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Screening of the anticonvulsant activity of some plants from Fabaceae family in experimental seizure models in mice

[*1] Sayyah, M., [1,2] Khodaparast, A., [3] Yazdi, A., [4] Sardari, S.

[1]Department of Physiology and Pharmacology, Pasteur Institute of Iran, [2]Department of Biotechnology, Research & Science Campus, Azad University, [3]Department of Biology, Shahed University, [4]Department of Medical Biotechnology, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

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ABSTRACT

Background and purpose of the study: Fabaceae is the third largest family of flowering plants. Lack of essential oils in the plants of this family can be an advantage in search for safe and effective medicines. In this study the anticonvulsant effect of the leaves of *Albizzia julibrissin, Acacia juliflora, Acacia nubica* and aerial parts of *Astragalus obtusifolius* was evaluated in pentylenetetrazole (PTZ) and maximal electroshock (MES) seizure tests.

Methods: The hydroalcoholic extracts of the plants were obtained by percolation. Different doses of the extracts were injected to the mice intraperitoneally (i.p.) and occurrence of clonic seizures induced by PTZ (60 mg/kg, i.p.) or tonic seizures induced by MES (50 mA, 50Hz, 1sec) were monitored up to 30 min after administration. Acute toxicity of the extracts was also assessed. The safe and effective extract was then fractionated by dichloromethane and anticonvulsant activity of the fractions was determined. Finally, the constituents of the extract and the fractions were screened by thin layer chromatography.

Results: Among the extracts, only *A. obtusifolius* extract showed low toxicity and protective effect against clonic seizures with ED50 value of 3.97 g/kg. Fractionation of the extract led to increase in anticonvulsant activity and ED50 value of 2.86 g/kg was obtained for the aqueous fraction. Phytochemical screening revealed the presence of alkaloids, flavonoids, anthrones and saponins in the aqueous fraction.

Major conclusion: The presence of anticonvulsant compounds in *A. obtusifolius* suggests further activity-guided fractionation and analytical studies to find out the potential of this plant as a source of anticonvulsant agent.

Keywords: Pentylenetetrazole, Maximal electroshock, *A. obtusifolius.*

INTRODUCTION

Epilepsy is the third most common neurological disorder after stroke and Alzheimer's disease. Although new antiepileptic drugs have been available since late 1980s, refractoriness to treatment is still an important issue in epilepsy care. Current available anticonvulsant drugs are able to control epileptic seizures efficiently in about 50% of the patients and lead to improvement in another 25% whereas the remainder do not benefit significantly (1). Furthermore, undesirable side effects of the drugs used clinically often render treatment difficult; so that a demand for new types of anticonvulsants exists. One of the approaches to search for new antiepileptic drugs is investigation of naturally-occurring compounds, which belong to new structural classes.

Fabaceae or Leguminosae is a large and economically important family of flowering plants, which is commonly known as the legume family, pea family or bean family. Fabaceae is the third largest family of flowering plants, behind Orchidaceae and Asteraceae, with 730 genera and over 19400 species, according to the Royal Botanical Gardens. The largest genera are *Astragalus* with more than 2000 species, *Acacia* with more than 900 species, and *Indigofera* with around 700 species (2).

Essential oils have often high toxicity and narrow therapeutic index. Furthermore, their particular chemical structures have weak potential for modification, which renders them not good candidate for drug design (3). Most of the plants of Fabaceae family have either no or negligible amounts of the essential oils (2), which may be considered as an advantage in search for safe and effective medicines pertaining to new structural classes. Several plants

