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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: $S = 1 -$ (control latency / 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

platform facing an open arm and observed for 5 min [19,20]. The number of entries by each animal into the open or closed arms and the time spent by each animal on either open or closed arms (conventional parameters) were recorded with stopwatches by two trained experimenters. The center platform time and some ethological parameters like rearing and head dipping were also recorded.

2.8.4. Open-field test with naïve mice

One hour after appropriate treatment administration, naïve mice were placed in the center of an open field. The open field used was a wooden square box: 40 cm × 40 cm × 45 cm, and the floor was divided into 16 smaller squares of equal dimensions (10 cm × 10 cm). Animals placed one by one in the center of the box could explore the box for 5 min. Mice were observed for 5 min in order to evaluate the effects of the plant both on the exploratory activity and on anxiety [19,21]. Hand-operated counters and stopwatches were used to score the number of crossings (number of square floor units entered), rearing (number of times that the animal stood on its hind legs), grooming, and defecation. The positive control group received diazepam at a dose of 0.3 mg/kg.

2.9. Biochemical tests

Following the behavioral testing with kindled mice, the mice were decapitated under ether anesthesia and the brains were quickly removed, cleaned with ice-cold saline, and stored at −80 °C.

2.9.1. Tissue preparation

The whole brain of each mouse was dissected out and divided into two cerebral hemispheres for biochemical estimations. From one half, 10% (w/v) homogenate was prepared with ice-cold 0.1 M phosphate buffer (pH 7.4), and the lipid peroxidation product and reduced glutathione were assessed. With the other half, 10% (w/v) homogenate was prepared with 0.1 M Tris–HCl buffer containing 1% Triton-X and was used to assess cholinesterase activity.

2.9.2. Brain lipid peroxidation

Malondialdehyde (MDA), a measure of lipid peroxidation, was measured as described by Jainkang et al. [22]. The reagents 1.5 mL acetic acid (20%), pH 3.5, 1.5 mL thiobarbituric acid (0.8%), and 0.2 mL sodium dodecyl sulfate (8.1%) were added to 0.1 mL of processed tissue samples and then heated at 100 °C for 60 min. The mixture was cooled with tap water and 5 mL of *n*-butanol/pyridine (15:1), and 1 mL of distilled water was added. The mixture was vortexed vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was separated and absorbance was measured at 532 nm using a spectrophotometer. The concentration of MDA is expressed as nmol/g tissue.

2.9.3. Reduced brain glutathione

Reduced brain glutathione (GSH) was measured according to the method of Ellman [23]. An equal quantity of homogenate was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.01 mL of this supernatant, 2 mL of phosphate buffer (pH 8.4), 0.5 mL of 5′5-dithiobis (2-nitrobenzoic acid), and 0.4 mL of double-distilled water were added. The mixture was vortexed and the absorbance read at 412 nm within 15 min. The concentration of reduced glutathione was expressed as mg/g tissue.

2.9.4. Brain cholinergic status

The cholinergic markers acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were estimated in the mouse brain according to the method of Ellman [24]. The assay is a spectrophotometric method that involves two linked reactions to produce a colored compound. Briefly, 10 mM DTNB in 0.1 M Tris–HCl buffer (pH 8.0), 100 μL of supernatant, and 30 mM acetylthiocholine iodide as substrate were added for AChE estimation. For BChE estimation, 10 mM DTNB in 0.1 M Tris–HCl buffer (pH 8.0), 300 μL of supernatant, and 7.5 mM butyrylthiocholine iodide as substrate were added. Absorbance was measured at 412 nm for 3 min at 30-second intervals using a spectrophotometer. Acetylcholinesterase activity is expressed as μmol of acetylthiocholine iodide hydrolyzed/mg of protein/min, while BChE activity is expressed as μmol of butyrylthiocholine iodide hydrolyzed/mg of protein/min.

2.10. Statistical analysis

Data were expressed as mean ± standard error of the means (S.E.M.) per group. Statistical differences between control and treated groups were tested by two-way analysis of variance (ANOVA), followed by the Newman–Keuls post hoc test. The differences were considered significant at $p < 0.05$. The statistical package used for the analysis was GraphPad Prism 5.01 for Windows (GraphPad Prism Software, San Diego, CA, USA).

3. Results

3.1. Preliminary qualitative phytochemical analysis of the lyophilized aqueous extract of F. apodanthera

The aqueous extract of *F. apodanthera* contained flavonoids, alkaloids, saponins, tannins, glycosides, anthraquinones, and phenols but not cardiac glycosides and lipids.

3.2. Effects of F. apodanthera on the development of PTZ kindling

Repeated administration of a subconvulsant dose of PTZ (30 mg/kg) on alternate days resulted in increasing convulsive activity leading to generalized tonic–clonic seizures (stage 5) after 30.00 ± 1.67 days (16 injections) in negative control mice. One-way ANOVA showed a significant difference in the development of kindling among the treated groups [$F(7, 32) = 51.46$, $p < 0.05$]. The aqueous extract of *F. apodanthera* at doses of 50 and 100 mg/kg did not modify the course of the development of kindling induced by PTZ. However, the higher doses of the aqueous extract (150 and 200 mg/kg) inhibited the development of kindling ($p < 0.05$) since none of the animals could achieve stage 5 even after 43 days (22 injections of PTZ 30 mg/kg). The dose 300 mg/kg of sodium valproate also significantly ($p < 0.05$) delayed the course of the development of kindling induced by PTZ similarly to that in the *F. apodanthera* (150 and 200 mg/kg) groups (Fig. 1).

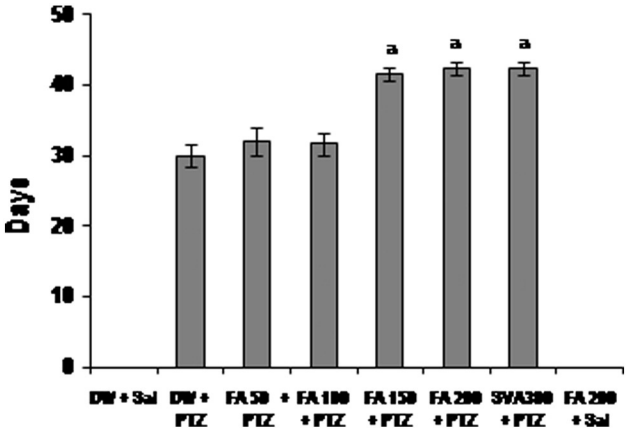

Fig. 1. Development of pentylenetetrazol-evoked kindling. Histograms represent the latency (in days) to reach generalized tonic–clonic seizures (stage 5). Results are expressed as mean ± S.E.M. for 6 animals. [a] $p < 0.05$ compared to the saline-treated PTZ group (two-way ANOVA followed by Newman–Keuls post hoc test). DW = distilled water, FA100 = lyophilized aqueous extract of *Feretia apodanthera* 100 mg/kg, Sal = saline, and SVA = sodium valproate.

