



Antiepileptic activity of hydroalcoholic extract of basil in mice

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ABSTRACT

Introduction: Epilepsy has been always considered as one of the most prevalent disorders of nervous system and despite various drugs available, there are still some patients who have drug resistance. In traditional medicine, basil (*Ocimum basilicum*) has been used in treating epilepsy. To achieve an effective antiepileptic drug with few side effects, the antiepileptic effect of *O. basilicum* was investigated in pentylenetetrazole (PTZ)-induced epilepsy model.

Methods: In this experimental research, 48 laboratory female mice (for removing gender factor) were designated in 6 equal groups. The experimental groups comprised control, sham, and four treatment groups receiving the extract at 100, 250, 300, and 350 mg/kg doses (intraperitoneally; ip), 65 minutes before PTZ injection and the factors of epilepsy symptoms and the symptoms frequency were studied.

Results: The obtained results of using different doses (100, 250, 300, 350 mg/kg) of the extract indicated that the mice in 100 mg/kg dose group exhibited the highest frequency of epileptic attacks. The samples receiving the extract at 100 and 250 mg/kg doses exhibited the highest and lowest frequency of myoclonic twitches, respectively. In the group treated with 250 mg/kg dose, the symptoms of epilepsy, frequency of epilepsy, and mortality rate respectively increased, decreased, and decreased ($p < 0.05$).

Conclusion: By the obtained results, the hydroalcoholic extract of *O. basilicum* at 250 mg/kg dose could be recommended as an effective drug for preventing epilepsy in the animal model.

Implication for health policy/practice/research/medical education:

The hydroalcoholic extract of *Ocimum basilicum* is able to prevent the attacks in the animal epilepsy model and might be beneficial in epileptic patients.

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Introduction

In traditional medicine, there is an attempt to introduce suitable alternatives to synthetic drugs by means of the chemical compounds existing in plants or through combining different parts of a number of plants. In other words, the purpose in traditional medicine is to improve the function of a system in relation to other systems and hence herbal medicines enjoy a more extensive range of effects than existing synthetic ones in pharmacotherapy (1). Use of antiepileptic drugs can lead to numerous complications including decreased white blood cells and hepatic toxicity. Also, taking these drugs in pregnancy can have teratogenic effects and cause fetal anomalies. Therefore,

herbal medicines with fewer complications could be a very appropriate alternative for treating this disease (2).

Ocimum basilicum has long been used as sedative, analgesic, and anticonvulsant (3). *O. basilicum* is a herbaceous, annual, and aromatic plant, reaching a height of 60 cm (4). In a study by Li et al, 1-8 cineole, linalool, and geraniol together comprising 93% of the essential oils were introduced as the main components of *O. basilicum* (3,5). In India, *O. basilicum* leaves are used as soothing and their sap as febrifuge and drug bust in bronchitis. The powder of its dried leaves is blown into the nose for atrophic rhinitis which is manifested by excessive dryness of the nose, ample and smelly scabs, and broad nasal cavity

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(3). The boiled root of the plant is taken as diaphoretic in malaria fever cases. The leaves and stems of this plant are crushed and put on the mosquito bite. Its essence is applied as disinfectant, insecticide, and insect repellent especially at the onset of typhus. This plant is also anti *Bacillus typhosus* (3). Epilepsy is one of prevalent disease (6) as more than 50 million people are suffering from it worldwide (7).

Epilepsy is a chronic and mainly progressive disorder and is manifested as an unpredictable and periodical attack, developed by abnormal discharge of brain neurons (8). Regarding that no study has been yet conducted on antiepileptic effect of *O. basilicum* in pentylenetetrazole (PTZ) model, the present study was designed and conducted to study the antiepileptic effect of *O. basilicum* in an animal model to take a small step in treatment of epilepsy, and relief of pain, suffering, and concerns of human.

