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Macrophage activation induced by *Orbignya phalerata* Mart.

Flávia R.F. Nascimento [a,*], Elizabeth S.B. Barroqueiro [b], Ana Paula S. Azevedo [b], Adelson S. Lopes [c], Susanne C.P. Ferreira [d], Lucilene A. Silva [d], Márcia C.G. Maciel [d], Dunia Rodriguez [e], Rosane N.M. Guerra [a]

[a] *Department of Pathology, Federal University of Maranhão, Av. dos Portugueses, s/n Campus Universitário do Bacanga, Integrado, Bloco I, CEP 65085-580, São Luís, MA, Brasil*
[b] *Department of Physiologic Sciences, Federal University of Maranhão, São Luís, MA, Brazil*
[c] *Department of Morphology, Federal University of Maranhão, São Luís, MA, Brazil*
[d] *Department of Biology, Federal University of Maranhão, São Luís, MA, Brasil*
[e] *Department of Immunology, Biomedical Science Institute, São Paulo University, São Paulo, SP, Brasil*

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Abstract

Babassu is the popular name of *Orbignya phalerata* Mart. [Arecaceae (Palmae)], which fruits mesocarp has been used in Brazil as medicine for the treatment of pains, constipation, obesity, leukemia, rheumatism, ulcerations, tumors and inflammations. In this study, we investigated the effect of babassu mesocarp flour aqueous extract (BM) on C3H/HePas mice peritoneal cellular migration and macrophage activation by measuring the nitric oxide (NO), hydrogen peroxide (H_2O_2) and tumor necrosis factor (TNF) release, spreading activity and major histocompatibility complex (MHC) class II expression. Our results demonstrate that BM injected once ip in mice at 10 and 20 mg/kg increased the cellular influx to the peritoneal cavity, the MHC class II expression and the spreading ability, and also induced the production of NO, TNF and H_2O_2. The increase in NO-production and MHC expression was also observed after the addition of BM to resident macrophage cultures (100 μg/ml). Thus, BM-treatment was able to activate peritoneal macrophages in vitro and in vivo inducing the production of inflammatory and cytotoxic metabolites, which could justify the popular use of babassu mesocarp in the treatment of tumor diseases, but not in inflammatory pathologies.

Keywords: Macrophages; Babassu mesocarp flour; *Orbignya phalerata*; Nitric oxide; MHC; TNF

1. Introduction

Babassu is the popular name of *Orbignya phalerata* Mart. (syn. *Attalea glasmanii* Zona Palms) [Arecaceae (Palmae)]. This palm has been used by Apinaye and Guajajara Indians of northeastern Brazil yielding a variety of important products. Babassu palms provide food, fuel, shelter, fiber, construction materials, medicine, magic and other basic necessities of life for those people (Balick, 1988).

The babassu mesocarp is extracted, powdered and dissolved in water for use by Brazilian people as food supplement, since it is rich in carbohydrates and mineral salts (Gaitan et al., 1994). In the same way it is also used in the folk medicine for the treatment of menstrual pains, constipation, obesity, rheumatism, ulcerations, leukemia and tumors, and finally, in inflammatory related diseases such as colitis and arthritis (Silva and Parente, 2001; Caetano et al., 2002). The use of babassu mesocarp flour as medicine is empiric and based on popular knowledge that recommends to dissolve about two soup spoons of babassu mesocarp flour in 1 l

Abbreviations: BM, babassu mesocarp aqueous extract; NO, nitric oxide; H_2O_2, hydrogen peroxide; TNF, tumor necrosis factor; MHC, major histocompatibility complex (IAk); BCG, bacille Calmette-Guerín; PBS, phosphate buffered solution; Con A, concanavalin A; iNOS, induced nitric oxide synthase; PMA, phorbol myristate acetate; ip, intraperitoneal

* Corresponding author. Tel.: +55 98 321 78548; fax: +55 98 323 16844.
E-mail address: nascimentofrf@yahoo.com.br (F.R.F. Nascimento).

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of water, and to use it twice a day by topical or oral route, depending on the disease.

Anti-inflammatory and analgesic properties of babassu mesocarp were confirmed experimentally using a chloroform extract of the dried fruits (Maia and Rao, 1989; Silva and Parente, 2001). The effect can be related to the triterpenes present in *Orbignya phalerata* (Garcia et al., 1995). Others compounds have also been demonstrated in babassu, such as tannins, sugars, saponins and steroid compounds (Bandeira et al., 1986).

