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Iso-6-spectaline effects on convulsions induced in epilepsy models

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ABSTRACT

The central nervous system (CNS) and anticonvulsant activities of iso-6-spectaline (SPEC) from *Senna spectabilis* were investigated in animal models. The SPEC from *Senna spectabilis* var. *excelsa* (Schrad) (0.1, 0.5 and 1.0 mg/ kg) injected by oral route (p.o.) in mice caused a significant decrease in the motor activity up to 24 h after the administration and in the dose of 1.0 mg/kg significantly reduced the remaining time on the Rota-rod apparatus. Additionally, SPEC (0.1, 0.5 and 1.0 mg/kg, p.o.) was also capable of promoting increase of latency for development of convulsions induced by pentylenetetrazole. This SPEC was also capable of promoting an increase of latency for development of convulsions induced by picrotoxin (PIC) only at highest dose. In the same way, the anticonvulsant effect of SPEC was affected by pretreatment with flumazenil, a selective antagonist of the benzodiazepine site of the $GABA_A$ receptor. These results suggest possible anticonvulsant activities in mice that needs further investigation.

Key words: Fabaceae, open field, pentylenetetrazole, picrotoxin, *senna spectabilis*

INTRODUCTION

Several herbal medicines are recognized as active in the central nervous system (CNS), and they have at least a hypothetical potential to affect neurodegenerative conditions including epilepsy, that do not respond well to conventional treatments. Thus, iso-6-spectaline (SPEC) may possess a neuromodulatory role in the treatment of seizures, since this piperidine alkaloid compound can interrupt cellular oxidative processes and monoaminergic system changes in the hippocampus and striatum. The effects of SPEC on these cerebral areas have not yet been determined, therefore, would be important to conduct these studies to clarify its brain action mechanism.

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Piperidine alkaloids are abundant in nature and many of them are known to exhibit some biological activity. In our search for potential anxiolytic, antidepressant or anticonvulsant agents employing a mechanism-based yeast bioassay for CNS-modifying agents,[1]

we have isolated iso-6-spectaline (SPEC; 14-[(2R,3R,6R) -3-hydroxy -2-methylpiperidine]-tetradecan-13-one), piperidine alkaloids, is a heterocyclic organic not aromatic compound [Figure 1] found in many plant species. It was isolated for the first time from *Cassia sp.*, species previously known as *Cassia excelsa*, hence the term cassine.[2] Piperidine alkaloid derivatives with CNS effects include SPEC which exerts neuroprotective effects against depression model.[2]

Senna spectabilis is used as anti-inflammatory, analgesic, laxative, purgative, antimicrobial and antiulcerogenic.[3-5] Studies have shown that the extract of Senna sp. inhibits excessive production of free radicals, and the imbalance between the concentrations of these and the antioxidant defenses may be related to the pathogenesis of seizures.[6-8]

The genus *Cassia* possesses about 600 species distributed worldwide, being well known due to its diverse biological and pharmacological properties.[4] *Senna spectabilis* (DC) Irwin and Barneby var. spectabilis *(Cassia spectabilis* DC) is widely grown as an ornamental plant in tropical and subtropical areas, and has been commonly used in traditional medicine for many years. It has also been used in traditional Brazilian medicine for the treatment of flu and cold, as a laxative and purgative.[9,10]

Previous studies about behavioral screening realized with the SPEC demonstrates that it produces antioxidant effects in vitro and reduces lipid peroxidation in hippocampus of adult mice after pilocarpine-induced seizures, increasing survival rate and reducing number of seizures in mice. Additionally, there is no work demonstrating SPEC effects in neurodegenerative diseases in animal on epilepsy models.

MATERIALS AND METHODS

Plant material and chemistry study

The plant was collected in September 2003, at Boa Viagem, State of Ceará, Brazil, and was identified by Prof. A.G. Fernandes, in the Department of Biology of the Federal University of Ceará. The voucher specimen is deposited at the Prisco Bezerra Herbarium under the voucher number 33013.

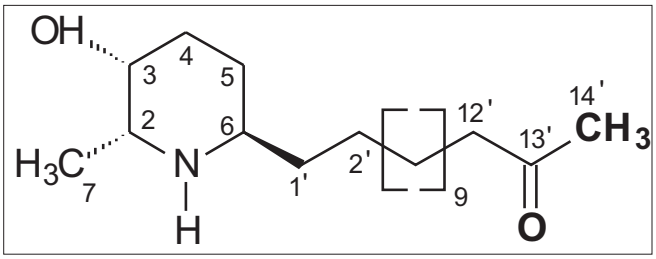

Figure 1: Chemical structure of iso-6-spectaline (SPEC; 14-[(2R,3R,6R)-3-hydroxy-2-methylpiperidine]-tetradecan-13-one)

The botanical material, leaves (3.0 kg), stem (10.2 kg) and roots (7.6 kg), were triturated and exhaustingly extracted with ethanol and concentrated in rotative evaporator, producing 78 g, 55 g and 84 g respectively. The leaf extract (78 g) was then submitted to technical of selective extraction of alkaloids a fraction rich in alkaloids (FA) and a non alkaloids (FNA) were obtained. The alkaloid fraction was submitted to chromatography on SEPHADEX, with methanol as movable phase. The dichloromethane fraction (15.2 g) was submitted to the same chromatography process.

The analysis of the fractions was made in chromatography in thin layer (CCD), which revealed the purity of the rich fraction in SPEC (30 mg) which when subjected to the test with the reagent Dragendoff revealed an orange stain, and was thus positive for alkaloids.[11] Its spectra of NMR RMN^{1}H, RMN^{13}C, DEPT 135, COSY, HMBC e HSQC[1] were obtained and compared with the data from the literature for identification. The iso-6-spectaline is an amorphous white solid with M.P. 130.8-132.3ºC; the value of TLC in MeOH/EtOAc (1:1), Rf = 0.58; NMR spectra description of ^{1}H is ^{1}H NMR (MeOD, 500 MHz) δ_H 3.83 (1H, H-3); 3.30 (1H, H-6); 3.23 (1H, H-2); 2.47 (2H, H-12'); 2.15 (3H, H-14'); 1.44 (3H, H-7); 1.29-1.33 (12H, H-4' - H-9'); NMR spectra description of ^{13}C is ^{13}C NMR (MeOD, 125 MHz) δ_C – 212.4 (C, C-13'); 66.1 (CH, C-3); 58.8 (CH, C-2); 57.7 (CH, C-6); 44.4 (CH_2, C-12'); 34.9 (CH_2, C-1'); 31.2 (CH_2, C-4); 29.9 (CH_3, C-14'); 30.9 (CH_2, C-3'); 30.5 (CH_2, C-10'); 30.5-30.9 (CH_2, C-4'-C-9'); 26.4 (CH_2, C-2'); 23.8 (CH_2, C-5); 23.8 (CH_2, C-11'); 16.1 (CH_3, C-7). In the present work, the iso-6-spectaline was suspended in 0.5% Tween 80 distilled in water, and sonicated before use. Agents were administrated orally (p.o.) and intraperitoneally (i.p.) at a dose volume of 0.1 ml/10 g.

Animals

Male Swiss mice (25-30 g), two months of age were used. The animals were randomly housed in appropriate cages at 23 ± 2°C on a 12-h light/dark cycle (lights on 08:00 a.m. – 18:00 p.m.) with free access to food (Purina®) and water. All experiments were carried out between 08:00 a.m. and 18:00 p.m. in a quiet room. Experimental protocols and procedures were approved by the Ethics Committee on Animal Experiments at the Federal University of Piaui (CEEA/UFPI # 44/09).