Biomedicine & Pharmacotherapy 101 (2018) 181–187

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

The anticonvulsant effect of a polysaccharide-rich extract from *Genipa americana* leaves is mediated by GABA receptor

Dayanne Terra Tenório Nonato[a], Silvânia Maria Mendes Vasconcelos[c], Mário Rogério Lima Mota[b], Paulo Goberlânio de Barros Silva[b], Arcelina Pacheco Cunha[d], Nágila Maria Pontes Silva Ricardo[d], Maria Gonçalves Pereira[a,e], Ana Maria Sampaio Assreuy[a], Edna Maria Camelo Chaves[a,*]

[a] Superior Institute of Biomedical Sciences, State University of Ceara, Av. Dr. Silas Munguba, 1700, Itaperi, 60714-903, Fortaleza, CE, Brazil
[b] Department of Oral Pathology and Clinical Stomatology of Federal University of Ceara, Rua Coronel Nunes Valente, 1127, Rodolfo Teófilo, 607430-970, Fortaleza, CE, Brazil
[c] Departament of Physiology and Pharmacology, Federal University of Ceara, Rua Coronel Nunes Valente, 1127, Rodolfo Teófilo, 607430-970, Fortaleza, CE, Brazil
[d] Department of Organic and Inorganic Chemistry, Federal University of Ceara, Rua Humberto Monte, S/N, Campus de PICI, 60440554, Fortaleza, CE, Brazil
[e] Faculty of Education Science and Letters of the Hinterland, Rua José de Queiroz Pessoa, 2554 – Planalto Universitário, 63.900-000, Quixadá, CE, Brazil

ARTICLE INFO

Keywords:
Plant polysaccharide
"Jenipapo"
Seizures
Black neurons
Brain oxidative stress

ABSTRACT

Background: This study aimed to chemically characterize a polysaccharide-rich extract (PRE) obtained from *Genipa americana* leaves and evaluate its neuroprotective effect in the brain morphology and oxidative markers using mice behavioral models.

Methods: Dry powder (5 g) of *G. americana* leaves were submitted to depigmentation in methanol. PRE was obtained by extraction in NaOH and precipitation with absolute ethanol and characterized by infrared spectroscopy (FTIR) and nuclear magnetic resonance (^{1}H and ^{13}C NMR). Swiss mice (25–35 g) received saline (0.9% NaCl) or PRE (1–27 mg/kg) by intraperitoneal (i.p.) route, 30 min before evaluation in behavioral models (open field, elevated plus maze, sleeping time, tail suspension, forced swimming, seizures induced by pentylenetetrazole-PTZ). Animal's brain were dissected and analyzed for histological alterations and oxidative stress.

Results: FTIR spectrum showed bands around 3417 cm^{-1} and 2928 cm^{-1}, relative to the vibrational stretching of O—H and C—H, respectively. ^{1}H NMR spectrum revealed signals at δ 3.85 (methoxyl groups) and δ 2.4 (acetyl) ppm. ^{13}C NMR spectrum revealed signals at δ 108.0 and δ 61.5 ppm, corresponding to C1 and C5 of α-L-arabinofuranosyl residues. PRE presented central inhibitory effect, increasing the latency for PTZ-induced seizures by 63% (9 mg/kg) and 55% (27 mg/kg), and the latency to death by 73% (9 mg/kg) and 72% (27 mg/kg). Both effects were reversed by the association with flumazenil.

Conclusions: PRE, containing a heteropolysaccharide, presents antioxidant and anticonvulsant effect in the model of PTZ-induced seizures via gamma-aminobutyric acid (GABA), decreasing the number of hippocampal black neurons.

1. Introduction

Epilepsy is the second most common neurological disorder after stroke, affecting from 0.5% to 1% individuals at all ages of the world population [1]. The progression of epilepsy underlies a sequential cascade of events that includes cognitive impairment, accompanied by abnormal behavior and generation of reactive oxygen species in the brain, considered one of the leading causes of generalized epilepsy associated with recurrent seizures [2]. Furthermore, formation of dark neurons and morphological changes may occur depending on duration and intensity of seizures [3].