3.3. Effects of F. apodanthera on seizures in PTZ-kindled mice

The aqueous extract of *F. apodanthera* and sodium valproate caused a dose-dependent increase in the latency to myoclonic jerks [$F(7, 34) = 109.52$, $p < 0.001$] as well as the latency to generalized tonic–clonic seizures [$F(7, 26) = 97.13$, $p < 0.001$] and a decrease in the number of myoclonic jerks [$F(7, 42) = 116.24$, $p < 0.001$] compared to the distilled water-treated PTZ mice.

The latency to myoclonic jerks increased from 45.17 ± 4.78 s in the distilled water-treated PTZ mice to 110.17 ± 5.22 s ($p < 0.05$) and 160.50 ± 5.17 s ($p < 0.001$) in the groups administered with *F. apodanthera* 150 and 200 mg/kg, respectively (Fig. 2).

The number of myoclonic jerks decreased from 55.33 ± 3.55 in the distilled water-treated PTZ mice to 18.17 ± 2.94 ($p < 0.001$), 12.83 ± 3.17 ($p < 0.001$), and 7.00 ± 1.33 ($p < 0.001$) in the groups administered with *F. apodanthera* 100, 150, and 200 mg/kg, respectively (Fig. 3).

An increase in the onset of clonic seizures was observed [$F(7, 41) = 135.74$, $p < 0.001$]. *Feretia apodanthera* significantly increased the latency to clonic seizures from 61.83 ± 8.61 s in the distilled water-treated PTZ mice to 176.17 ± 7.55 s ($p < 0.05$), 273.50 ± 9.83 s ($p < 0.01$), and 365.33 ± 11.1 s ($p < 0.001$) in *F. apodanthera* 100-, 150-, and 200-mg/kg-treated groups, respectively (Fig. 2).

The aqueous extract of *F. apodanthera* also increased the latency to generalized tonic–clonic seizures from 175.83 ± 15.55 s in the distilled water-treated PTZ mice to 350.67 ± 26.11 s ($p < 0.05$), 399.33 ± 11.22 s ($p < 0.01$), and 496.50 ± 31.33 s ($p < 0.01$) in the groups administered with *F. apodanthera* 100, 150, and 200 mg/kg, respectively (Fig. 2).

There was a significant difference in the duration of generalized tonic–clonic seizures among the different groups [$F(7, 42) = 109.71$, $p < 0.001$]. The duration of generalized tonic–clonic seizures decreased from 18.33 ± 1.33 s in the distilled water-treated PTZ mice to 7.17 ± 0.55 s ($p < 0.05$), 4.50 ± 0.67 s ($p < 0.01$), and 3.33 ± 0.44 s ($p < 0.001$) in the groups administered with 100, 150, and 200 mg/kg of the aqueous extract, respectively (Fig. 3).

Seizure scores among the groups were also significantly different [$F(7, 32) = 172.41$, $p < 0.001$]. It increased from 0 in the distilled water-treated PTZ mice to 0.56 ± 0.04 ($p < 0.05$) and 0.64 ± 0.05 ($p < 0.01$) in the groups administered with *F. apodanthera* 150 and 200 mg/kg, respectively (Fig. 4).

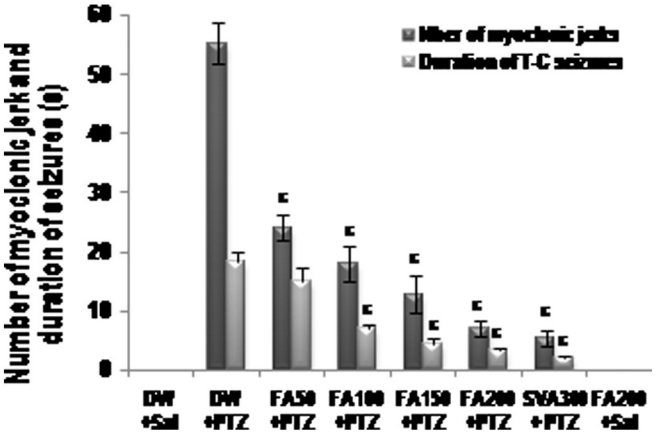

Fig. 3. Effects of the lyophilized aqueous extract of *Feretia apodanthera* or sodium valproate on the number of myoclonic jerks and the duration of tonic–clonic seizures in PTZ-kindled mice. Results are expressed as mean ± S.E.M. for 6 animals. $^{c}p < 0.001$ compared to the distilled water-treated PTZ mice (two-way ANOVA followed by Newman–Keuls post hoc test). DW = distilled water, FA100 = lyophilized aqueous extract of *F. apodanthera* 100 mg/kg, Sal = saline, and SVA = sodium valproate.

3.4. Effects on cognitive impairment

3.4.1. Effects of F. apodanthera on the elevated plus-maze test in PTZ-kindled mice

There was no significant difference in initial transfer latency [$F(7, 25) = 79.41$, $p > 0.064$] from the open arm to the closed arm among the groups, whereas there was a significant difference in retention transfer latency [$F(7, 35) = 114.62$, $p < 0.001$]. Pentylenetetrazole kindling caused a significant increase in the retention transfer latency from 9.67 ± 1.22 s in the distilled water + saline-treated group to 39.33 ± 1.89 s in the distilled water-treated PTZ mice (Fig. 5). The aqueous extract of *F. apodanthera* produced a dose-dependent reversal of the effect of PTZ-induced kindling on the retention transfer latency. Thus, the retention transfer latency decreased from 39.33 ± 1.89 s in the distilled water-treated PTZ mice to 19.33 ± 3.11 s ($p < 0.01$), 18.83 ± 3.22 s ($p < 0.001$), and 11.33 ± 1.67 s ($p < 0.001$) in groups administered with *F. apodanthera* 100, 150, and 200 mg/kg, respectively. Sodium valproate reduced the retention transfer latency to 8.83 ± 1.28 s. The

