

Research Article

Anticonvulsant Activity of Extracts of *Plectranthus barbatus* Leaves in Mice

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Plectranthus barbatus is a medicinal plant used to treat a wide range of disorders including seizure. However, the anticonvulsant activity of this plant has not been studied in depth. We therefore sought to evaluate the anticonvulsant activity of a hydroalcoholic extract of *P. barbatus* leaves on seizures induced by strychnine sulphate (2.0 mg/kg) and pilocarpine (600 mg/kg) in mice. The extract was administered orally at 1, 10, 30, and 100 mg/kg. We report that the *P. barbatus* extract had marked anticonvulsant activity against strychnine-induced convulsions, but was quite ineffective against pilocarpine-induced convulsions. Further experiments will be required to identify the active molecules(s) and their mechanism(s) of action.

1. Introduction

Plectranthus barbatus Andr. is a perennial shrub that is thought to have originated in Africa and is used as a medicinal plant to treat a wide range of disorders. The plant is also known by the synonyms *Plectranthus forskohlii* Briq., *Plectranthus forskalaei* Willd., *Plectranthus kilimandschari* (Gürke) H.L. Maass., *Plectranthus grandis* (Cramer) R.H. Willemse, *Coleus forskohlii* Briq., *Coleus kilimandschari* Gürke ex Engl., *Coleus coerulescens* Gürke, and *Coleus barbatus* (Andr.) Benth [1]. The medicinal uses of *P. barbatus* include digestive, respiratory, circulatory, nervous disorders [1] and infections [2] and has been reported to have abortifacient, contraceptive [3], and antipain [4] properties. The plant is also used to treat gastritis and intestinal spasms [5], nausea [6], stomach ache, and as a purgative [7]. Uses in respiratory disorders include the relief of colds [8], cough, [4] and bronchitis [9], and in the circulatory system uses include myalgia, angina, and hypertension [4].

P. barbatus is also used for the treatment of epilepsy and convulsions [10]; however, only limited data are available concerning the anticonvulsant activity of this plant. The present work was undertaken to evaluate the anticonvulsant activity of an extract of *Plectranthus barbatus* leaves.

2. Methods

2.1. Plant Extract Preparation. Fresh leaves of cultivated *Plectranthus barbatus* were obtained from the Medicinal and Toxic Plants Garden, Sector of Seedlings Production, Universidade Federal Rural do Semi-Árido—UFERSA, Mossoró, RN, Brazil. A reference specimen is deposited in the Prisco Bezerra Herbarium at the Universidade Federal do Ceará, Fortaleza, CE, Brazil, under number 24408. Leaves were air-dried at 35°C, pulverized, and the powder was extracted with ethanol:water (20:80), filtered, and dried by evaporation. The dry extract was dissolved in distilled water at concentration of 50 mg dried *P. barbatus* solids per ml.

2.2. Animals. Swiss albino male mice, weighing between 25 to 30 g, two months old, were kept under controlled conditions (21–25°C on a 12 h:12 h light:dark cycle). Animals were maintained on a standard mouse diet; before the experiments they were fasted overnight with water *ad libitum*.

Ethical procedures were based on the Brazilian law 6638 (May 8, 1979) “Normas para Prática Didático-Científica da Viviseção de Animais” and “Ethical Principles for

TABLE 1: Anticonvulsant effect of *Plecthranthus barbatus* leaf extracts on strychnine-induced seizures in mice.

Dose (mg/kg) p.o.	Time to seizure onset (s)	Death latency (s)	Death rate (%)
0 (control)	217 ± 23.9	282.0 ± 46.7	100
1	461.3 ± 68.1*	518.8 ± 48.6*	100
10	236.4 ± 51.3	353.0 ± 46.7	100
30	317.4 ± 45.4	452.3 ± 52.6	87.5
100	no seizures	no seizures	0

* $P < .05$, compared to control (ANOVA followed by Dunnett's multiple comparisons test).

TABLE 2: Anticonvulsant effects of *Plecthranthus barbatus* extract on pilocarpine-induced seizures in mice.

Dose (mg/kg) p.o.	Time to seizure onset (s)	Death latency (s)	Death rate (%)
0 (control)	117.1 ± 14.8	194.7 ± 19.9	100
1	300.9 ± 53.3*	364.5 ± 59.4*	100
10	373.9 ± 18.7*	468.1 ± 39.6*	100
30	276.4 ± 20.6*	317.1 ± 26.9	100
100	261.0 ± 29.1*	334.0 ± 25.2*	100

* $P < .05$, significant difference compared to control (ANOVA followed by Dunnett's multiple comparisons test).

Use of Experimental Animals” from Colégio Brasileiro de Experimentação Animal (COBEA), Brazil, which are in accordance with the “European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” (Strasbourg, March 18, 1986).

2.3. Evaluation of Anticonvulsant Activity. For each seizure-inducing drug tested, 40 mice were divided into five groups of eight animals; *P. barbatus* extract was administered by oral gavage at doses of 0, 1, 10, 30, and 100 mg/kg. Thirty minutes after administration, each mouse was injected intraperitoneally with strychnine (2.0 mg/kg) or with pilocarpine (600 mg/kg). The doses of used drugs were defined in a pilot study. The latency to first convulsion and the latency and percentage mortality were recorded for a period of 30 min. Animals surviving more than 30 min were considered to be protected.

