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Effects of a lyophilized aqueous extract of *Feretia apodanthera* Del. (Rubiaceae) on pentylenetetrazole-induced kindling, oxidative stress, and cognitive impairment in mice

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ABSTRACT

Feretia apodanthera Del. (Rubiaceae) is extensively used in ethnomedicine in Cameroon and Nigeria for epilepsy, febrile convulsions, and rheumatic pains and for enhancing cognitive performance. The aim of the present study was to examine the effects of a lyophilized aqueous extract of *F. apodanthera* on the course of kindling development, kindling-induced learning deficit, oxidative stress markers, and cholinesterase activity in pentylenetetrazole (PTZ)-kindled mice. Pentylenetetrazole, 30 mg/kg, induced kindling in mice after 30.00 ± 1.67 days. The aqueous extract of *F. apodanthera* showed dose-dependent antiseizure effects. *Feretia apodanthera* (150–200 mg/kg) significantly increased the latency to myoclonic jerks, clonic seizures, and generalized tonic–clonic seizures. The extract also improved the seizure score and decreased the number of myoclonic jerks. Pentylenetetrazole kindling induced significant oxidative stress and cognitive impairment which were reversed by pretreatment with *F. apodanthera* in a dose-dependent manner. The significant decrease in cholinesterase activity observed in the PTZ-kindled mice was reversed by pretreatment with the *F. apodanthera* extract. The results indicated that pretreatment with the aqueous extract of *F. apodanthera* antagonizes seizures, oxidative stress, and cognitive impairment in PTZ-kindled mice. The aqueous extract of *F. apodanthera* also showed anxiolytic activities, but the inhibition of memory impairment was not attributed to the anxiolytic activities of the plant. These results thus suggest the potential of *F. apodanthera* as an adjuvant in epilepsy both to prevent seizures as well as to protect against seizure-induced oxidative stress and memory impairment.

1. Introduction

Epilepsy is the second most common neurological disorder after stroke, affecting at least 50 million persons worldwide [1]. It shows a prevalence rate of 1–2% of the world population [2]. Various anticonvulsant agents are available to grapple with this neurological disorder. Dose-related neurotoxicity, cognitive impairment, and a range of systemic side effects are the major problems caused by antiepileptic drugs [3]. Despite treatment with available antiepileptic drugs, epilepsy remains refractory in one-third of patients. Further, adverse effects associated with antiepileptic drugs and recurrent seizures limit their use. Increasing data from experimental and clinical reports suggest the involvement of oxidative stress in the pathophysiology of epilepsy [4]. Ongoing research on certain plant products has paved the way towards the development of a newer category of antiepileptic drug therapies [5,6]. The stem bark of *Feretia apodanthera* Del. (Rubiaceae) is being used empirically in traditional medicine in Cameroon to treat epilepsy and diseases related to the brain like agitation, anxiety, infantile convulsions, headaches, insomnia, pains, and schizophrenia according to our traditional healers and the literature [7–10]. In Senegal, the leaves of *F. apodanthera* are used to treat different urinary and renal infections. The plant is also used to treat stomach aches, nausea, and syphilis, as a calming agent for agitated mental conditions, and for enhancing cognitive performance [11]. The aim of this study was to evaluate the effects of a lyophilized aqueous extract of *F. apodanthera* on the course of kindling development, kindling-induced learning deficit, and oxidative stress in PTZ-kindled mice. Its effect on brain cholinesterase activity was also evaluated. Part of the results was published in abstract form [12].

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2. Materials and methods

2.1. Plant material

The stem bark of *F. apodanthera* was collected from the North Region of Cameroon in April 2009. The botanical identification of the plant was done by the National Herbarium of Cameroon where the voucher specimen was conserved under the reference number 31225/HNC.

2.2. Preparation of the aqueous extract

The stem bark was separated, cleaned, sun-dried, and pulverized using a mechanical grinder. The powdered material was extracted with distilled water (50 g powder per 375 mL water) by cold maceration for 24 h, filtered through Whatman no. 1 filter paper, and freeze-dried (FreeZone® Dry 4.5, USA). This gave a yield of 8.62% (w/w). The freeze-dried extract was then subsequently reconstituted in distilled water at appropriate concentrations for the various experiments and administered orally in a volume of 10 mL/kg of body weight.

2.3. Preliminary qualitative phytochemical analysis

Preliminary phytochemical analysis of the aqueous extract of *F. apodanthera* was done using the following chemicals and reagents: flavonoids (NaCl and HCl), alkaloids (with Mayer's and Dragendorff's reagents), saponins (frothing test), tannins ($FeCl_3$), glycosides ($NaCl_3$ and Fehling's solutions A and B), cardiac glycosides (Salkowski test), anthraquinones (Borntrager's reaction), phenols ($FeCl_3$ and $K_3Fe(CN)$), and lipids (filter paper) [13].