Pentylenetetrazole (PTZ) is widely accepted as an experimental animal model for the investigation of epileptogenesis and also to test the effectiveness of antiepileptic drugs [4]. Administration of PTZ by

Abbreviations: NaOH, sodium hydroxide; PTZ, pentylenetetrazole; PRE, polysaccharide-rich extract; NMR, nuclear magnetic resonance; GABA, gamma-aminobutyric acid; H, hydrogen; C, carbon; FTIR, infrared spectroscopy

* Corresponding author.
E-mail addresses: dayanne.nonato@aluno.uece.br (D.T.T. Nonato), silvania@pesquisador.cnpq.br (S.M.M. Vasconcelos), mariolmota@ufc.br (M.R.L. Mota), coordodontopesquisa01@unichristus.edu.br (P.G. de Barros Silva), arcelinapacheco@yahoo.com.br (A.P. Cunha), naricard@ufc.br (N.M.P.S. Ricardo), maria.pereira@uece.br (M.G. Pereira), ana.assereuy@uece.br (A.M.S. Assreuy), edna.chaves@uece.br (E.M.C. Chaves).

https://doi.org/10.1016/j.biopha.2018.02.074
Received 9 January 2018; Received in revised form 31 January 2018; Accepted 19 February 2018

intravenous or intraperitoneal routes enhances excitatory neuronal responses in the central nervous system via blockade of the inhibitory responses of gamma amino butyric acid (GABA) [5].

The current pharmacological treatment of epilepsy often fails, being in most cases palliative, achieving the effective symptoms relief in about two-third of patients. Additionaly, numerous adverse effects are manifested, such as drowsiness, amnesia, tolerance and physical dependence [6]. In this context, biomolecules of plant origin could be considered as an alternative therapy.

The literature has been reported neuroprotective effects of plant polysaccharide extracts, such as antidepressant [7], antioxidant [8], anticonvulsant [9], also in cognitive impairment [10] and cerebral ischemic injury [11].

Genipa americana L. (Rubiaceae), "jenipapo" or "jenipapeiro", is widely distributed in Brazil, being its macerated leaves traditionally used by some native tribes to treat fever [12]. Phytochemical analysis of the extract from *G. america* leaves revealed the presence of flavonoids, among others [13], compounds recognized for their antioxidant effects [14,15].

The objective of this study was to chemically characterize the polysaccharide-rich extract obtained from *G. americana* leaves and evaluate its neuroprotective effects in the brain morphology and oxidative markers using mice behavioral models.

2. Materials and methods

2.1. Collection of G. americana leaves and polysaccharide extraction

Leaves were collected at Custodio-Quixada, Ceara (Brazil) and a voucher specimen (n° 46794) was deposited in the Herbarium Prisco Bezerra of Federal University of Ceará, Brazil. For polysaccharide extraction, leaves were dried at 40 °C and grounded into powder (5 g), which was suspended in absolute methanol (1:50, w/v), homogenized (2 h, 70 °C) and filtered (procedure repeated 2×) for partial remotion of methanol-soluble material. Residue was suspended at 0.1 M NaOH (1:50 w/v), homogenized (2 h, 97 °C) and centrifuged (5421 × *g*, 30 min, 25 °C) (procedure repeated 3×). Supernatant S1 (highly pigmented) was discarded and supernatants S2 and S3 were pooled, neutralized with 1 M HCl, precipitated with four volumes of 96% ethanol (24 h, 4 °C) and centrifuged (5241 × *g*, 30 min, 25 °C). The pellet was dialyzed against distilled water for 48 h, re-centrifuged and the lyophilized supernatant, containing 54.6% total carbohydrates (including 21.1% uronic acid) and 12% protein [16] was named PRE (polysaccharide-rich extract; 6.5% yield).

