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Extracts of *Feretia apodanthera* Del. demonstrated anticonvulsant activities against seizures induced by chemicals and maximal electroshock

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ABSTRACT

Extracts of *Feretia apodanthera* Del. (Rubiaceae) have been extensively used in traditional Cameroonian medicine to treat a variety of diseases, including some neurological disorders. The present study was aimed to tests the anticonvulsant properties of the aqueous extract and the alkaloid fraction of the stem barks of *Feretia apodanthera*. The anticonvulsant investigation was carried out against bicuculline-, picrotoxin-, pentylenetetrazol-, Methyl-β-carboline-3-carboxylate-, *N*-Methyl-*D*-aspartate-, 4-aminopyridine-, and maximal electroshock-induced seizures or turning behavior in mice. The aqueous extract protected mice against bicuculline-, picrotoxin-, pentylenetetrazol-, Methyl-β-carboline-3-carboxylate-, *N*-methyl-*D*-aspartate –, 4-aminopyridine- and maximal electroshock-induced seizures or turning behavior. Also, *N*-Methyl-*D*-aspartate-, 4-aminopyridine- and maximal electroshock- induced seizures or turning behavior, were significantly antagonized by the alkaloid fraction (80 mg/kg) from *Feretia apodanthera*. The total protection of mice provided by the aqueous extract against convulsions induced by pentylenetetrazol or picrotoxin was anagonized by flumazenil, a specific antagonist of the benzodiazepine site in the $GABA_A$ receptor complex.

The aqueous extract of *Feretia apodanthera* (but not the alkaloid fraction) increased the brain GABA content and inhibited the GABA transaminase activity.

In conclusion, *Feretia apodanthera* was revealed possessing anticonvulsant effects in mice, likely via the GABAergic neurotransmission.

1. Introduction

Epilepsy is the second most common neurological disorder after stroke, affecting at least 50 million persons worldwide (Scheuer and Pedley, 1990). It shows a prevalence rate in 1–2% of the world population (Kamboj et al., 2009). Various anticonvulsant drugs are available to grapple with this neurological disorder. Dose-related neurotoxicity, cognitive impairment, and systemic side effects are the major problems caused by antiepileptic drugs (Reynolds and Trimble, 1985). Despite treatment improvement has occurred with the panel of available antiepileptic drugs, epilepsy remains refractory in one third of patients. Furthermore, it should be mentioned that adverse effects associated with antiepileptic drugs and recurrent seizures limit their use (Maertns et al., 1995). Therefore, the search for new therapeutic agents continues, and medicinal plants have emerged as a crucial source for the development of drugs to treat neurological disorders and play an important role for patients who respond poorly to conventional treatments (Herrera-Ruiz et al., 2006; Carlini, 2003). *Feretia apodanthera* Del. (Rubiaceae) is a medium-sized flowering tree distributed in some countries of Western Africa. The stem barks of *Feretia apodanthera* is being used

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empirically in Cameroonian traditional medicine to treat infantile convulsions, epilepsy, agitations, anxiety, schizophrenia, various forms of pain and headaches (Adjanohoun et al., 1996; Kerharo and Adams, 1974; Dalziel, 1937). In Senegal, the leaves of *Feretia apodanthera* are used to treat different urinary and renal infections. The leaves are also used to treat stomach aches as well as nausea and syphilis (Adjanohoun et al., 1996; Kerharo and Adams, 1974). Phytochemical studies of *Feretia apodanthera* allowed the isolation and identification of seven iridoid glucosides: feretoside, gardenoside, geniposide, desacetyl asperulosidic acid, 11-methyl ixoside, apodanthoside and 10-ethyl apodanthoside (Bailleul et al., 1980, 1977). The first three occur in the flowers. Feretoside and gardenoside were isolated from the root barks of *Feretia apodanthera* (Bailleul et al., 1980). Only few studies pointed to the possible neuropharmacological effects of *Feretia apodanthera*. Stem and root barks extracts of *Feretia apodanthera* decreased the exploratory and spontaneous motor activities in mice, increased hexobarbital sleeping time in mice and protected rabbits against pentylenetetrazol-induced seizures (Bailleul et al., 1981, 1980). The present study was undertaken to explore the antiepileptic effects of both the aqueous extract and the alkaloid fraction from the stem barks of *Feretia apodanthera*, by using chemicals and maximal electroshock induced seizures. Part of the results was published in abstract form (Taiwe et al., 2014).

2. Material and methods

2.1. Plant material

The stem barks of *Feretia apodanthera* used in this study was harvested from the north region of Cameroon in April 2009. The species was authenticated and a voucher was deposited at the National Herbarium, Yaoundé (Num. 31225/HNC).

2.2. Preparation of the aqueous extract and alkaloid extraction

The stem barks were separated and cleaned, then sun-dried and crushed using a mechanical grinder. The powdered material was extracted with distilled water (50 g of powder per 375 ml water) by cold maceration for 24 h, then filtered through Whatman n°. 1 filter paper and freeze-dried (FreeZone® Dry 4.5, USA). This procedure resulted in a yield of 8.62% (w/w).

