method for apoptosis, and examined under an Olympus BX53F light microscope (Olympus, Tokyo, Japan).

$GABA_A$ Receptor alpha immunohistochemistry

Rabbit polyclonal to $GABA_A$ Receptor alpha 1 (Abcam, Cat: ab33299) was used as the primary anticore. Staining was performed in the presence of appropriate positive controls using the Ventana Benchmark XT immunohistochemistry automatic staining system and Ventana Ultraview Dab Detection Kit. Positive staining cell counting was done with images taken from 5 selected similar fields (at 200X magnification).

TUNEL Assay

DNA fragmentation was analyzed using the TUNEL kit (ApopTag ® Plus Peroxidase In Situ Apoptosis Detection Kit), following the manufacturer's guidelines. At least 10 areas of each section were analyzed at 200X magnification with a light microscope Olympus BX53F (Olympus, Tokyo, Japan).

Statistical analysis

Statistical significance was established using the Kruskal-Wallis and Tukey tests. A value of p < 0.05 was considered statistically significant. Data are presented as the mean ± SEM. Statistical Package for the Social Sciences software, version 24, was used for the statistical evaluations.
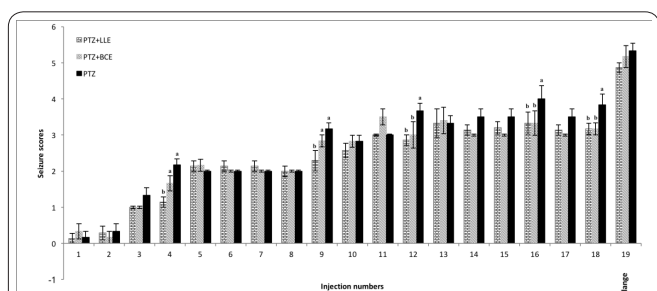

Figure 1. Effect of LLE or BCE administration on the development of PTZ-induced kindling. Data are presented as the mean ± SEM. Different letters show significant differences between the groups (p < 0.05).
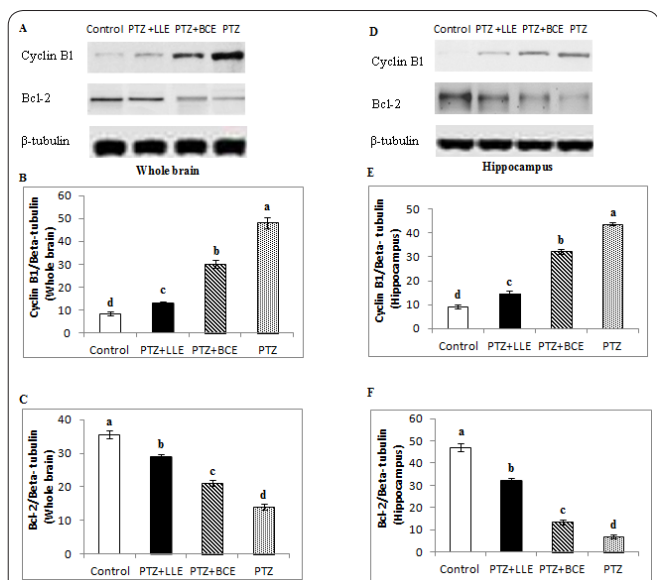

Figure 2. The bcl-2 expression levels were decreased and cyclin-B1 levels were increased in both the whole brain and hippocampus of the PTZ group: A) The cyclin-B1 and bcl-2 expression levels in the whole brain of the control, PTZ+LLE, PTZ+BCE, and PTZ groups. B) Effects of PTZ, LLE, or BCE administration on the expression levels of cyclin-B1 in the whole brain (p < 0.01). C) Effects of PTZ, LLE, or BCE administration on the bcl-2 expression levels in the whole brain (p < 0.01). D) The cyclin-B1 and bcl-2 expression levels in the hippocampus of the control, PTZ+LLE, PTZ+BCE, and PTZ groups. E) Effects of PTZ, LLE, or BCE administration on the expression levels of cyclin-B1 in the hippocampus (p < 0.01). F) Effects of PTZ, LLE, or BCE administration on the bcl-2 expression levels in the hippocampus (p < 0.01). Data are presented as the mean ± SEM. Different letters show significant differences between the groups (p< 0.01).

