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PROTEINS OBTAINED FROM *Calotropis procera* (CpLP) MODULATE THE RELEASE OF MEDIATORS DERIVED FROM MURINE MACROPHAGES.

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INTRODUCTION

The proteins isolated from the latex of *Calotropis procera* (CpLP) have demonstrated a high healing potential in vivo by stimulating the inflammatory phase of wound healing, promoting the release of essential cytokines for the other stages of the healing process. Macrophages are fundamental cells in this inflammatory response, providing incentives for the migration and proliferation of fibroblasts and keratinocytes in order to promote injury contraction, matrix production, collagen deposition, and finally the formation of new tissue. This research aimed to investigate the profile of mediators released by murine macrophages *in vitro* after treatment with the CpLP fraction.

MATERIAL AND METHODS

In order to evaluate the viability of cell lines treated with CpLP, the Sulforodamine B (SRB) staining method was used, which binds to the terminal portions of the amino acids of the cells, so the higher the staining intensity, the larger the number of viable cells. The absorbance was measured at 564 nm. To assess the release profile of mediators, macrophages were pretreated with CpLP (0,78; 1,56; 3,12 and 6,25 µg/mL) and stimulated with LPS for 24 hours in order to evaluate nitrite levels by Griess and TNF-α, IL-β, IL-6, IL-8 (KC), IL-10, TGF-β and VEGF by enzyme-linked immunosorbent assay (ELISA).

RESULTS

Pretreatment of CpLP with subsequent addition of LPS (100ng / mL) stimulated the proliferation of macrophages at 6.25 and 12.5 µg / mL and reduced nitrite synthesis at all concentrations. CpLP stimulated the release of mediators essential for the innate immune response, such as IL1-β, IL-6 and TNF-α (3.12 and 6.25 µg / mL), which in the inflammatory phase signal the elimination of debris and microorganisms pathogenic. Proteins (0.78 µg / mL) were also able to increase levels of IL-10, which promotes the end of the inflammatory phase, and TGF-β, which stimulates chemotaxis and proliferation of fibroblasts and endothelial cells. These events characterize the proliferative phase. There wasn't increase in VEGF levels after treatment and levels of IL-8 were not detected in this experimental model.

CONCLUSIONS

CpLP modulates the release of important cytokines in the inflammatory and proliferative phases of wound healing, and may be one of the mechanisms involved in healing activity. The study of this fraction has been deepened in clinical studies for the treatment of plantar ulcer.

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