Activity of a traditional South African epilepsy remedy in the GABA-benzodiazepine receptor assay

Anna K. Jägera,∗ Seemole P. Mohoto b, Fanie R. van Heerden c, Alvaro M. Viljoen b

a Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, 2 Universitetsparken, 2100 Copenhagen O, Denmark
b Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa
c Department of Chemistry and Biochemistry, Rand Afrikaans University, P.O. Box 524, Auckland Park 2006, South Africa

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Abstract
Aqueous and ethanol extracts of six plants, Acrotome inflata, Aptosimum indivisum, Asparagus suaveolens, Barleria bolusii, Commiphora marlothii and Sesamum triphyllum, which constitute an ancient Northern Sotho remedy for epilepsy, Sehlare sa Seebana, was tested in the GABA A-benzodiazepine receptor binding assay. Both aqueous and ethanol extracts of Aptosimum indivisum and Asparagus suaveolens and the aqueous extract of Commiphora marlothii showed good dose-dependent activity. The ethanol extract of all six plants extracted together was more active than the aqueous extract. The results did not suggest a synergistic effect of the plant mixture.

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1. Introduction
Traditional health care is utilized by the majority of the population in Southern Africa, this is especially true of treatment for mental health problems. This is partly due to a severe lack of facilities for treatment of mental disease in the Western health care system, but also because these diseases in their cultural context are better handled by a traditional healer. In African culture, diseases, mental or otherwise, are the result of disregard for the ancestors, who can inflict an individual. This means that cultural/psychological/spiritual elements nearly always are a part of the treatment.

There are reports in the literature on the use of herbal drugs for the treatment of epilepsy in African traditional medicine (Watt, 1967; Iwu, 1993; van Wyk et al., 1997). As far back as the seventeenth century, a herbal remedy, Sehlare sa Seebana, for treatment of epilepsy was used by the Northern Sotho ancestors of one of the authors (S. Mohoto). The recipe for this herbal remedy has been passed from generation to generation and the remedy is still used to treat family members and other patients. The remedy is administered via inhalation of smoke. Equal parts of the plants are placed in a red-hot clay pot and the patient inhales the smoke. In most cases only a single treatment is required to relieve the patient of his fits.

Epilepsy is a syndrome of different cerebral disorders of the central nervous system and it affects approximately 50 million people worldwide. Epilepsy can be caused by imbalance in the GABAergic system. γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system, where it exerts its physiological effects by binding to three different receptor types in the neuronal membrane: GABA A, GABA B and GABA C receptors. The GABA A receptor, which is involved in epilepsy, has a binding site for compounds such as benzodiazepine...
diazepines, β-carbolines, barbiturates and certain steroids, that allosterically modify the chloride channel gating of GABA. In addition, it has been found that flavones bind with high affinity to the benzodiazepine site of the GABA<sub>A</sub> receptor (Kahnsberg et al., 2002). The pharmacological effects of benzodiazepines (anxiolytic, anticonvulsant, muscle relaxant and sedative-hypnotic) make them the most important GABA<sub>A</sub> modulating drugs (Doble and Martin, 1996).

In the present study, we investigated the ancient Sehlare se Sefram herbal remedy for GABA<sub>B</sub> benzodiazepine receptor activity.

2. Materials and methods

2.1. Plant materials

Aerial parts of Acrotome inflata Benth. (Labiatae) (Voucher no.: SMPP 784), Aptoismum indivisum Burch. Ex. Benth. (Scrophulariaceae) (SMPP 783), Asparagus suaveolens Burch. (Liliaceae) (SMPP 780), Barleria bolusii Oberm. (Acanthaceae) (SMPP 785), Commiphora marlothii Engl. (Burseraceae) (SMPP 781) and Sesamum triphyllum Engl. (Pedaliaceae) (SMPP 782) were collected at Ramongona village, 25 km north west of Polokwane, Limpopo Province, South Africa. Voucher specimens are stored at the Department of Pharmacy and Pharmacology, University of the Witwatersrand. The plant material was dried indoors at ambient temperature.