Results

Repeated administration of PTZ (35 mg/kg), resulted in increasing convulsive activity in the PTZ group (Figure 1). LLE treatment (200 mg/kg) significantly decreased the seizure scores with the 4th, 9th, 12th, 16th, and 18th injections (p < 0.05) compared to that in the PTZ group (Figure 1). BCE administration significantly decreased the seizure scores with the 12th, 16th, and 18th injections (p < 0.05) compared to that in the PTZ group (Figure 1).

The expression levels of cyclin-B1 protein in the whole brain (Figures 2A, B) and hippocampus (Figures 2D, E) were significantly increased (Table 1) in the PTZ, PTZ+BCE, and PTZ+LLE groups when compared to the control (p < 0.01). Compared to the PTZ group, the expression level of cyclin-B1 protein in the PTZ+LLE and PTZ+BCE groups significantly decreased in the whole brain (p < 0.01) and hippocampus (p < 0.01) (Figures 2B, E). The expression of cyclin-B1 protein in the PTZ+LLE group was significantly decreased compared to that in the PTZ+BCE group in the hippocampus (p < 0.01) and whole brain (p < 0.01).

As shown in Figures 2A and C (in the whole brain), and 2D and F (in the hippocampus), the bcl-2 expression levels were significantly decreased in the other groups when compared to the control (Table 1) (p < 0.01). When compared to the PTZ group, the bcl-2 expression levels in the PTZ+BCE and PTZ+LLE groups were increased in the whole brain (p < 0.01) and in hippocampus (p < 0.01). The bcl-2 expression levels were significantly increased by LLE pretreatment compared to the PTZ+BCE group in the hippocampus (p < 0.01) and in whole brain (p < 0.01).

As shown in Figure 3, PTZ treatment significantly increased the MDA levels, in the brain tissue when compared to the Control (p < 0.01) (Table 2). Both pretreatment with LLE or BCE reversed (p < 0.01) the PTZ-induced increase in the MDA level (Figure 3A, Table 2). PTZ treatment decreased the SOD levels in the brain tissue when compared to the control (p < 0.01) (Figure 3B, Table 2). Both pretreatment with LLE or BCE significantly increased the SOD levels when compared to the PTZ group (p < 0.01) (Figure 3B, Table 2). Neither
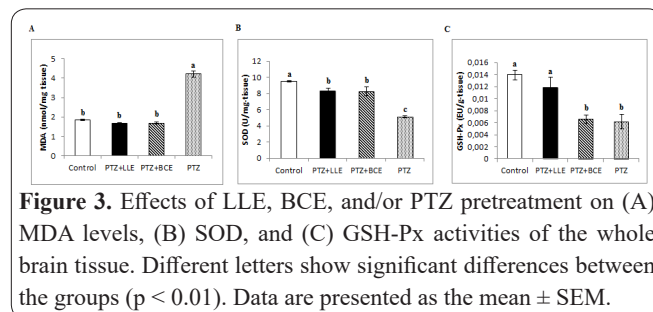

Figure 3. Effects of LLE, BCE, and/or PTZ pretreatment on (A) MDA levels, (B) SOD, and (C) GSH-Px activities of the whole brain tissue. Different letters show significant differences between the groups (p < 0.01). Data are presented as the mean ± SEM.

Table 1. Effects of LLE, BCE, and/or PTZ on cyclin-B1 and bcl-2 levels of the whole brain and hippocampus tissue.

