Journal of Ethnopharmacology 136 (2011) 518–524

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Antinociceptive and antiinflammatory activities of *Adiantum latifolium* Lam.: Evidence for a role of IL-1β inhibition

Fabiana Regina Nonato[a], Tâmara Magalhães Oliveira Nogueira[a], Taís Adelita de Almeida Barros[a,b], Angélica Maria Lucchese[b], Carlos Eduardo Cordeiro Oliveira[b], Ricardo Ribeiro dos Santos[a,c], Milena Botelho Pereira Soares[a,c], Cristiane Flora Villarreal[a,d,*]

[a] *Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Rua Waldemar Falcão 121, CEP 40296-710 Salvador, Bahia, Brazil*
[b] *Programa de Pós-Graduação em Biotecnologia, Universidade Estadual de Feira de Santana, Avenida Transnordestina s/n, CEP 44036-900 Feira de Santana, Bahia, Brazil*
[c] *Hospital São Rafael, Av. São Rafael 2152, CEP 41253-190 Salvador, Bahia, Brazil*
[d] *Faculdade de Farmácia, Universidade Federal da Bahia, Rua Barão de Geremoabo s/n, CEP 40170-290 Salvador, Bahia, Brazil*

ARTICLE INFO

Article history:
Received 20 January 2010
Received in revised form 25 May 2010
Accepted 31 May 2010
Available online 8 June 2010

Keywords:
Adiantum latifolium
Ferns
Antinociception
Antiinflammatory
Interleukin-1β

ABSTRACT

Aim of study: Adiantum, one of the most widely distributed genera of the family Pteridaceae, is employed in folk medicine worldwide. *Adiantum latifolium* Lam. has been used in Latin American traditional medicine as anxiolytic, analgesic and antiinflammatory. The present study investigates the antinociceptive and antiinflammatory properties of the methanolic extract of *Adiantum latifolium* (MEA) in animal models of pain and inflammation to confirm its medicinal use.

Material and methods: The antinociceptive and antiinflammatory activities of MEA were evaluated using the writhing, formalin, and tail-flick tests, carrageenan-induced paw edema and arachidonic acid-induced ear edema. Mice motor performance was evaluated in the rota rod test and the acute toxicity evaluated over 14 days.

Results: Intraperitoneal (1–100 mg/kg) or oral (100–400 mg/kg) administration of MEA produced a dose-related inhibition of acetic acid-induced writhing in mouse. Furthermore, treatment with MEA (100 mg/kg) inhibited both the early and late phases of formalin-induced hypernociception. In contrast, MEA (100 mg/kg/IP) did not prevent the thermal nociception in the tail-flick test. In addition, MEA (100 and 200 mg/kg/IP) inhibited important events related to the inflammatory response induced by carrageenan or arachidonic acid, namely local edema and increase in tissue interleukin-1β levels. MEA (300 mg/kg/IP)-treated mice did not show any motor performance alterations. Over the study period of 14 days, there were no deaths or toxic signs recorded in the group of mice given 1000 mg/kg of MEA.

Conclusion: The results demonstrate that *Adiantum latifolium* has antinociceptive and antiinflammatory activities, acting through the inhibition of IL-1β production.

1. Introduction

Non-steroidal antiinflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain and inflammatory conditions (IMS Health, 2005). However, the long-term administration of NSAID may induce gastro-intestinal ulcers, bleeding, and renal disorders due to their non-selective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenases enzymes (Robert, 1976; Peskar, 1977; Tapiero et al., 2002). On the other hand, fully selective COX-2 inhibitors with reduced gastro-intestinal toxicity have been associated with adverse cardiovascular effects (Dogné et al., 2005). Due of the deleterious side effects attributed to the prolonged use of NSAID and their ineffectiveness in some cases, the control of inflammatory pain is still a major challenge. Nowadays, there has been an enhanced interest in the development of disease-modifying drugs. It is well accepted that cytokines constitute a link between cellular injuries and signs of inflammation, e.g. cell migration, edema, fever, and hyperalgesia (Ferreira et al., 1988; Faccioli et al., 1990; Dinarello, 2000). In contrast to NSAID, inhibitors of cytokine production can exhibit disease-modifying activities (Geiger et al., 1994), representing an improved therapeutic strategy for the control of inflammatory diseases.

Ferns have been considered an excellent source of medicines for a long time (Barros and Andrade, 1997). However, few stud-

* Corresponding author at: Laboratório de Engenharia Tecidual e Imunofarmacologia, Centro de Pesquisa Gonçalo Moniz, Fundação Oswaldo Cruz, Waldemar Falcão 121 Candeal, CEP 40296-710 Salvador, Bahia, Brazil. Tel.: +55 71 31762260; fax: +55 71 31762327.
E-mail address: cfv@ufba.br (C.F. Villarreal).

