EuroSciCon Conference on Pediatrics

Anticonvulsant and anti-inflammatory effects of the polysaccharide rich extract from *Genipa americana* leaves

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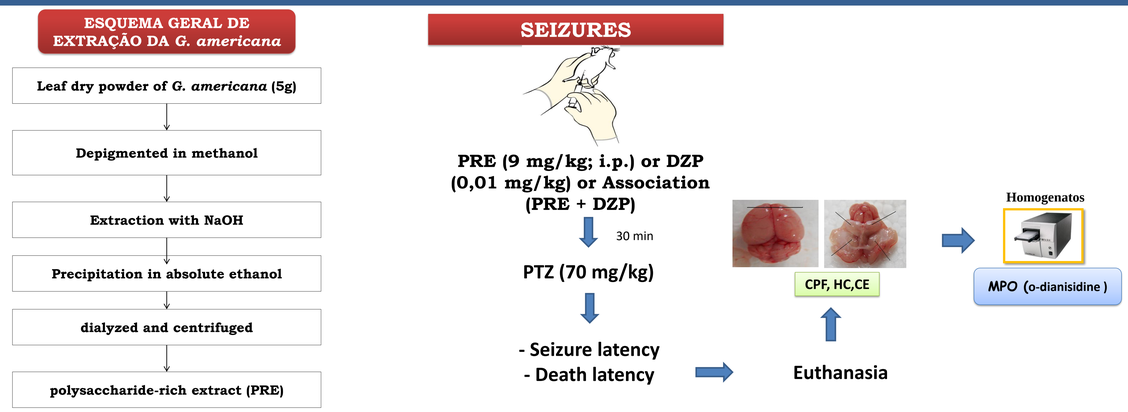
Abstract

Plant polysaccharides present some activities involving the central nervous system, such as neuroprotective, antidepressant, antioxidant and anti-inflammatory. We aim to evaluate the anticonvulsant and anti-inflammatory effects of the polysaccharide rich extract from *G. americana* leaves in mice. The leaf dry powder (5 g) was depigmented in methanol and the polysaccharide-rich extract (PRE) obtained by extraction with NaOH followed of precipitation in absolute ethanol. PRE was dissolved in 0.9% NaCl and administered (9 mg/kg) in male Swiss mice (25-35 g) by intraperitoneal (i.p.) route, 30 min before seizures induced by a single dose of pentylenetetrazole (PTZ: 70 mg/kg, i.p), n=7/group. The synergism of PRE effect was evaluated by its association with diazepam (DZP: 0.01 mg/kg). After euthanasia, the prefrontal cortex (CPF), hippocampus (HC) and striatum (EC) were removed for the quantification of myeloperoxidase levels (MPO) by o-dianisidine method. Experimental protocols was approved by Animal Ethics Committee (UECE Nº 2451142/2014). The PRE increased the seizure latency (9 mg/kg: 171,7 ± 29,62 *versus* saline: 62.00 ± 4,709 s) and death latency (9 mg/kg: 597.4 ± 101,5 *versus* saline: 150.0 ± 14.52). The association of PRE with diazepam potentiated the protective effect of DZP, increasing seizure latency (DZP: 128,3 ± 24,62 *versus* PRE + DZP: 222.4 ± 47.57), without altering in death latency. MPO levels was reduced in hippocampus (PRE: 34.24 ± 7.167, DZP: 42.27 ± 9.559 and DZP + PRE: 31.26 ± 5.726 *versus* saline + PTZ: 81.91 ± 11.70) and striatum (PRE: 17,89 ± 3,310, DZP + PRE: 18.69 ± 3.776 *versus* saline + PTZ: 37.27 ± 5.169). However there was no difference between groups (DZP, PRE or DZP + PRE) in each brain area. We conclude that PRE of *G. americana* leaves protects against seizures and promote anti-inflammatory effects in brain.

