BIOLOGICAL AND ENZYMATIC CHARACTERIZATION OF FRACTIONS FROM BOTHROPS PAULOENSIS SNAKE VENOM

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Snake venoms are complex mixtures of toxic enzymes including proteases that cause several biological effects. The present work aimed to characterize coagulant, hemorrhagic and fibrinogenolytic activities present in fractions from Bothrops pauloensis snake venom. Crude venom was submitted to CM-Sepharose with ammonium bicarbonate buffer 0.05M pH 7.9 and five fractions were isolated (CM1 to CM5). CM1 was submitted to DEAE-Sephacel at the same buffer and five fractions were obtained (D1 to D5). For fibrinogenolytic activity 50µl of fibrinogen (1,5mg/ml) were incubated with 10µg of each fraction at 37° C for two hours and analyzed by SDS-PAGE. All fractions degraded the Aa and Bß fibrinogen chains, however only D1 presented high proteolytic activity being capable to degrade γ chain. Hemorrhagic activity was tested by intradermical injection in Swiss male mice. After three hours the animals were sacrificed, their skins were removed, and the presence of hemorrhagic halos was measured. Only the fraction D3 presented hemorrhagic activity. Coagulant activity on bovine plasma was tested at 37° C at coagulometer apparatus using each fraction. The appearing of fibrin net was evidenced only in fraction D3. The procedure allowed separating different biological and enzymatic fractions in Bothrops pauloensis snake venom.

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