Groups	Cyclin-B1 (whole brain)	Cyclin-B1 (hippocampus)	Bcl-2 (whole brain)	Bcl-2 (hippocampus)
Control	8.4836 ± 0.7334[d]	9.1869 ± 0.7957[d]	35.6290 ± 1.1781[a]	47.0616 ± 1.7758[a]
PTZ+LLE	13.1103 ± 0.7184[c]	14.5900 ± 1.2882[c]	28.9698 ± 0.9072[b]	32.2976 ± 1.1581[b]
PTZ+BCE	30.2407 ± 1.6594[b]	32.3570 ± 1.0864[b]	21.2438 ± 0.8639[c]	13.5422 ± 1.1020[c]
PTZ	48.1650 ± 2.3837[a]	43.8667 ± 0.4618[a]	14.1583 ± 0.8626[d]	7.1000 ± 0.8234[d]

Data are presented as the mean ± SEM. Different letters show significant differences between the groups (p < 0.01).

Table 2. Effects of LLE, BCE, and/or PTZ on oxidative stress.

Groups	TAS (micromol H_2O_2Eq/L)	TOS (micromol H_2O_2Eq/L)	OSI (arbitrary Unit)	MDA (nmol/mg-tissue)	SOD (U/mg-tissue)	GSH-Px (EU/g-tissue)	NO (µmol/g)
Control	2.857 ± 0.360	5.505 ± 0.521[b]	0.021 ± 0.003 [b]	1.8515 ± 0.0467[b]	9.5290 ± 0.0981[a]	0.0140 ± 0.0008[a]	192.17 ± 27.18
PTZ+LLE	2.342 ± 0.034	5.189 ± 1.295[b]	0.022 ±0.005 [b]	1.6656 ± 0.0266[b]	8.3005 ± 0.3834[b]	0.0118 ± 0.0018[a]	251.54 ± 47.07
PTZ+BCE	2.121 ± 0.143	5.782 ± 0.406 [b]	0.028 ± 0.003[b]	1.6826 ± 0.0593[b]	8.2990 ± 0.5399[b]	0.0066 ± 0.0007[b]	284.44 ± 60.54
PTZ	2.218 ± 0.053	21.060 ± 5.250[a]	0.096 ± 0.025[a]	4.2132 ± 0.1591[a]	5.1547 ± 0.0956[c]	0.0062 ± 0.0012[b]	278.89 ± 21.97

Data are presented as the mean ± SEM. Different letters show significant differences between the groups (p < 0.01).
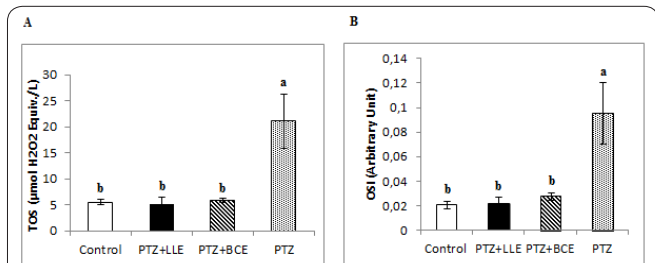

Figure 4. Effects of LLE, BCE, and/or PTZ pretreatment on the (A) TOS levels of the serum and (B) OSI values. Different letters show significant differences between the groups (p < 0.01). Data are presented as the mean ± SEM.
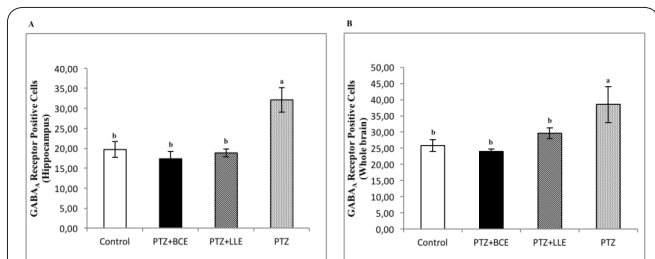

Figure 5. Quantitative comparison of $GABA_A$ receptor positive cells of the A) hippocampus and B) whole brain in the control, PTZ, PTZ+BCE, and PTZ+LLE groups. Different letters show significant differences between groups (p < 0.05). Data are presented as the mean ± SEM.

