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Anticonvulsant activities of *Sambucus nigra*

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Abstract. – OBJECTIVE: ***Sambucus (S)*** **spp. is reported to possess a variety of activities and has been used in traditional medicine for many years. In spite of CNS activity of this genus, nothing is known about the anticonvulsant activity of *S. nigra*.**

MATERIALS AND METHODS: Anticonvulsant activities of methanolic extracts of bark, fruit and leaf of *S. nigra* at doses of 250, 500 and 1000 mg kg^{-1} were determined by pentylenetetrazole (PTZ) induced and maximal electroshock (MES) induced convulsions in mice.

RESULTS: Normal saline treated mice showed tonic hind limb extension for a duration of 6.58 ± 1.24 s in MES model. Administration of extracts significantly and dose-dependently increased the delay of the onset of seizures and decreased significantly the duration of tonic hind limb extension. Bark extract at 500 and leaf extract at 1000 mg kg^{-1} gave 100% protection against seizures. They inhibited induction of convulsion and gave 100% protections against mortality. PTZ (100 mg kg^{-1}) induced tonic seizures in all of the control mice. Pretreatment with leaf extract at 500 and 1000 mg kg^{-1} significantly decreased duration of tonic hind limb extension ($p < 0.05$ and $p < 0.001$).

CONCLUSIONS: GABA receptors were involved in epilepsy. Reduction of mortality and increase the onset of convulsion in MES model was comparable with that of diazepam. Extracts might possibly be producing an antiepileptic action by increasing the level of GABA.

Key Words:
Anticonvulsant activity, *S. nigra,* Pentylenetetrazole, Maximal electroshock.

Introduction

Epilepsy is a chronic disease of the CNS and fifty million people are suffering from epilepsy worldwide[1]. Eighty percent of patients are living in the developing countries, where 3/4 of the patients are not receiving adequate treatment. Herbal plants are valuable sources of biologically active constituents. Because of some adverse effects and ineffectiveness of chemical drugs, there is an increasing acceptance of the herbal medicine as alternative medicine. Studies have shown that several medicinal plants have been documented for the treatment of CNS problems. These plants could serve as sources of readily accessible, inexpensive and effective medication and thus would be helpful in improving the present status[2].

Genus of *Sambucus* has eighteen species. Of these, *S. nigra* extensively growth in the forest margins on Northern coast of Caspian Sea[3]. *S. nigra* is a good source of anthocyanins, vitamins, calcium and iron. It also contains tannins, sterols and essential oils[4]. In the traditional medicine, *S. nigra* has been used for their diaphoretic, laxative and diuretic properties and used for treatment of various illnesses such as stomach ache, sinus congestion, diarrhea, sore throat, common cold, and rheumatism[5]. The flowers are said to have diaphoretic, anti-catarrhal, expectorant, stimulant, diuretic, and topical anti-inflammatory actions[4]. Leaves and the inner bark have been used for their purgative, emetic, diuretic, laxative, expectorant and diaphoretic actions[4]. Its antioxidant and antidepressant activities have been reported[4,5]. Because of its good antioxidant, nitric oxide scavenging[4] and CNS activities[5], this plant was selected for assay of anticonvulsant activity. The present study was carried out to evaluate anticonvulsant activities of methanolic extracts of *S. nigra* on maximal electroshock (MES) and pentylenetetrazole (PTZ)-induced seizures in mice.

Materials and Methods

Chemicals

Pentylenetetrazole (PTZ) was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Diazepam was purchased from Roche (Johannesburg, South Africa).

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Experimental Animals

The protocol for the study was approved by Animal Ethical Committee of Mazandaran University of Medical Sciences, Iran. Swiss male albino mice (20-25 g, Institute Pasteur of Iran) were used. The animals were housed in standard cages with free access to food and water. The temperature was maintained at 23± 1°C with a 12-h light/12-h dark cycle. Each animal was tested once. All of the experiment conducted between 10:00 and 14:00 h.