2.4. Statistical Analysis. Results are expressed as means ± standard error of the mean (SEM). Comparisons between the averages of series of values were performed by ANOVA followed by Dunnett's multiple comparisons test. Data analysis employed Graphpad INSTAT version 2.0 software; statistical significance was set at $P < .05$.

3. Results

P. barbatus extract at doses of 1 and 100 mg/kg significantly ($P < .05$) delayed the onset of strychnine-induced seizures (Table 1). At a dose of 1 mg/kg, there was a significant ($P < .05$) increase in the latency to death, but the extract failed to protect the animals against drug-induced death. At a dose of 10 mg/kg, there was no significant difference in time before onset of seizure or death latency. However, *P. barbatus* extract at dose of 30 mg/kg protected 12.5% (1/8) of treated animals; although time to seizure onset and latency to death were increased by extract administration the difference failed to achieve statistical significance ($P > .05$). At 100 mg/kg,

all treated animals (8/8) were now protected against drug-induced death ($P < .0001$).

For pilocarpine-induced seizures, all *P. barbatus* extract doses (1, 10, 30, and 100 mg/kg) significantly ($P < .05$) delayed seizure onset, and doses of 1, 10, and 100 mg/kg significantly ($P < .05$) increased the latency to death. However, no significant retardation of death latency at a dose of 30 mg/kg was seen compared to the control group, and no dose protected animals from convulsions or death (Table 2).

4. Discussion

We report that a hydroalcoholic extract of leaves of *Plecthranthus barbatus* exerts anticonvulsant effects against both strychnine- and pilocarpine-induced seizures. All tested doses of *P. barbatus* extract retarded pilocarpine-induced seizures but failed to protect against death. By contrast, no seizure activity and no death were observed in strychnine-treated animals pretreated with the highest tested dose of *P. barbatus* extract (100 mg/kg).

The mechanism underlying strychnine-induced seizures is thought to involve direct antagonism of strychnine-sensitive glycine receptors not only in higher brain areas but also in the spinal cord and brainstem, thereby abrogating spinal reflexes and causing motor disturbance, increased muscle tone, hyperactivity of sensory, visual and acoustic perception, tonic convulsions, and death through respiratory or spinal paralysis or by cardiac arrest [11]. Our results demonstrate that strychnine-induced seizures are partly suppressed by *P. barbatus* treatment.

Pilocarpine is a nonspecific muscarinic acetylcholine receptor agonist; it has been suggested that cerebral structures with a high density of muscarinic receptors are likely to represent the sites of origin of acute pilocarpine seizures [12]. Pilocarpine administration to rodents leads to repetitive limbic seizures and status epilepticus, replicating several features of human temporal lobe epilepsy [13]. It was previously suggested that extracts of *P. amboinicus* might

antagonize cholinergic action at muscarinic receptors [14]. On the other hand, *P. barbatus* extracts were previously reported to inhibit acetylcholinesterase activity [15]. In the present work, the administration of *P. barbatus* extracts failed to inhibit pilocarpine-induced seizures, even though it retarded the onset of the seizures. Thus, it appears likely that *P. barbatus* extract presented slight antagonist action in the muscarinic acetylcholine receptors.

Phytochemical studies on *P. barbatus* have revealed numerous bioactive compounds: notably diterpenoids including forskolin [16], plectranthone J, plectrin, (16S)-coleon E, coleon F, (16R)-plectrinone A, plectrinone B [17], cyclobarbatusin, barbatusin, and 7 β -acetyl-12-desacetoxycyclobarbatusin [18]. Of these, forskolin is a promising candidate for the anticonvulsant activity of *P. barbatus* because this molecule was shown to suppress seizures induced by pentylentetrazol [19]. Forskolin activates adenylate cyclase and increases intracellular cAMP levels. It was previously reported that forskolin can elicit membrane depolarization in medium spiny neurons of the mouse nucleus accumbens; this was mediated by cAMP stimulation of inward rectifier K⁺ currents [20]. Forskolin increases the amplitude of glycinergic miniature inhibitory postsynaptic currents in the presence of low concentrations of extracellular glycine in isolated rat substantia gelatinosa neurons [21].

In addition to direct effects on ion channels, forskolin treatment is likely to exert neuroprotective effects by increasing cellular levels of neurotrophin-3 (NT-3) [22], a member of the neurotrophin family of neurotrophic factors that support cell survival, axonal growth, and neuronal plasticity [23, 24]. Brain injury decreases NT-3 synthesis in the hippocampus [25], whereas NT-3 supplementation can protect neurons against excitotoxic insult [26]. Seizures induced by kainic acid [27] or pilocarpine [28] injection decreased NT-3 expression in the brain of mice, whereas intraventricular infusion of NT-3 in rats inhibited kindling epileptogenesis, mossy fiber sprouting, and increases in hilar area [29], arguing that NT-3 upregulation by forskolin is likely to inhibit seizure development and seizure-related synaptic reorganization.

5. Conclusion

In conclusion, *P. barbatus* extract was found to have marked anticonvulsant activity against strychnine-induced convulsions, but was quite ineffective against pilocarpine-induced convulsions. Although forskolin is a promising candidate molecule for the anticonvulsant activity of *P. barbatus* extracts, it is possible that the bioactivity is mediated by a combination of two or more molecules. Further experiments will be required to identify the active molecule(s) and their mechanism(s) of action.

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