Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Ethnopharmacology 100 (2005) 145–152
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Antihemorrhagic, antinucleolytic and other antiophidian properties of the aqueous extract from *Pentaclethra macroloba*

Jocivânia O. da Silva[a,1], Juliana S. Coppede[b,1], Vanessa C. Fernandes[b,1], Carolina D. Sant'Ana[a,1], Fábio K. Ticli[a], Maurício V. Mazzi[a], José R. Giglio[c], Paulo S. Pereira[b], Andreimar M. Soares[a,*], Suely V. Sampaio[a,*]

[a] *Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, FCFRP/USP, Brazil*
[b] *Unidade de Biotecnologia, Universidade de Ribeirão Preto, UNAERP, Ribeirão Preto-SP, Brazil*
[c] *Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, USP, Ribeirão Preto-SP, Brazil*

Received 3 November 2004; received in revised form 3 November 2004; accepted 31 January 2005
Available online 20 April 2005

Abstract

Several Brazilian plants have been utilized in folk medicine as active agents against various effects induced by snake venoms. The inhabitants of the Amazon region use, among others, the macerated bark of a plant popularly named "Pracaxi" (*Pentaclethra macroloba* Willd) to combat these effects. We report now the antihemorrhagic properties against snake venoms of the aqueous extract of *Pentaclethra macroloba* (EPema). EPema exhibited full inhibition of hemorrhagic and nucleolytic activities induced by several snake venoms. Additionally, partial inhibition of myotoxic, lethal, phospholipase and edema activities of snake venoms and its isolated PLA_2s by EPema is reported. In vivo tests showed that EPema is able to totally inhibit a *Bothrops jararacussu* metalloprotease (BjussuMP-I) induced hemorrhage, suggesting interaction of the extract compounds with this high molecular weight protein. The extract did induce neither hemorrhage nor death in mice when administered alone by i.m. route. When administered separately by i.m. route, the extract did not induce death in mice at 12.5–300 mg/kg doses. Other assays demonstrated that EPema was unable to inhibit fibrinogenolytic and coagulant activities of *Bothrops atrox* venom. Although the mechanism of action of EPema is still unknown, the finding that no visible change was detected in the electrophoretic pattern of snake venom after incubation with the extract excludes proteolytic degradation as a potential mechanism. The search for new inhibitors of venom metalloproteases and DNAases are a relevant task. Investigation of snake venom inhibitors can provide useful tools for the elucidation of the action mechanisms of purified toxins. Furthermore, these inhibitors can be used as molecular models for development of new therapeutical agents in the treatment of ophidian accidents.

Keywords: *Pentaclethra macroloba*; Antiophidian Brazilian plants; Antihemorrhagic; Antinucleolytic activity; Antimyotoxic; Antilethal; *Bothrops*; Snake venoms

Abbreviations: Batx, *Bothrops atrox* venom; Basp, *Bothrops asper* venom; Balt, *Bothrops alternatus* venom; Bjar, *Bothrops jararaca* venom; Bjussu, *Bothrops jararacussu* venom; BjussuMP-I, *Bothrops jararacussu* metalloprotease I; Bmoo, *Bothrops moojeni* venom; Bneu, *Bothrops neuwiedi* venom; Bpir, *Bothrops pirajai* venom; Catx, *Crotalus atrox* venom; Cdt, *Crotalus durissus terrificus* venom; Crho, *Calloselasma rhodostoma* venom; CK, creatine kinase; EPema, aqueous extract of *Pentaclethra macroloba*; PBS, phosphate buffered saline

* Corresponding authors. Tel.: +55 16 603 6706; fax: +55 16 633 1936.
E-mail addresses: andreims@fcfrp.usp.br (A.M. Soares), suvilela@fcfrp.usp.br (S.V. Sampaio).
[1] These authors contributed equally to this work.

0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.01.063

1. Introduction

Snakebite envenomations constitute a relevant public health hazard in Latin America. Most accidents are inflicted by species of the genus *Bothrops*, although envenomations by *Crotalus* sp. occur throughout the region, having particular relevance in South America, where they are usually associated with severe systemic effects (Azevedo-Marques et al., 1987).

Snake venoms are complex mixtures of proteins, including phospholipases A_2 (PLA_2s), myotoxins, hemorrhagic metalloproteases and other proteolytic enzymes, cytotoxins, cardiotoxins and others (Gutiérrez, 2002; Rosenberg, 1990; Soares et al., 2004a). The pathophysiology of snake envenomations involves a complex series of events that depend on the combined action of these venom components (Ownby, 1998).

