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ORIGINAL ARTICLE / ÖZGÜN MAKALE

EFFECT OF *HELIOTROPIUM HIRSUTISSIMUM, HELIOTROPIUM DOLOSUM* AND *HELIOTROPIUM LASIOCARPUM* METHANOLIC EXTRACTS IN PENTYLENTETRAZOL INDUCED CONVULSIONS ON MICE

HELIOTROPIUM HIRSUTISSIMUM, *HELIOTROPIUM DOLOSUM* VE *HELIOTROPIUM LASIOCARPUM* METANOL EKSTRELERİNİN FAREDE PENTİLENTETRAZOL İNDÜKLÜ NÖBETLERE ETKİSİ

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ABSTRACT

Objective: *Epilepsy is a common neurological problem known since ancient times. Modern treatment of epilepsy includes treatment with drugs to avoid seizures. Search for new antiepileptic drugs continues due to the presence of resistant patients to drug treatment. Plants are important sources of new drug discovery for epilepsy. We aimed to evaluate anticonvulsive activity of methanolic extracts of Heliotropium (Scorpion herb, Herb for warts in Turkish) species namely Heliotropium hirsutissimum Grauer, H. dolosum De Not and H. lasiocarpum Fisch. et Mey. on mice.*

Material and Method: *PTZ (pentylentetrazol) is used for the induction of convulsions. Experimental groups are PTZ and 3 groups of extracts of aforementioned Heliotropium species+PTZ.*

Result and Discussion: *In H. hirsutissimum and H. lasiocarpum groups, number of animals having tonic-clonic convulsions was decreased compared to PTZ and H. dolosum groups however difference was found insignificant. Latency time for first myoclonic convulsion and latency time for first tonic-clonic convulsion were*

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prolonged in all Heliotropium groups compared to PTZ group (p>0.05). Number of ex animals following tonic-clonic convulsions decreased in Heliotropium groups (p>0.05). Tonic-clonic convulsion period decreased in H. hirsutissimum and H. lasiocarpum (p>0.05).
When results are evaluated together Heliotropium species may have an ethnopharmacological relevance for its anticonvulsive usage.

Keywords: *convulsion; epilepsy; Heliotropium; PTZ; pentylentetrazol*

ÖZ

Amaç: *Epilepsi antik dönemlerden bu yana bilinen yaygın bir nörolojik problemdir. Epilepsinin modern tedavisi nöbetleri engelleyici ilaçlar ile yapılmaktadır. İlaç tedavisine dirençli hastaların varlığı nedeniyle yeni antiepileptik ilaçların arayışı devam etmektedir. Bitkiler yeni ilaçların bulunabilmesi için önemli bir kaynaktır. Bu çalışmada Heliotropium (Türkçe; Akrep otu, Siğil otu) türlerinin; Heliotropium hirsutissimum Grauer, H. dolosum De Not ve H. lasiocarpum Fisch. et Mey.'in faredeki etkilerini değerlendirmeyi amaçladık.*

Gereç ve Yöntem: *Nöbetlerin oluşması için PTZ (pentilentetrazol) kullanıldı. Deney grupları PTZ ve bahsedilen 3 Heliotropium türünün ekstreleri+PTZ olarak belirlendi.*

Sonuç ve Tartışma: *H. hirsutissimum ve H. lasiocarpum gruplarında, tonik-klonik nöbete giren hayvan sayısı PTZ ve H. dolosum gruplarına göre azaldı ancak istatistik anlamlılık gözlenmedi. İlk miyoklonik nöbet ve ilk tonik-klonik nöbet için gereken süre tüm Heliotropium gruplarında PTZ grubuna göre uzadı (p>0.05). Bu tonik klonik nöbetler sonrasında ölen hayvan sayısı tüm Heliotropium gruplarında azaldı (p>0.05). Tonik-klonik nöbet süresi H. hirsutissimum ve H. lasiocarpum gruplarında azaldı (p>0.05).*
Sonuçlar birlikte değerlendirildiğinde Heliotropium türlerinin antikonvülsif olarak etnobotanik kullanımının bir karşılığını olabileceğini düşündürmektedir.

Anahtar Kelimeler: *epilepsy; Heliotropium; nöbet; PTZ; pentilentetrazol*

INTRODUCTON

Epilepsy is an important neurological disease which has been known since ancient times and mostly interpreted with metaphysical impacts. Hippocrates on the other hand defined epilepsy as a brain disorder and tried to heal it with diet and plant based medication. Almost more than 2 millennia later, modern approach to epilepsy treatment includes diets such as ketogenic diet [1] and medication with chemicals. Some of those chemicals are closely related to plant ingredients such as valproic acid which has a close structural relation with the components of Valerian root [2]. Currently, some of the patients are still facing uncontrolled seizures despite being treated with drugs. Therefore, the need to identify new antiepileptic molecules continue and plants constitute an important field of research due to the diversity of their chemical components.

