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Investigation of antioxidant and anticonvulsant activity of *Hypericum triquetrifolium* Turra

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ABSTRACT: Epilepsy is a state characterized by sudden, recurrent epileptic seizures that are not initiated by an identifiable event. There are various studies has been shown that *Hypericum* species may be used for their anticonvulsant potentials. Besides, the relationship between anticonvulsant activity and antioxidant effect has already been shown in the literature. In the current study, *H. triquetrifolium* was investigated for the first time for its potential antioxidant and anticonvulsant potential using in vitro and in vivo test models. *H. triquetrifolium* extracts were tested with DPPH assay, FRAP assay, copper (II) ion reducing antioxidant capacity assay, and acetylcholinesterase inhibitory activity assay to understand their antioxidant potential. Especially, methanolic extract of *H. triquetrifolium* was shown the highest antioxidant activity. Moreover, a pentylenetetrazole (PTZ, 80 mg/kg, i.p.)-induced seizure model was conducted to analyze the anticonvulsant activities of *H. triquetrifolium* extracts in mice. In addition, this study revealed that *H. triquetrifolium* decreased the ratio of severe seizures and increased the mean onsite of mortality and survival rate in a dose-dependent manner. It is thought that the anticonvulsant effect may be either related to the antioxidant potential of *H. triquetrifolium* or its interference in the GABAergic system.

KEYWORDS: *Hypericum triquetrifolium* Turra; antioxidant activity; anticonvulsant activity; epilepsy; Hypericaceae.

1. INTRODUCTION

The genus *Hypericum L.* is a member of the Hypericaceae family. This genus, which includes more than 400 species in the world, is distributed in Africa, North America, Europe, and Asia as well as in the Mediterranean, tropical, and subtropical regions, and it is distributed in the Near East Regions [1]. *Hypericum* species contain many bioactive components and essential oils, including naphthodianthronenes, which are characterized by proanthocyanins, flavonoids, biflavonoids, xanthones, phenylpropanoids, and their relatives [2,3]. Hypericum species have been widely utilized in traditional/modern medicine as a medicinal herb for the treatment of wounds, depression, inflammation, burn, and eczema for years. In Türkiye, the genus is represented by approximately 100 taxa gathered under 19 sections. Moreover, it is known that 49 Hypericum taxa are endemic [4]. The most common species in Türkiye are represented by *Hypericum perforatum L.*, (St. John's Wort) *Hypericum triquetrifolium* Turra, *Hypericum calycinum L.* (great flowering Hypericum herb), *Hypericum empetrifolium* Willd. (puree, yellow puree), *Hypericum scabrum L.* (yeast herb, cappirotu), and *Hypericum tedrapetum* Fries [4,5].

Hypericum triquetrifolium is native mainly to the eastern Mediterranean region. In addition, it can be seen in Spain, France, Italy, Malta, Libya, Montenegro, Albania, Greece Cyprus, and Türkiye [6]. The species has recently received much scientific attention as a source of various biologically active compounds such as polyphenols, hyperoside, quercetin, quercitrin, chlorogenic acid, rutin, kaempferol, and flavonoids [7,8]. The plant *Hypericum triquetrifolium* traditionally has soothing, anti-helminthic, anti-inflammatory, and antiseptic effects [8–10]. In addition, several studies have shown that its essential oil and its crude extracts are mainly used in the treatment of burn and gastroenteritis with its anti-nociceptive and anti-oxidant effects [8,11–13].

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Epilepsy is a condition characterized by sudden, recurrent epileptic seizures that are not triggered by an identifiable event, resulting from abnormal and excessive electrical discharge in cortical neurons [14,15]. The disease, which can be seen in all age groups, can be diagnosed in the clinic with various seizure types associated with different pathophysiology and symptoms [15]. Epilepsy affects 50 million people on a worldwide scope and this ratio makes the disease one of the most prevalent neurological diseases globally [16]. It is known that seizure control is around 65-75% with the proper antiepileptic agents given in this disease [14,16]. Today, studies continue to reduce the side effects of antiepileptic drugs commonly used and to increase their antiepileptic effects [14,15]. In addition, *Hypericum scabrum* and *Hypericum perforatum* were found to have important anticonvulsant effects [17,18]. As the relationship between anticonvulsant activity and antioxidant effect has already been shown, examination of the antioxidant potential of the extract is crucial [19,20]. In the current study, *H. triquetrifolium* was investigated for its potential antioxidant and anticonvulsant activities using in vitro and in vivo test models.