2.4. Drugs and chemicals

Diazepam (DZP), pentylenetetrazole (PTZ), sodium valproate (SVA), reduced glutathione, thiobarbituric acid, *n*-butanol, pyridine, sodium dodecyl sulfate, 5′5-dithiobis (2-nitrobenzoic acid) (DTNB), trichloroacetic acid, acetylthiocholine iodide, butyrylthiocholine iodide, and all other chemicals and reagents used in biochemical and preliminary phytochemical estimations were obtained from Sigma Chemical, USA.

2.5. Animal

The experiments were conducted using male Swiss mice (26–30 g). All animals were housed in a controlled environment, with free access to food and water, and were maintained on a 12 h light–dark cycle. All experiments were performed according to the Cameroon National Ethical Committee (ref. no FW-IRB00001954) for animal handling and experimental procedure. Twelve hours before behavioral testing, the mice were deprived of food to enhance their motivation to perform the test [14]. All behavioral tests were performed between 8:00 a.m. and 6:00 p.m.

2.6. Experimental design

Animals were randomly divided into eight groups of six animals each. The first group received saline intraperitoneally while the second to seventh groups were administered PTZ (30 mg/kg; i.p.) dissolved in saline on every second day (48 ± 2 h). One hour before administration of PTZ, the first and second groups received distilled water and the third to sixth groups were administered aqueous extract of *F. apodanthera* (50, 100, 150, and 200 mg/kg, respectively) orally through an intragastric feeding tube. Group seven animals were administered sodium valproate (300 mg/kg) intraperitoneally. Pentylenetetrazole was administered up to day 43 or until stage 5 seizures on two consecutive trials were achieved. In group eight, aqueous extract of *F. apodanthera* (200 mg/kg) was administered alone to study its *per se* effect, if any, on cognitive functions and biochemical parameters. Elevated plus-maze and T-maze tests were performed 24 and 48 h after the last administration of PTZ. Animal behaviors were manually recorded by two blinded experimenters holding stopwatches. Following the behavioral test, the animals were sacrificed and the whole brain was dissected for the estimation of markers of oxidative stress and brain cholinergic status. In addition, elevated plus-maze and open-field tests were done in naïve mice.

2.7. Kindling induction

For PTZ kindling, a subconvulsant dose of PTZ (30 mg/kg in a volume of 10 mL/kg of body weight) was injected intraperitoneally on every second day (*i.e.*, day 1, day 3, day 5, …). Pentylenetetrazole was administered up to day 43 (22nd injection) or until stage 5 seizures on two consecutive trials were achieved. Seizure activity was evaluated using the following scale [15]: stage 0: no response; stage 1: hyperactivity and vibrissal twitching; stage 2: head nodding, head clonus, and myoclonic jerk; stage 3: unilateral forelimb clonus; stage 4: rearing with bilateral forelimb clonus; and stage 5: generalized tonic–clonic seizures with loss of righting reflex. The number of myoclonic jerks and the latencies to myoclonic jerks and generalized tonic–clonic seizures were recorded. The latencies were transformed into seizure scores [16] which were calculated using the following formula: $S = 1 - (\text{control latency} / \text{drug seizure latency})$.

Animals were considered kindled if they exhibited stage 5 seizures on two consecutive trials. The score was zero for animals which developed seizures and one for animals that did not develop seizures. Animals were also observed for 24-h mortality.

2.8. Behavioral tests

2.8.1. Elevated plus-maze test with PTZ-kindled mice

Cognitive impairment in mice was assessed using an elevated plus maze. The apparatus was made up of two open arms (16 cm × 5 cm) and two closed arms (16 cm × 5 cm × 10 cm) that extended from a common central platform (5 cm × 5 cm). The entire maze was elevated to a height of 50 cm above the floor level [17]. In the first trial, the time that the animal took to enter a closed arm with all four limbs when placed at the end of one open arm facing away from the central platform was recorded as the initial transfer latency. A 60-s cutoff was set. The mouse was then allowed to move freely in the maze regardless of open and closed arms for another 10 s. Twenty-four hours later, a retention transfer latency test was performed in the same way as in the acquisition trial. The mice were again put into the elevated plus maze. If the mice did not enter the enclosed arm within 60 s on the second trial, the transfer latency was assigned 60 s.

2.8.2. T-maze test with PTZ-kindled mice

The T-shaped maze was made of gray wood and consisted of a start arm and two choice arms. Each arm was 30 cm × 10 cm × 20 cm (length × width × height). A recessed black plastic cup (3 cm in diameter, 1 cm in depth) containing food was placed on the floor at the end of each choice arm. A day before the experiment, each animal was placed in the start position (at the end of the start arm) for a 10-min exploration phase with one arm of the maze open and then returned to their home cage. After a delay of 1 day, the animals were reintroduced to the T-maze for a 5-min testing period. During the retrial (the two choice arms were opened), animals were randomly placed in a start arm and the number of visits and the time spent in the two arms were assessed [18].

2.8.3. Elevated plus-maze test with naïve mice

The apparatus was the same as in Section 2.8.1. Mice were treated with distilled water for the negative control group, with diazepam (3 mg/kg) for the positive control group, and with different doses of the aqueous extract of *F. apodanthera* for the tested groups. One hour after treatment, mice were individually placed on the EPM center