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Afrormosin, an isoflavonoid from *Amburana cearensis,* inhibits inflammatory mediators in stimulated human neutrophils

TM Pierdoná[1], AA Lopes[2], LM Kabeya[3], AESC Azzolini[3], YM Lucisano-Valim[3], HIF Magalhães[1], ER Silveira[4], GSB Viana[1], LKAM Leal[2]. *[1]Federal University of Ceará, Pharmacology 60420-270, Brazil, [2]Federal University of Ceará, Pharmacy 60430-160, Brazil, [3]University of São Paulo, Physical and Chemical 14040-903, Brazil, [4]Federal University of Ceará, Organic and Inorganic Chemistry 60455-760, Brazil*

Introduction and objectives: *Amburana cearensis* A. C. Smith (Fabaceae) is native to dry regions of Brazil where is locally known as “cumaru”. Its trunk bark is popularly used in the treatment of asthma. Previous studies (Leal et al., 2009) from our laboratory showed the some pharmacological activities of the extract and molecules from *A. cearensis*. The aims of this study were to investigate the toxic and anti-inflammatory effects of afrormosin (AFM) on elastase activity and TNF-α levels in human neutrophils. Methods and Results: Polymorphonuclear (PMNs, 2.5 $x10^6$ cells/mL), predominantly neutrophils (80-90%) with cell viability of 95 % (technical Trypan Blue exclusion) were isolated from human blood (Lucisano & Mantovani, 1984) and incubated (37 °C) with AFM (5, 10, 25, 50 and 100 µg/mL), DMSO (1 % in water, control) or HBSS. The anti-inflammatory activity was investigated by the measure of elastase (EL) activity (Kanashiro et al., 2007) and tumoral necrosis factor (TNF) -α levels. To measure EL activity aliquots of PMNs were challenged by the addition of cytochalasin b (1 µM) and fMLP (1 µM) prior to incubation of AFM and the enzyme activity was measured by spectrophotomer (620 nm) after the addition of N-succinyl-l-alanyl-l-alanyl-l-valine-p-nitroanilide (SAAVNA - 1mM) at the supernadants. The production of TNF-α was challenge by the activation to the cells of PMA (0.1 mol/L) after incubation with AFM (5-100 µg/mL) and assayed using a TNF-α colorimetric assay Kit. The results were expressed as mean ± SEM of percentage of inhibition for elastase acivity or TNF-α levels, and the results were considered significant when $p< 0,05$ (ANOVA, Tukey, post hoc). To investigate the cytotoxic potential of AFM, the methodology of flow cytometry was applied the using propidium iodide (PI) and annexin V as stainings (Vermes et al., 1995). AFM (5 - 100 µg/mL) was not able to inhibit the EL activity, however AFM (5-100 µg/mL) caused an inhibition of TNF-α levels of the order of 44 %. In the PI assay, AFM (1, 10, 50 and 100 µg/mL) showed a relative toxicity (61.87 ± 3.62, 59.37 ± 1.72, 51.83 ± 3.25, 53.86 ± 7.59 %, respectively) compared to HBSS (non-trated cells, 82.03 ± 2.40 %). In annexin V assay, AFM (1, 10, 50 and 100 µg/mL) showed a reduction of viable cells (86.71 ± 3.70, 77.25 ± 1.60, 79.09 ± 0.21, 72.10 ± 0.67, respectively) compared to HBSS (non-treated cells, 94.33 ± 0.45). Conclusion: The anti-inflammatory activity could be attributed, at least in part, to the regulation of inflammatory mediators as TNF-α. This study also showed that AFM has a relative toxicity to human neutrophils.

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