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Anticonvulsant Activity of *Otostegia persica* (Burm.) Boiss

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Investigation of the anticonvulsant activity *Otostegia persica*. The anticonvulsant activity of *O. persica* total extract was assessed in pentylenetetrazole (PTZ)-induced convulsion in mice, with Diazepam as standard drug. While mechanistic studies were conducted using flumazenil, a GABAA-benzodiazepine receptorcomplex site antagonist. the extractproduced protectionagainst convulsion at800mg/kg, compared with protection with benzodiazepine.The mean onset and percentage protection against convulsion in extract-treated mice werereduced by flumazenil. These results suggest that *O. pesica* extractpossesses biologically active constituent(s) that haveanticonvulsant activity which supports the ethnomedicinal claims of the use of the plant in the management of seizure.

Key words: *Otostegia persica,* Lamiaceae, Anticonvulsant activity, Clonic seizure**.**

Described as a chronic disorder of the central nervous system, epilepsy is a major medical and social problem which is characterized by recurrent seizures due to excessive discharge of cerebral neurons (Gaustaut, 1973; Senanayake and Roman, 1993). According to the WHO (WHO, 2001) around 450 million people in the world have affected mental, neurological, or behavioral problems at some time in their life of which about 50 million suffer epilepsy (WHO fact sheet dated January 2009). Extensive research on plants and their derivatives and investigations into natural sources of effective drugs that may be more readily accessible have taken place in recent years that could provide some new alternative treatments and therapeutic uses for diseases of the central nervous system (CNS) especially epilepsy and seizure. Interest in medicinal plants reflects the recognition of the validity of many traditional claims regarding the value of natural products in healthcare (Nair *et al*., 2005).

The medicinal use of plants has been known since the early times. Some are used in the control of emotions and mood, for their anticonvulsant properties and sedative, anxiolytic and antidepressant effects. Some studies suggest that they act by modulating the central neurotransmission (Leite *et al*., 2008).

The genus *Otostegia* (Lamiaceae) consists of about 33 species growing mainly in the Mediterranean region and adjoining Asia Minor (Ahmad *et al*., 2004). *O. persica* locally called "Golder" is widely distributed in south and south eastern of Iran. It is traditionally used for alleviate opium withdrawal syndrome and treatment of malaria, fever, seizure and diabetes (Hajhashemi *et al*., 2004).The aerial part of *O. persica* is reported to have high antioxidant activity which is related to the flavonoids (Yassa *et al*., 2005) and its hydro-alcoholic extract is effective on morphine

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withdrawal syndrome in mice (Hajhashemi *et al*., 2004). Methanolic extract of the aerial parts of *O. persica* has shown positive activity in the brine shrimp lethality test. Additional biological screening of the methanol extract has revealed strong antioxidant as well as antibacterial activities against various strains of Gram-positive and Gram-negative bacteria (Ayatollahi *et al*., 2007; Meyer *et al*., 1982; Rahman *et al*., 1999).

Due to the folklore use of this plant in Iranian traditional medicine for relief and treatment of seizure, we prompted to evaluate the anticonvulsant activity of its total methanol extract and investigate the pharmacological basis for the folkloric use of it as an anticonvulsant agent. This study explores the anticonvulsant property of *O. persica*by investigating their suppression of seizures induced by Pentylenetetrazole (PTZ) which enhances excitatory responses in the central nervous system by inhibiting inhibitory responses to glycine and gamma amino butyric acid respectively (Purves *et al*., 2008).

MATERIALS AND METHODS

Plant material

Flowering aerial parts of *O. persica*were collected from Jabr-Abad (Sistan-Beluchistan Province)in May 2012. The plant was identified by Dr. G. Amin. Voucher specimen has been deposited at the herbarium of Department of Pharmacognosy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran, under code number 311. The plants were air dried in the shade and powdered.

Extraction Procedure

300 g of dried ground materials were extracted by percolator apparatus using methanol (Merck). The extraction was repeated for 3 times. The extract was concentrated by rotary evaporator apparatus and the solvent removed to produce a dark green gummy solid. The resulting extract was kept in a clean vial in a dark and cool place for further investigations.

Experimental animals

Albino mice of either sex (20–25 g) were used throughout this study. Animals were housed in groups of 5-6 and were allowed free access to food and water except for the short time that animals were removed from their cages for testing. All experiments were conducted during the period between 10.00 a.m. and 13 p.m. with normal room light (12 h regular light/dark cycle) and temperature (22 ± 1°C). All procedures were carried out in accordance with the institutional guidelines for animal care and use (ethical approval number: 3183). Each mouse was used only once.

