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Antiepileptic Activity of Four Selected Skullcap (Scutellaria) Species on Mice

Seçilmiş Dört Kaside (Scutellaria) Türünün Farede Antiepileptik Etkisi

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ABSTRACT

Epilepsy is a common neurological disorder which has different types. Epileptic convulsions are decreasing life quality of patients and can even cause death. Medical treatment of epilepsy includes drugs and surgical interventions. Plant originated substances offers new molecules in this issue. In this study we have studied 4 different Skullcap (Scutellatia) species. Methanolic extracts of aerial parts of Scutellaria brevibracteata subsp brevibracteata, S. galericulata, S. megalapsis and S. orientalis subsp pichleri were tested on mice in pentylentetrazol induced convulsions. Animals were administered 5 days with 200 mg/kg i.p. plant extracts dissolved in physiological saline. Last day they were injected with 80 mg/kg pentylentetrazol. Pentylentetrazol group was administered solely with pentylentetrazol. Results showed a decrease in number of animals having tonic clonic convulsion ($p>0.05$), a decrease in number of ex animals due to convulsions ($p>0.05$), an increase in latency to have tonic-clonic convulsions ($p>0.05$) in all tested Scutellaria groups and a significant increase in latency for first myoclonic convulsion ($p<0.05$) in S. orientalis subsp pichleri species against pentylentetrazol induced convulsions. Evaluation of present results may suggest a potential anticonvulsive activity of tested Scutellaria species. Further studies are needed to determine related molecules from this activity.

Key Words: Anticonvulsant activity, Skullcap, Scutellaria, pentylentetrazol

ÖZET

Epilepsi farklı tipleri olan nörolojik bir bozukluktur. Epileptik nöbetler hastanın hayat kalitesini düşürür ve ölüme dahi neden olabilir. Epilepsinin tıbbi tedavisi ilaçlar ve cerrahi müdahaleler ile gerçekleştirilir. Bitkisel kökenli maddeler bu konuda yeni moleküller sunabilmektedir. Bu çalışmada 4 farklı Kaside (Scutellaria) türü araştırılmıştır. Scutellaria brevibracteata subsp brevibracteata, S. galericulata, S. megalapsis ve S. orientalis subsp pichleri türlerinin topraküstü kısımlarının metanollü ekstresi pentilentetrazol indüklü konvülsiyonlarda denenmiştir. Hayvanlara 5 gün boyunca 200 mg/kg i.p. bitki ekstresi serum fizyolojik içinde çözünerek uygulanmıştır. Son gün 80 mg/kg pentilentetrazol enjekte edilmiştir. PTZ grubuna sadece pentilentetrazol verilmiştir. Sonuçlar test edilen Scutellaria türlerinin hepsinde tonik-klonik nöbete giren hayvan sayısında azalma ($p>0.05$), nöbet nedeniyle ölen hayvan sayısında azalma ($p>0.05$), tonik-klonik nöbete girme süresinde uzama ($p>0.05$) ve miyoklonik nöbete girme süresinde S. orientalis subsp pichleri türünde anlamlı uzama ($p<0.05$) olduğunu göstermektedir. Mevcut sonuçların birlikte değerlendirilmesi test edilen Scutellaria türlerinin potansiyel antikonvülsif özellik taşıyabileceğini düşündürmektedir. Bu aktivite ile ilgili moleküllerin belirlenebilmesi için ileri çalışmalar ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Antikonvülsan aktivite, Kaside, Scutellaria, pentilentetrazol

Introduction

Epilepsy is a brain disorder which is characterized with spontaneous seizures invoked by neurotransmitter system complex. It is among the most common neurological diseases in the world which affects about 50 million people (1). Although there are surgical interventions and chip implants for the treatment, current chemical treatment relies on prevention of convulsions with antiepileptic drugs. However some of the patients still have convulsions during this drug administration. In addition in almost 20% of the cases, insufficient drug activity, serious side effects and chronic toxicities are reported (2). Therefore search for new molecules continues in this disorder. Plants have various metabolites which are subject of new drug candidates (3). Some plants have potent impact on GABA receptors and voltage-gated