Plant Material and Preparation of Freeze-Dried Extract

S. nigra fruit, leaf and bark were collected from the botany garden of Urmia University, in June 2013. The sample was authenticated by Dr. B. Siami and the voucher specimen was deposited (No. 1216) in the Sari School of Pharmacy herbarium. Plant materials were dried under the dark conditions at the room temperature. The dry material was coarsely ground (2-3 mm) and then extracted by methanol for 24 h at the room temperature. The extracts were then separated from the sample residues by filtration. Extraction was repeated thrice. The resulting extracts were concentrated over a rotary vacuum at 35-40 °C until a crude solid extracts were obtained which then were freeze-dried for complete solvent removal.

Anticonvulsant Activity

Pentylenetetrazole Induced Convulsions

PTZ induced convulsions was performed to evaluate anticonvulsant property of extracts. Seventy seven male mice were divided into eleven groups, each group comprised seven mice. Different groups were treated with normal saline (10 ml kg^{-1}), diazepam (4 mg kg^{-1}), and three extracts of *S. nigra* at doses of 250, 500, and 1000 mg kg^{-1} separately. Thirty minutes later, convulsions were induced by the intraperitoneally administration of 100 mg kg^{-1} of PTZ. Following the administration of PTZ, mice were placed in separate transparent Plexiglas cages (25 × 15 × 10 cm) and were observed for the occurrence of seizures over a 30 min time period. The latency of first convulsive episode, duration of tonic convulsions, and percentage of deaths were recorded[6].

Maximal Electroshock (MES) Induced Convulsions

Sixty six male mice were divided into eleven groups, each group comprised six mice. Different groups were treated with normal saline (10 mm kg^{-1}), diazepam (4 mg kg^{-1}), and three extracts of *S. nigra* at doses of 250, 500, and 1000 mg kg^{-1} separately. Thirty minutes later, convulsions were induced in all the groups of animals using electro-convulsometer. A 50 Hz alternating current of 60 mA for 0.2 s was delivered through the ear electrodes[7]. The occurrence and duration of tonic hind limb extension was recorded.

Statistical Analysis

Data were presented as mean ± SD. Analysis of variance (ANOVA) was performed. Duncan's new multiple-range test was used to determine the differences in means. All p values less than 0.05 were regarded as significant.

Results

Maximal electroshock produced hind limb tonic extension in all of animals. Normal saline treated mice showed tonic hind limb extension for duration of 6.58 ± 1.24 s. The onset was 2.90±0.35 s. Administration of extracts (250-1000 mg kg^{-1}) significantly and dose dependently increased in onset of seizures induced by MES and also decreased significantly duration of tonic hind limb extension (Table I). The effects of bark and leaf extracts were especially significant. Bark extract at 500 mg kg^{-1} and leaf at 1000 mg kg^{-1} gave 100% protection against seizures. They inhibited induction of convulsion and gave 100% protections against mortality. Fruit extract at 500 mg kg^{-1} also protected 83.2% of mice from mortality. This extract significantly delayed the onset of seizures ($p < 0.001$) and significantly decreased duration of tonic hind limb extension ($p < 0.001$) respect to control group (Table I). No convulsion and a 100% protection were found in mice treated by diazepam at 4 mg kg^{-1}. PTZ (100 mg kg^{-1}) induced tonic seizures in 100% of control mice. Pretreatment with leaf extract at 500 and 1000 mg kg^{-1} significantly decreased duration of tonic hind limb extension ($p > 0.05$ and $p > 0.001$, respectively). These extracts also delayed the onset of seizures induced by PTZ (27.01 ± 8.36 and 33.33±9.92 s respect to control group, 18.44 ± 6.26 s) but these increases were not statistically significant ($p > 0.05$). Percentage of mortality, also was not decreased. Diazepam showed a significant anticonvulsant activity at 4 mg kg^{-1}. It caused a 22.22% protection in mortality of mice.