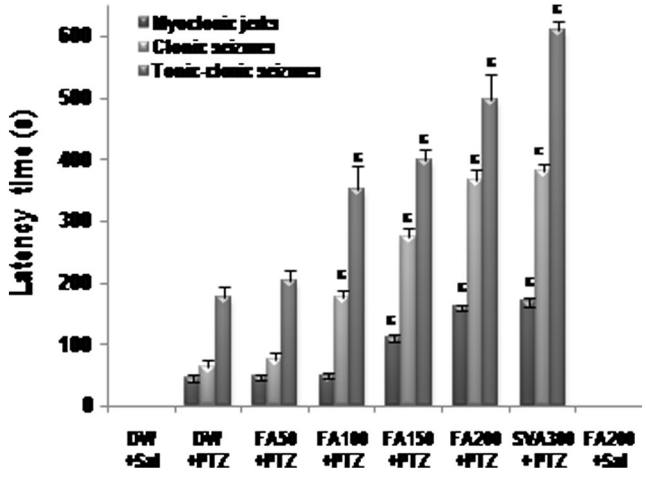

Fig. 2. Effects of the lyophilized aqueous extract of *Feretia apodanthera* or sodium valproate on the latencies to myoclonic jerks, clonic seizures, and generalized tonic–clonic seizures in PTZ-kindled mice. Results are expressed as mean ± S.E.M. for 6 animals. $^{c}p < 0.001$ compared to the distilled water-treated PTZ mice (two-way ANOVA followed by Newman–Keuls post hoc test). DW = distilled water, FA100 = lyophilized aqueous extract of *F. apodanthera* 100 mg/kg, Sal = saline, and SVA = sodium valproate.

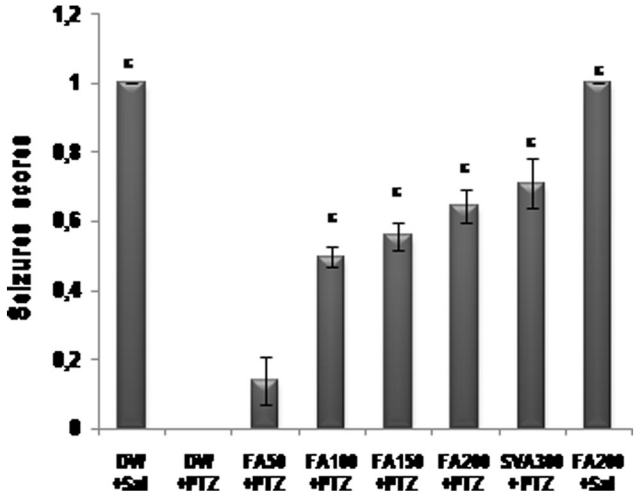

Fig. 4. Effects of the lyophilized aqueous extract of *Feretia apodanthera* or sodium valproate on the seizure scores in PTZ-kindled mice. Results are expressed as mean ± S.E.M. for 6 animals. $^{c}p < 0.001$ compared to the distilled water-treated PTZ mice (two-way ANOVA followed by Newman–Keuls post hoc test). DW = distilled water, FA100 = lyophilized aqueous extract of *F. apodanthera* 100 mg/kg, Sal = saline, and SVA = sodium valproate.

aqueous extract of *F. apodanthera* alone at the dose of 200 mg/kg also significantly reduced ($p < 0.001$) the retention transfer latency (Fig. 5).

3.4.2. Effects of F. apodanthera on the T-maze test in PTZ-kindled mice

On the retrial, animals first entered the familiar arm that contained food and after they started exploring the novel arm. The time spent in the novel arm was significantly increased compared with the time spent in the start and other arms in the groups administered with the aqueous extract of *F. apodanthera*. The time spent in the novel arm was 134.67 ± 5.78($p < 0.05$) and 136.33 ± 7.33($p < 0.05$) in the groups administered with 150 and 200 mg/kg of *F. apodanthera*, respectively, that is about 2-fold of that in the distilled water-treated PTZ mice (59.50 ± 6.67). This time slightly increased in the groups administered with 50 mg/kg (100.33 ± 4.55; $p < 0.05$) and 100 mg/kg (112.00 ± 9.67; $p < 0.05$). The effect of the plant extract was similar to the effect of sodium valproate, a known anticonvulsant compound (Fig. 6A). *Feretia apodanthera* also increased the number of entries into the novel arm from 6.83 ± 2.11 in the distilled water-treated PTZ mice to 16.16 ± 0.83 ($p < 0.01$) and 18.00 ± 1.33 ($p < 0.01$) in the groups administered with 150 and 200 mg/kg of the aqueous extract, respectively (Fig. 6B).

3.5. Effects on anxiety and locomotion

3.5.1. Effects of F. apodanthera on the elevated plus-maze test in naïve mice

The aqueous extract of *F. apodanthera* increased the number of entries in the open arms ($p < 0.001$), the % of entries in the open arms ($p < 0.001$), and the % of time spent in the open arms ($p < 0.001$) from 0.17, 2.57%, and 0.17% in the control group to 1.33, 66.67%, and 66.89% in the group treated with the aqueous extract of *F. apodanthera* at the dose of 200 mg/kg, respectively. Diazepam (3 mg/kg, i.p.) also induced an increase in the % of entries and time spent in the open arms of the EPM (Table 1, Fig. 7A). Like diazepam, *F. apodanthera* induced a significant reduction in the number of closed arm entries ($p < 0.001$) and the % of closed arm time ($p < 0.001$) from 6.33 and 95.44% in the control group to 0.67 and 3.39% in the group treated with the *F. apodanthera* aqueous extract at the dose of 200 mg/kg, respectively (Fig. 7B). In addition, the ratio of open entries/total entries *vs.* closed entries/total entries strongly and significantly increased with the doses of *F. apodanthera* from 2.63 in the control group to 200.00 in the group treated with *F. apodanthera* at the dose of 200 mg/kg ($p < 0.001$). The number of

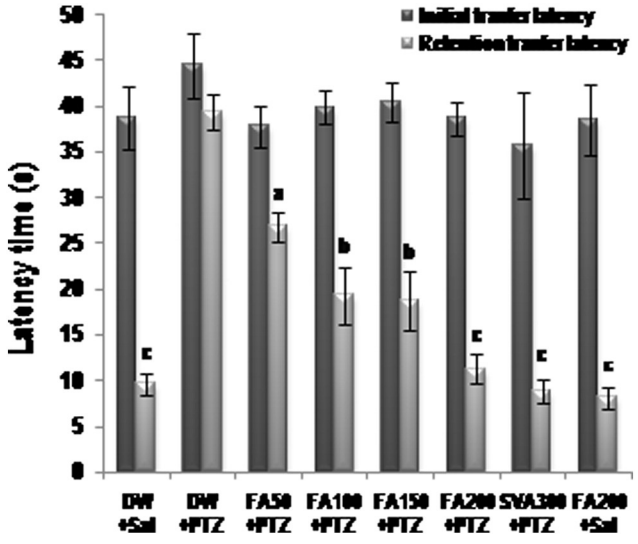

Fig. 5. Effects of the lyophilized aqueous extract of *Feretia apodanthera* or sodium valproate on initial and retention transfer latencies in the elevated plus-maze test in PTZ-kindled mice. Results are expressed as mean ± S.E.M. for 6 animals. [a]$p < 0.05$, [b]$p < 0.01$, and [c]$p < 0.001$ compared to the distilled water-treated PTZ mice (two-way ANOVA followed by Newman–Keuls post hoc test). DW = distilled water, FA100 = lyophilized aqueous extract of *F. apodanthera* 100 mg/kg, Sal = saline, and SVA = sodium valproate.