Table 3. Effects of LLE, BCE, and/or PTZ pretreatment on the number of $GABA_A$ receptor in whole brain and hippocampus.

Groups	$GABA_A$ Receptor Positive Cells (hippocampus)	$GABA_A$ Receptor Positive Cells (whole brain)
Control	19.67 ± 1.96[b]	25.83 ± 1.78[b]
PTZ+BCE	17.33 ± 1.86[b]	24.00 ± 0.73[b]
PTZ+LLE	18.86 ± 0.96[b]	29.57 ± 1.69[b]
PTZ	32.17 ± 3.07[a]	38.50 ± 5.50[a]

Data are presented as the mean ± SEM. Different letters show significant differences between the groups (p < 0.05).

the PTZ, LLE, nor BCE treatments changed the NO levels in the brain when compared to the control (Table 2). PTZ treatment decreased the GSH-Px levels in the brain tissue when compared to the control (p < 0.01). LLE administration reversed the PTZ induced decrease in the GSH-Px levels (p < 0.01). BCE administration did not change the PTZ-induced decrease in the GSH-Px level when compared to the PTZ group (Figure 3C, Table 2).

TOS levels were increased by PTZ and restored by the LLE and BCE pretreatments. OSI values were increased by PTZ and restored by the LLE and BCE pretreatments (Table 2, Figure 4)

A significant increase was also found in the number of neurons that expressed $GABA_A$ receptors in the PTZ group when compared to the control, in both the hip-

pocampus (Figure 5A) and whole brain (Figure 5B) (p < 0.05) (Table 3). Differences between the number of neurons that expressed $GABA_A$ receptors in the whole brain and hippocampus were not significant between the control, PTZ+LLE, and PTZ+BCE groups (Table 3, Figures 5A, 5B, 6, 7).

Using the TUNEL assay, there was no statistically significant difference in the number of apoptotic neurons in the brain among the control, PTZ, PTZ+ BCE, and PTZ+LLE groups (data not shown).

Discussion

We investigated the effects of *L. leontopetalum* and *B. chrysogonum* on oxidative stress markers, cyclin-B1 and bcl-2 levels, and the number of neurons that expressed $GABA_A$ receptors. Results suggest a possible anticonvulsant effect of LLE and BCE against PTZ-induced kindling. It has been reported that lupanine, saponin, and tannin alkaloids, which have known anticonvulsant activities (24-26), are present in *L. leontopeta-*
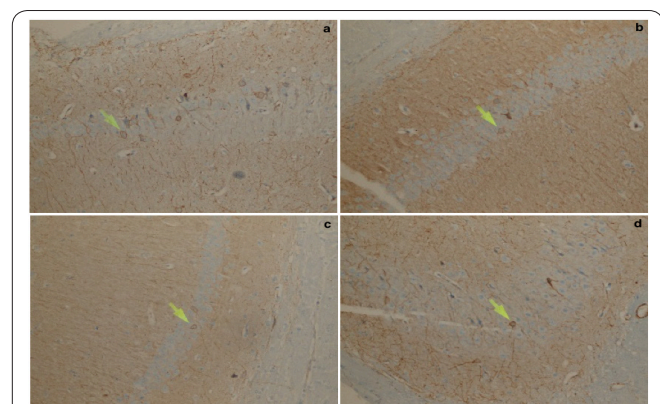

