Biochemical and Biological Characterization of a PLA_2 from Crotoxin Complex of *Crotalus durissus cumanensis*

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Introduction and Objectives. In Colombia are reported about 3,000 snakebites every year, one per cent of which are inflicted by *Crotalus durissus cumanensis* rattlesnake. In this report, we describe the purification, biochemical, biological and preliminary structural characterization of a PLA_2 (Cdcum6) from crotoxin complex of Colombian *Crotalus durissus cumanensis* rattlesnake.

Results and Conclusions. A new PLA_2 (Cdcum6) from crotoxin complex of Colombian *Crotalus durissus cumanensis* rattlesnake was purified using molecular exclusion chromatography and RP-HPLC. The molecular mass of Cdcum6 was determined by SDS-PAGE ~14 KDa and confirmed by MALDI-TOF (14321.98 Da). The enzyme showed K_m 6.0 mM, V_{max} 3.44 nmol/min, optimum pH was 8.0 and temperature was between 30 - 45°C and, and it had a strict requirement of Ca^{2+} for its activity. The N-terminal sequence of PLA_2 was SLVQF EKMIK EVAGK NGVPWY. Comparison of amino acid sequence data with other PLA_2 from South American *Crotalus durissus* rattlesnakes showed that Cdcum6 shares the highest sequence identity with Cdr13 an isoform PLA_2 from *Crotalus durissus ruruima*, nevertheless, Cdcum6 showed high content of basic and hydrophobic amino acids. In mice, Cdcum6 presented higher LD_{50} than crotoxin complex from *Crotalus durissus cumanensis*. Additionally, Cdcum6 induced a conspicuous local myotoxic effect, and moderate footpad edema; In vitro, it was anticoagulant in doses as low as 0.5 µg/ml, and it was not cytotoxic on myoblast but Cdcum6 was able to lise myotubes.

Key words: *Crotalus durissus cumanensis*, crotoxin, myotoxin, phospholipase A_2.