Procoagulant and fibrinogenolytic activity of cysteine proteases from *Calotropis procera* latex

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**Introduction:** Studies have shown that latex of plants is a rich source of enzymes with proteolytic activities. The *C. procera* latex proteins are well known for their pharmacological properties, such as anti and pro-inflammatory, anti-cancer and procoagulant activity. In view of these findings, this work reports the isolation, characterization and procoagulant activities of three new cysteine proteases purified from *Calotropis procera* latex. **Material and Methods:** These proteases, named as CpCP-1, CpCP-2 e CpCP-3, were purified using two sequential steps of ion exchange chromatography. They were firstly fractionated in CM-Sepharose column and secondly in Resource S column coupled to FPLC system. The samples purity was confirmed by mass spectrometry and SDS-PAGE. The N-terminal amino acid sequences were determined by Edman degradation. The proteolytic activity was performed using azocasein, BANA and BApNA as substrates. The procoagulant assays were performed using human plasma and fibrinogen as well as coagulation kits APTT (activated partial thromboplastin time – Labtest®) and PT (prothrombin time – Labtest®). **Results and Discussion:** According to ESI-Q-TOF, proteases molecular masses are CpCP-1 = 26.213, CpCP-2 = 26.133 and CpCP-3 = 25.086. Their N-terminal sequences were identical and composed by 30 amino acid residues. In proteolytic assays, the proteases were capable of digesting azocasein and BANA substrates and were inhibited by E-64, a specific cysteine protease inhibitor, which confirmed that they are cysteine proteases. The proteases were capable of inducing faster plasma clotting, acting in intrinsic pathway coagulation cascade. They also digested fibrinogen similarly to thrombin in a time and dose dependent manner. Fibrin clot was partially dissolved in a dose dependent manner by these proteases. **Conclusions:** These results confirm fibrinogenolytic activities associated to cysteine proteases of latex and show new perspectives about the pharmacological effects of *C. procera*.

Keywords: *C. procera*, cysteine proteases, latex, plasma clotting.
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