Correspondence: sayyahm2@pasteur.ac.ir

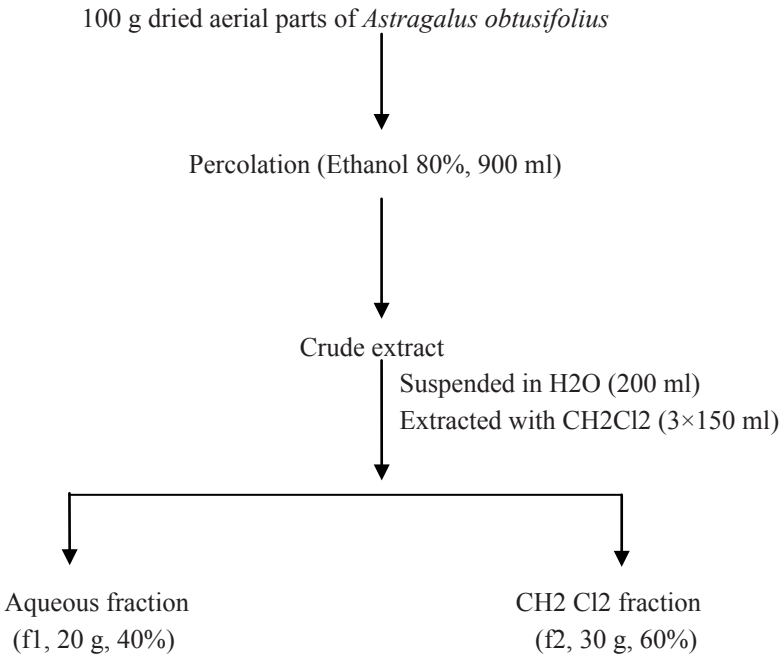

Figure 1. Flow diagram of fractionation of the crude extract obtained from the aerial parts of *Astragalus obtusifolius*.

from Fabaceae family have shown anticonvulsant activity in animal models (4-6).

In this study the possible anticonvulsant and toxic effects of four plants pertaining to Fabaceae family including *Albizzia julibrissin, Acacia juliflora, Acacia nubica* and *Astragalus obtusifolius* that are widely grown in Iran, were evaluated in mice.

MATERIAL AND METHODS

Animals

Adult male NMRI mice, weighing 20-28 g, from Pasteur Institute of Iran were used throughout this study. The animals were housed in standard cages with free access to food (standard laboratory rodent›s chow) and water. The animal house temperature was maintained at 23±1 °C with a 12-h light/12-h dark cycle (light on from 06:00 AM to 18:00 PM). The study was approved by the ethics committee of Pasteur Institute of Iran and conforms to the European Communities Council Directive of 24th of November, 1986 (86/609/EEC). All animal experiments were carried out in a way that minimizes the number of animals and their sufferings. Each animal was tested once. All the injections were intraperitoneal (i.p.) in volume of 0.1 ml/10 g of mice body weight.

Chemicals

Pentylenetetrazole (PTZ), phenytoin sodium and ethosuximide were purchased from Sigma-Aldrich (Pool, UK). Tween 80, Dimethyl sulfoxide (DMSO), ethanol, dichloromethane, antimony trichloride, Dragendorrf's reagent, potassium hydroxide, glacial acetic acid, vanillin, sulphuric acid, ferric chloride, hydrochloric acid and sodium hydroxide were all from Merck (Darmstadt, Germany).

Plant materials

Botanical data of the plants are demonstrated in table 1. The plants were authenticated by Soroush Sardari and the voucher specimens were deposited in the herbarium of Pasteur Institute of Iran, Tehran.

Extract preparation

Air-dried parts (100 g) of each plant were grounded and extracted at the room temperature for 48 hrs, by percolation method using 80% ethanol (900 ml). The extracts were then concentrated by a rotary evaporator apparatus at temperature not exceeding 50°C. The yields of the extracts are demonstrated in table 1. The extracts were stored at 4°C throughout experiments.

Fractionation

The crude extract of *A. obtusifolius* was suspended in 200 ml of distilled water and extracted with dichloromethane (3×150 ml). The aqueous (f1) and dichloromethane (f2) fractions were collected separately, dried by rotary evaporator at 40 °C and stored at 4°C throughout experiments (Fig. 1).

PTZ-induced seizures

The minimal i.p. dose of PTZ at which 99% of the animals showed general clonus was determined by a dose-percent effect curve. General clonus was considered as the criteria of clonic seizure, which was characterized by clonus of four limbs with transient loss of righting reflex (8). The extracts, fractions and

Table 1. Botanical data of the plants.

Scientific name	Voucher No.	Place and date of collection	Parts of the plant extracted	Yield of the extract (W/W)
A. juliflora	74-41	Around Behbahan to Ramhormoz road, May 2008	Leaves	25%
A. nubica	74-32	Around Behbahan to Ramhormoz road, May 2008	Leaves	15%
A. obtusifolius	74-18	Around Behbahan to Ramhormoz road, May 2008	Aerial parts	25%
A. julibrissin	74-85	Garden of Pasteur Institute of Iran, May 2008	Leaves and flowers	30%

Table 2. Effect of intraperitoneal injection of hydroalcoholic extracts of *A. julibrissin, A. juliflora, A. nubica and A. obtusifolius* and fractions on clonic seizures induced by pentylenetetrazole in mice.

