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Anticonvulsant activity of *Pseudospondias microcarpa* (A. Rich) Engl. hydroethanolic leaf extract in mice: The role of excitatory/inhibitory neurotransmission and nitric oxide pathway

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Diazepam (PubChem CID: 3016)
Pentylenetetrazole (PubChem CID: 73422)
Picrotoxin (PubChem CID: 5311359)
Isoniazid (PubChem CID: 3767)
Strychnine (PubChem CID: 441079)
4-AP (PubChem CID: 1727)
L-NAME (PubChem CID: 39836)
L-Arginine (PubChem CID: 6322)
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ABSTRACT

Ethnopharmacological relevance: *Pseudospondias microcarpa* (A. Rich) Engl. is a plant used for managing various diseases including central nervous system disorders.
Aim of the study: This study explored the anticonvulsant activity of *P. microcarpa* hydroethanolic leaf extract (PME) as well as possible mechanism(s) of action in animal models.
Methods: Effects of PME was assessed in electroconvulsive (the maximal electroshock and 6-Hz seizures) and chemoconvulsive (pentylenetetrazole-, picrotoxin-, isoniazid-, 4-aminopyridine-, and strychnine-induced seizures) models of epilepsy. In addition, effect of the extract on the nitric oxide pathway and $GABA_A$ receptor complex was evaluated.
Results: The extract (30, 100 and 300 mg kg^{-1}, *p.o.*) significantly delayed the onset as well as decreased the duration and frequency of pentylenetetrazole-, picrotoxin- and strychnine-induced seizures. In addition, PME pre-treatment significantly improved survival in the 4-aminopyridine- and isoniazid-induced seizure tests. Furthermore, the extract protected against 6-Hz psychomotor seizures but had no effect in the maximal electroshock test. The anticonvulsant effect of PME (100 mg kg^{-1}, *p.o.*) was also reversed by pre-treatment with flumazenil, L-arginine or sildenafil. However, L-NAME or methylene blue (MB) augmented its effect.
Conclusion: Results show that PME has anticonvulsant activity and may probably be affecting GABAergic, glycinergic, NMDA, K^+ channels and nitric oxide-cGMP pathways to exert its effect.

1. Introduction

Epilepsy, a brain disorder, is characterized by occurrence of more than one seizure with a persistent predisposition to generate subsequent epileptic seizures. This is associated with neurobiological, cognitive, psychological, and social disturbances (Raol and Brooks-Kayal, 2012). Epilepsy is the second most common neurological disorder, with 0.5% prevalence, and a 2–3% life time risk of being diagnosed of it (Browne and Holmes, 2001; Löscher, 2002a). More than 80% of people with epilepsy live in developing countries, where

Abbreviations: AED, Antiepileptic drug; CNS, Central Nervous System; PME, Pseudospondias microcarpa extract; CBZ, Carbamazepine; FMZ, Flumazenil; VPA, Valproic acid; KNUST, Kwame Nkrumah University of Science and Technology; PTZ, Pentylenetetrazole; PTX, Picrotoxin; Hz, Hertz; GABA, Gamma amino butyric acid; NO, Nitric oxide; 4-AP, 4-aminopyridine; STN, Strychnine; INH, Isoniazid; cGMP, cyclic GMP; cAMP, cyclic AMP; sGC, soluble guanylyl cyclase; PDE5, Phosphodiesterase V; L-NAME, L-Nitro Arginine Methyl Ester; MB, Methylene Blue; DZP, Diazepam; NOS, Nitric oxide synthase; ANOVA, Analysis of variance; GAD, Glutamic acid decarboxylase; L-ARG, L-Arginine; BZD, Benzodiazepine; NMDA, N-Methyl D-Aspartate; MES, Maximal electroshock seizure

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the condition remains largely undiagnosed, poorly untreated and hence results in a poor prognosis (de Boer et al., 2008).

Despite the availability of many antiepileptic drugs (AEDs), nearly one in three patients with epilepsy who have access to current AEDs show less than satisfactory prognosis and a similar proportion experience unacceptable AED-related adverse effects (Brodie, 2005; Löscher, 2002b). Thus, the need to source for clinically efficacious and safer AEDs with improved clinical profiles is still valid. Plant extracts are attractive sources of new drugs, and have shown to produce promising results for the treatment of epilepsy. Examples of plants that have shown promise as a source of good pharmacological properties include: *Passiflora incarnate* (LINN.), *Berberis vulgaris* (LINN.), *Butea monosperma* (Lam.) Taub. and *Cymbopogon winterianus* (Jowitt.) (Bhutada et al., 2010; Kasture et al., 2002; Nassiri-Asl et al., 2007; Quintans-Junior et al., 2008).

Pseudospondias microcarpa has been extensively used in Ghana and other parts of Africa as medication for different diseases. The plant is suspected of having a sedative effect on those who sit or sleep under it, hence the Ghanaian name *katawani*, literally meaning "close your eyes". It is therefore used traditionally as a sedative and for treating general central nervous system (CNS) disorders (Burkill, 1985). Preliminary studies from our laboratory showed that the hydroethanolic leaf extract of *P. microcarpa* (PME) possesses sedative effects (Adongo et al., 2014), confirming the traditional use of the plant. Moreover, in this study, we indicated the presence of some phytochemical constituents which were reported earlier (Yondo et al., 2009). The antioxidant (Yondo et al., 2009), antimicrobial (Kisangau et al., 2008), cytotoxic and antiplasmodial (Malebo et al., 2009) effects of the plant have also been reported. We also showed in our preliminary studies that PME protected against convulsions induced by pentylenetetrazole (PTZ) (Adongo et al., 2014). Therefore, this study further explored the anticonvulsant activity of PME and possible mechanism(s) in mice models.