Figure 6. Immunohistochemical staining of the $GABA_A$ receptors in the hippocampus of the a) PTZ, b) Control, c) PTZ+BCE, and d) PTZ+LLE groups. Arrow indicates stained cells. (Original magnification, 200X).
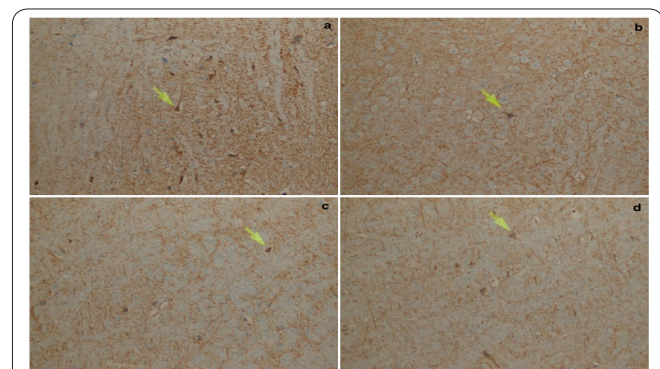

Figure 7. Immunohistochemical staining of the $GABA_A$ receptors in the whole brain of the a) PTZ, b) Control, c) PTZ+BCE, and d) PTZ+LLE groups. Arrow indicates stained cells. (Original magnification, 200X).

lum plant (27).

Neuronal cell death in the central nervous system is closely linked to a cell division process (28). We investigated the effects of *L. leontopetalum* and *B.* chrysogonum on the cyclin-B1 levels of the whole brain and hippocampus. We found a significant increase in the cyclin-B1 levels of the PTZ, PTZ+BCE, and PTZ+LLE groups when compared to the control. Consistent with our results, it was reported that neuronal cyclin B expression occurred in TLE patients (6) and in PTZ-kindled rats (4). In addition, Neuronal death that was accompanied by cyclin b1 expression was reported in PTZ kindled rats (4). Similarly, our results may indicate that PTZ caused neuronal loss via an aberrant cell cycle in the whole brain and hippocampus. In the present study, cyclin-B1 levels were significantly low in the PTZ+LLE and PTZ+BCE groups when compared to the PTZ group. These results suggest an attenuating effect of the extract administrations on PTZ induced cyclin-B1 expression.

Bcl-2 is a protein that can inhibit apoptosis (29). In the present study, we also observed a significant decrease in the bcl-2 levels of both the hippocampus and whole brain in the PTZ group. In the whole brain and hippocampus, both the BCE and, especially, LLE pretreatments significantly increased the bcl-2 levels when compared to the PTZ group. Consistent with our results, Ullah *et al.* (30) reported that epileptic seizures decreased bcl-2 levels in the rat brain. An increase in bcl-2 levels inhibits apoptosis (31). The ameliorating effects of LLE and BCE on bcl-2 levels is promising because the extracts may decrease seizure-triggered apoptotic neuronal cell death. A significant increase in the anti-apoptotic bcl-2 levels and a significant decrease in the cell cycle marker cyclin-B1 levels were observed with the BCE and LLE pretreatments in the whole brain and hippocampus when compared to the PTZ group, which may be due to the neuroprotective properties of the extracts.

We also investigated the effects of LLE and BCE on the oxidative stress markers. Several rodent studies have demonstrated that MDA levels were significantly increased with PTZ (9, 32). In the present study, we found a significant increase in the MDA levels in the PTZ group when compared to the control. We also found that pretreatment with LLE and BCE resulted in a significant decrease in the MDA levels in the rat brain. It was reported that Valproate (an antiepileptic drug) decreased brain MDA levels when compared to a PTZ-kindled group (33). It was reported that significant increase in brain MDA levels results in free-radical formation and oxidative stress (32). Our results were consistent with the study by Rahmati *et al.* (32), which indicated that oxidative stress may be a factor involved in the epilepsy pathophysiology of PTZ kindling. Both LLE and BCE have exerted a protective effect against lipid peroxidation.