Heliotropium L. species (Boraginaceae) are commonly used in traditional medicine. Among these, antipyretic, cholagouge [3], anticancer [4] wound healing, stomachic, anti-ulcer, asthma, bronchitis, sunburn, baldness, eczema, nail problems, gastritis can be mentioned [5]. Infusion of the aerial parts is used against asthma [6]. Studies also reveal that pharmacologically important activities of *Heliotropium* species such as anti-inflammatory and antimicrobial activities are mainly due to flanovoid content of these plants [7, 8]. Other activities include spasmolytic, vasorelaxant, bronchodilator

antihyperlipidemic and antidiabetic activities [9, 10]. Although *Heliotropium* species are important medicinal plants, they are also known for their toxic effects [11]. Serious intoxications and deaths were reported with the exposure of livestock to toxic pyrrolizidine alkaloids during grazing or through ingestion of contaminated food [12].

In Turkey, *H. europeum* L. is also used against scorpion bites and this usage of the plant is an indicator of its commonly used name in Turkey (Scorpion plant) [13]. This fact gives a clue for the possible effectiveness of plant on central nervous system. However, as far as we know, no study has been conducted to examine the possible anticonvulsive activity of *Heliotropium* species in Turkey until now.

There are several methods to test pro or anticonvulsive property of different chemicals and plant based extracts. Chemical convulsion model induced with pentylentetrazol (PTZ) is one of the most common methods used and accepted as a valid model in experimental convulsion models [14]. There are several studies which evaluate antiepileptic activity of plant extracts with PTZ [15]. PTZ can be used to trigger kindling model via administration of long term low dose PTZ. On the other hand, acute high dose injection [most commonly 75 or 80 mg/kg intraperitonally (i.p.)] cause myoclonic jerks, clonus and tonic extensions [16].

The aim of this study is to examine the anticonvulsive activity of *Heliotropium hirsutissimum*, *H. dolosum* and *H. lasiocarpum* species administered in an acute fashion.

MATERIAL AND METHOD

Plant material

The plants were collected by authors from the below mentioned locality and identified by Prof. Dr. Hayri Duman (Gazi University, Faculty of Science, Department of Biology). Voucher specimens are kept in AEF (Herbarium of Ankara University Faculty of Pharmacy):

H. dolosum: B4: Ankara, Ankara University Tandoğan Campus, roadsides, 863 m, 18/9/2013, AEF 26354

H. hirsutissimum: C2: Antalya, Manavgat, Çolaklı, 1 km to Ankara University Antalya facilities, roadsides, 31 m, 14/9/2013, AEF 26355

H. lasiocarpum: B1: İzmir, Dikili Bankacılar Sitesi, roadsides, 28 m, 2/8/2013, AEF 26353.

Preparation of plant extracts

Air-dried and powdered aerial parts were extracted with methanol. The extracts obtained were filtered from filter paper and dried in a freeze dryer.

Animals

Male Swiss-albino mice weighing between 25-30 grams were kept in Van Yuzuncu Yil

University Animal Facility. The animals were housed in standard cages with water and provided pelleted food *ad libitum*. The approval of Animal Ethics Committee was obtained. Relevant procedures involving animals were performed in accordance with the guidelines and international rules considering the animal experiments and rights.

Experimental design

4 groups including 6 animals each was set as follows:

PTZ group was administered with 80 mg/kg, i.p. PTZ (sigma) at 5th day. *H. hirsutissimum* group was administered 5 days of single *H. hirsutissimum* methanolic extract (200mg/kg, i.p.) and last day injected with PTZ (80 mg/kg, i.p.). *H. dolosum* group was administered 5 days of single *H. dolosum* methanolic extract (200mg/kg, i.p.) and last day injected with PTZ (80 mg/kg, i.p.). *H. lasiocarpum* group was administered 5 days of single *H. lasiocarpum* methanolic extract (200mg/kg, i.p.) and last day injected with PTZ (80 mg/kg, i.p.).

Anticonvulsive activity

Following PTZ injection animals were observed for myoclonic jerks, tonic clonic convulsion, number of animals having tonic-clonic convulsions and number of ex animals due to PTZ injection. An increased latency for initiation of myoclonic convulsion or tonic-clonic convulsion, decrease in number of animals having tonic-clonic convulsion and decrease in number of ex animals compared to lone PTZ group are accepted as anticonvulsive activity and vice versa.