2. Materials and methods

2.1. Collection of plant material and extraction

Fresh leaves of *P. microcarpa* were collected from the campus of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi (6° 40.626′N, 1° 34.041′W). The plant was authenticated at the Department of Herbal Medicine, KNUST, Kumasi, Ghana. A voucher specimen (KNUST/HM1/2013/L005) was subsequently kept at the herbarium of the Faculty. Leaves of the plant were room-dried for seven days and pulverised into fine powder. The powder was extracted by cold percolation with 70% (v/v) ethanol in water over a period of 72 h and the resulting extract concentrated into a syrupy mass under reduced pressure at 60 °C in a rotary evaporator. It was further dried in a hot air oven at 50 °C for 7 days and kept in a refrigerator for use with a yield of 20.5% (w/w). The crude extract is subsequently referred to as (*Pseudospondias microcarpa* extract*)* PME or simply, extract.

2.2. FT-IR analysis of crude extract

To identify the possible functional groups that may be present in the sample, a triplicate FT-IR (PerkinElmer UATR Two) spectra was generated and baseline corrected. The spectra between 400 and 1400 cm^{-1} is usually considered as the unique region for every compound/compound mixtures and hence can be used for identification and quality control.

2.3. Animals

Male ICR mice (20–25 g) were purchased from the Noguchi Memorial Institute for Medical Research, Accra, Ghana and kept in the vivarium of the Department of Pharmacology, KNUST. The animals were housed in groups of five (5) in stainless steel cages (34 cm×47 cm×18 cm) with soft wood shavings as bedding. Housing conditions of mice were as follows: controlled–temperature maintained at 24–25 °C, relative humidity 60–70%, and 12 h light-dark cycle. All mice had free access to food and water ad libitum. A period of at least one week for acclimatization to the laboratory environment was allowed. All laboratory procedures were conducted in accordance with accepted principles for laboratory animal use and care (NRC, 2010). Approval for this study was obtained from the Faculty Ethics Committee.

2.4. Drugs and chemicals

Pentylenetetrazole (PTZ), picrotoxin (PTX), 4-aminopyridine (4-AP), strychnine (STN), isoniazid (INH), N-nitro-L-arginine methyl ester (L-NAME), L-arginine (L-arg) and methylene blue (MB) (Sigma-Aldrich Inc., St. Louis, MO, USA); diazepam, DZP (INTAS, Gujarat, India); sildenafil, SIL (Pfizer, U.S.A); carbamazepine, CBZ (Tegretol®, Novartis, Basel, Switzerland); flumazenil, FMZ (Anexate®, Roche products Ltd., Herts, England); sodium valproate, VPA (Epilim®, Sonofi-Synthelabo Ltd-UK).

2.5. Pentylenetetrazole-induced seizures

Clonic convulsions was induced using Pentylenetetrazole (60 mg kg^{-1}, s.c.) according to methods described by Oliveira et al. (2001). Mice were divided into 7 groups (n=8) and received PME (30, 100 or 300 mg kg^{-1}, *p.o.*), diazepam (0.1, 0.3 or 1 mg kg^{-1}, i.p.) or vehicle (normal saline; 10 mL kg^{-1} i.p.) 30 min (i.p.) or 1 h (*p.o.*) before subcutaneous injection of pentylenetetrazole (PTZ), respectively. Immediately after subcutaneous injection of PTZ, animals were placed in Perspex-walled testing chambers (15 cm×15 cm ×15 cm) with a mirror angled at 45° below the floor of the chamber to allow a complete view of convulsive events, if present. The convulsive behaviour was captured with a camcorder placed at a favourable distance directly opposite to the mirror. Video outputs of each 30 min session was later scored using JWatcher™ Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sidney, Australia available at http://www.jwatcher.ucla.edu/.) for behavioural parameters including: latency, frequency and duration of clonic convulsions. The observed clonic seizures were characterized for the appearance of facial myoclonus, forepaw myoclonus and forelimb clonus. The ability of a drug/extract to reduce or prevent the seizures or delay/prolong the latency or onset of the clonic convulsions was considered as an indication of anticonvulsant activity.

2.6. Picrotoxin-induced seizures

Anticonvulsant testing method by Leewanich et al. (1996) was modified and adopted for this test. Briefly, mice were divided into 7 groups (n=8) and received PME (30, 100 or 300 mg kg^{-1}, *p.o.*), diazepam (0.1, 0.3 or 1 mg kg^{-1}, i.p.) or vehicle (normal saline; 10 mL kg^{-1} i.p.) 30 min (i.p.) or 1 h (*p.o.*) before the injection of picrotoxin (3 mg kg^{-1}, i.p.) respectively. The latency to, frequency and duration of clonic convulsions were recorded for 30 min.

2.7. Isoniazid-induced seizures

Mice were divided into seven groups (n=10) and received PME (30, 100 or 300 mg kg^{-1}, *p.o.*), vehicle or the standard drug diazepam (0.1, 0.3 or 1.0 mg kg^{-1}, i.p.). One hour (*p.o.*) or 30 min (i.p.) after administration of test compounds, animals were injected with isoniazid (300 mg kg^{-1}, s.c.). Thereafter, mice were observed for 120 min for characteristic behavioural signs, such as intermittent forelimb extension, clonic seizures, tonic seizures and death. The latencies to the