Anticonvulsant activity

Pentylenetetrazole (PTZ)-induced convulsion in mice

Myoclonic seizure induced by Pentylenetetrazole (PTZ) is a standard experimental model of clinical myoclonic petit-mal seizures with both face and construct validity. To assess the seizure susceptibility, the more sensitive method of IV administration of PTZ that allows better detection of modulatory effects on convulsive tendency (Endres *et al*.,1998). The threshold of PTZ was determined by infusion of PTZ (0.5%) at a constant rate of 0.5 ml/min into the tail vein of unrestrained freely moving mice. Infusion was halted when forelimb clonus followed by full clonus of the body was observed (Shafaroodi *et al*., 2012).

Treatments

The method of IV administration of PTZ to assess the seizure susceptibility was used. 30 mice were divided into 6 groups each containing 5 mice. The first group received the vehicle, salin (IP), the second, third, fourth and fifth groups received 100, 200, 400 and 800 mg/kg IP of the extract, while the sixth groups was injected with diazepam 0.025 mg/kg IP. Thirty minutes after treatment, mice in all the groups received PTZ 0.5 mg/kg (IV). Mice were placed into separate individual plastic cage for observation lasting 1 h. The onset of a general clonus was used as the endpoint. The general clonus was characterized by forelimb clonus followed by full clonus of the body. The time taken before the onset of clonic convulsions, the duration of clonic convulsions, and the percentage of seizure and mortality protection were recorded (Vogel and Vogel, 1997).

Statistical analysis

Data were expressed as mean ± S.E.M. The one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons were used to analyze the data of clonic seizures. $P<0.05$was considered the significant level between the groups.

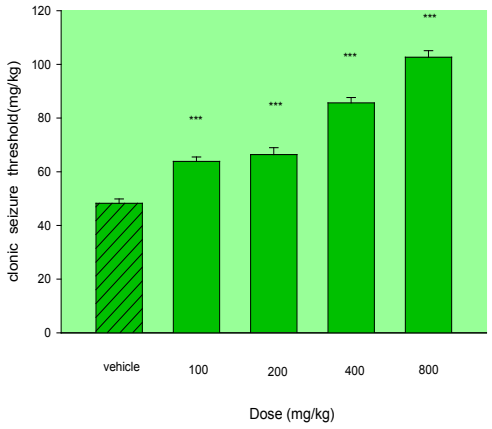
RESULTS

Screening for anticonvulsant activity

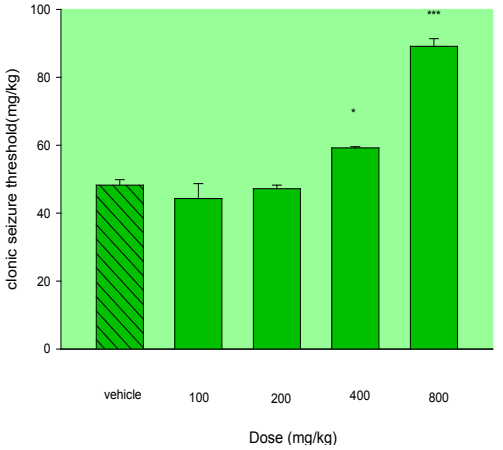
The anticonvulsant activity of the extractwas determined using chemically induced (PTZ) convulsion in mice. Fig. 1 shows the effect of acute IP administration of different doses of each extract (100, 200, 400 and 800 mg/kg) on the clonic seizure threshold induced by intravenous PTZ. Different doses of the extract were administered 30 and 60 min prior to PTZ to distinct groups of mice. One-way Anova revealed a significant effect for the extracts ($P< 0.05$).

At 800 mg/kg, the extract provided 93.75% protection ($P< 0.05$). The mean clonic seizure latency (662.20 ± 1.73 s) in the mice treated with 800 mg/kg 30 min before PTZ injection was statistically significant from control (102.66 + 2.44 s). The mean clonic seizure latency in the mice treated with 800 mg/kg 60 min before PTZ injection was also statistically significant from control (89.12 + 2.27s) and the extract provided 93.05% protection ($P< 0.05$).

Fig 2 shows the effect of *O. persica* extract (800 mg/kg) 30, 60 and 240 min pirior to PTZ injection. As it is mentioned from Fig. 2, the effects of the extracts got weaker by longitude time of PTZ injection. The best result is observed by administration of 800 mg/kg of *O. persica* extract 30 min before PTZ injection.



a) IP administration of *O. persica* extract 30 min pirior to PTZ injection.Z



b) IP administration of *O. persica* extract 60 min pirior to PTZ injection.