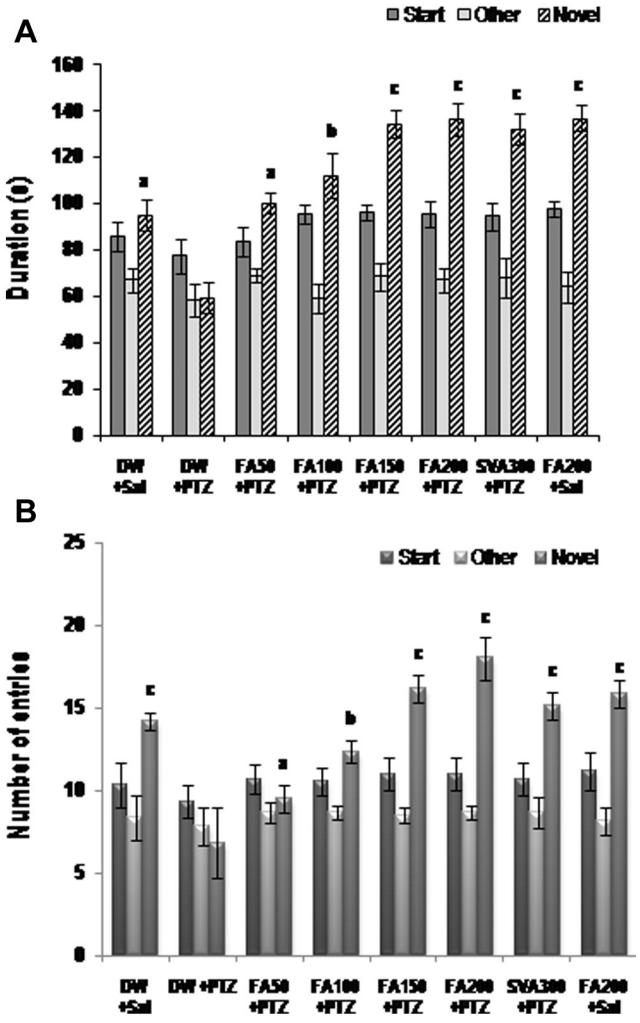

Fig. 6. Effects of the lyophilized aqueous extract of *Feretia apodanthera* or sodium valproate on the duration of time spent (A) and number of entries (B) in each T-maze arm in PTZ-kindled mice. Results are expressed as mean ± S.E.M. for 6 animals. [a]$p < 0.05$, [b]$p < 0.01$, and [c]$p < 0.001$ compared to the distilled water-treated PTZ mice (two-way ANOVA followed by Newman–Keuls post hoc test). DW = distilled water, FA100 = lyophilized aqueous extract of *F. apodanthera* 100 mg/kg, Sal = saline, and SVA = sodium valproate.

rearings and that of head dipping were also reduced by both diazepam and *F. apodanthera* (Table 1).

3.5.2. Effects of F. apodanthera on the open-field test in naive mice

Like in the EPM test, the number of rearings was decreased ($p < 0.001$) by both *F. apodanthera* and diazepam. They also decreased ($p < 0.001$) the mass of fecal boli. Controversially, the aqueous extract of *F. apodanthera* increased crossing ($p < 0.001$), grooming ($p < 0.001$), and the time spent by mice in the center ($p < 0.001$) (Table 2).

3.6. Biochemical measurements

3.6.1. Effects of F. apodanthera on brain MDA levels in PTZ-kindled mice

Pentylenetetrazole kindling significantly increased ($p < 0.001$) the brain MDA level from 128.754 ± 16.193 nmol/g wet tissue in the distilled water + saline-treated mice to 427.493 ± 18.157 nmol/g wet tissue in the distilled water-treated PTZ mice. *Feretia apodanthera* antagonized the increased brain MDA levels due to PTZ kindling in a dose-dependent manner. The MDA levels significantly decreased from 427.493 ± 18.157 nmol/g wet tissue in the distilled water-treated PTZ mice to 178.374 ± 22.633 nmol/g wet tissue ($p < 0.01$), 137.518 ± 18.491 nmol/g wet tissue ($p < 0.001$), and 127.842 ± 21.352 nmol/g wet tissue ($p < 0.001$) in groups administered with *F. apodanthera* 100,

Table 1
Effects of the lyophilized aqueous extract of *Feretia apodanthera* on open arm entries, closed arm entries, total arm entries, rearing, head dipping, and ratio of open entries/total entries (OE/TE) *versus* closed entries/total entries (CE/TE) of mice in the elevated plus-maze test.

Treatments	Open arm entries	Closed arm entries	Total arm entries	Ratio of OE/TE *vs.* CE/TE	Rearing	Head dipping
DW	0.17 ± 0.28	6.33 ± 1.10	6.50 ± 1.33	2.63 ± 0.01	15.50 ± 0.71	6.17 ± 1.44
FA50	0.50 ± 0.50	5.50 ± 1.17	6.00 ± 1.33	9.09 ± 5.19	11.67 ± 1.14	2.83 ± 0.47**
FA100	0.67 ± 0.44	1.67 ± 0.67**	2.33 ± 0.44**	40.00 ± 7.36*	9.67 ± 2.33	1.33 ± 1.00***
FA150	1.17 ± 0.28*	1.0 ± 0.00**	2.17 ± 0.28**	116.67 ± 12.87***	2.50 ± 0.83***	1.17 ± 0.28***
FA200	1.33 ± 0.44*	0.67 ± 0.44***	2.00 ± 0.67**	200.00 ± 10.87***	1.17 ± 0.89***	0.67 ± 0.89***
DZP3	1.50 ± 0.50**	0.50 ± 0.50***	2.00 ± 0.67**	300.00 ± 40.46***	0.50 ± 0.50***	0.17 ± 0.28***

Results are expressed as mean ± S.E.M. for 6 animals. DW = distilled water, DZP3 = diazepam 3 mg/kg, and FA50 = lyophilized aqueous extract of *F. apodanthera* 50 mg/kg.
$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared to the distilled water-treated mice, (two-way ANOVA, followed by Newman-Keuls post hoc test).