For fractionation, the dried and powdered stem barks of *Feretia apodanthera* (1000 g) were extracted with acetone/H_2O (7/3; 5 l) at room temperature. The extract was evaporated in vacuo to afford a dark residue (649.17 g). The residue was suspended in warm water (1 l) and then extracted successively with ethyl acetate (0.5 l × 3) and *n*-butanol (0.5 l × 3), and concentrated to give residue A (153.71 g) and B (392.54 g), respectively. The latter was resolved in warm water (1 l), acidified with 1 mol/l HCl to pH between 4 and 5, and extracted with $CHCl_3$ (0.5 l × 3). The aqueous layer was neutralized with 1 mol/l NaOH to pH 9–10 and extracted with $CHCl_3$ (0.5 l × 3) once again and concentrated *in vacuo* to obtain the crude base (alkaloid fraction; 195.18 g) (Taïwe et al., 2012a,b; Taiwe et al., 2014). The freeze-dried extract (aqueous extract) and the alkaloid fraction from the stem barks of *Feretia apodanthera* were dissolved in saline 0.90% containing dimethyl sulfoxyde 2% (vehicle) at the appropriate concentrations as indicated in the various experiments and administered orally in a volume of 10 ml/kg.

2.3. Chemicals

Bicuculline, picrotoxin, pentylenetetrazol, clonazepam, flumazenil, methyl-β-carboline-3-carboxylate, diazepam, glacial acetic acid, *N*-Methyl-*D*-aspartate, *D*-2-amino-7-phosphonoheptanoate, 4-aminopyridine and phenobarbital were obtained from Sigma Chemical, USA. Diazepam was obtained from Roche. All other chemicals and reagents used in the brain gamma-aminobutyric acid (GABA) content estimation and in the determination of brain GABA-T activity are from Sigma Chemical, USA.

2.4. Animals

Swiss albino mice (25–30 g) of either sex were used in this study. The animals were randomly housed in appropriate cages at 22 ± 2 °C on a 12 h light/dark cycle with free access to food and water. In all the experimental studies, each group consisted of six to ten animals. Each animal was used only once. The investigation conforms to the Guide for the Care and Use of Laboratory Animal, according to the ethical guidelines of Cameroon Bioethics Committee (Ref n○ FW-IRB00001954) and the US National Institutes of Health (NIH No. 85-23, revised 1996).

2.5. Pharmacological tests

2.5.1. Bicuculline-induced seizures

Briefly, ten groups of six mice were administered graded doses of the aqueous extract of *Feretia apodanthera* (50, 100, 150 and 200 mg/kg; p.o.), the alkaloid fraction from *Feretia apodanthera* (10, 20, 40 and 80 mg/kg; p.o.), diazepam (positive control; 3 mg/kg, i.p.) or vehicle (10 ml/kg p.o.). One hour later, all animals were injected intraperitonealy with bicuculline (4 mg/kg) and placed in isolated cages. The time to onset of clonic or tonic seizures was recorded. A threshold convulsion lasting for at least 5 s has been considered as an episode of clonic spasms. Absence of this threshold convulsion over 30 min indicated that the animal was protected from the convulsant-induced seizures (Masereel et al., 1998).

2.5.2. Picrotoxin-induced seizures

Mice were divided into twelve groups of six mice each, and received the aqueous extract of *Feretia apodanthera* (50, 100, 150 and 200 mg/kg; p.o.), the aqueous extract + flumazenil (200 mg/kg, p.o. + 10 mg/kg, i.p.), the alkaloid fraction from *Feretia apodanthera* (10, 20, 40 and 80 mg/kg; p.o.), the alkaloid fraction + flumazenil (80 mg/kg, p.o. + 10 mg/kg, i.p.), clonazepam (1 mg/kg, i.p.) or vehicle (10 ml/kg p.o.). One hour later, clonic seizures were induced in mice by intraperitoneal injection of 7.5 mg/kg picrotoxin. Mice were observed for 15 min and the protective effect of the different treatments was recorded. Animals that did not convulse within the 15 min of observation were qualified as protected (Ngo Bum et al., 2001). The time to onset of clonic or tonic seizures was recorded. In the antagonistic experiment, flumazenil was administered 30 min before the test.

2.5.3. Pentylenetetrazol-induced seizures

Twelve groups of six mice were treated as discussed previously. However, the positive control group received 0.1 mg/kg clonazepam i.p. Clonic seizures were induced in mice by the i.p. injection of 70 mg/kg pentylenetetrazol. The protective effect of the different treatments given 1 h before pentylenetetrazol injection was recorded. Animals that did not convulse within the 10 min of observation were qualified as protected (Schmutz et al., 1990; Ngo Bum et al., 2009a). The time to onset of clonic seizures was recorded.

2.5.4. Methyl-β-carboline-3-carboxylate-induced seizures

Ten groups of six mice were administered graded doses of the aqueous extract of *Feretia apodanthera* (50, 100, 150 and 200 mg/kg; p.o.), the alkaloid fraction from *Feretia apodanthera* (10, 20, 40 and 80 mg/kg; p.o.), diazepam (positive control; 4 mg/kg, i.p.) or vehicle (10 ml/kg p.o.). Seizures were induced in mice by the i.p. injection of