

Anticonvulsant activity of *Hypericum scabrum* L.; possible mechanism involved

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Abstract. – OBJECTIVES: *Hypericum* (*H.*) spp. has been used in traditional medicine for their anti-convulsant effect for many years. In spite of many works on this genus, little is known about *H. scabrum*. In this work, anticonvulsant activity of *H. scabrum* was investigated.

MATERIALS AND METHODS: Anticonvulsant activity of aqueous extract was evaluated by pentylenetetrazole (PTZ) induced convulsion and picrotoxin induced convulsion. Also, nitric oxide radical scavenging was investigated as a possible mechanism involved.

RESULTS: Extract (125-500 mg kg⁻¹, i.p.) significantly delayed the onset of PTZ induced convulsion. At 500 mg kg⁻¹, 100% protection against mortality was observed. At this dose, it significantly prolonged the onset of picrotoxin induced convulsion in mice, too. It showed significant nitric oxide radical scavenging activity.

CONCLUSIONS: Mechanism of anticonvulsant activity may be through GABA and/or nitric oxide pathway.

Key Words:

Anticonvulsion, *Hypericum scabrum*, Pentylenetetrazole, Picrotoxin.

Introduction

Despite the technological advancement in modern medicine, many people from all over the world still rely on traditional medicine and medicinal plants for their daily healthcare needs because they are safe¹. *Hypericum* (*Hypericaceae*) genus which contains more than 400 species occurs throughout the world and is well represented in the Mediterranean and the Near East Areas distributed across tropic and subtropic regions, as well as across Europe and Asia². Recently, there has been increasing interest in the genus *Hypericum*, because it is a source of a variety of compounds². Modern studies have been focused on the activity of extracts of these plants against certain viruses and bacteria and on their possible applications as medicines for various diseases³.

Many reports have been published for antimicrobial, antifungal, antiviral, antioxidant and anticonvulsant activities of *Hypericum* species^{4,5}. Previous reports showed that *H. scabrum* L has antimicrobial activity⁶. Also, it used to its sedative effect and has antiseptic, antidiarrhea, anti-hemorrhoid, antieczema, antipsoriasis, anthelmintic and antifungal activities⁷. Essential oil composition, fatty acid composition, antimicrobial and antiulcerogenic activities of *H. scabrum* have been reported⁸. We have recently published good antihypoxic and antidepressant activities of this plant⁹. In continuation of our research program, in order to scientifically evaluation of ethnomedical uses of *H. scabrum*, its anticonvulsant activity in mice was investigated. In addition its nitric oxide scavenging model, as a possible mechanism involved, was evaluated.

Materials and Methods

Chemicals

Pentylenetetrazole and picrotoxin were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride were purchased from Merck (Darmstadt, Germany). Diazepam was purchased from Roche, Johannesburg, South Africa.

Experimental Animals

The protocol for the study was approved by Animal Ethical Committee of Mazandaran University of Medical Sciences, Sari, Iran. Swiss male albino mice, weighing 20-25 g (Institute Pasteur of Iran) were used in this study. The animals were housed in standard cages with free access to food and water. The animal house 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

H. scabrum aerial part was obtained in summer of 2008 from Golestanak area, northern of Iran. Plant material was dried under dark conditions at room temperature. The dry material extracted by water for 24 h at room temperature. The extract was then separated from the sample residue by filtration through Whatman No.1 filter paper. The resulting extracts were concentrated over a rotary vacuum at 45°C until a crude solid extract was obtained which then was freeze-dried for complete solvent removal (yields 29%).

Anticonvulsant Activity

Pentylentetrazole (PTZ)-Induced Convulsion

The method of Swinyard et al¹⁰ was employed to induce convulsion in mice. Fifty male mice were divided into five groups each containing ten mice. The first group received normal saline 10 ml/kg *i.p.*; the second, third and fourth groups received 125, 250 and 500 mg kg⁻¹ *i.p.* of extract; while the fifth group was injected with diazepam 1 mg/kg *i.p.* Thirty minutes after treatment, mice in all the groups received PTZ 100 mg kg⁻¹ *i.p.* Mice were observed over a period of 30 min, hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period were noted.

Picrotoxin-Induced Convulsions

Vellucci and Webster method¹¹ was used to assess the anticonvulsant effect of extract. Mice were kept individually in transparent mice cages (25×15×15 cm) for 60 min to acclimatize to their new environment before the commencement of the experiment. Seizure was induced in picrotoxin (10 mg kg⁻¹, *i.p.*). Animals were observed for convulsion for a period of 30 min. Hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period were noted. Experiments were repeated following the pretreatment of animals either extract or diazepam (0.5 mg kg⁻¹ *i.p.*) or vehicle prior to the administration of picrotoxin. The ability of extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity. Onset and duration of convulsions in the

mice were noted and recorded and protection percentages were determined. Normal saline (3 ml kg⁻¹ *i.p.*) were used as control.

Assay of Nitric Oxide-Scavenging Activity

For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline (PBS), was mixed with different concentrations of extract dissolved in water and incubated at r. t. for 150 min. The same reaction mixture, without the extracts but with an equivalent amount of water, served as control. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm (UV – Visible EZ201, Perkin Elmer, Waltham, MA, USA). Quercetin was used as positive control¹².