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ion channels. In addition they have neuroprotective effects (4). World Health Organization mentions that nearly 80% of the World's population relies on traditional medicinal practices which brought a field for scientific studies to find new molecules from plants. For assessment of antiepileptic activity of plants pentylentetrazolyum (PTZ) induced epilepsy forms a widely used model. PTZ can be administered at low doses for prolonged period to induce kindling or at high doses to induce tonic-clonic convulsions. A study by Loscher (5) presents reproducible results of this model. There are several studies using PTZ induced convulsion models to test activity of plant based compounds (6). Plants belonging to Scutellaria genus are traditionally used plants. Scutellaria genus is known as skullcap. S. orientalis is used in traditional medicine as blood stopper, for wound healing and regulation of intestinal activities in Turkey (7). Essential oils of Scutellaria genus found in Turkey are also studied (8). There are antiepileptic uses of Scutellaria species in the World (9). Zhang et al (10) identified phenolic compounds as the predominant compounds from the aerial part of Scutellaria lateriflora L. (American Skullcap) which is used for sedative and anticonvulsive purposes traditionally by Native Americans and Europeans. This species was also shown to alleviate mood via its anxiolytic effects (11). A study performed by Senol et al (12) evaluated acetylcholine esterase and buthylcholine esterase activity of different Scutellaria species. Results of this study showed that some of the species have such activity which makes them important for evaluation for their potential use in Alzheimer's disease. However no record exists in literature about pro/anticonvulsive effect of Scutellaria brevibracteata subsp brevibracteata (SB), S. galericulata (SG), S. megalapsis (SM) and S. orientalis subsp pichleri (SO) species in the scientific literature. Therefore methanolic aerial parts of those selected Scutellaria species which are found in Turkey are tested for this activity in mice.

Materials and Methods

Animals: Male adult Swiss-albino mice weighing 25-30 grams were used in experiments. Prior to experiments, animals were kept in rooms in standard temperature and humidity conditions. Standard mice pellet and tap water was given ad libitum. An ethical permission was obtained.

Drugs: Pentylenetetrazol (PTZ) 80 mg/kg (Sigma) was used. Methanolic extracts of SB, SG, SM and SO aerial parts were dissolved in saline. Drugs and plant extracts were administered via i.p. route.

Experimental design: 30 mice were separated into 5 groups randomly. Each group contained 6 animals.

1. PTZ (80 mg/kg, i.p.)
2. SB group. 5 days of daily SB methanolic extract (200mg/kg, i.p.) + last day PTZ (80 mg/kg, i.p.)
3. SG group. 5 days of daily SG methanolic extract (200mg/kg, i.p.) + last day PTZ (80 mg/kg, i.p.)
4. SM group. 5 days of daily SM methanolic extract (200mg/kg, i.p.) + last day PTZ (80 mg/kg, i.p.)
5. SO group. 5 days of daily SO methanolic extract (200mg/kg, i.p.) + last day PTZ (80 mg/kg, i.p.)

Plants were extracted with methanol. Following extraction, methanol was evaporated and remaining crude powder was dissolved in physiological saline and administered to animal at 200 mg/kg i.p. dose for 5 days. In each group latency for first myoclonic and tonic-clonic convulsions, convulsion period, number of animals having tonic-clonic convulsions and number of ex animals due to convulsions were recorded. In this model 80 mg/kg i.p. PTZ was used to induce convulsions. Kruskal-wallis, Mann-Whitney U test, post hoc Bonferonni correction and Fisher's exact tests were used for statistical evaluation.

Results

No toxic effects of plant administrations were observed in this period. In alone PTZ and PTZ+plant extract groups myoclonic (muscular jerks and twitches) and tonic-clonic (in which hind legs extent to rear of the body with a 180 degree angle to body axis) convulsions were observed. In plant administered groups a prolongation of latency for having both types of convulsions was observed. Although no statistically difference was found in latency for tonic-clonic convulsion, a significant increase in latency for myoclonic convulsion was observed in SO species compared to PTZ group ($p<0.05$). For convulsion period no significant difference was observed among groups. Number of animals having tonic-clonic convulsions and number of ex animals due to convulsion was decreased in all tested Scutellaria species ($p>0.05$) (Table 1).