Snake venom hemorrhagic components are zinc-dependent metalloproteases that display pronounced local hemorrhage and tissue damage. Despite the fact that the precise mechanism of action of hemorrhagic metalloproteases has not been fully elucidated, several studies have been described that enzymatic degradation of basement membrane components of capillary vessels is a key step. Catalytic function of most metalloproteases depends on a zinc ion in their active site. In addition, several zinc-peptidases enzymes, including thermolysin, astacins, serralysins, adamalysins and matrixins families contain structural characteristics topologically conserved in these molecules (Stöcker et al., 1995).

In human accidents, *Bothrops* venoms cause local tissue damage, as well as hemorrhage, proteolysis, myonecrosis and edema. Muscle necrosis is an important local effect induced by several snake venoms, sometimes resulting in an irreversible loss of tissue and occasionally requiring amputation of the affected limb. Myonecrosis may be due to an indirect action as a consequence of vessel degeneration and schemia caused by hemorrhagic metalloproteases or by a direct effect of myotoxic PLA_2s homologues upon plasma membranes of muscle cells (Gutiérrez and Lomonte, 1997; Soares et al., 2004a). *Bothrops atrox* represents a serious medical problem in the Brazilian Amazon region. More than 90% of snakebite patients receiving treatment in Manaus (Amazon State, Brazil), were bitten by *Bothrops atrox* specimens.

The search for natural venom inhibitors is substantial to complement the traditional therapy, particularly regarding treatment of local tissue damage. Medicinal plant extracts, rich source of natural inhibitors and pharmacologically active compounds, have been shown to antagonize the activity of some venoms and toxins (Gowda, 1997; Martz, 1992; Mors et al., 2000; Soares et al., 2004b; Ticli et al., 2005). *Casearia sylvestris*, *Eclipta prostrata*, *Mandevilla* sp., *Mimosa pudica*, *Tabernaemontana catharinensis* and others are known to inhibit a variety of snake venom activities (de Almeida et al., 2004; Batina et al., 2000; Borges et al., 2000, 2001; Veronese et al., in press). The use of natural products, derived from plants and animals, in alternative therapy for anti-snake venoms drugs has been increased (Esmeraldino et al., 2005; Lizano et al., 2003; Mors et al., 2000; Soares et al., 2004b).

The use of plants to combat the effects of snakebites is spread out among Amazon population. Natives and "caboclos" (local people) use the macerated bark of *Pentaclethra macroloba* Willd, applied in the form of cataplasm, on the site of snakebite. In the present study, the ability of the aqueous extract of *Pentaclethra macroloba* (EPema) against the hemorrhagic and nucleolytic activities induced by snake venoms (*Bothrops* spp., *Lachesis muta*, *Crotalus atrox* and *Calloselasma rhodostoma*) and isolated metalloprotease was evaluated. Additionally, inhibition of myotoxic and lethal activities of *Bothrops atrox* venom by EPema was reported in this work.

2. Materials and methods

2.1. Materials

Swiss male mice (20–25 g) were used for biological assays. The crude lyophilized *Bothrops* spp. and *Crotalus durissus terrificus* venoms were obtained from Serpentário Bioagents, Batatais, SP and Instituto Butantan, São Paulo, SP, Brazil. *Crotalus atrox* and *Calloselasma rhodostoma* venoms were purchased from Sigma Chem. Co. (USA). *Bothrops jararacussu* BjussuMP-I and BthTXs were isolated as previously described (Andrião-Escarso et al., 2000; Mazzi et al., 2004). Normal human plasma from healthy donors was obtained from the blood bank of Hemocentro, Ribeirão Preto, SP, Brazil.

2.2. Plant material

Pentaclethra macroloba Willd (Mimosaceae) barks were collected in Macapá, Amapá, Brazil. The botanical identification was performed by Prof. Dr. Jorge Y. Tamashiro from Laboratório de Taxonomia, Departamento de Botânica, Universidade Estadual de Campinas (UNICAMP-SP), Brazil. A voucher specimen (UEC 119264) was deposited at the Herbarium of this University.

2.3. Preparation of plant extract

Dry and worn-out stem barks (800 g) were extracted with distilled water (3300 mL), maintained in infusion for 24 h at room temperature and then vacuum filtered. The aqueous extract (EPema) was lyophilized and stored at $-18\,^{\circ}C$. Before use, it was weighed and dissolved in PBS.

2.4. Inhibition of venom and toxin activities

Desiccated venoms and freeze-dried isolated toxins were weighed and dissolved in PBS at 10 $\mu g/\mu L$. For inhibition experiments, solutions containing a fixed amount of venoms or