Statistical Analysis

Experimental results are expressed as means ± SD. Data were analyzed by one-way ANOVA followed by Tukey-Kramer tests. The percentage of mortality was assessed by Fisher's exact test. Results were considered significant at $p < 0.05$.

Results

PTZ (100 mg kg⁻¹, *i.p.*) elicited tonic convulsion in 100% of the control animals used. Extract (125-500 mg kg⁻¹, *i.p.*) significantly delayed the onset of PTZ induced tonic convulsion. At 500 mg kg⁻¹, 10% protection against convulsion and 100% protection against mortality were observed. Diazepam (1 mg kg⁻¹, *i.p.*) protected 50% of the animals against tonic convulsion and 100% against mortality (Table I). Picrotoxin (10 mg kg⁻¹, *i.p.*) elicited seizures in all mice used in control group. Extract at 500 mg kg⁻¹, *i.p.* significantly prolonged the onset of convulsion in mice. At 500 mg kg⁻¹, 20% protection against seizures and 60% protection against mortality was observed. At 125 and 250 mg kg⁻¹ no effect were observed on convulsion. Diazepam, at 1 mg kg⁻¹ (*i.p.*), significantly prolonged the onset of picrotoxin induced tonic convulsion and significantly decreased the appearance of the convulsion by protecting 60% of the picrotoxin treated animals and 100% protection against mortality (Table I). Extract exhibited potent nitric oxide-scavenging activity between 10 and 160 µg ml⁻¹. The percentage of inhibition was increased with increasing concentration of the extract. IC₅₀ was 57.5 ± 2.3 µg ml⁻¹. Quercetin showed more potent activity than that of extract (17 ± 1.5 µg ml⁻¹).

Table I. Effect of *H. scabrum* aqueous extract on onset of pentylenetetrazole (PTZ) and picrotoxine (PCT)-induced seizures in mice.

Onset of tonic convulsion (mean \pm SD) (second)	Mortality protection	No. of animal death/used	Animals not convulsed	No. of animals convulsed/used	Dose Diazepam	Dose extract	Dose PCT	Dose PTZ
67.5 \pm 3**	20%	8/10	0	10/10	–	125	–	100
96.8 \pm 7.5***	50%	5/10	0	10/10	–	250	–	100
142.5 \pm 6.6***	100%	0/10	10%	9/10	–	500	–	100
54.3 \pm 2.8	0	10/10	0	10/10	–	–	–	100
421.8 \pm 7.9***	100%	0/10	50%	5/10	1	–	–	100
348.1 \pm 15.8*	0	10/10	0	10/10	–	125	10	–
401.5 \pm 31.9***	20%	8/10	0	10/10	–	250	10	–
537.3 \pm 31.3***	60%	4/10	20%	8/10	–	500	10	–
206.9 \pm 20.2	0	10/10	0	10/10	–	–	10	–
582.3 \pm 9***	100%	0/10	60%	4/10	1	–	10	–

Doses are in mg/kg. Each group represents the mean \pm SD (n = 10). ** p < 0.01, *** p < 0.001 vs. control

Discussion

Results show the onset of tonic convulsion produced by PTZ was significantly delayed by *H. scabrum*. According to the report of De Sarro et al¹³ PTZ exerts its convulsant effect by inhibiting the activity of GABA which is implicated in epilepsy. Delay in occurrence of PTZ convulsion by extract improved that interfering with GABA can be regarded as a possible mechanism for its anticonvulsant activity. Extract reduced mortality and increase the onset of convulsion but incidence of seizures did not changed as compared to diazepam (Table I). According to Amabeoku et al¹⁴ picrotoxin exerts its convulsant effect by blocking the GABA_A receptor-linked chloride ion channel which normally opens to allow increased chloride ion conductance into the brain cells following the activation of GABA_A receptors by GABA. Our results showed picrotoxin induced convulsion in mice, diazepam and *H. scabrum* but not phenytoin could attenuated the convulsion. It seems *H. scabrum* attenuated picrotoxin convulsion by enhancing GABA neurotransmission. This further supports the hypothesis that *H. scabrum* may be affecting GABAergic mechanism to exert its anticonvulsant activity.

NO has been associated with a variety of physiologic processes in the human body since it was identified as a novel signal molecule. It transmits signals from vascular endothelial cells to vascular smooth muscle cells and plays an important role in vital physiologic functions many systems. In the nervous system, NO works as an atypical

neural modulator that is involved in neurotransmitter release, neuronal excitability, and learning and memory¹⁵. The scavenging of NO is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of NO compete with oxygen, leading to reduced production of nitrite ions. Extract exhibited potent nitric oxide-scavenging activity between 10 and 160 $\mu\text{g ml}^{-1}$. There are some evidences that strongly suggest involvement of NO signaling pathway in CNS disorders¹⁶. Anticonvulsant activity of this plant may be partially mediated by NO pathway.

Conclusions

Results suggest *H. scabrum* aqueous extract has very good anticonvulsant activity and thus, lend pharmacological justification to the use of the plant extract by traditional medicine practitioners in the treatment of epilepsy. GABAergic pathway and/or NO pathway are proposed for its effect. It is promising for further pharmacological and biochemical experiments, which will be focused on evaluating other activities.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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