Materials and Methods

This experimental research was conducted in 2010-2011 in Payam-e-Noor University of Isfahan, Iran. In this research, we used small female Balb/C mice weighing 30 ± 5 gr. The adult female mice under study were kept in laboratory conditions for one week to get adapted to the environment. The animals were kept in a room with normal light conditions and $24-26$ °C temperature and water and food were freely placed at their disposal. The laboratory mice under this study comprised 48 mice randomly assigned into six groups of eight each and each group was kept in a separate cage.

To prepare the hydroalcoholic extract of *O. basilicum*, we divided the supplied leaves into small pieces and pulverized them using mill. 30 gr of this powder was poured into a sterile erlen and 40 ml ethyl alcohol was introduced. The mixture was kept in a cool environment for 24 hours and then the contents of the erlen were completely mixed using a shaker for five minutes. After the sample was filtrated using Whatman paper and the remaining extract in the solution (2.34 gr) was calculated, *O. basilicum* concentration was determined in the mother solution and the required doses were prepared through diluting by injectable normal saline. The hydroalcoholic extract was intraperitoneally injected to the experimental groups as follows:

- A) Control group received injectable normal saline intraperitoneally at 0.5 ml.
- B) Sham group: For comparison between the extract's effect and diazepam, this group received injectable diazepam at 2 mg/kg dose.
- C) The group receiving injection at 100 mg/kg
- D) The group receiving injection at 250 mg/kg
- E) The group receiving injection at 300 mg/kg
- F) The group receiving injection at 350 mg/kg

All samples were injected with the epilepsy-causing PTZ at 130mg/kg dose, 65 minutes after receiving the extract at determined doses (9-11). Then, the factors of epilepsy symptoms and symptoms frequency were investigated. In this research, the mice were checked for one hour after the

extract and PTZ injection and all manifested movements were recorded by a camera, so that the type of movements could be closely interpreted and measured. Epileptic responses were classified as follows:

Stage zero: no response, stage one: immobility, stage two: body stretching, stage three: myoclonic twitches, and stage four: general tonic-clonic seizures. The obtained data were analyzed by SPSS 11.5 using Tukey's test, Duncan's test, and ANOVA.

Results

The control and C groups developed body stretching most frequently. The sham and D groups developed fewest attacks of body stretching ($p < 0.05$). The control and C groups developed myoclonic twitches most frequently. Also, the D group developed the fewest myoclonic twitches ($p < 0.05$; Table 1).

The effect of 250 mg/kg dose on absence of various attacks was greater than that of other doses. In addition, none of the used doses had a significant effect on absence of immobility ($p > 0.05$). The 250 mg/kg dose could be introduced as the best dose with a function similar to that observed in the B group. The highest reduction in mean frequency of myoclonic twitches was noted in the F group. The 300 mg/kg dose had the greatest effect, after diazepam, on decreasing mean frequency of general tonic-clonic seizures. Also, the 350 mg/kg dose had the highest effect on decreasing mean frequency of myoclonic twitches compared to the other doses ($p < 0.05$). There was no significant difference in mean frequency of general tonic-clonic seizures between the A group and other groups ($p > 0.05$; Table 1).

Discussion

The analysis of the data obtained in this study indicated that intraperitoneal injection of *O. basilicum* hydroalcoholic extract was effective on the epileptic behaviours parameters in the mice and confirmed the antiepileptic property of this plant leaves. The obtained data indicated that the effect of *O. basilicum* extract is dose-dependent and different concentrations could exert inhibitory effect on or delay a different series of epileptic behaviors.

Herbal medicines have long been addressed as therapeutic compounds with fewer complications and used as very appropriate alternatives for treating diseases (2). *O. basilicum* has been much used as a very suitable sedative, analgesic, and anticonvulsant herbal medicine (3). The important issue in using herbal medicines is determination of the appropriate dose with the fewest complications; therefore we studied the effect of 100, 250, 300, and 350 mg/kg dose of *O. basilicum* hydroalcoholic extract on treating the epilepsy induced by PTZ injection in this research project. It has been well confirmed that PTZ has adverse effects on neuronal membrane. PTZ affects potassium and calcium channels and discharges the intracellular repertoires of calcium ions. In addition, PTZ decreases the neurotransmitter-induced chloride conductance (9).