2. RESULTS

2.1. Antioxidant Activity

2.1.1. DPPH assay

The free radical scavenging activities of the extracts obtained from the *H. triquetrifolium* were determined using the DPPH method. The antioxidant activities of the extracts and ascorbic acid used as a standard were evaluated by comparing their IC50 values. The results obtained were shown in Table 1. When the obtained results were compared, it was found that the methanol extract showed the highest DPPH radical scavenging activity. It was also observed that all extracts had lower radical scavenging activity than ascorbic acid.

Table 1. DPPH radical scavenging activities of extracts from *H. triquetrifolium*

Extracts	**DPPH (IC_{50}: mg/mL)**
Methanol	0,0158±0,008
Ethyl acetate	0,0553±0,0013
Petroleum ether	0,9909±0,2773
Ascorbic acid	0,004± 0,007

2.1.2. FRAP assay

The iron reducing power is based on the reduction of Fe+3 to Fe+2 of the herbal extract and its spectrophotometric examination at 593 nm. In this method, high absorbance indicates high iron reduction potential. When the FRAP values obtained as a result of this study were compared among themselves, methanol extracts had the highest FRAP values. In addition, it was determined that the *H. triquetrifolium* had lower FRAP values than the ascorbic acid compound (Table 2).

Table 2. FRAP values of extracts obtained from *H. triquetrifolium*

Extracts	**FRAP (mM $FeSO_4$/mg extract)**
Methanol	34,432±1,723
Ethyl acetate	13,6284±1,467
Petroleum ether	4,7267±0,940
Ascorbic acid	37.925±1,02

2.1.3. Determination of copper (II) ion reducing antioxidant capacity (CUPRAC)

The copper (II) ion reducing the antioxidant capacity of the extracts obtained from the *H. triquetrifolium* was evaluated comparatively and the results were shown in Table 3. When the results were evaluated, it was determined that the methanol extract had a stronger reduction potential of Cu(II) to Cu(I) than the other extracts, and the petroleum ether extract had the lowest activity (Table 3).

Table 3. CUPRAC values of extracts obtained from *H. triquetrifolium*

Extracts	CUPRAC value (mM trolox equivalent/mg extract)
Methanol	91,7035±2,923
Ethyl acetate	47,903±1,19
Petroleum ether	10,25±1,29
Galantamine	104,521±3,91

2.2. Determination of AChE inhibitory activities

AChE enzyme inhibition percentages of different plant extracts were compared according to the Ellman method (Table 4). According to the results obtained, the methanol extract showed the highest enzyme inhibition value when the extracts were compared among themselves. When the enzyme inhibition percentages of all extracts were compared with galantamine, it was observed that all extracts had very significant inhibition properties.

Table 4. Anticholinesterase activities of extracts obtained from *H. triquetrifolium* plant.

Extracts	Enzyme Inhibition (%) (500 µg/mL)
Methanol	97,754 ± 0,9556
Ethyl acetate	86,015 ± 0,1326
Petroleum ether	89,440 ± 0,8762
Galantamine	96.54±0,9

2.3. Anticonvulsant activity

PTZ-induced tonic-clonic seizures were created in all the animals used in the test. All experimental groups were compared with the control group. Mice pretreated once with *H. triquetrifolium* extracts (500 mg/kg and 1000 mg/kg, p.o.) were compared for the onset of convulsion and onset of mortality. Current study results have revealed that none of the extracts delayed the onset of convulsion compared to the control group ($p>0.05$) (Table 5). In the positive control group, carbamazepine (100 mg/kg) inhibited the severe tonic-clonic convulsions, especially considerably decreased the ratio of grade IV to 15.8% compared to the control group (32.0%) and mortality in mice although it could not significantly delay the onset of convulsion ($p>0.05$). Besides, a similar pattern of the decrease in severe seizures (ratio of Grade IV, V) has been observed in both dosages of *H. triquetrifolium* extracts. It decreased the ratio of grade V to 3.3 % at 1000 mg/kg dosage compared to the control group (5.1 %), whereas, this ratio was 2.1 % for the positive control group. On the other hand, although the results were not statistically significant ($p>0.05$), the mean onsite of mortality increased in *H. triquetrifolium* extract in a dose-dependent manner compared to the control group. Furthermore, *H. triquetrifolium* extract at the dose of 1000 mg/kg has shown a higher survival rate (%80) than the control and positive control groups (%40 and %70, respectively) (Table 5).

Table 5. Effects of *H. triquetrifolium* extract on pentylenetetrazole (PTZ)-induced seizures in mice (n=10).

