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Original article

Anticonvulsant activity of *Astragalus squarrosus* Bunge

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Abstract

The anticonvulsant activity of *Astragalus squarrosus* total extract was assessed in pentylenetetrazole (PTZ)-induced convulsion in mice, with diazepam as the standard drug, while mechanistic studies were also conducted using flumazenil, a GABA A-benzodiazepine receptor complex site antagonist. The extract produced protection against convulsion at 400 mg/kg, comparable with protection of benzodiazepine (diazepam). The mean onset and percentage protection against convulsion in extract-treated mice were reduced by flumazenil. The results suggest that *A. squarrosus* extract possesses biologically active constituent(s) that have anticonvulsant activity which supports the ethnomedical claims of its use in the management of seizure.

Keywords: anticonvulsant activity, *Astragalus squarrosus,* clonic seizure, Fabaceae

Introduction

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 [1,2]. According to WHO [3], around 450 million people in the world have been affected by mental, neurological, or behavioral problems some time in their lives and about 50 million of these people suffer from epilepsy [4]. Extensive research on plants and their derivatives and investigations of natural sources for effective drugs have taken place in recent years in order to provide some new alternative treatments and therapeutic agents 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 [5].

The medicinal use of plants has been known since the early times and they have been used for controlling emotion and mood, anticonvulsant, sedative, anxiolytic and antidepressant properties. Some studies suggest that they act by modulating the central neurotransmissions [6].

Astragalus L. (Fabaceae) is generally considered as the largest genus of vascular plants with an estimated 2500 to 3000 species. *Astragalus* is widely distributed in temperate regions of the Northern hemisphere. The greatest numbers of species are found in the arid, continental regions of Western North America (400 species) and

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central Asia (2000 to 2500 species) [7]. Many species of *Astragalus* are useful as forage plants, to control erosion, as ornamentals or as medicinal plants [8,9]. The interest in chemical constituents of various species of the genus *Astragalus* has been increasing during the recent years. Many species have been investigated for flavonoids, non-protein amino acids, saponins, alkaloids, nitro compounds, mucilage, sterols, etc. [10].

Due to the folklore use of this plant for relief and treatment of seizure, we were prompted to evaluate the anticonvulsant activity of its methanol extract and to investigate the pharmacological basis for its folklore use as an anticonvulsant agent. This study has explored the anticonvulsant property of *A. squarrosus* by investigating suppression of seizures induced by pentylenetetrazole (PTZ) which enhances excitatory responses in the central nervous system by preventing the inhibitory responses to glycine and gamma amino butyric acid, respectively [11].

Experimental

Plant material

Flowering aerial parts of *A. squarrosus* were collected from Mutteh (Isfahan Province) in May 2012. The plant was identified by Dr. G. Amin. A voucher specimen has been deposited at the Herbarium of the Department of Pharmacognosy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran (number 216). The aerial parts were air dried in shade and powdered.

Extraction

300 g of the dried ground material were extracted by maceration method using methanol. The extraction was repeated 3 times. The extract was concentrated by rotary evaporator apparatus and the solvent was 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 housed in groups of 5 and were allowed free access to food and water except for the short time that the animals were removed from their cages for testing. All experiments were conducted during the period between 10 a.m. and 13 p.m. with normal room light (12 h regular light/dark cycle) and temperature (22±1 ˚C). The 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 was used [12]. 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 [13].

Treatments

The method of IV administration of PTZ to assess the seizure susceptibility was used. 25 mice were divided into 5 groups each containing 5 mice. The first group received the vehicle, salin (*IP*), the second, third and fourth groups received 100, 200 and 400 mg/kg *IP* of the extract, while the fifth group was injected with diazepam 0.025 mg/kg *IP*. Thirty minutes after treatment, the mice in all the groups received PTZ. Mice were placed into separate individual plastic cages 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 [14].

We also studied the effects of flumazenil, a selective benzodiazepine receptor antagonist site in the GABAA-BZD receptor complex, on the anticonvulsant activity of *A. squarrosus* 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 (400 mg/kg) or diazepam (0.025 mg/kg).