Fig. 1. The effect of different doses of *O. persica* extract on PTZ-induced clonic seizure threshold in mice. *** *P<0.05* compared to vehicle control group.

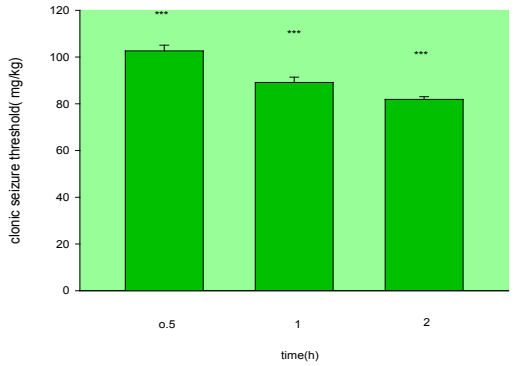


Fig. 2. The effect of *O. persica* extract (800 mg/kg) on PTZ-induced clonic seizure threshold in mice

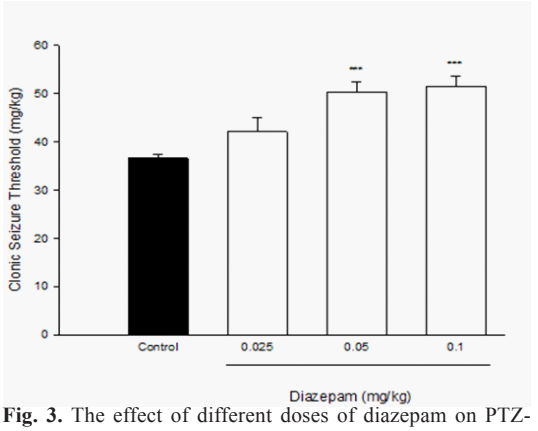


Fig. 3. The effect of different doses of diazepam on PTZ-induced clonic seizure threshold in mice. *** *P<0.01*compared to vehicle control group

We also studied the effects of flumazenil, a selective benzodiazepine receptor antagonist site in the GABAA-BZD receptor complex, on the anticonvulsant activity of *O. persica* extract in order to elucidate the mechanism involved in extract-induced protection of mice from PTZ-induced seizure. Flumazenil (0.5 mg/kg) was administered 5 min prior to injection of the extract (800 mg/kg) or diazepam (0.025 mg/kg). Flumazenil reversed the effect of the extract in prolonging seizure latency. Also, flumazenil could reverse the anticonvulsant activity of diazepam.

DISCUSSION

Recent studies on medicinal plants and their main components have attracted the attention of many scientists and encouraged them to screen any of these natural products to study their chemical and pharmacological aspects that might potentially lead to the development of new anticonvulsant-like compounds with advantages over current therapeutic drugs (Almeida *et al*., 2003). The present study investigated the anticonvulsant effect of *O. persica* methanol extract using myoclonic seizure induced by Pentylenetetrazole (PTZ) model which is a standard experimental model of clinical myoclonic petit-mal seizures with both face and construct validity. This was demonstrated by the activity against PTZ-induced seizures which correlate with anti-absence activity (Delgado and Remers, 1998). The extract (800 mg/kg) could suppress onset and duration of clonic seizure in PTZ model and it seems that this effect increased dose dependently.

Clonic seizure was induced by γ-aminobutyric acid (GABA) transmission blocker PTZ (Riazi *et al*., 2004). Regarding the possible contribution of GABAergic system in the anticonvulsant activity of the extrat, flumazenil, a benzodiazepine receptor antagonist, was used (File and Pellow, 1986). Flumazenil decreased the prolongation of seizure latency induced by the extract and it also antagonized the effect of the extract on decreasing the duration of clonic seizures in the PTZ model. It is noteworthy that since the anticonvulsant effect of the extract is blocked by an antagonist of benzodiazepine receptor, its effect seems to be related to benzodiazepine receptor activation

The major components of the aerial parts of the investigated plant have been reported as clerodane and tetracyclic diterpenoids (Ayatollahi *et al*., 2009).

Psychopharmacological evaluation of diterpenes in mice revealed that these compounds have marked sedative effects at the CNS, including protection against PTZ and electroshock induced convulsions (Baird-Lambert *et al*., 1980; Okokon and Nwaford, 2009; Daló e al., 2007).So it could be concluded that the anticonvulsant activity of *O. persica* extract was related to its high content of diterpenes.

The results of the study have demonstrated that *O. persica* extract possessed anticonvulsant activity on the animal model investigated and this provides a rationale for its use in traditional medicine for the management of epilepsy.

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