Introduction

Epilepsy is the second most common neurological disorder, affecting from 0.5% to 1% individuals at all ages of the world population (Trinka et al., 2010). The current pharmacological treatment of epilepsy often fails, being in most cases palliative (Bidwell et al., 2010). The experimental animal model for the investigation the effectiveness of antiepileptic drugs was Pentylenetetrazole (PTZ) that widely accepted (Fisher, 1989). In this context, biomolecules of plant origin could be considered as an alternative therapy. Plant polysaccharides present some activities involving the central nervous system, such as neuroprotective, antidepressant, antioxidant and anti-inflammatory. Nonato, 2018 identified a heteropolysaccharide in the polysaccharideric extract (PRE) obtained from *G. americana*, that possesses central inhibitory effect, anticonvulsant and antioxidant activity. The objective of this study was evaluate the synergism when associating with diazepam and anti-inflammatory effects of the polysaccharide rich extract from *G. americana* leaves in mice.

Methods and Materials



Results

The PRE increased the seizure latency (9 mg/kg: 171,7 ± 29,62 *versus* saline: 62.00 ± 4,709 s) and death latency (9 mg/kg: 597.4 ± 101,5 *versus* saline: 150.0 ± 14.52). The association of PRE with diazepam potentiated the protective effect of DZP, increasing seizure latency (DZP: 128,3 ± 24,62 *versus* PRE + DZP: 222.4 ± 47.57), without altering in death latency.
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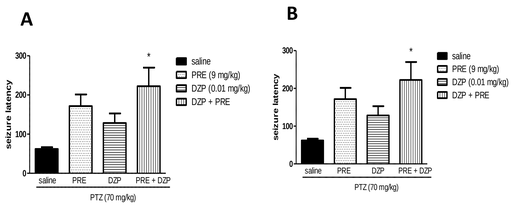

Fig. 1 PRE protects PTZ-induced seizures. Mice received i.p. 0.9% NaCl (Saline), PRE, DZP or association 30 min before evaluation. (A) seizure latency (B); death latency. Mean ± SEM (n=8). ANOVA and Newman-Keuls test.*p < 0.05 vs. Saline. PRE: polysaccharide-rich extract of *G. americana*; DZP: diazepam

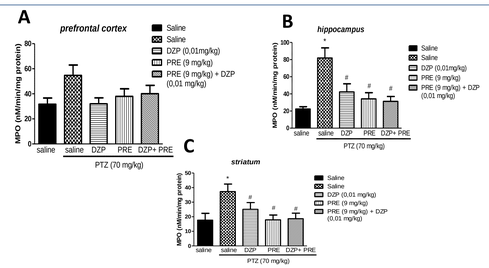

Fig. 2 PRE reduced MPO levels PTZ-induced. Mice received i.p. 0.9% NaCl (Saline), PRE, DZP or association 30 min before evaluation. (A) MPO levels in prefrontal cortex; (B) MPO levels in hippocampus; (C) MPO levels in striatum Mean ± SEM (n=8). ANOVA and Newman-Keuls test.*p < 0.05 vs. Saline. PRE: polysaccharide-rich extract of *G. americana*; DZP: diazepam

Discussion

- Epilepsy is a chronic neurological disease with significative impact on life quality of patients. In general, drugs used to control this condition possessing central inhibitory effects and produce sedation as its major adverse effect (Leão et al., 2015).
- The epileptogenesis induced by PTZ is a great model to study the new therapeutic drugs engaged in epilepsy and with a few adverse effects (Dhir, 2012).
- Natural products, such as plant polysaccharides, are a good alternative source of different substances with inhibitory action on central nervous system.
- Here, we showed, for the first time, the anticonvulsant effect of *G. americana* in PTZ mice model. Additionally, we observed that this effect is caused in part by its anti-inflammatory action.
- In fact, the injection of *G. americana* increased the seizure and death latency, besides reducing the MPO levels in hippocampus and striatum.
- MPO is an enzyme present in neutrophils and its mensuration is an indirect toll to evaluate migration of these cells to focus of inflammation (Hickey et al., 2011).
- A single injection of PTZ induces the blood-brain barrier (BBB) disruption and neutrophils infiltration, which contributes to epileptogenesis (Lenz et al., 2014) .
- The anti-inflammatory action of *G.americana* could be attributed to flavonoids present in its composition (Alves et al., 2017), which can be investigated in future studies.

Conclusions

We conclude that PRE of *G. americana* leaves protects against seizures and promote anti-inflammatory effects in brain.

Future Directions

More investigations can be carried out to elucidate the action mechanisms of *G. americana* in the protection of seizures, such as cytokine levels on central nervous system and oxidative stress.

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Acknowledgements

Sponsor's names: (CNPq/ CAPES/FUNCAP)