Statistical analysis

Data were expressed as mean±SEM. 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 and Discussion

The anticonvulsant activity of the extract was determined using chemically induced (PTZ) convulsion in mice. figure 1 shows the effect of acute *IP* administration of different doses of the extract of *A. squarrosus* (100, 200 and 400 mg/kg) on the clonic seizure threshold induced by intravenous PTZ. Different doses of the extract were administered 30 min prior to PTZ to distinct groups of mice. One-way Anova revealed a significant effect of the extract in doses of 200 and 400 mg/kg ($P< 0.05$) compared to the group which received just the vehicle.

Figure 2 shows the effect of *A. squarrosus* extract (400 mg/kg) 30, 60 and 240 min prior to PTZ injection. As it is demonstrated, the effects of the extracts have been decreased by longitude time of PTZ injection. The best result was observed by administration of 400 mg/kg of *A. squarrosus* extract 30 min before PTZ injection.

Flumazenil reversed the effect of the extract in prolonging seizure latency. It could also reverse the anticonvulsant activity of diazepam.

The extract (400 mg/kg) could increase clonic seizure threshold in PTZ model and it seems that this effect has increased dose dependently. Clonic seizure was induced by γ-aminobutyric acid (GABA) transmission blocker PTZ [15]. Regarding the possible contribution of GABAergic system in the anticonvulsant activity of the extract, flumazenil, a benzodiazepine receptor antagonist, was used [16]. 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. Since the anticonvulsant effect of the extract was blocked by an antagonist of benzodiazepine receptor, its effect seems to be related to benzodiazepine receptor activation.

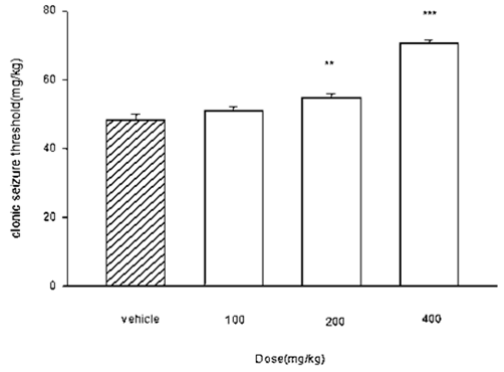

Figure 1. The effect of different doses of *A. squarrosus* extract on PTZ-induced clonic seizure threshold in mice 30 min prior to PTZ injection. * $P<0.05$ compared to vehicle control group

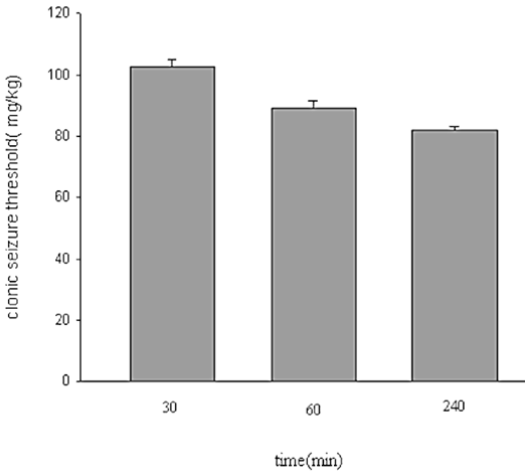

Figure 2. The effect of *A. squarrosus* extract (400 mg/kg) on PTZ-induced clonic seizure threshold in mice

The major components of the aerial parts of the investigated plant have been reported as flavonoids and phenolic compounds [7].

Psychopharmacological evaluation of flavonoids in mice have revealed that these compounds have marked sedative effects in CNS, including protection against PTZ and electroshock induced convulsions [17,18]. It could be concluded that the anticonvulsant activity of *A. squarrosus* extract could be correlated to its high content of flavonoids [7].

Recent studies on medicinal plants and their main components have attracted the attention of many scientists and encouraged them to screen these natural sources for their chemical and pharmacological aspects that might potentially lead to the development of new anticonvulsant compounds. The present study has investigated the anticonvulsant effect of *A. squarrosus* 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 constructs validity. The results of the present study have demonstrated that *A. squarrosus* extract possessed anticonvulsant activity on the animal model investigated which provides a rationale for its use in folklore medicine for management of epilepsy.

Acknowledgments

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