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Methanol extract of *Ficus platyphylla* ameliorates seizure severity, cognitive deficit and neuronal cell loss in pentylenetetrazole-kindled mice

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ABSTRACT

Decoctions of *Ficus plathyphylla* are used in Nigeria's folk medicine to manage epilepsy for many years and their efficacies are widely acclaimed among the rural communities of Northern Nigeria. In this study, we examined the ameliorative effects of the standardized methanol extract of *Ficus platyphylla* (FP) stem bark on seizure severity, cognitive deficit and neuronal cell loss in pentylenetetrazole-kindled mice. The ^{35}S-GTPγS, glutamate and γ-aminobutyric acid receptors binding properties of the extract were also evaluated. Male CD-1 mice were kindled with an initial subeffective dose of pentylenetetrazole (PTZ, 37.5 mg/kg, i.p.) for a total of 13 convulsant injections and the treatment groups concurrently received FP (100 and 200 mg/kg). Control animals received the same number of saline injections. Twenty-four h after kindling completion the animals' learning performance was tested in a two-way shuttle-box. The animals were challenged with another subeffective dose of PTZ (32.5 mg/kg, i.p.) on day 7 after kindling completion. Animals were sacrificed a day after the challenged experiment and the brains were processed for histological investigation. FP ameliorates seizure severity, cognitive deficits and neuronal cell loss in PTZ kindled mice. Components of the extract showed affinity for GABAergic and glutamatergic receptors. Glutamate release was diminished and the ^{35}S GTPγS binding assay revealed no intrinsic activity at glutamatergic receptors. Our results revealed that FP contains psychoactive secondary metabolites with anticonvulsant properties, thus supporting the isolation and development of the biologically active components of this medicinal plant as antiepileptic agents.

Introduction

Epilepsy is a neurological disorder characterized by recurrent seizures that vary from the briefest lapses of attention or muscle jerks to severe and prolonged convulsions. Between 1% and 3% of the World's population suffers from epilepsy (Patsalos 1999; Löscher 2002a), making it the most prevalent neurological disorder. This heterogeneous and debilitating neurological disorder that may either be symptomatic of various disorders or idiopathic in nature, is often accompanied by psychiatric comorbidities (Motamedi and Meador 2003; Lagae 2006; Schmidt 2009; Eddy et al., 2011; Kanner et al., 2012).

Despite the availability of an armoury of antiepileptic drugs, the current treatment of epilepsy is not satisfactory in terms of drug associated deleterious effects (Elger et al., 2004). The development of new, affordable and accessible pharmacological agents that can overcome these limitations has become a major goal in epilepsy research (Chindo et al., 2009). The plant kingdom has become a major target in the search of new drugs and lead compounds to treat this debilitating neurological disorder (Bienvenu et al., 2002; Adeyemi et al., 2007; Bum et al., 2009; Chindo et al., 2009, 2014).

Ficus platyphylla Del.-Holl (Family: Moraceae) is a deciduous plant found mainly in the savanna regions of the West African coast. Decoctions of the plant are used in Nigeria's folk medicine to manage epilepsy, depression, psychoses, pain and inflammation for many years, and their efficacies are widely acclaimed among the rural communities of Northern Nigeria (Audu 1989).

The cold water extract or decoctions of the stem or root bark are usually taken orally, while the powder is often mixed with food and eaten, or placed in burning charcoal and inhaled (Audu

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1989). Our previous studies revealed that the methanol extract of *Ficus platyphylla* stem bark is safe in rodents (Chindo et al., 2012) with potential central nervous system activity, analgesic and anti-inflammatory properties (Amos et al., 2002; Chindo et al. 2003). We have examined and reported the psychopharmacological and anticonvulsant effects of the saponins rich faction (Chindo et al., 2008, 2009); and the HPLC fingerprint, the intraperitoneal LD_{50}, the behavioral and anticonvulsant properties of the standardized and relatively safer methanol extract of *Ficus platyphylla* stem bark (Chindo et al., 2014) to scientifically describe the potential values of this important medicinal plant in the Nigeria's folk medicine for the management of CNS disorders. The preliminary phytochemical screening revealed the presence of saponins, flavonoids and tannins (Chindo et al., 2014). Flavonoids, coumarins and 1, 4-dimethylbenzenedicarboxylic acid ester had earlier been isolated and characterized from this important medicinal plant (Sayed et al., 1986; Sayed et al., 1991; Chindo et al., 2011). In this study, we examined the ameliorative effects of the standardized methanol extract of *Ficus platyphylla* on seizure severity, cognitive deficits and neuronal cell loss in pentylenetetrazole kindled mice in order to complement our existing knowledge on the antiepileptic potential of FP. The ^{35}S GTPγS, glutamate and GABA receptor binding properties of FP were also studied.

Materials and methods

Plant material

The plant material was collected, identified, authenticated, chopped, cleaned, air dried, milled and extracted; and the phytochemical screening and the HPLC analysis conducted as described previously (Chindo et al., 2014).

