Mycobacteria research in water buffalo milk

Jordão Junior, C. M. **Mycobacteria research in water buffalo milk**. Araraquara – SP. 2004. [Dissertação de Mestrado – Área: Ciência dos Alimentos - Faculdade de Ciências Farmacêuticas – UNESP Araraquara] Orientadora: Profª. Drª. Clarice Queico Fujimura Leite.

Milk is an important nutritional source to man, but it can also carry pathogenic mycobacteria, especially *Mycobacterium bovis.* In this study, we tried to evaluate the classical technique (culture) and molecular technique PCR (*Polymerase Chain Reaction*) and PRA (*PCR - Restriction Enzyme Analysis*) in the isolation and identification of mycobacteria from water buffalo´s milk samples. First of all, it was standardized an efficient PCR method for *M. bovis* identification from milk. In this sense, a known number of *M. bovis* AN5 were inoculated in the milk that was submitted to a serial dilution. Then, two different PCR protocols were done to evaluate the threshold of *M. bovis* detection. Two pair of primers were tested, one to detect *Mycobacterium* spp (INS1 e INS2) and other to detect *M. bovis* (JB21 e JB22). Dilutions were also submitted for culture using Stonebrink medium and incubated at 37°C/90 days, to determine the number of bacilli in the milk. After this, 23 samples of milk collected from water buffalos (*Bubalus bubalis*), 7 PPD + and 16 PPD-, were analyzed by PCR technique and culture (Stonebrink medium) to detect the presence of *M. bovis.* Isolation of other mycobacteria were done by culture (Lowenstein-Jensen medium) and identified by mycolic acids and PRA. The results of PCR showed that the protocol using primer par INS1/ INS2 was positive until 10^{-3} dilution (800 CFU/mL). The protocol with primers JB21/JB22 was more sensitive and specific, detecting *M. bovis* until 10^{-4} dilution (80 CFU/mL). *M. bovis* could not be identified by PCR and isolated in culture from milk samples of water buffalo. Other species of mycobacteria were identified as: *M. flavenscens* (1 sample), *M. simiae* (3 samples) and *M. intracellulare* (1 sample). The last two species are considered pathogenic mycobacteria to human.

Dissertação está disponível na Biblioteca da
Faculdade de Ciências Farmacêuticas – UNESP Araraquara.
e-mail: clesojunior@uol.com.br

Immunological activity evaluation of *Alchornea* spp in vitro on the production of hydrogen peroxide, nitric oxide and tumor necrosis factor-α by murine macrophages

Lopes, F.C.M. **Immunological activity evaluation of *Alchornea* spp in vitro on the production of hydrogen peroxide, nitric oxide and tumor necrosis factor-a by murine macrophages.** Araraquara – SP. 2004. [Dissertação de Mestrado – Área: Análises Clínicas - Faculdade de Ciências Farmacêuticas – UNESP Araraquara] Orientadora: Profª. Drª. Iracilda Zeppone Carlos.

The use of natural resources as treatment and healing for diseases is as old as the human species. However, most of all plant species were not investigated chemistry or biologically. Many plants used in the traditional medicine modulate the immunological response. The immune system is a remarkably adaptive defense system that has evolved in vertebrates to protect them from invading pathogenic microorganisms and cancer. Macrophages play an important role in this system because they are cells capable to secrete many biological active products such as reactive nitrogen and oxygen species and

cytokines. In this work, methanolic extract and ethyl acetate fraction obtained from *Alchornea triplinervia* and *Alchornea glandulosa* were studied in the murine immune system using peritoneal macrophages cultures from Swiss mice. Cell viability assays were realized to assure the experimental development. Hydrogen peroxide (H_2O_2) and nitric oxide (NO) were determined by espectrophotometric procedures and enzyme-linked immunosorbent assay (ELISA) was used to detect tumor necrosis factor (TNF-α). The ability of methanolic extract and ethyl acetate fraction to stimulate or inhibit the murine immune system was evaluated. These plants didn't show immunostimulating properties, once liberation of H_2O_2, NO and TNF-α were not observed. However, extracts and fractions from both plants, strongly inhibited NO and H_2O_2 production induced by LPS and PMA, respectively. Production of TNF-α by LPS-stimulated macrophages was partially inhibited. The concentration of 15,62 μg/mL from *A. triplinervia* methanolic extract (cellular viability > 95%) showed to inhibit 88,35% of H_2O_2, 52,54% of NO and 10,41% of TNF-α production. The ethyl acetate fraction of the same plant and concentration (cellular viability > 90%), inhibited 72,25% of H_2O_2, 47,80% of NO and 16,41% of TNF-α production. Regarding the *A. glandulosa* methanolic extract in the concentration of 15,62μg/mL (cellular viability > 91%), there was a production inhibition of 88,62% of H_2O_2, 32,40% of NO and 11,61% of TNF-α. The ethyl acetate fraction of the same plant and concentration (cellular viability > 92%), inhibited 70,56% of H_2O_2, 21,67% of NO and 12,21% of TNF -α production. In the NO and TNF-α assays, the inhibition percentage grows according to increasing concentrations, but this fact was not noticed in H_2O_2 determination. According to these results, it is suggested that methanolic extracts and ethyl acetate fractions from *A. triplinervia* and *A. glandulosa* can present anti-inflammatory activity, confirming their traditional use.

Dissertação está disponível na Biblioteca da
Faculdade de Ciências Farmacêuticas – UNESP Araraquara.
e-mail: fcmlopes@terra.com.br