150, and 200 mg/kg, respectively. *Feretia apodanthera per se* (200 mg/kg) caused a decrease in oxidative stress ($p < 0.001$), based on whole brain MDA levels,compared with the distilled water-treated PTZ mice (Table 3).

3.6.2. Effects of F. apodanthera on brain glutathione levels in PTZ-kindled mice

The brain GSH was significantly lower ($p < 0.001$) in the group administered with distilled water + PTZ compared to the groups administered with distilled water + saline. *Feretia apodanthera* reversed the decreased brain GSH levels. At doses of 150 and 200 mg/kg, *F. apodanthera* significantly increased the levels of brain GSH to 173.147 ± 16.328 μg/g wet tissue($p < 0.05$) and 191.532 ± 12.492 μg/g wet tissue($p < 0.05$), respectively. *Feretia apodanthera* (200 mg/kg) alone also caused a significant increase (198.713 ± 13.715 μg/g wet tissue; $p < 0.05$) in the brain GSH levels (Table 3).

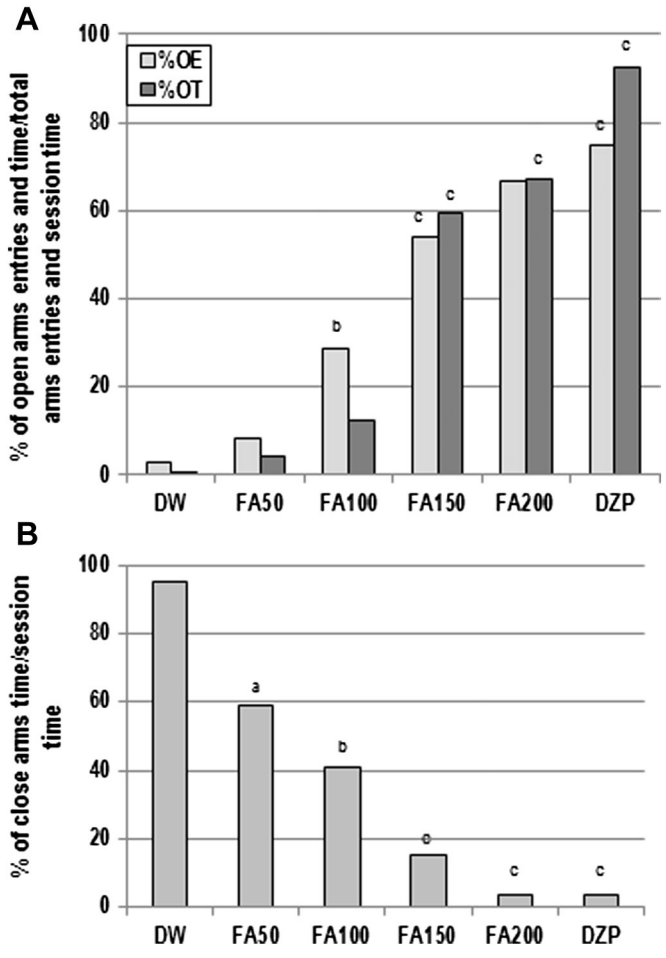

Fig. 7. Effect of *Feretia apodanthera* on mice placed on the EPM: % of open arm entries and % of open arm time (A) and % of closed arm time (B). N = 6 per dose. $^{a}p < 0.01$, $^{b}p < 0.01$, and $^{c}p < 0.001$(ANOVA followed by Dunnett's (HSD) test). DW = distilled water, DZP0.3 = diazepam 0.3 mg/kg, DZP3 = diazepam 3 mg/kg, and FA100 = lyophilized aqueous extract of *F. apodanthera* 100 mg/kg.

3.6.3. Effects of F. apodanthera on cholinesterase activity

The activity of AChE and BChE significantly decreased in the distilled water-treated PTZ mice ($p < 0.01$) compared with the distilled water + saline-treated group. A significant increase of AChE and BChE activity was observed in mice pretreated with *F. apodanthera* (100, 150, and 200 mg/kg) and sodium valproate. *Feretia apodanthera* (200 mg/kg) alone also significantly increased ($p < 0.01$) AChE and BChE activity (Table 3).

4. Discussion

Kindled seizures are widely accepted as an animal model of temporal lobe epilepsy, wherein repeated subthreshold brain stimulation (electrical or chemical) leads to behavioral signs of tonic and clonic seizures [25]. In the present study, a subconvulsant dose of PTZ when given intraperitoneally on alternate days induced kindling in mice after 30 days. The groups which were administered with the aqueous extract of *F. apodanthera* showed dose-dependent protection against seizures. *Feretia apodanthera* increased the latencies to myoclonic jerks, clonic seizures, and generalized tonic–clonic seizures as well as the duration of generalized tonic–clonic seizures. *Feretia apodanthera* in a dose-dependent manner decreased the number of myoclonic jerks and the seizure score. The protection against PTZ-induced kindling in mice and seizures in kindled mice suggest that *F. apodanthera* may have anticonvulsant properties against partial epilepsy, especially temporal lobe epilepsy [26]. The seizure protection offered by *F. apodanthera* (200 mg/kg, p.o.) was comparable to the standard antiepileptic drug sodium valproate (300 mg/kg, p.o.). Valproic acid and its salts, sodium or magnesium valproate, are effective anticonvulsants with a broad spectrum of activity [27,28]. Valproic acid increased PTZ thresholds in different seizure types [29,30]. The protective effect of valproic acid on PTZ-induced kindling is believed to be achieved through different neural mechanisms including inhibition of the voltage-dependent sodium channels, augmentation of the concentration of the natural inhibitor GABA in CNS synapses, facilitation of GABAergic neurotransmission, reduction of N-methyl-D-aspartate receptor-mediated glutamate excitation, augmentation of serotonergic inhibition, and attenuation of neurogenic inflammation [31–36]. The effect of *F. apodanthera* could be mediated by at least one of valproic acid's mechanisms of action.

Epilepsy has been described as a condition of excessive neuronal discharge associated with or resulting from oxidative stress [37,38]. In addition, high oxidative stress observed during epilepsy yields a consequent limitation on the learning and memory capabilities of patients suffering from seizures [39]. Thus, chemical kindling reflects the convulsive component of this disease as well as secondary alterations in the field of cognition [40]. The results showed that PTZ caused a significant increase in the retention transfer latency in the elevated plus-maze test which

Table 2
Effects of the lyophilized aqueous extract of *Feretia apodanthera* on rearing, crossing, grooming, center time, and quantity of fecal boli in the OF test.