Statistical analysis

Results were reported as mean ± standard error of mean (SEM). Myoclonic convulsions were analyzed by performing Kruskal-Wallis and Mann-Whitney U. For comparison of tonic clonic convulsions Fisher's exact test was used. Significance was accepted as $p<0.05$. SPSS 20 was used in data evaluation.

RESULT AND DISCUSSION

There were no deaths due to injection of the extracts. In *H. hirsutissimum* and *H. lasiocarpum* groups, number of animals having tonic-clonic convulsions was decreased compared to PTZ and *H. dolosum* groups however difference was found insignificant. Latency time for first myoclonic convulsion and latency time for first tonic-clonic convulsion were prolonged in all *Heliotropium* groups compared to PTZ group ($p>0.05$). Number of animals become ex following such tonic-clonic convulsions decreased in *Heliotropium* groups ($p>0.05$). Tonic-clonic convulsion period decreased in *H. hirsutissimum* and *H. lasiocarpum* ($p>0.05$). Results concerning anticonvulsive activity of plant extracts and drugs are presented in Table 1.

Table 1. Effect of *Heliotropium* species on PTZ induced convulsion

Administration	Latency for FMC (s)	Latency for FTCC (s)	NC (#)	CP (s)	Ex
PTZ	99.5±16.6^{ns}	281.0±57.0^{ns}	6^{ns}	15.7±2.7^{ns}	6^{ns}
HH	156.8±20.2^{ns}	353.3±78.4^{ns}	3^{ns}	10.7±1.5^{ns}	2^{ns}
HD	117.7± 13.2^{ns}	393.0±33.5^{ns}	6^{ns}	17.2±0.9^{ns}	2^{ns}
HL	147.0±14.5^{ns}	384.8±44.4^{ns}	4^{ns}	11.0±1.4^{ns}	2^{ns}

^{ns}: non-significant ($p>0.05$)
Results are given as mean±S.E.M. FMC: Latency for first myoclonic convulsion in seconds, FTCC: Latency for first tonic-clonic convulsion in seconds, NC: number of animals having tonic-clonic convulsions, CP: convulsion period in seconds, Ex: Number of ex animals due to tonic-clonic convulsions, PTZ: Pentylenetetrazol, HS: *Heliotropium hirsutissimum* group, HD: *Heliotropium dolosum* group, HL: *Heliotropium lasiocarpum* group.

In this study possible anticonvulsive activity of 3 *Heliotropium* species (*Heliotropium hirsutissimum, H. dolosum* and *H. lasiocarpum*) was evaluated.

Plant originated chemicals such as valerianic acid are well known for their anticonvulsive activity. In addition literature records reveal potent anticonvulsive property for certain plant species [17]. Some of those studies administered similar dosage (200 mg/kg) with our study and anticonvulsive activity was observed [18].

Pyrrolizidine alkaloids are known for their toxic effects on different animals including livestock. Grazing on *H. europeum* is known to result in toxicity in 42 to 63 days [19]. In experimental animals, toxicity of different *Heliotropium* species also was examined. Dosages of 0.5 to 12.0g/kg bodyweight administered to Swiss albino mice showed LD50 as 9.78 g/kg b.wt [20]. Among species examined in our study, literature records show that *H. dolosum* cause toxicity on broiler chickens in a period between 10 to 52 days of age [21]. In another study [22], *H. dolosum* was tested on Swiss albino mice and untoward effects on mice were observed within a period of 24 weeks. However, our administration scheme was limited to 5 days, therefore no such significant intoxication was observed in our study probably due to this short time of exposure. *Heliotropium* species are used in ayurvedic treatment practices. Among other uses such as for asthma and snake poisoning, *Heliotropium* species are also used traditionally for treatment of epilepsy in India [23-25].

Although none of our tested parameters show a statistical significance, when they are evaluated together, increase in latency for first myoclonic convulsion in each *Heliotropium* group and increase in latency for first tonic-clonic convulsion in each *Heliotropium* group; decrease in number of animals having tonic-clonic convulsions in *H. hirsutissimum* and *H. lasiocarpum*; decrease in number of ex animals following tonic-clonic convulsions in each *Heliotropium* group and decrease in convulsion period in *H. hirsutissimum* and *H. lasiocarpum* suggest a possible ethnopharmacological relevance for traditional use of *Heliotropium* species as anticonvulsive. Literature records reveal that different *Heliotropium* species have been tested for their anticonvulsive activities. *H. strigosum* was reported to exert no anticonvulsive activity at 50, 100 and 200 mg/kg i.p. doses in PTZ induced convulsions [26].

Though our results also show no significant difference, performing the study on a higher number of animals may provide significant results since decrease in number of animals having tonic-clonic convulsions attenuate the number of animals for statistical evaluation.

Conflict of Interest

Authors declare no conflict of interest.

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