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PROTEOMIC PROFILE, BIOLOGICAL ACTIVITIES AND ANTIGENIC ANALYSIS OF THE VENOM FROM BOTHRIOPSIS BILINEATA SMARAGDINA ("LORO MACHACO"), A PIT VI-PER SNAKE FROM PERU

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In Peru, snakebite is a public health problem, especially in the rain forest, as a result of progressive colonization of this geographical area. This country is the second in Latin America, after Brazil, to exhibit the largest variety of venomous snakes. B. atrox and B. b. smaragdina snakes are sympatric species in Peruvian Amazon region and are responsible for approximately 95% of the envenomings reported in this region. B. b. smaragdina may cause a smaller share (3 to 38%) of those accidents, due to its arboreal habits, that make human encounters with these snakes less likely to happen. Despite B. b. smaragdina recognized medical importance, its venom composition and biological activities have been poorly studied. In order to determine Bothriopsis bilineata smaragdina venom (BbsV) composition, proteomic approaches were performed. Venom components were analyzed by RP-HPLC, SDS-PAGE and nano LC on line with LTQ Orbitrap XL. Results showed a total of 189 identified proteins, grouped into 11 different subgroups which include snake venom metalloproteinases (SVMPs, 54.67%), snake C-type lectins (Snaclecs, 15.78%), snake venom serine proteinases (SVSPs, 14.69%), cystein-rich secretory proteins (CRISP, 2.61%), phospholipases A2 (PLA2, 1.14%), phosphodiesterase (PDE, 1.17%), venom endothelial growth factor (VEGF, 1.06%) 5'nucleotidases (0.33%), L-amino acid oxidases (LAAOs, 0.28%) and other proteins. In vitro enzymatic activities (SVMP, SVSP, LAAO, Hyal and PLA2) of BbsV were also analyzed. BbsV showed high SVSP activity but low PLA2 activity, when compared to other Bothrops venoms. In vivo, BbsV induced haemorrhage and edema in mice and showed intraperitoneal median lethal dose (LD50); 92.74 $(\pm 0.15) \mu q/20 q$ of mice. Furthermore, BbsV reduced cell viability when incubated with VERO cells. Peruvian and Brazilian bothropic antivenoms recognize BbsV proteins, as detected by ELISA and Western Blotting. Both antivenoms were able to neutralize in vivo edema and haemorrhage.