0378-8741/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2010.05.065

ies concerning Brazilian pteridophyte pharmacological properties have been reported and, in most reports, these plants are called merely ferns or "avenca" (in Portuguese language). *Adiantum* L. is popularly called "maiden hair fern" because of the shiny black rachis of the leaves. It is one of the most widely distributed genera of the family Pteridaceae and is represented by 63 species in Brazil (Winter et al., 2007). The plants called "avenca" in Brazil represent species of *Adiantum* L. which are employed in folk medicine worldwide as antiinflammatory, analgesic, antiinfectious, and diuretic (Cambie and Ash, 1994; Barros and Andrade, 1997; Christensen, 1997; Gogoi, 2002; Bresciani et al., 2003). Infusions and compresses of *Adiantum latifolium* Lam. have been used in Latin American traditional medicine as anxiolytic, analgesic, and antiinflammatory (Barros and Andrade, 1997; Lopez et al., 2001; Bourbonnais-Spear et al., 2007). *Adiantum latifolium* has been used in Brazil to treat different types of pain, whereas in Colombia it was used for the treatment of cutaneous conditions associated with inflammation and infection (Lopez et al., 2001). It is a terrestrial widespread fern in Tropical Americas that ranges from Mexico to South America, including Central America, Antilles, Colombia, Venezuela, Guayanas, Ecuador, Peru, Bolivia, Brazil, and Paraguay (Moran and Yatskievych, 1995). The present study was undertaken to establish the antinociceptive and antiinflammatory properties of the methanolic extract of *Adiantum latifolium* leaves (MEA) and to investigate the mechanisms responsible for its effects. In order to discard possible non-specific muscle-relaxant or sedative effects of MEA, mice motor performance and toxicity of MEA were also evaluated.

2. Materials and methods

2.1. Plant material

Plant specimens were collected in the Atlantic Forest region at Salvador, Bahia State, Brazil, in August, 2006, in authorized areas by IBAMA (Brazilian Institute for the Environment and Natural Resources), and was botanically identified by Dr. Fabiana R. Nonato. A voucher specimen has been deposited at the Herbarium of Universidade Estadual de Feira de Santana, Bahia, Brazil (HUEFS 142949).

2.2. Preparation of the methanolic extract of Adiantum latifolium leaves

Air-dried and powdered blades (87 g) were exhaustively extracted with methanol at room temperature. The solvent was removed under reduced pressure on a rotary evaporator at 40–45 °C, resulting in the crude extract (4 g, 4.6% yield).

2.3. Animals

Experiments were performed on male Wistar rats (180–200 g) or male Swiss Webster mice (30–35 g) from the Animal Facilities of Centro de Pesquisas Gonçalo Moniz. Animals were housed at 24 ± 1 °C, under a 12:12 h light–dark cycle (lights on at 07:00 AM), with free access to chow and tap water until the day of the experiment, when only water was made available to them. Each animal was used only once. Animal care and handling procedures were in accordance with International Association for the Study of Pain guidelines for the use of animals in pain research (Zimmermann, 1983) and the Institutional Animal Care and Use Committee FIOCRUZ (L-029/09). All efforts were made to minimize the number of animals used and any discomfort. All behavioral testing was performed between 8:00 a.m. and 4:00 p.m.

2.4. Nociceptive tests

In the present study, the term hypernociception was used to define the decrease in the nociceptive withdrawal threshold, since the pain perception in animals is not obvious.

2.4.1. Writhing test

The intraperitoneal and oral antinociceptive doses of MEA were determined in mice using the writhing test. Acetic acid (0.8%, v/v, 10 ml/kg) was injected into the peritoneal cavities of mice, which were placed in a large glass cylinder and the intensity of nociceptive behavior was quantified by counting the total number of writhes occurring between 0 and 30 min after the stimulus injection (Collier et al., 1968). The writhing response consists of a contraction of the abdominal muscle together with a stretching of the hind limbs. The antinociceptive activity was expressed as the writhing scores over 30 min.

2.4.2. Formalin test

Rats were placed in an open Plexiglas observation chamber for 30 min to accommodate to their surroundings, and then removed for formalin administration. Rats were gently restrained while the dorsum of the hind paw was subcutaneously administered with 50 μl of formalin 1% (1:100 dilution of stock formalin solution, 37% formaldehyde in 0.9% saline) using a 30 gauge needle. Following injection, the rat was returned to the observation chamber for a 60 min observation period. A mirror was placed behind the chamber to enable unhindered observation of the formalin-injected paw. Rats were observed from 0 to 10 min (early phase) and from 10 to 60 min (late phase) and a nociception score was determined for each period by counting the number of flinches of the injected limb during the observation time (Dubuisson and Dennis, 1977). Flinches were discrete and easily quantifiable.

2.4.3. Tail-flick test

The tail-flick test (Analgesiometer, Insight, Brazil) in rats was conducted as described elsewhere (D'Amour and Smith, 1941), with minor modifications. Each animal was placed in a ventilated tube with the tail laid across a wire coil which was at room temperature (23 ± 2 °C). The coil temperature was then raised by the passage of electric current and the latency for the tail withdrawal reflex was measured. The heating was applied to a portion of the ventral surface of the tail 4 cm from the tip. Each trial was terminated after 6 s to minimize the probability of skin damage. Tail-flick latency was measured before (basal) and after the treatment.

2.5. Paw edema induced by carrageenan in mice

Carrageenan (200 μg) was injected by intraplantar administration in the right hind footpad of mice. The volume of the mice paws was measured with a plesthismometer (Ugo Basile, Comerio, Italy) before (Vb, baseline) and 2, 4, 24, and 48 h after (Vt), as described previously (Winter et al., 1962). The amount of paw swelling was determined for each mouse and the difference between Vt and Vb was taken as the edema value.

2.6. Ear edema induced by arachidonic acid in mice

Arachidonic acid (2 mg/ear) was subcutaneously injected into the mice right ear. The thickness of the ears was measured before and 1 h after the inflammation induction (Young et al., 1984; Crummey et al., 1987) using a digital caliper (Mitutoyo 500-136 CD-6"). The caliper was applied near the tip of the ear just distal to the cartilaginous ridges and the thickness was recorded in μm. Edema was expressed as