2.2. Preparations of extracts

Five hundred milligrams of material was extracted with 10 ml of water or ethanol for 60 min on an ultrasound bath, whereafter the extracts were filtered. The ethanol extracts were taken to dryness under reduced pressure and the aqueous extracts freeze dried. For the extracts of the mixture of plants, 100 mg of each plant was mixed and the mixture extracted with 12 ml solvent. The extracts were redissolved at 10 mg/ml in their respective extraction solvents.

2.3. Preparation of rat brain homogenate

Preparations were performed at 0–4°C unless otherwise indicated. Cerebral cortices of rats were removed immediately after decapitation. Tissues were homogenized in 20 volumes of 50 mM Tris-citrate buffer (pH 7.1) by ultra-turraxing immediately after decapitation. Tissues were homogenized in 20 volumes of 50 mM Tris-citrate buffer and incubated at 37°C for 30 min to remove endogenous GABA, followed by centrifugation at 27,000 x g for 10 min. The final pellets were resuspended to yield 2 mg fresh brain tissue/ml buffer and frozen in aliquots at −20°C.

2.4. <sup>3</sup>H-Ro 15-1788 binding assay

The assay was carried out as described by Risa et al. (2003). The membrane preparation was thawed, resuspended in Tris-citrate buffer and centrifuged at 0–4°C for 10 min at 27,000 x g. The pellet was resuspended in Tris-citrate buffer (500 ml buffer per gram of original tissue) and used for binding assays. Five hundred microliters of membrane suspension was added to 25 µl of test solution and 25 µl of <sup>3</sup>H-Ro 15-1788 (flumazenil) (0.5 nM in assay), mixed and incubated at 0–4°C for 40 min. Nonspecific binding was determined using clonazepam (1 µM in assay). After incubation, 5 ml ice-cold Tris-citrate buffer was added to each sample, which was filtered through Advantec GC-50 glass fiber filters under suction, and immediately washed with a further 5 ml buffer. The amount of radioactivity on the filters was determined by scintillation counting. Specific binding was calculated as total binding minus non-specific binding. All experiments were done in triplicate.

3. Results and discussion

The results of the screening of plant extracts in the GABA<sub>B</sub> benzodiazepine binding assay are shown in Fig. 1. Both aqueous and ethanol extracts of Aptoismum indivisum and Asparagus suaveolens and the aqueous extract of Commiphora marlothii showed good dose-dependent activity.

In order to investigate whether there might be beneficial effects of administering the six plants together, aqueous and ethanol extracts of a mixture of the plants were prepared. The ethanol extract of all six plants extracted together was more active that the aqueous extract. The results did not suggest a synergistic effect of the plant mixture.

Traditionally, the remedy is administered by inhaling the smoke from heated plant material. This is a common way of administration in Southern Africa. This raises the question whether active artifacts could be produced by the high temperatures.

Resin of other Commiphora species is used as myrrh. The resin of C. guollitti is also used against convulsions in traditional African medicine (Neuwinger, 2000).

Three metabolites, acteoside (verbascoside), pinocembrin 7-neohesperidoside and shanzhiside methyl ester were isolated from Aptoismum indivisum (Aptoismum indivisum was misidentified as Crotorecapna tarsodes, personal communication) (van Heerden et al., 2002). Barleria bolusii is known to contain acteoside as a major compound (van Heerden et al., 2002), which is reported to bind to the benzodiazepine receptor (Dael-Rakotsourison et al., 2000). It is therefore surprising that the extracts of Barleria bolusii did not show more activity in the assay, when both plants contain acteoside. A 90% methanol extract of the Indian Barleria lupulina was found to have CNS-activity in mice and rats, where it affected the spontaneous activity, sound, pain and touch responses and at higher doses produced depression in...
Fig. 1. Percent binding of Ro 15-1788 (flumazenil) to the GABA_A-benzodiazepine receptor in the presence of various concentrations of (♦) aqueous and ((circle) ethanol extracts of individual plants and a mixture of the plants used traditionally to treat epilepsy.
patterns concerned with alertness and awareness (Suba et al., 2002). The extracts also increased the sleeping time.

The results of this study show that the traditional remedy Sehlare sa Seebana has effect on the GABA_A-benzodiazepine receptor, which rationalizes its use in traditional medicine against epileptic fits.

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References


Doble, A., Martin, J.L., 1996. The GABA_A/Benzodiazepine Receptor as Target for Psychoactive Drugs. Springer-Verlag, Heidelberg, Germany.


