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Original Article

Anticonvulsant effect of ethanolic extract of *Cyperus articulatus* L. leaves on pentylenetetrazol induced seizure in mice

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ABSTRACT

Cyperus articulatus (CA) rhizomes have demonstrated different properties on nervous system. However, the leaves still have not studied to treat epilepsy. The aim of this study was to determine the effect of CA ethanolic extract on pentylenetetrazol (PTZ) induced seizures in mice as well as measuring its antioxidant activity *in vivo* and *in vitro*. Mice were divided into five groups: (1) control (PTZ 80 mg/kg; i.p.), (2) PTZ-Diazepam (1 mg/kg; i.p.), (3–5) PTZ-CA 50, PTZ-CA 150 and PTZ-CA 300 (50, 150 and 300 mg/kg of CA extract, 30 min prior to each PTZ injection). The PTZ-CA 150 group showed lower seizure scores ($P < 0.01$), latency ($P < 0.01$), frequency ($P < 0.01$) and duration ($P < 0.01$) than control group. The antioxidant activity of CA extract scavenged DPPH radical showed IC 50 = 16.9 ± 0.1 μg/mL and TEAC = 2.28 ± 0.08, mmol trolox/g of extract, the content of gamma amino butyric acid (GABA) and malondialdehyde (MDA) were significantly high ($P < 0.01$) at dose of 150 mg/kg (82 ± 1.2 ng/g tissue; 1.0 ± 2.2 mol/g tissue, respectively). The present research demonstrated that CA extract possesses a potential effect to prevent PTZ induced seizures, antioxidant activity in addition to increase GABA levels.

1. Introduction

Epilepsy is a chronic disease characterized by recurrent seizures and other complications, which has been demonstrated to cause oxidative stress by reactive oxygen species (ROS) in the brain.[1] Approximately, more than 50 million people worldwide have been diagnosed with epilepsy, representing a public health problem of all ages, genera and social groups. Medical reports have shown an incidence rate of epilepsy in developed countries of 50 per 100,000 otherwise in developing countries is 100 per 100,000.[2]

The modern concept of epilepsy proposed by the International League Against Epilepsy (ILAE) involves the occurrence of at least one or more epileptic seizures.[3] The etiology can be genetic or sporadic, by consequence, cerebral damage could be linked to congenital cortical abnormality, traumas, neurocysticercosis, infections or cerebral tumor. The pathophysiology of epilepsy is known by depolarization disorders from the neural membrane, ionic medium and neuronal morphology.[4,5] These alterations produce an imbalance of excitatory and inhibitory neurotransmitters like excitatory aminoacids, (glutamate or aspartate) or inhibitory neurotransmissions (gamma amino butyric acid – GABA).[6]

One of the experimental models for inducing epileptic seizures is pentylenetetrazol (PTZ), this chemical agent produces primary generalized discharges and acts as a noncompetitive agonist of $GABA_A$ receptors, also induces oxidative stress on the brain, altering the normal metabolism of phospholipids and proteins. Oxidative stress is an associated factor that leads to epilepsy.[7] Brain is extremely susceptive to an oxidative damage by free radicals, due to a high aerobic metabolism, blood perfusion and a significant concentration of polyunsaturated fatty acids compared with others organs with deficient antioxidant systems.[8]

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Several drugs are used by physicians to manage epilepsy. However, such drugs show serious side effects like ischemia, hepatotoxicity depression, cognitive disorders, and motor disability.[9] Additionally, 20–30% of those patients are resistant to treatments with synthetic drugs so, it is necessary to discover new treatments by using the natural medicine to reduce the complications or side effects of antiepileptic drugs.

Cyperus articulatus L. (Cyperaceae) commonly called "piri-piri" in Peru is a rhizome-bearing herb found in Africa, Latin America, Asia and Oceania. In Cameroon, Central Africa Republic, Gabon, and Senegal use the decoction of its rhizome for the treatment of headaches, migraines, etc. In addition, Indians of Amazonia use the plant in a similar way.[10] However, the leaves of this plant have not been studied, an experimental study based on previous studies was designed in order to determine the anticonvulsant effect of *C. articulatus* L leaves extract, using pentylenetetrazol as chemical inductor of seizures in mice and evaluate the gamma amino-butyric acid (GABA) and malondialdehyde (MDA) levels on mice brain.