Table I. Effect of *S. nigra* extracts on pentylenetetrazole (PTZ) and maximal electroshock (MES) induced convulsions in mice.

Test	Treatment (mg kg^{-1})		Onset of convulsion (sec)	Duration of convulsion (sec)	No of animals convulsed/used	Protection (%)
MES	Bark	250	4.36 ± 0.01***	3.98 ± 0.97***	5/6	16.6
		500	----------	----------	0/6	100
	Leaf	250	3.82 ± 0.46***	4.25 ± 0.92***	5/6	16.6
		500	3.99 ± 0.49***	4.03 ± 0.16***	2/6	66.66
		1000	----------	----------	0/6	100
	Fruit	250	4.13 ± 0.49***	4.25 ± 0.55***	4/6	23.23
		500	5.25 ± 0.21***	4.17 ± 0.35***	1/6	83.23
	Diazepam	4	----------	----------	0/6	100
	Control		2.90 ± 0.35	6.58 ± 1.24	6/6	0
				Time of death (s)	Mortality/used	
PTZ	Bark	250	16.71 ± 4.38	114.0 ± 50.58	7/7	0
		500	25.04 ± 5.34	132.1 ± 55.82	7/7	0
		1000	34.02 ± 18.08	143.3 ± 71.95	7/7	0
	Leaf	250	26.2 ± 3.29	156.0 ± 61.07	7/7	0
		500	27.01 ± 8.36	255.8 ± 122.5**	7/7	0
		1000	33.33 ± 9.92	604.1 ± 282.3***	7/7	0
	Fruit	250	15.71 ± 5.34	44.00 ± 3.69	7/7	0
		500	22.43 ± 3.82	68.01 ± 22.23	7/7	0
		1000	24.80 ± 3.54	97.58 ± 44.25	7/7	0
	Diazepam	4	117.1 ± 27.69***	810.0 ± 127.3***	2/9	22.22
	Control		18.44 ± 6.26	85.67 ± 19.63	9/9	0

Data are expressed as mean ± SD (n = 6 or 7). $^{**}p < 0.01$, $^{***}p < 0.001$ respect to control.

Discussion

Medicinal plants have served as sources of readily accessible, inexpensive, and effective medication. Several ethnomedicinal plants have been found to possess neurobehavioral profile and serve as alternative to modern medicine[2]. The present study was proposed to assess anticonvulsant effects of methanolic extracts of bark, leaf and fruit of a medicinal plant. MES and PTZ as the most popular and widely used animal seizure models were done for assay of anticonvulsant activities. The results of the present study indicate that these extracts possess anticonvulsant activity. The effect of most of antiepileptic agents (such as diazepam) is to enhance the response to $GABA_A$ by facilitating the opening of GABA-activated chloride channels[8]. Benzodiazepines increase the frequency of channel opening events, which leads to an increase in chloride ion conductance and inhibition of the action potential of the receptor results in an increase in $GABA_A$. This may explain the mechanism of action of our tested extract as well, because it is clear that the effect of the extract was similar to diazepam. Norepinephrine (NE) appears to protect against electroshock induced convulsions, whereas 5-HT protects against PTZ-induced convulsions. Thus it can be postulated that CNS depressant action in mice induced by the *S. nigra* might be related to the increased concentration of NE in the mouse brain, too.

MES-induced tonic seizures and seizures induced by PTZ can be prevented either by drugs that inhibit voltage-dependent Na^+ channels or by drugs that block glutaminergic excitation mediated by the N-methyl-D-aspartate receptor[9]. Antiepileptic drugs, such as sodium valproate, which is effective in both types of seizure tests, possess multiple mechanisms of action and display the broadest therapeutic utility.

Conclusions

It seems that *S. nigra* extracts have multiple mechanisms for its anticonvulsant action.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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