Treatments	Rearing	Crossing	Grooming	Fecal boli (g)	Center time (s)
DW	11.67 ± 1.33	8.83 ± 2.11	0.83 ± 0.28	0.28 ± 0.15	4.33 ± 1.67
FA50	5.67 ± 1.67**	11.83 ± 2.22	1.67 ± 0.44	0.18 ± 0.14	3.33 ± 1.33
FA100	2.33 ± 0.78***	21.17 ± 4.55*	1.67 ± 0.44	0.11 ± 0.04	7.33 ± 1.33*
FA150	2.67 ± 0.67***	30.67 ± 3.11***	2.17 ± 0.83*	0.02 ± 0.03*	18.17 ± 0.83*
FA200	1.83 ± 0.56***	47.17 ± 3.44***	1.67 ± 0.44	0.02 ± 0.04*	32.83 ± 8.83***
DZP0.3	1.17 ± 0.56***	48.17 ± 7.83***	1.83 ± 0.28	0.02 ± 0.03*	39.17 ± 8.17***

Results are expressed as mean ± S.E.M. for 6 animals. DW = distilled water, DZP0.3 = diazepam 0.3 mg/kg, and FA50 = lyophilized aqueous extract of *F. apodanthera* 50 mg/kg.
*p < 0.05, **p < 0.01, ***p < 0.001, compared to the distilled water-treated mice, (two-way ANOVA, followed by Newman-Keuls post hoc test).

indicates impairment of memory in mice. However, *F. apodanthera* shortened retention transfer latencies, suggesting that *F. apodanthera* improved the cognition in kindled mice since the mice appeared to remember that there is a closed arm where they could stay safely [41,42]. Because the reduction of the retention transfer latencies can also be caused by hyperlocomotion, behavioral tests with naïve mice were done in the EPM and OF. *Feretia apodanthera* reduced closed and total arm entries in the EPM. It also reduced rearing and head dipping in the OF. These results suggested that locomotion is not involved in the reduction of transfer latencies by the plant. In the EPM, the aqueous extract of *F. apodanthera* showed anxiolytic activities by increasing the number of entries in the open arms, the % of entries and time spent into the open arms, and the ratio of open entries/total entries *vs.* closed entries/total entries and by reducing the number of closed arm entries and the % of closed arm time. These anxiolytic activities of *F. apodanthera* were confirmed in the OF by the increase of crossing, grooming, and center time. The presence of the anxiolytic properties in *F. apodanthera* did not affect the retention transfer latencies. The results in the T-maze test with PTZ-kindled mice showed that *F. apodanthera* reduced the retention transfer latencies but not the initial transfer latencies. If the reduction of the retention transfer latencies was caused by the presence of anxiolytic properties, even the initial transfer latencies could have been reduced. That was not the case. It could then be suggested that *F. apodanthera* certainly improved memory, for the animals appeared to remember that there was a safe area somewhere (closed arms). The memory improvement induced by *F. apodanthera* was dose-dependent, with the maximum benefit at the dose of 200 mg/kg. *Feretia apodanthera* showed antiseizure activity which may be at least partially responsible for the improvement in cognitive function.

To confirm the effect of the aqueous extract of *F. apodanthera* on spatial short-term memory impairment in PTZ-treated mice, the T-maze test was used to measure spontaneous alternation in which the mice must remember the arm most recently entered to alternate the arm choice [43,44]. The number of entries and the amount of time spent into the novel arm were lower in the distilled water-treated PTZ mice than in the distilled water + saline-treated mice, and this reduced PTZ-induced spontaneous alternation was reversed by *F. apodanthera* and sodium valproate. It has been proposed that the time animals spend in the novel arm reflects exploratory behavior while the number of entries into the novel arm reflects inquisitive behavior [45,46]. Since the elevated T-maze did not have open arms, the measurement of anxiolytic effects could not be very appropriate in this test; thus, the exploratory activity in the T-maze induced by *F. apodanthera* is not attributed to the anxiolytic properties of the plant. Moreover, on the retrial, the fact that animals first entered the familiar arm that contained food before starting to explore the novel arm to search for food suggested that *F. apodanthera* significantly prevented the spatial short-term memory deficits induced by PTZ.

Free radicals are normal by products of cellular aerobic metabolism involved in the development of seizures [47]. However, when the production of free radicals increases or the defense mechanism of the body decreases, they cause cellular dysfunction by attacking the polyunsaturated sites of the biological membranes causing lipid peroxidation. In the present study, PTZ kindling increased the level of MDA and decreased the level of GSH in the mouse brain. Pentylenetetrazole thus caused an imbalance between antioxidant and oxidant defense systems which may be at least partially responsible for seizures and cognitive impairment [48]. *Feretia apodanthera* prevented the rise of brain MDA levels in a dose-dependent manner. The decrease in brain MDA levels by *F. apodanthera* indicated an inhibition of lipid peroxidation since its increase is a marker of lipid peroxidation [49]. *Feretia apodanthera* alone also decreased the MDA level which supports its antioxidant property.

Table 3
Effects of the lyophilized aqueous extract of *Feretia apodanthera* on the lipid peroxidation product, reduced brain glutathione, and cholinesterase activity in the whole brain of PTZ-kindled mice.

Treatments	Dose (mg/kg)	Brain lipid peroxidation	Reduced brain glutathione	Brain cholinergic status	
		MDA (nmol/g wet tissue)	GSH (μg/g wet tissue)	AChE (units)	BChE (units)
DW + saline	– + –	128.754 ± 16.193	199.342 ± 12.471	0.165 ± 0.029	0.078 ± 0.002
DW + PTZ	– + 30	427.493 ± 18.157[a]	101.163 ± 14.294[b]	0.072 ± 0.005[c]	0.027 ± 0.003[c]
FA + PTZ	50 + 30	415.628 ± 19.416[b]	138.385 ± 13.358[b]	0.091 ± 0.007[b]	0.032 ± 0.002[b]
FA + PTZ	100 + 30	178.374 ± 22.633**	142.528 ± 11.275*	0.113 ± 0.003*	0.058 ± 0.004*
FA + PTZ	150 + 30	137.518 ± 18.491***	173.147 ± 16.328*	0.125 ± 0.018**	0.064 ± 0.003**
FA + PTZ	200 + 30	127.842 ± 21.352***	191.532 ± 12.492*	0.139 ± 0.023**	0.068 ± 0.005**
SVA + PTZ	300 + 30	131.575 ± 16.217***	196.145 ± 12.356*	0.145 ± 0.014**	0.066 ± 0.003**
FA + saline	200 + –	110.529 ± 17.143***	198.713 ± 13.715*	0.151 ± 0.026**	0.062 ± 0.002**

Results are expressed as mean ± S.E.M. for 6 animals. DW = distilled water, SVA = sodium valproate, and FA = lyophilized aqueous extract of *F. apodanthera*. Cholinesterase activity in the whole mice brain was expressed as μmol of acetylthiocholine iodide hydrolyzed/mg protein/min and μmol of butyrylthiocholine iodide hydrolyzed/mg protein/min for AChE and BChE, respectively.
*p < 0.05, **p < 0.01, ***p < 0.001, compared to the distilled water-treated PTZ mice, (two-way ANOVA, followed by Newman-Keuls post hoc test).
[a]p < 0.05, [b]p < 0.01, [c]p < 0.001, compared to the distilled water + saline-treated mice (two-way ANOVA, followed by Newman-Keuls post hoc test).