Experimental groups	Mean onsite of seizure (sec)	Grade I %	Grade II %	Grade III %	Grade IV %	Grade V %	Mean onsite of mortality (sec)	Survival %
Control	**65.5±5.9**	**7.3**	**38.5**	**17.1**	**32.0**	**5.1**	**469.3±106.8**	**40**
Carbamazepine 100 mg/kg	**67.7±3.6**	**9.5**	**53.2**	**19.4**	**15.8**	**2.1**	**425.7±157.4**	**70**
***H. triquetrifolium* 500 mg/kg**	**63.2±4.2**	**4.4**	**46.5**	**26.7**	**17.3**	**5.1**	**474.3±88.9**	**40**
***H. triquetrifolium* 1000 mg/kg**	**61.9±5.7**	**3.8**	**43.1**	**33.5**	**16.3**	**3.3**	**560.0±100.0**	**80**

Values are expressed in Mean ± SEM. One-way ANOVA was carried out followed by post hoc Dunnet's multiple comparison test.

3. DISCUSSION

Epilepsy is a progressive chronic disorder that may cause brain damage, impaired cognition, and other neurological problems [15]. Although there are many pharmacotherapy options in the drug market, 35% of the patients experience recurrent seizures [14]. Besides antiepileptic drugs may cause severe adverse drug reactions such as impaired memory and mood disorders [14,15]. Given these efficacy and safety issues, research based on new antiepileptic alternatives still remains important. *Hypericum* species have been the subject of research in many indications, including epilepsy. However, the antioxidant and anticonvulsant properties of *H. triquetrifolium* were demonstrated for the first time in this article.

Oxidative stress can be defined as a disproportion between oxidants (free radicals) which may cause the potential for organic damage. Alteration of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and decreased bioavailability of nitric oxide (NO) has associated with oxidative stress. It is fact that oxidative stress has a responsibility on neurological diseases such as epilepsy and Alzheimer's disease [19,20]. In addition to these, it is thought that there is a relationship between the above-mentioned neurological diseases being more common in the geriatric group and the decrease in antioxidant potential in this population [19,21]. According to this data, it is vital to know the antioxidant capacity of the substance, while investigating the anticonvulsant effect. Previous studies have shown that rutin, hypericin, and other phenolic compounds, which are the main active ingredients of *Hypericum* species, have strong antioxidant properties [4,7,13,22]. In addition to this, various researches were conducted to demonstrate the high antioxidant capacities of *Hypericum* species and related therapeutic approaches [2,4,11,17]. Similar to the literature, the high antioxidant capacity of *H. triquetrifolium*, especially its methanolic extract, has been determined by various tests in this study.

It is known that extracts of *Hypericum* species are beneficial in central nervous system disorders[4,22]. Although studies on this subject generally focus on antidepressant activity, *Hypericum* species have also been shown to be effective in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease [23,24]. Reduction of oxidation is the highlighted mechanism of action according to the investigations based on neurodegenerative diseases and *Hypericum* species. Furthermore, experimental studies stated that oxidative stress is a contributing factor to the epileptogenesis [19]. Similar to other neurodegenerative diseases, antioxidant extracts may have the potential to contribute onset and evolution of epilepsy. In accordance, various studies stated that *Hypericum* species may be beneficial in epilepsy treatment. *Hypericum perforatum* and *H. scabrum* are shown anticonvulsant activity in the literature. In both studies, the potential mechanism of action is based on potent nitric oxide-scavenging activity [17,18]. Moreover, water and butanol extracts of *H. perforatum* have reduced the excitability of neurons in a kindling model of epilepsy. Despite that, an ether extract of *H. perforatum* has shown pro-epileptic effects [25]. Besides those, PTZ performs its convulsant effect by inhibiting the GABAergic activity, which is important in the epileptogenesis [26]. Interfering with GABA from *Hypericum* species may be the second potential mechanism of action. In addition to all data, this study demonstrated that, although it is not statistically significant, *H. triquetrifolium* decreased the ratio of severe seizures and increased the mean onsite of mortality and survival rate in a dose-dependent manner.

4. CONCLUSION

In conclusion, antioxidant and anticonvulsant activities of *H. triquetrifolium* have been indicated by the current study. *H. triquetrifolium* is shown antioxidant potential and it is demonstrated anticonvulsant activity in higher doses. It is thought that the anticonvulsant effect may be either related to the antioxidant potential of *H. triquetrifolium* or its interferation of the GABAergic system. Future work should also reveal the effect of chronic administration and multiple doses of *H. triquetrifolium* extract on epilepsy. Further well-designed studies are needed to enlighten the responsible compounds as well as their mechanisms of anticonvulsant activity.