The inflammatory response is a complex reaction that includes the secretion of a number of mediators involved in a sequence of events. The inflammatory response is important for the establishment of immunological reactions to different stimulus. Some of the inflammatory mediators are produced by macrophages. Macrophages are central to cell-mediated and humoral immunity, and they have an important role in the immune system as a part of the host defense mechanism. Various agents, including interferon-γ (IFN-γ), lipopolysaccharide (LPS), or other microbial products, lectins and vegetal extracts are known to stimulate the macrophages (Ma et al., 2003; Fujihara et al., 2003).

We have previously demonstrated that activated macrophages can inhibit the growth of a variety of tumor cells and micro-organisms due to an increase in spreading and phagocytic ability, nitric oxide (NO), hydrogen peroxide (H_2O_2) and cytokine production, and major histocompatibility complex (MHC) expression (Nascimento et al., 1998, 2002).

In this study, we investigated the effect of an aqueous extract of babassu mesocarp flour (BM) in promoting macrophage migration and activation.

2. Material and methods

2.1. Mice

Groups of male C3H/HePas mice (10/group), 8–12 weeks old were originally obtained as breeding units from Institute Pasteur (Paris, France), and have been maintained for many generations in the Animal Breeding Unit (Biotério de Camundongos Isogênicos, ICB, USP, São Paulo, SP, Brazil) under standard pathogen-free conditions. Animals were age and sex matched, fed sterilized food and water and treated according to ICB/USP Animal Welfare guidelines.

2.2. Plant material

The flour of babassu mesocarp was purchased from Hensa Farma, São Luís, MA (Brazil). This commercial product was previously submitted to analysis of authenticity, integrity and purity, by physical–chemical tests, such as standard chromatographic techniques. Similarities in all the botanical and phytochemical aspects were demonstrated when it was compared with the mesocarp flour obtained in our laboratory (Batista and Ribeiro, 2003). In these tests, *Orbignya phalerata* fruits were collected from Pedreiras, MA, Brazil, and an authenticated voucher specimen is kept on file in Herbário Ático Seabra under the number 1135.

Twenty grams of the mesocarp flour were extracted during 1 h with 1000 ml of hot water under constant stirring. The aqueous extract was filtered, and then diluted to the appropriate concentration for the in vivo or in vitro assays.

2.3. Treatment

For the in vivo assays the babassu mesocarp aqueous extract (BM) was diluted in phosphate buffered solution (PBS). Doses of 10 or 20 mg/kg were injected ip, and the mice were sacrificed 24 h later. To be able to evaluate the results obtained with BM, two other groups of mice were treated ip with two reference drugs BCG (80 mg/kg) and Concanavalin A (0.5 mg/kg), and were sacrificed after 8 days or 24 h, respectively.

For the in vitro experiments the BM solution was added to macrophage cultures at a concentration of 100 μg/ml for 48 h.

2.4. Peritoneal cell harvesting

Mice were killed by CO_2 asphyxia, and the peritoneal cells were aseptically collected by washing the peritoneal cavity with 5 ml sterile ice-cold PBS devoid of calcium and magnesium ions. For total cell determination, nine volumes of peritoneal cells were added to 1 volume of 0.05% crystal violet dissolved in 30% acetic acid, and counts were performed using a bright-line hemocytometer (Sigma, St. Louis, MO, USA). Differential cell counts were determined by cytospin preparations stained with Instant-Prov (Newprov, Pinhais, Brazil).

2.5. Spreading assay

The peritoneal cell suspensions containing 2×10^6 cells were centrifuged and suspended in 1 ml of Dulbecco's PBS containing 5 mM glucose. A volume of 50 μl of cellular suspension were layered on glass cover slips and incubated for 1 h at 37 °C in a humid atmosphere with 5% CO_2. The cover slips were gently rinsed in PBS. The glass adherent cells were fixed in 2.5% glutaraldeyde and examined with a phase contrast microscope at 400× magnification. Two hundred cells were examined and classified as either round or spread, as previously described (Rabinovitch et al., 1977).

2.6. Sequential analyses of macrophage functions using a single sample of macrophages

To determine H_2O_2-release, NO-production and IA^k-expression in a single macrophage sample a method described previously by Nascimento et al. (2003) was used.

Briefly, to evaluate H_2O_2-release, a horseradish peroxidase-dependent phenol red oxidation micro-assay