Oxidative stress caused by extreme free-radical release is probably involved in epileptogenesis (2). The brain has antioxidant protection enzymes (such as SOD and GSH) that protect it from oxidative injury (34). The antioxidant enzyme GSH-Px prevents the production of hydroxyl radicals (33). We also investigated the GSH-Px and SOD levels and found that GSH-Px activ-ity was significantly decreased in the PTZ group when compared to the control. Consistent with our results, it was reported that PTZ kindling leads to a significant decrease in GSH-Px levels (33). The decreased levels of GSH-Px may be attributed to the GSH-Px was depleted during the struggle against oxidative stress (33). We also found that pretreatment with LLE ameliorated the GSH-Px levels. GSH-Px detoxifies the excessive reactive oxygen species (ROS) ; hence, GSH-Px may hinder lipid peroxidation (35). Indeed, our results indicated that the MDA values were also ameliorated in the LLE-treated group. Pretreatment with LLE may protect the antioxidant enzyme activity of GSH-Px, thus preventing the generation of free-radicals and extreme ROS production and lipid peroxidation. We also found that PTZ administration caused a significant decrease of brain SOD activity in the PTZ group and pretreatment with LLE and BCE significantly increased the SOD activity when compared to the PTZ group. Consistent with our results, a significant decrease was reported in the SOD activity in the PTZ-kindled group (33, 36). Ilhan *et al.* (33) reported that valproate prevented a PTZ kindling-induced reduction in SOD activity. SOD is an antioxidant enzyme (33). SOD catalyzes the reaction which superoxide is converted to hydrogen peroxide (36). It was concluded that LLE and BCE pretreatment might protect the antioxidant enzyme activity of SOD.

We investigated the serum TAS, TOS levels, and calculated the OSI values. Neither PTZ, LLE, nor BCE altered the TAS levels of the serum. We found that PTZ kindling significantly increased the serum TOS levels and OSI when compared to the control. Both the LLE and BCE pretreatments decreased the TOS and OSI values (the same level as the control) when compared to the PTZ group. Consistent with our findings, a study by Tutanc *et al.* (37) reported increased TOS and OSI levels in epileptic children.

We also investigated the number of neurons that expressed $GABA_A$ receptors in the whole brain and hippocampus. We found a statistically significant increase in the number of neurons that expressed $GABA_A$ receptors in the hippocampus and whole brain of the PTZ group when compared to the control, PTZ+LLE, and PTZ+BCE groups. An upregulation of $GABA_A$ receptor subtypes in TLE patients was reported (38). It was also reported that an acute injection of PTZ results in temporary changes in the mRNA levels of GABA receptors, but not the number of receptors (39), while chronic administration of PTZ significantly increased the number of neurons that expressed $GABA_A$ receptors (40). Surviving neurons might increase the number of $GABA_A$ receptors for combating the repeated waves of excitation, thus augmenting their response to the inhibitor neurotransmitter (38). In conclusion, both LLE and BCE have antioxidant activity and both may produce their effects mainly through their antioxidant properties. LLE and BCE pretreatment kept the number of $GABA_A$ receptors close to that of the control, decreased the cyclin-B1 levels, and increased the bcl-2 levels in both the hippocampus and whole brain, despite the PTZ application. Since we used only a 200 mg/kg dose of the extracts, more studies are required to determine whether LLE and BCE alter the investigated parameters in a dose-dependent fashion.

Acknowledgments

Conflict of interest

None declared.

References

1. Taiwe GS, Moto FC, Ayissi ER, Ngoupaye GT, Njapdounke JS, Nkantchoua GC, et al. Effects of a lyophilized aqueous extract of Feretia apodanthera Del. (Rubiaceae) on pentylenetetrazole-induced kindling, oxidative stress, and cognitive impairment in mice. Epilepsy Behav. 2015;43:100-8.

2. Shin E-J, Jeong JH, Chung YH, Kim W-K, Ko K-H, Bach J-H, et al. Role of oxidative stress in epileptic seizures. Neurochemistry international. 2011;59(2):122-37.

3. Ekonomou A, Smith AL, Angelatou F. Changes in AMPA receptor binding and subunit messenger RNA expression in hippocampus and cortex in the pentylenetetrazole-induced 'kindling' model of epilepsy. Molecular brain research. 2001;95(1-2):27-35.