Discussion

This study was conducted to test pro/anticonvulsive effects of methanolic extracts of aerial parts of SB, SG, SM and SO species in mice. All plants showed prolongation of latency for tonic-clonic convulsions ($p>0.05$) and an attenuation in number of animals having convulsion and number of ex animals due to

Table 1. Effect of different administrations on PTZ induced convulsion

Administration	Latency for First Myoclonic Convulsion (s)	Latency for First Tonic-clonic Convulsion (s)	Tonic-clonic Convulsion Period (s)	Number of animals (TC)	Number of ex animals
PTZ	62.00±10.02^{b}	322.60±16.11^{ns}	6.60±1.23^{ns}	6/6^{ns}	6/6^{ns}
SB	128.8±25.0^{ab}	478.0±101.4^{ns}	10.8±1.7^{ns}	4/6^{ns}	4/6^{ns}
SB	67.6±6.0^{b}	532.3±76.29^{ns}	8.0±2.4^{ns}	3/6^{ns}	3/6^{ns}
SM	71.5±5.0^{b}	388.2±55.1^{ns}	9.4±0.9^{ns}	5/6^{ns}	5/6^{ns}
SO	523.0±297.6^{a}	621.0±120.5^{ns}	8.3±0.7^{ns}	3/6^{ns}	3/6^{ns}

Results are given as mean±S.E.M. PTZ: Pentylenetetrazol, SB: Scutellaria brevibracteata subsp brevibracteata, SG: Scutellaria galericulata, SM: Scutellaria megalapsis, SO: Scutellaria orientalis subsp pichleri. ns:non-significant. TC: Tonic clonic convulsion. P<0.05. Statistically different groups in each column are given with letters a and b.

convulsion (p>0.05) however only significant effect was observed in SO extracts in delay for the latency of myoclonic convulsions (p<0.05). Further experiments with more samples sizes in each groups may provide significant results because decrease of animals having tonic-clonic convulsions decrease number of animals for statistical evaluation. Genus Scutellaria contains about 350 species and its distribution encompasses from East Asia to North America. They have a wide pharmacological activity list such as antioxidant, hepatoprotective and anticonvulsant (13). Anticonvulsant activity of such plants can be related with flavonoids, coumarins, phenylpropanoids and terpenoids which are commonly found ingredients of this genus. Flavonoids and coumarins interact with GABA receptor and certain voltage gated ion channels which are also target of synthetic antiepileptic drugs (14). Studies performed on Scutellaria species focus on two species mostly; S. laterifolia and S. baicalensis. S. laterifolia is used against epilepsy in North American Indians (15). S. baicalensis is a traditional Chinese medicinal herb used for various purposes including epilepsy. Both of their anticonvulsive properties were tested on different convulsion models and results link their activity with phenolic ingredients such as baicalin, oroxylin and wogonin. Baicalin is a flavonoid compound found in Scutellaria baicalensis. This compound was shown to exert anticonvulsant and neuroprotective effects in rats administered with pilocarpine to induce epilepsy (16). Yoon et al (17) investigated anticonvulsive activity mechanism of flavone compounds of S. baicalein in an in vitro model. Results showed that anticonvulsant activity of baicalein is due to benzodiazepine binding site of $GABA_A$ receptor. However it also has GABAergic activity without involvement of benzodiazepine sites which gives the plant anxiolytic and sedative activity in mice (18). Park et al (9) showed that i.p. wogonin injection exerts anticonvulsant activity by GABAergic neuronal involvement. We did not perform any chemical isolation of plants since it was a preliminary testing of anticonvulsive property of selected Scutellaria species. However results of this study showed that ethnopharmacological use of Scutellaria species against epilepsy may be valid also for SO. Further studies focusing on active substances related with this activity in this plant species as well as other potential species in this genus are needed.

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