Animals

The animals used were male CD-1 [Crl: CD1 (ICR)] Mice (24 – 29 g), obtained from Charles River, Sulzfeld, Germany. Animals were housed in groups of 8 in Macrolon III cages padded with wood shavings, under standard conditions of temperature (20 ± 2 (C) and 12/12 h light/dark cycle; and fed with commercial pellets (ssniff R/M-H, ssniff Spezialdiäten GmbH, Soest, Germany) and tap water *ad libitum*. The mice were aged 8 – 9 weeks at the beginning of the experiments. All experiments were performed between 8:00 am and 3:00 pm. Injection volume was 10 ml/kg body weight. The work was conducted in accordance with EC regulations and those of the National Act on the use of Experimental Animals (Germany). The experimental protocol was approved by the Saxony-Anhalt Committee on Animal Care. All efforts were made to minimize the number of mice used and their suffering.

Kindling induction

Kindling is an accepted model for the study of the underlying processes of epileptogenesis, epilepsy-related alterations of behavior; and to test potential antiepileptic drugs (Löscher 2002b; Bertram, 2007). The animals were kindled with a total of 13 convulsant injections of an initial subeffective dose of pentylenetetrazole (PTZ, Carl Roth, Karlsruhe, Germany). This subeffective dose of PTZ (37.5 mg/kg, i.p.), is an ED_{16} related to clonic-tonic seizures that was established in a preceding experiment. Control animals received the same number of saline injections. The test system was suitable for detection of the potency of standard antiepileptic drugs like diazepam and phenytoin (Becker et al., 1991). The treatment groups concurrently received FP (100 and 200 mg/kg, i.p.). Whereas the lower dose of FP used in this study was found to be ineffective against acute PTZ seizures, the higher dose showed a significant anticonvulsant effect (Chindo et al., 2014). FP and saline (sal) were administered 30 min prior to PTZ and the convulsive behavior observed for 20 min after each injection. Injections were administered on Monday, Wednesday and Friday. The resultant seizures were classified according to a modified Racine scale as follows (Becker et al., 1995):

Stage 0: no response;
Stage 1: ear and facial twitching;
Stage 2: myoclonic jerks without rearing;
Stage 3: myoclonic jerks, rearing;
Stage 4: turning over into side position, clonic-tonic seizures;
Stage 5: turning over into back position, generalized clonic-tonic seizures.

One week after the last PTZ kindling injection, the mice received a challenge dose of PTZ (32.5 mg/kg, i.p.) to check the persistence of enhanced susceptibility to the convulsant. Resultant seizures were scored as mentioned above.

Learning performance

Mice were tested for learning performance in a two-way shuttle-box, 24 h after the final kindling injection, when the drugs were expected to have been cleared. The computer-controlled automatic shuttle box (TSE, Bad Homburg, Germany) was located in a sound-attenuating enclosure ventilated by an extractor fan. The shuttle box is divided into two compartments ($13 \times 15 \times 10\,cm^3$) separated by a 4 cm hurdle. The conditioned stimuli (CS) were light (40 W bulbs located on the central ceiling of each compartment) and a sound produced by a buzzer (90 dB). The unconditioned stimulus (UCS) was an electric foot shock of 0.1 – 0.4 mA (50 Hz, impulse widths 10 ms, pulsatile direct current, rectangular pulses), depending on the individual sensitivity of the animals and below vocalisation threshold, delivered through stainless-steel rods on the floor of the apparatus. The interval between the conditioned stimuli and the unconditioned stimulus was 4 s; and each trial was limited to 20 s if the animal failed to react earlier. If the animal did not avoid or escape from the compartment where the shock was applied, the trial was repeated after a 30 s interval. Intertrial intervals lasted for randomised periods of 15 – 45 s. Each session consisted of 30 trials (avoidance reactions or escape reactions) and was repeated on four consecutive days. Sessions were performed during the light part of the 12:12 h cycles at about the same time ±1 h. Prior to the first session, the mice were allowed to explore the box for 5 min, and on the following days 1 min was provided. The number of escapes refers to crossing into the other compartment when the CS and the UCS are presented simultaneously, reaction time 4 – 20 s, while the conditioned avoidance reactions refers to the actual learning, moving into the other compartment by recognising the CS alone, reaction time <4 s. The inter trial crossings were also recorded for evaluation.

Histology

The animals were processed for histological examination 24 h after challenged experiment. The mice were anasthetized with isoflurane and decapitated. The brains were removed, frozen in 2-methylbutane (−45 °C, Carl Roth, Karlsruhe, Germany) and placed in a −70 °C freezer until cutting. Prior to cutting the brains were embedded in OCT compound (Jung, Nussloch, Germany). Using a Cryo Cut CM3050 (Leica, Bensheim, Germany), sections of 10 μm thick were cut in the plane of the nucleus habenulae. The sections were stained with toluidine blue and embedded in DPX (Sigma-Aldrich, Taufkirchen, Germany). Cells in hippocampal CA1and CA3 were counted in squares of 500 μm × 500 μm using a counting net. There was an average of five fields in the left and right

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