Treatment	Dose	Incidence of clonic seizures (%)	Latency to occurrence of clonic seizures (sec)
Control 1	10 ml/kg	100	176.2 ± 40.3
Control 2	10 ml/kg	90	180.2 ± 20.3
Ethosuximide	150 mg/kg	0***	-
A. julibrissin	0.5 g/kg	70	375.7 ± 69.3*
A. juliflora	0.1 g/kg	90	213.3 ± 36.2
A. nubica	0.1 g/kg	100	220.3 ± 33.1
A. nubica	0.4 g/kg	80	165.2 ± 22.0
A. nubica	1 g/kg	90	220.0 ± 28.3
A. nubica	3 g/kg	70	310.0 ± 39.2*
A. nubica	6 g/kg	60	580.0 ± 82.9**
A. obtusifolius	2 g/kg	90	627.3 ± 122.7**
A. obtusifolius	3 g/kg	50*	638.7 ± 201.1*
A. obtusifolius	4 g/kg	20***	666.0 ± 366.0
f1	1 g/kg	80	293.5 ± 65.2
f1	3 g/kg	60	270.8 ± 52.3
f1	4 g/kg	20***	559.0 ± 113.0*
f2	1 g/kg	70	513.5 ± 121.3*

n=10, *P<0.05, **P<0.01 and ***P<0.001 compared to control value. Control 1: Saline, solvent of Ethosuximide; Control 2: Tween 80 (25%): DMSO (2:1, v/v), solvent of the extracts and fractions. f1: Aqueous fraction of *A. Obtusifolius* hydroalcoholic extract, f2: dichloromethane fraction of *A. Obtusifolius* hydroalcoholic extract.

the solvent of the extract [Tween 80 (25%): DMSO (2:1, v/v) 10 ml/kg], as control, ethosuximide (150 mg/kg), as positive control and saline (10 ml/kg), as control were injected to groups of 10 mice. After 30 min, PTZ (60 mg/kg) was injected to animals. If no general clonus occurred during a 30-min period of observation, the animals were considered protected.

MES-induced seizure

Electro-convulsive shock, inducing Hind Limb Tonic Extension (HLTE) in 99% of the animals (8) was determined by a current intensity-percent effect curve. The electrical stimulus (50 mA; 50 Hz; 1sec duration) was applied through ear-clip electrodes using a stimulator apparatus (MGH-777, Development of Electronic Industry, Iran). Different groups of 10 mice were pretreated i.p. with the extracts, the solvent of the extracts [Tween 80 (25%): DMSO (2:1, v/v) 10 ml/kg, as control], phenytoin sodium (25 mg/kg), as positive control and saline (10 ml/kg), as control. After 30 min the animals received transauricular electroshock. If no HLTE was observed within 10 sec after delivery of the electroshock, the animals were considered protected.

Acute toxicity

Groups of 10 mice each were treated i.p. with the solvent of the extracts, different doses of the extracts or the fractions. The mortality rate was recorded after 24 hrs.

Preliminary phytochemical screening

The crude extract of *A. obtusifolius* and its fractions, f1 and f2, were screened for the presence of triterpens/sterols, alkaloids, flavonoids, anthraquinones, anthrones, coumarines, valepotriates, essential oils and tannins by thin layer chromatography using silica gel G (Merck) plates of 0.25 mm thickness. The extract and the fractions were dissolved in Tween 80 (25%): DMSO (2:1v/v). Development

Table 3. Effects of intraperitoneal injection of *A. julibrissin, A. juliflora, A. nubica and A. obtusifolius* hydroalcoholic extracts on tonic seizures induced by Maximal electroshock in mice.

Treatment	Dose	Incidence of tonic seizures (%)
Control 1	10 ml/kg	100
Control 2	10 ml/kg	90
Phenytoin	150 mg/kg	0***
A. julibrissin	0.5 g/kg	70
A. nubica	6 g/kg	80
A. obtusifolius	4 g/kg	100

n=10, ***P<0.001 compared to control value. Control 1: Saline, solvent of Phenytoin; Control 2: Tween 80(25%): DMSO (2:1, v/v), solvent of the extracts.