Table 1. Comparison of effect of basil hydro-alcoholic extract against pentylenetetrazole-induced seizure in mice

Parameters	Groups					
	A	B	C	D	E	F
The number of attacks (stretching)	6.80±0.20	6.65±0.33	6.80±0.30*	3.80±0.20*	3.70±0.30	4.75±0.30
The number of myoclonic contractions	6.90±0.50	3.90±0.54	6.60±0.33*	3.70±0.23*	4.80±0.60	4.85±0.20
The absence of attacks	6.80±0.60	6.90±0.50	6.80±0.30	3.60±0.40*	6.80±0.40	6.90±0.70
Frequency of myoclonic contractions	3.60±0.20	2.50±0.20	2.60±0.70	2.50±0.20	2.60±0.30	1.50±0.20*
Frequency of generalized tonic-clonic seizures	6.40±0.70*	3.10±0.50	6.40±0.80*	6.60±0.20*	3.81±0.30	6.50±0.20*

A: Control, B: Sham, C, D, E, and F: The groups receiving the extract at doses of respectively 100, 250, 300, and 350 mg/kg; The data are shown as Mean±SD; * significant at p<0.05.

As the drugs effective on PTZ-induced epilepsies are clinically very useful in treating small epilepsy or absence (10,11). Therefore, the extract of *O. basilicum* leaves has also exhibited good effects on inhibiting the PTZ-induced epilepsies and could be effective on absence seizure treatment (12).

Since seizure mechanism is thought to be associated with decreased cerebral GABA levels, Castor *et al.* studied the effect of benzene ethyl acetate fraction of *Sesbania grandiflora* on the cerebral GABA contents in the mice after demonstrating antiepileptic property of this fraction in the PTZ-induced seizure animal model. The results indicated an increase in cerebral GABA content (13). Analysis of *O. basilicum* essential oil indicated that terpenes were the main existent compounds in this extract, with linalool, (44.18%), cineole (13.65%), eugenol (8.59%), isocaryophyllene (3.10%), and methyl cinnamate (4.26%) as the main terpenes (14). As linalool is one of the main components of *O. basilicum*, the mechanism of linalool effect has been mentioned on modulation of GABAergic system (15). Therefore, the antiepileptic effect of *O. basilicum* extract is likely to be exerted also in the PTZ-induced seizure model thanks to increased cerebral GABA content.

Linalool is a monoterpene which is present in many aromatic oil essences as a main component and could be a candidate for antiepileptic effect of *O. basilicum*. Many of the plants containing linalool are used as antiepileptic agents in traditional medicine, of which juniper, scientifically named *Aeollanthus* from family Labiatae, could be mentioned. Also, oil essence of *Laurus nobilis* leaves containing linalool was demonstrated to be effective on PTZ-induced seizure. Linalool exerts its antiepileptic effect through inhibiting glutamate binding in rats' cortex and impacting GABAergic and glutamatergic connections (16). Linalool is one of the main components of the extract and has dose-dependent effects. This component has been used for dyspepsia and epilepsy, which its effects were attributed to its local anesthetic activity (2, 17). The local anesthesia developed by linalool is attributed to its inhibitory effect on discharging acetylcholine and the duration within which the channel remains open in muscular neural functions. One of the causes of this dose's toxicity is increased linalool which disrupts vital functions of the cells because of excessive

inhibition of acetylcholine transmission and leads to the animal death (18).

Conclusion

In view of the obtained results, the effect *O. basilicum* hydroalcoholic extract at 250 mg/kg dose on developing myoclonic twitches is more pronounced than that of this extract at other doses and this dose is recommended as the best one, with a function similar to that observed in the sham group. Therefore, the notable point, in view of the effect's dose-dependency, in using this herbal medicine is to select the exact dosage based on the need. This should be further investigated in complementary works.

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Authors' contributions

All the authors wrote the manuscript equally.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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