5. MATERIALS AND METHODS

5.1. Plant materials and preparation of *H. triquetrifolium* extracts

The *H. triquetrifolium* was collected from Türkiye. Specimens were identified by Bahar Gürdal and the voucher specimens were deposited at the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE No.: 117306). The plant sample was dried in room conditions. The samples were consumed by the maceration method. The plant sample was extracted using petroleum ether, ethyl acetate, and methanol at

room temperature, respectively. The solvents were evaporated using a rotary evaporator at low temperature and pressure. The raw extracts were kept at + 4 °C in the refrigerator.

5.2. Antioxidant Activity Determination Methods

5.2.1. DPPH assay

The free radical scavenging capacity of extracts was determined by using the previously described method [27]. The data gained from the investigation were given as IC_{50} = mg/mL.

5.2.2. FRAP assay

The FRAP assay was conducted according to Benzie and Strain method [28]. FRAP values of the extracts were given as mM Fe^{2+}/mg extract.

5.2.3. Cupric ion reducing/antioxidant power (CUPRAC) assay

In brief, 60 µL Cu(II)x2H2O, 60 µL neocuproine, and 60 µL NH4Ac (1 M) were mixed. Then 60 µL of the extract and 10 µL of ethanol were added to the mixture. After the duration time of 60 min, the mixture absorbance was spectrophotometrically measured at 450 nm. CUPRAC values of the extracts were given as mM Trolox/mg extract [29].

5.3. Determination of acetylcholinesterase inhibitory activities

The inhibition of acetylcholinesterase (AChE) of extracts was evaluated according to the Ellman method using a 96-well microplate reader [30]. The findings from this study were given as percent AChE enzyme inhibition.

5.4. In vivo animal test

The study protocol (permission number: 17.2021.mar) was approved by the Marmara University Animal Experiments Local Ethics Committee. Female BALB/c mice weighing 22-30 g were used to examine anticonvulsant effects. The animals were housed in the standard cages (48 cm × 35 cm × 22 cm) at room temperature (22 ± 2 °C), supplied with artificial light from 7:00 a.m. to 7:00 p.m.; the mice were provided with pelleted food and water ad libitum. The procedures followed were by animal rights as per the Guide for the Care and Use of Laboratory Animals.

5.5. Experimental design for anticonvulsant activity

Mice were divided into 4 groups for the anticonvulsant activity test of 10 mice in each group. Group I was named as the control group and mice received isotonic saline solution (ISS) with oral gavage once (10 ml/kg). Group II, which is chosen as the positive control, was given carbamazepine in a 100 mg/kg dosage [31,32]. The mice in Groups III and IV were treated with *H. triquetrifolium* extracts at doses of 500 mg/kg and 1000 mg/kg for evaluating their anticonvulsant activities. For carbamazepine (Group II) and *H. triquetrifolium* extract groups (Group III and IV) vehicle was ISS.

5.6. Induction of epileptic seizure and assessment of anticonvulsant activity

The anticonvulsant activities of *H. triquetrifolium* extracts at doses of 500 mg/kg and 1000 mg/kg were investigated and compared by a PTZ-induced seizure model in mice [33]. This rodent model is widely used as a standard method for predicting protection against tonic-clonic seizures in humans [34]. The effective dose 50 (ED50) value for PTZ which was calculated in the literature was chosen as 80 mg/kg [35]. Firstly, mice were fasted overnight, and then, ISS (as vehicle 10 ml/kg, p.o.), carbamazepine (100 mg/kg, p.o.), *H. triquetrifolium* extracts (500 mg/kg and 1000 mg/kg, p.o.) were administered to different groups 30 min before the administration of PTZ (80 mg/kg, i.p.) [36]. After PTZ injection, each mouse was placed in a separate observation box and their behaviors were observed for 30 min for frequency of convulsions, latency to the onset of seizures, percentage of grades, and mortality [37]. The animals that survived after that period were considered protected. Moreover, each seizure was graded according to a modified Racine scale as follows: 1-No movements; 2-Head nodding and myoclonic jerks (MKJ); 3-Forelimb clonus; 4-Rearing; 5-Falling and generalized convulsions with tonic extension [38].

5.7. Statistical Analyses

Statistical analysis was evaluated by using SPSS 22.0 program. Results were reported as mean ± SEM (standard error of the mean) and as a percentage (%). One-way analysis of variance (One-Way ANOVA, post hoc Dunnet's test) was conducted for statistical analyses. Probability levels of less than 0.05 were considered significant.

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