2. Materials and methods

2.1. Animals

A total of 30 Balb/C albino mice (20–30 g) of male sex obtained from the National Institute of Health (Lima-Peru) were used in this study. Mice were placed in Plexiglas cage with access to pelletized food and water *ad libitum*, housed in animal room with controlled temperature (22–24 °C) and 12 h light/dark cycle. All mice were divided into five groups of six animals each, acclimatized to the laboratory 15 days previous to the experiments. The experimental protocol was carried out according to the guidelines established by the European Union on Animal Care (CCE Council 86/609), and approved by the Ethics Committee of the National Institute of Health (Reg. 21433-13; ET-060-13).

2.2. Plant collection

1 kg of "Piri-piri" leaves were collected from Pucallpa, Ucayali, Peru and kept in kraft paper to be transported to the laboratory. The botanical identification of the plant material was classified and a voucher specimen (13-2014-USM-MHN) was deposited at the National Herbarium of National University of San Marcos (UNMSM), Lima, Peru.

2.3. Preparation of extract

The extraction of *C. articulatus* leaves was carried out by using a maceration process with alcohol 96%. The extract was concentrated on a rotavap and lyophilized, finally 75 g of extract was obtained and stored at 4 °C until further studies.

2.4. Phytochemical screening

The extract of *C. articulatus* was analyzed for the presence of phytochemical constituents, such as alkaloids, terpenoids, quinones, flavonoids, saponins, steroids and phenolic compounds, with the standard qualitative phytochemical methods described.[11]

2.5. Drugs and chemicals

Pentylenetetrazol (PTZ), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Trolox, 2,2′-Azinobis-3-ethylbenzotiazilone-6-sulphonic acid (ABTS), (Sigma Chemical Co), Diazepam injection (Medifarma Pharmaceuticals). All other chemicals used were of analytical grade.

2.6. Antioxidant activity by DPPH assay

The antioxidant activity of extract and positive control (Trolox) were estimated, based on the method of Brand-Williams et al.[12] Aliquots (100 μL) of extract at various concentrations and Trolox were mixed with 900 μL of DPPH solution. The solution was shaken and stored at room temperature for 30 min in the dark. The absorbance of the reactive solution was measured by spectrophotometric method at 517 nm. The percentage of antioxidant activity was calculated according to the equation:

$$\%\ \text{antioxidant activity} = [(\text{ABS control} - \text{ABS sample})/\text{ABS control}] \times 100.$$

2.7. 2,2′-Azinobis-3-ethylbenzotiazilone-6-sulphonic acid ($ABTS^{\bullet+}$) assay

The assay was performed as previously described by Arnao et al.[13] The stock solution included 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution to react for 12 h at room temperature in the dark. The solution was diluted to obtain an absorbance of 0.7 ± 0.02 units at 734 nm. Extract (40 μL) was mixed with 1960 μL of ABTS to react for 7 min. UV/Vis spectrophotometer was blanked with distilled water, thus absorbance was recorded at 734 nm. Each assay was made by triplicated. The trolox equivalent antioxidant capacity (TEAC) was calculated as mmol trolox per gram of sample from a calibration standard curve with Trolox.

2.8. Anticonvulsant effect

Mice were randomly divided to five groups (n = 6) and treated according to the experimental protocol: Group I: Control group (distilled water); Group II: Standard group, reference drug (Diazepam, 1 mg/kg i.p.); Group III, IV, V: Test groups, *C. articulatus* extract (50, 150, 300 mg/kg p.o. respectively).

All the drugs were administered 30 min prior to the administration of PTZ (80 mg/kg, i.p.). Vehicle (distilled water; 10 mL/kg, p.o.) and diazepam (1 mg/kg, i.p.) were administered 15 min respectively before PTZ injection. The animals were placed in Plexiglas cage (20 × 20 × 20 cm) and observed for 30 min for latency of seizure onset, frequency of convulsions, mortality and duration of a seizure. The animals that survived after that period of time were considered to be protected. Furthermore, each seizure was classified according to a modified Racine scale as follows: 1-Mouth and facial movements; 2-Head nodding; 3-Forelimb clonus; 4-Rearing; 5-Rearing and falling.[14,15]

2.9. Biochemical parameters

Two biochemical parameters were determined by spectrophotometry in order to determine the effect of the extract on mice brain.

2.9.1. Estimation of GABA by spectrophotometry

GABA (gamma amino butyric acid) was determined from whole brain and was isolated immediately to be transferred to homogenization tube containing 5 mL of 0.01 M hydrochloric acid. Brain homogenate was transferred to bottle containing 8 mL of ice cold absolute alcohol and kept for 1 h at 0 °C. The content was centrifuged for 10 min at 16,000 rpm, supernatant was collected in petridish. Precipitate was washed with 5 mL of 75% alcohol for three times and washes were combined with supernatant. Next, samples were evaporated to dryness at 70 °C on water bath. To the dry mass

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