Glutathione is an endogenous antioxidant which gets converted to its oxidized form. This oxidized form of reduced glutathione reacts with free radicals and prevents the generation of most toxic hydroxyl radicals [50]. The decrease in reduced glutathione in mouse brain tissue induced by PTZ indicated that during kindling, there was excessive oxidative stress and, as a consequence, glutathione levels were depleted while combating oxidative stress [51,52]. However, *F. apodanthera* in all doses antagonized this decrease and restored the reduced glutathione level in the brain tissues of PTZ-kindled mice.

Seizures decrease choline acetyltransferase and acetylcholinesterase activity in the brain, which may result in cognitive deficit in mice with epilepsy [53]. Thus, significant decreases in acetylcholinesterase and butyrylcholinesterase activity were observed in the distilled water-treated PTZ mice, which might be also related to the development of cognitive impairment. *Feretia apodanthera* provided protection against the development of seizures as a result of which acetylcholinesterase and butyrylcholinesterase activity was not decreased but instead increased. This finding is in accordance with a previous study in which AChE activity was reported to decrease PTZ-induced seizures [54,55]. Taken together, we suggest that *F. apodanthera* had ameliorating effects on memory dysfunction and antagonized cognitive impairment. No mortality was observed for 24 h.

To conclude, a lyophilized aqueous extract of *F. apodanthera* offered protection against PTZ kindling in mice and antagonized oxidative stress and cognitive impairment in mice. This study thus suggests the potential of *F. apodanthera* as an adjuvant to antiepileptic drugs for patients with epilepsy with dual advantages of better seizure control as well as reduced cognitive impairment. In clinical practice, the administration of *F. apodanthera* to a patient with epilepsy could result first in stopping the memory impairment process and second in repairing memory impairment. However, further biochemical, molecular, and clinical studies are required to ascertain its effectiveness and mechanism of action during epilepsy.

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Conflict of interest

The authors declare no conflict of interest.