Table 5. Components of the hydroalcoholic extract and the fractions of the leaves of *A. obtusifolius.*

Components	Hydroalcoholic extract	f1	f2
Triterpens/sterols	+	-	+
Alkaloids	+++	++	+
Flavonoides	+	+	-
Saponins	+	+	-
Anthrones	++	+	+
Anthraquinones	-	-	-
Coumarines	-	-	-
Valepotriates	-	-	-
Essential oil	-	-	-
Tannin	-	-	-

+: positive; -: negative, f1: Aqueous fraction, f2: dichloromethane fraction

was carried out with ethyl acetate: methanol: water (100: 13.5: 10 v/v/v) and ethyl acetate: toluene (93: 7). After development, the plates were sprayed with the following reagents for detection of the respective classes of compounds: antimony trichloride (triterpens/sterols), Dragendorrf's reagent (alkaloids), potassium hydroxide (anthraquinones, anthrones and coumarins), hydrochloric acid and glacial acetic acid mixture (valpotriates), Vanillin and sulfuric acid mixture (essential oil), ferric chloride (tannins). Reagents were prepared according to Stahl method (7). Detection was carried out visually in visible light and under UV light (λ=365 nm).

Data analysis

The dose of the extract required to produce an anticonvulsant effect (ED50) or death (LD50) in 50% of the animals was calculated (8) using a commercial computer program (GRAPHPAD INSTAT 3, version 2003). Data obtained from convulsive tests were expressed as the percentage of the animals showing convulsions and Fisher's exact test was used to analyze the data. *P* value less than 0.05 was the critical criterion for statistical significance.

Table 4. Acute toxicity of intraperitoneal injection *A. julibrissin, A. juliflora, A. nubica and A. obtusifolius* hydroalcoholic extracts and fractions in mice.

Treatment	dose	Incidence of mortality %
Control	10 ml/kg	0
A. julibrissin	1 g/kg	90***
A. juliflora	0.1 g/kg	0
A. juliflora	0.2 g/kg	100***
A. nubica	6 g/kg	20
A. obtusifolius	4 g/kg	10
A. obtusifolius	5 g/kg	10
A. obtusifolius	6 g/kg	60*
f1	4 g/kg	0
f1	6 g/kg	70**
f2	1 g/kg	0
f2	3 g/kg	70**

n=10. *P<0.05, **P<0.01 and ***P<0.001 compared to control value. Control: Tween 80 (25%): DMSO (2:1, v/v), solvent of the extract and the fractions. f1: aqueous fraction of *A. Obtusifolius* hydroalcoholic extract, f2: dichloromethane fraction of *A. Obtusifolius* hydroalcoholic extract.

RESULTS AND DISCUSSION

Among the extracts examined, only the extract of *A. obtusifolius* inhibited clonic seizures induced by PTZ and its protective value (ED50) was 3.97 g/kg (Tables 2 and 3). Fractionation of the extract led to increase in anticonvulsant potency and ED50 value for the aqueous phase was 2.86 g/kg (Table 2). Fractionation also resulted in decrease in toxicity of the extract as the LD50 value increased from 4.95 g/kg for the crude extract to 5.4 g/kg for the aqueous fraction (Table 4).

Prevention of seizures induced by PTZ and MES in laboratory animals is the most commonly used preliminary screening test to characterize potential anticonvulsant drugs. The MES test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures whereas the PTZ test represents a valid model for human generalized myoclonic and also absence seizures (8). Fabaceae, which is the third largest family of flowering plants, has been widely studied in search for safe and effective antiepileptic medicines (4-6). In line with these investigations, acceptable Therapeutic Index (TI= LD50/ED50) of 1.9 which was obtained for the aqueous fraction of *A. obtusifolius* in this study, might make it worthy for further studies.

The phytochemical tests revealed the presence of triterpens/sterols, alkaloids, flavonoides, anthrones, and saponins in the crude extract of *A. obtusifolius*

(Table 5). By fractionation, the entire flavonoids and saponins as well as the majority of alkaloids were transferred from the extract to the aqueous fraction while entire of terpen/sterols and negligible amount of alkaloids were transferred to the dichloromethane fraction. Anthrones were equally distributed in both fractions. It seems that alkaloids, flavonoids and saponins present in the extract and the aqueous fraction might be mainly responsible for the observed anticonvulsant activity. In agreement with this suggestion the anticonvulsant activity of flavonoids (9), saponins (10), and alkaloids (11) in experimental animals have been demonstrated. Further activity-guided fractionation and analytical studies are required to explore the anticonvulsant agents present in the plant.

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