4. Pavlova T, Stepanichev M, Gulyaeva N. Pentylenetetrazole kindling induces neuronal cyclin B1 expression in rat hippocampus. Neurosci Lett. 2006;392(1-2):154-8.

5. Popova M, Aniol V, Lazareva N, Stepanichev MY, Gulyaeva N. Neurodegenerative changes in rat hippocampal fields during the development of pentylenetetrazole kindling. Neuroscience and Behavioral Physiology. 2013;43(5):592-6.

6. Nagy Z, Esiri MM. Neuronal cyclin expression in the hippocampus in temporal lobe epilepsy. Exp Neurol. 1998;150(2):240-7.

7. Waldbaum S, Patel M. Mitochondria, oxidative stress, and temporal lobe epilepsy. Epilepsy Res. 2010;88(1):23-45.

8. Martinc B, Grabnar I, Vovk T. The role of reactive species in epileptogenesis and influence of antiepileptic drug therapy on oxidative stress. Curr Neuropharmacol. 2012;10(4):328-43.

9. Snehunsu A, Ghosal C, Kandwal M, Yadav PK, Nayak BS, Rao KR, et al. 1-Triacontanol cerotate; isolated from Marsilea quadrifolia Linn. ameliorates reactive oxidative damage in the frontal cortex and hippocampus of chronic epileptic rats. J Ethnopharmacol. 2015;172:80-4.

10. Pahwa P, Goel RK. Ameliorative effect of Asparagus racemosus root extract against pentylenetetrazol-induced kindling and associated depression and memory deficit. Epilepsy Behav. 2016;57(Pt A):196-201.

11. Özgökçe F, Özçelik H. Ethnobotanical aspects of some taxa in East Anatolia, Turkey. Economic Botany. 2004;58(4):697.

12. Kolak U, Hacibekiroglu I, Boga M, Özgökçe F, Ünal M, Choudhary M, et al. Phytochemical Investigation of Leontice leontopetalum L. subsp. ewersmannii with Antioxidant and Anticholinesterase Activities. Records of Natural Products. 2011;5(4):309.

13. Arslan A, Cakmak E, Ozaslan M, Cengiz B, Bagci C, Tarakcioglu M, et al. The effects of Bongardia chrysogonum (L) spach extract on the serum parameters and liver, kidney, and spleen tissues in rats. Biotechnology & Biotechnological Equipment. 2005;19(3):170-9.

14. Abuhamdah SMA, Abuirmeileh AN, Thaer F, Al-Olimat S, Abdel E, Chazot PL. Anti-convulsant Effects of Bongardia chrysogonum L. Tuber in the Pentylenetetrazole-induced Seizure Model. Int J Pharmacol. 2018;14(1):127-35.

15. Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol. 1972;32(3):281-94.

16. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem. 2004;37(4):277-85.

17. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38(12):1103-11.

18. Altindag O, Erel O, Soran N, Celik H, Selek S. Total oxidative/anti-oxidative status and relation to bone mineral density in osteoporosis. Rheumatol Int. 2008;28(4):317-21.

19. Yoshioka T, Kawada K, Shimada T, Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. Am J Obstet Gynecol. 1979;135(3):372-6.

20. Spitz DR, Oberley LW. An assay for superoxide dismutase activity in mammalian tissue homogenates. Anal Biochem. 1989;179(1):8-18.

21. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70(1):158-69.

22. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54.

23. Erkec OE, Arihan O, Colcimen N, Kara M, Karatas E, Demir H, et al. Effects of Cichorium intybus on serum oxidative stress, liver and kidney volume, and cyclin B1 and Bcl-2 levels in the brains of rats with ethanol induced damage. Cell Mol Biol. 2018;64(7):30-5.

24. García-López P, Garzón-de la Mora P, Meneses-Medina M, Ramírez Z, Moreno-Sandoval V, Toro-Ureña Id, et al., editors. Lupinus exaltatus obtained lupanine and its measured anticonvulsive activity. Lupin crops: an opportunity for today, a promise for the future Proceedings of the 13th International Lupin Conference, Poznań, Poland, 6-10 June 2011; 2011: International Lupin Association.