References

[1] Scheuer ML, Pedley TA. The evaluation and treatment of seizures. N Engl J Med 1990; 323:1468–74.
[2] Kamboj P, Singh I, Mahadevan N, Chaudhary G. Anticonvulsants from nature. Phcog Rev 2009;3:108–17.
[3] Reynolds EH, Trimble MR. Adverse neuropsychiatric effects of anticonvulsant drugs. Drugs 1985;29:570–81.
[4] Torbati D, Church DF, Keller JM, Pryor WA. Free radical generation in the brain precedes hyperbaric oxygen-induced convulsions. Free Radic Biol Med 1992;13:101–6.
[5] Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN. Screening of Indian plants for biological activity, part II. Indian J Exp Biol 1969;7:250–62.
[6] Ngo Bum E, Taïwe G, Moto FCO, Ngoupaye GT, Vougat RRN, Sakoue VD, et al. Antiepileptic medicinal plants used in traditional. In: Zaid Afawi, editor. Clinical and genetic aspects of epilepsy. Rijeka: InTech; 2011. p. 175–92.
[7] Dalziel JM. The useful plants of West Tropical Africa. London: The Crown Agency for the Colonies; 1937.
[8] Pousset JL. Plantes médicinales africaines. Paris: ACCT, Edition Marketing; 1989.
[9] Adjanohoun JE, Aboukakar N, Dramane K, Ebot ME, Ekpere JA, Enow-Orock EG, et al. Traditional medicine and pharmacopoeia. Contribution to ethnobotanical and floristic studies in Cameroon. Porto-Novo (Rep. Du Benin): Centre de Production de Manuels Scolaires; 1996.
[10] Arbonnier M. Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest. Paris: Centre de Coopération Internationale en Recherche Agronomique pour le développement/ Muséum national d'histoire naturelle/Union mondiale pour la nature (CIRAD/MNHN/ UICN); 2000.
[11] Kerharo J, Adams JG. La pharmacopée Sénégalaise traditionnelle. Paris: Vigot frère; 1974.
[12] Ngo Bum E, Taiwe SG, Goupaye G. Effects of a lyophilized aqueous extract of *Feretia apodanthera* Del. (Rubiaceae) on pentylenetetrazole-induced kindling, oxidative stress and cognitive impairment in mice. Epilepsy in Africa: bridging the gap. 2nd African Epilepsy Congress, p047, p41, 22nd–24th, Cape Town, South Africa; 2014.
[13] Trease GE, Evans WC. Pharmacognosy. London: Ballière Tindall Press; 1983.
[14] Ando S, Ohashi Y. Longitudinal study on age-related changes of working and reference memory in the rat. Neurosci Lett 1991;128:17–20.
[15] Racine RJ. Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroencephalogr Clin Neurophysiol 1972;32:281–94.
[16] Malhotra J, Gupta YK. Effect of adenosine receptors modulation on pentylenetetrazole-induced seizures in rats. Br J Pharmacol 1997;120:282–8.
[17] Sharma AC, Kulkarni SK. Evaluation of learning and memory mechanisms employing elevated plus-maze in rats and mice. Prog Neuropsychopharmacol Biol Psychiatry 1992;16:117–25.
[18] Deacon RMJ, Rawlins JNP. T-maze alternation in the rodent. Nat Protoc 2006;1:7–12.
[19] Ngo Bum E, Taiwe GS, Moto FCO, Ngoupaye GT, Nkantchoua GCN, Pelanken MM, et al. Anticonvulsant, anxiolytic and sedative properties of the roots of *Nauclea latifolia* Smith in mice. Epilepsy Behav 2009;15:434–44.
[20] Lister RG. The use of a plus-maze to measure anxiety in mouse. Psychopharmacology (Berl) 1987;92:180–5.
[21] Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 2003;463:3–33.
[22] Jainkang L, Rei E, Hideaki K, Akitane M. Antioxidant action of guilingi in the brain of rats with $FeCl_3$ induced epilepsy. Free Radic Biol Med 1990;9:451–4.
[23] Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys 1959;82:70–3.
[24] Ellman GL, Courtney KD, Andres Jr V, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88–95.
[25] Szyndler J, Piechal A, Blecharz-Klin K, Skórzewska A, Maciejak P, Walkowiak J, et al. Effect of kindled seizures on rat behavior in water Morris maze test and amino acid concentrations in brain structures. Pharmacol Rep 2006;58:75–82.
[26] Loscher W. Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. Epilepsy Res 2002;50: 105–23.
[27] El-Azab MF, Moustafa YM. Influence of calcium channel blockers on anticonvulsant and antinociceptive activities of valproic acid in pentylenetetrazole-kindled mice. Pharmacol Rep 2012;64:305–14.
[28] Aley KO, Kulkarni SK. GABAergic agents-induced antinociceptive effect in mice. Methods Find Exp Clin Pharmacol 1989;11:597–601.
[29] Loscher W, Nau H, Marescaux C, Vergnes M. Comparative evaluation of anticonvulsant and toxic potencies of valproic acid and 2-en-valproic acid in different animal models of epilepsy. Eur J Pharmacol 1984;99:211–8.
[30] Hoffmann K, Czapp M, Loscher W. Increase in antiepileptic efficacy during prolonged treatment with valproic acid: role of inhibition of histone deacetylases? Epilepsy Res 2008;81:107–13.
[31] Chapman A, Keane PE, Meldrum BS, Simiand J, Vernieres JC. Mechanism of anticonvulsant action of valproate. Prog Neurobiol 1982;19:315–59.
[32] Loscher W. Valproate: a reappraisal of its pharmacodynamics properties and mechanisms of action. Prog Neurobiol 1999;58:31–59.
[33] Jensen TS. Anticonvulsants in neuropathic pain: rationale and clinical evidence. Eur J Pain 2002;6:61–8.
[34] Loscher W. Basic pharmacology of valproate: a review after 35 years of clinical use for the treatment of epilepsy. CNS Drugs 2002;16:669–94.
[35] Owens MJ, Nemeroff CB. Pharmacology of valproate. Psychopharmacol Bull 2003;37: 17–24.
[36] Winkler I, Blotnik S, Shimshoni J, Yagen B, Devor M, Bialer M. Efficacy of antiepileptic isomers of valproic acid and valpromide in a rat model of neuropathic pain. Br J Pharmacol 2005;146:198–208.
[37] Waldbaum S, Patel M. Mitochondrial dysfunction and oxidative stress: a contributing link to acquired epilepsy? J Bioenerg Biomembr 2010;42:449–55.
[38] Shin EJ, Jeong JH, Chung YH, Kim WK, Ko KH, Bach JH, et al. Role of oxidative stress in epileptic seizures. Neurochem Int 2011;59:122–37.
[39] Liu SH, Chang CD, Chen PH, Su JR, Chen C, Chaung HC. Docosahexaenoic acid and phosphatidylserine supplementations improve antioxidant activities and cognitive functions of the developing brain on pentylenetetrazol-induced seizure model. Brain Res 2012;1451:19–26.
[40] Becker A, Grecksch G, Ruthrich HL, Pohle W, Marx B, Matthies H. Kindling and its consequences on learning in rats. Behav Neural Biol 1992;57:37–43.
[41] Itoh J, Nabeshima T, Kameyama T. Utility of an elevated plus-maze for the evaluation of memory in mice: effects of nootropics, scopolamine and electroconvulsive shock. Psychopharmacology (Berl) 1990;101:27–33.
[42] Miyazaki S, Imaizumi M, Machida H. The effects of anxiolytics and anxiogenics on evaluation of learning and memory in an elevated plus-maze test in mice. Methods Find Exp Clin Pharmacol 1995;17:121–7.
[43] Hauber W, Schmidt WJ. Effects of intrastriatal blockade of glutamatergic transmission on the acquisition of T-maze and radial maze tasks. J Neural Transm 1989;78: 29–41.
[44] Spowart-Manning L, van der Staay FJ. The T-maze continuous alternation task for assessing the effects of putative cognition enhancers in the mouse. Behav Brain Res 2004;151:37–46.
[45] Dellu F, Fauchey V, Le Moal M, Simon H. Extension of a new two-trial memory task in the rat: influence of environmental context on recognition processes. Neurobiol Learn Mem 1997;67:112–20.

[46] Dellu F, Contarino A, Simon H, Koob GF, Gold LH. Genetic differences in response to novelty and spatial memory using a two-trial recognition task in mice. Neurobiol Learn Mem 2000;73:31–48.
[47] Sejima H, Ito M, Kishi K, Tsuda H, Shiraishi H. Regional excitatory and inhibitory amino acid concentrations in pentylenetetrazole kindling and kindled rat brain. Brain Dev 1997;19:171–5.
[48] Mehla J, Reeta KH, Gupta P, Gupta YK. Protective effect of curcumin against seizures and cognitive impairment in a pentylenetetrazole-kindled epileptic rat model. Life Sci 2010;87:596–603.
[49] Liu CS, Wu HM, Kao SH, Wei YH. Phenytoin-mediated oxidative stress in serum of female epileptics: a possible pathogenesis in the fetal hydantoin syndrome. Hum Exp Toxicol 1997;16:177–81.
[50] Ono H, Sakamoto A, Sakura N. Plasma total glutathione concentrations in epileptic patients taking anti-convulsants. Clin Chim Acta 2000;298:135–43.
[51] Schulz JB, Lindenau J, Seyfried J, Dichgans J. Glutathione, oxidative stress and neurodegeneration. Eur J Biochem 2000;267:4904–11.
[52] Gupta YK, Kumar MHV, Srivastava AK. Effect of *Centella asiatica* on pentylenetetrazole-induced kindling, cognition and oxidative stress in rats. Pharmacol Biochem Behav 2003;74:579–85.
[53] Sales IM, Freitas RL, Saldanha GB, Souza GF, Freitas RM. Choline acetyltransferase and acetylcholinesterase activities are reduced in rat striatum and frontal cortex after pilocarpine-induced seizures. Neurosci Lett 2010;469:81–3.
[54] Visweswari G, Prasad KS, Chetan PS, Lokanatha V, Rajendra W. Evaluation of the anticonvulsant effect of *Centella asiatica* (gotu kola) in pentylenetetrazole-induced seizures with respect to cholinergic neurotransmission. Epilepsy Behav 2010;17:332–5.
[55] Pahuja M, Mehla J, Reeta KH, Joshi S, Gupta YK. Hydroalcoholic extract of *Zizyphus jujuba* ameliorates seizures, oxidative stress, and cognitive impairment in experimental models of epilepsy in rats. Epilepsy Behav 2011;21:356–63.