2.2. Polysaccharide chemical characterization

Infrared spectra (FTIR) was carried out on IR Prestige-21- Shimadzu spectrophotometer in the wave region of 4000–400 cm^{-1}, being the polysaccharide sample mixed and grounded with KBr (1:80). The spectrum was obtained from 120 scans at 4 cm^{-1}. The Nuclear Magnetic Resonance (NMR) spectra was obtained in a Brucker Avance-DRX 500 spectrometer equipped with an inverse detection probe and z-gradient accessory working at 499.9 (^{1}H) and 125 MHz (^{13}C), respectively. Approximately 30 mg of PRE were dissolved in 800 µl D_2O and sonicated for 20 min. For dimensional ^{1}H data, 120 transients were collected at 70 °C (2 s acquisition time, 20 ppm spectral window, 32 k data points). ^{1}H chemical shifts were referenced to residual D_2O at δ 4.78 ppm. For the one-dimensional ^{13}C spectrum, 20 k transients were collected at 70 °C (1 s acquisition time, 130 ppm spectral window, 32 k data points).

2.3. Drugs

Thiopental, diazepam (DZP), flumazenil (FLU), imipramine (IMP) and pentylenetetrazole (PTZ) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and solubilized in sterile saline (0.9% NaCl).

2.4. Animals

Male Swiss mice (25–30 g) were provided by the Central Animal House from the Federal University of Ceará -Brazil, maintained with free access to food and water, at 22 ± 2 °C and 12 h light-dark cycle and allowed to adapt to the laboratory for at least 1 h before experiments. The experimental protocols were performed during the light phase of the cycle, following the ethical standards in Directive 86/609/EEC, "European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes", 1986, in accordance with the Brazilian College of Animal Experimentation (COBEA) and the Ethics Committee for Animal Use (CEUA/UECE N°. 2451142/2014).

2.5. Experimental design

Animals (8–10) received saline (0.9% NaCl) or PRE (1–27 mg/kg) by intraperitoneal (i.p.) route, 30 min before evaluation in behavioral models (open field, elevated plus maze, sleeping time, tail suspension, forced swimming). After euthanasia, the entire brain was removed for histological analysis and dissected (prefrontal cortex -PFC, hippocampus – HC, striatum -ST) for oxidative stress evaluation.

2.6. Open field

Mice were individually placed in the open-field apparatus consisting of an acrylic box (30 × 30 × 15 cm) with the floor divided into 9 squares. The number of rectangles crossed with all paws (crossing) was counted during 6 min [17]. Animals received saline, PRE or the sedative drug diazepam (2 mg/kg; i.p.) 30 min before evaluation.

2.7. Elevated plus maze

Mice were placed in the center of the high-labyrinth test consisting of two opposing open arms (30 × 5 × 25 cm) and two closed arms (30 × 5 × 25 cm). The number of entries and the time spent on the open and closed arms were registered during 5 min. The increase in the parameters observed in the open arms reveals anxiolytic effect [18]. Animals received saline, PRE or the anxyolitic drug diazepam (1 mg/kg; i.p.) 30 min before evaluation.

2.8. Tail suspension

Mice were suspended 50 cm from the ground by a tape fixed at 1 cm from the tail tip and the immobility time was recorded during 5 min [19]. The decrease in immobility time reveals antidepressant activity. Animals received saline, PRE or the antidepressant drug imipramine (10 mg/kg; i.p.) 30 min before evaluation.

2.9. Forced swimming

Mice were placed individually in acrylic cylinders (35 cm × 24 cm) containing 13.5 cm of water for observation of immobility time during 5 min. Immobility is considered when the animals perform minimal movements necessary to keep his head out of the water [20]. The decrease in the immobility time reveals antidepressant effect. Animals received saline, PRE or the antidepressant drug imipramine (10 mg/kg; i.p.) 30 min before evaluation.

2.10. Sleep time

Mice received thiopental sodium (40 mg/kg; i.p.) and were left to sleep for observation of the sleep time (the corresponding time between righting reflex loss and recovery) and latency time [21]. The loss of