25. Pal D, Sannigrahi S, Mazumder UK. Analgesic and anticonvulsant effects of saponin isolated from the leaves of Clerodendrum infortunatum Linn. in mice. Indian J Exp Biol. 2009;47(9):743-7.

26. Kabuto H, Yokoi I, Mori A. Monoamine metabolites, iron induced seizures, and the anticonvulsant effect of tannins. Neurochem Res. 1992;17(6):585-90.

27. Eisenman SW, Zaurov DE, Struwe L. Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan: Springer Science & Business Media; 2012.

28. Herrup K, Neve R, Ackerman SL, Copani A. Divide and die: cell cycle events as triggers of nerve cell death. J Neurosci. 2004;24(42):9232-9.

29. Hoh NZ, Wagner AK, Alexander SA, Clark RB, Beers SR, Okonkwo DO, et al. BCL2 genotypes: functional and neurobehavioral outcomes after severe traumatic brain injury. J Neurotrauma. 2010;27(8):1413-27.

30. Ullah I, Badshah H, Naseer MI, Lee HY, Kim MO. Thymoquinone and vitamin C attenuates pentylenetetrazole-induced seizures via activation of GABAB1 receptor in adult rats cortex and hippocampus. Neuromolecular Med. 2015;17(1):35-46.

31. Luo Z, Fang Y, Zhang L. The effects of antiepileptic drug valproic acid on apoptosis of hippocampal neurons in epileptic rats. Pak J Pharm Sci. 2015;28(1 Suppl):319-24.

32. Rahmati B, Khalili M, Roghani M, Ahghari P. Anti-epileptogenic and antioxidant effect of Lavandula officinalis aerial part extract against pentylenetetrazol-induced kindling in male mice. J Ethnopharmacol. 2013;148(1):152-7.

33. Ilhan A, Gurel A, Armutcu F, Kamisli S, Iraz M. Antiepileptogenic and antioxidant effects of Nigella sativa oil against pentylenetetrazol-induced kindling in mice. Neuropharmacology.

2005;49(4):456-64.

34. Zeng LH, Zhang HD, Xu CJ, Bian YJ, Xu XJ, Xie QM, et al. Neuroprotective effects of flavonoids extracted from licorice on kainate-induced seizure in mice through their antioxidant properties. J Zhejiang Univ Sci B. 2013;14(11):1004-12.

35. Erdogan H, Ekici F, Katar M, Kesici H, Aslan H. The protective effects of endothelin-A receptor antagonist BQ-123 in pentylenetetrazole-induced seizure in rats. Hum Exp Toxicol. 2014;33(10):1008-16.

36. Kiasalari Z, Khalili M, Roghani M, Sadeghian A. Antiepileptic and Antioxidant Effect of Brassica nigra on Pentylenetetrazol-Induced Kindling in Mice. Iran J Pharm Res. 2012;11(4):1209-17.

37. Tutanc M, Aras M, Dokuyucu R, Altas M, Zeren C, Arica V, et al. Oxidative Status in Epileptic Children Using Carbamazepine.

Iran J Pediatr. 2015;25(6).

38. Loup F. GABAA Receptors in Human Temporal Lobe Epilepsy. Epileptologie; 2006.

39. Walsh LA, Li M, Zhao TJ, Chiu TH, Rosenberg HC. Acute pentylenetetrazol injection reduces rat GABAA receptor mRNA levels and GABA stimulation of benzodiazepine binding with No effect on benzodiazepine binding site density. J Pharmacol Exp Ther. 1999;289(3):1626-33.

40. Meral I, Esrefoglu M, Dar KA, Ustunova S, Aydin MS, Demirtas M, et al. Effects of Nigella sativa on apoptosis and GABAA receptor density in cerebral cortical and hippocampal neurons in pentylenetetrazol induced kindling in rats. Biotech Histochem. 2016;91(8):493-500.