Immunomodulation against *Paracoccidioides brasiliensis*: action of human monocytes stimulated with extract of *Plinia jaboticaba* leaves *in vitro*

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Abstract

Objectives: to evaluate the immunomodulatory action of extract from *Plinia jaboticaba* (VELL.) BERG (Myrtaceae) leaves on cultures of human monocytes, as well as the viability of the monocytes stimulated and challenged *in vitro* with the fungus *Paracoccidioides brasiliensis*. **Methods:** Human monocyte cultures of 17 healthy volunteers was used to evaluate the effects of the concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5mg/ml of *P. jaboticaba* extract. To quantify levels of nitric oxide production by the monocytes, the concentration of NO is evaluated indirectly after enzymatic reduction of nitrate (NO₃⁻) to nitrite (NO₂⁻) by nitrate reductase, using the Griess Colorimetric Reaction. After incubation, the absorbance (570 nM) was determined and the results expressed in nM. The cytotoxicity of the extract was verified by uptake and reduction of the MTT salt by viable monocytes. To obtain the monocyte cell viability rate, a specific formula was used [cell viability = (Absorbance of sample cells - Absorbance of white well) / (Control cell Absorbance - White well Absorbance) X 100]. To verify the normality of the data, the results were submitted to the Shapiro-Wilk Test, and then submitted to the Tukey test, for multiple comparison between concentrations. The level of significance was 95%.

Figure 2: Monocytes proliferation media after stimulation with different concentration of *Plinia jaboticaba* leaves extract - after 24-hour: increased cell proliferation in a dose-dependent manner. The significant concentration with highest growth was 100 mg/ml, with 176.01% of cell proliferation.

* # ANOVA statistical test p < 0,01

Figure 1: Extract effect from *Plinia jaboticaba* in monocyte cultures stimulated by *Paracoccidioides brasiliensis*.
(a) Cell viability experiments: extract concentrations showed no cytotoxicity in monocyte cultures; (b) Nitric oxide (NO) production by the monocytes: high levels in concentrations of 50mg/ml (mean: 0.088 μM) and 100mg/ml (mean: 0.096 μM) - none NO production was observed in the cultures stimulated only with the *P. brasiliensis* fungal suspension.

Conclusion

The use of different concentrations of *P. jaboticaba* leaf extract for the activation of human monocytes *in vitro* showed that, at 50 mg/ml and 100 mg/ml concentrations, the extract stimulated NO cell production to assist in the oxidative capacity against *P. brasiliensis*. Considering the absence of cytotoxicity and extract concentrations necessary for induction of cell proliferation, it is suggested that *P. jaboticaba* extract has an important potential in the therapeutic use.