Article

Venomics of *Trimeresurus (Popeia) nebularis*, the Cameron Highlands Pit Viper from Malaysia: Insights into Venom Proteome, Toxicity and Neutralization of Antivenom

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Abstract: *Trimeresurus nebularis* is a montane pit viper that causes bites and envenomation to various communities in the central highland region of Malaysia, in particular Cameron’s Highlands. To unravel the venom composition of this species, the venom proteins were digested by trypsin and subjected to nano-liquid chromatography-tandem mass spectrometry (LC-MS/MS) for proteomic profiling. Snake venom metalloproteinases (SVMP) dominated the venom proteome by 48.42% of total venom proteins, with a characteristic distribution of P-III: P-II classes in a ratio of 2:1, while P-I class was underdetected. Snake venom constituted the second most venomous protein family (19.43%), followed by snake venom serine proteases (SVSP) (14.28%), phospholipases A2 (5.04%), disintegrins (5.26%) and minor proteins including cysteine-rich secretory proteins, L-amino acid oxidases, phosphodiesterases, S'-nucleotidases. The venom profile correlates with local (painful progressive edema) and systemic (hemorrhage, coagulopathy, thrombocytopenia) manifestation of *T. nebularis* envenoming. As specific antivenin is unavailable for *T. nebularis*, the hetero-specific Thai Green Pit Viper Monovalent Antivenom (GPVAV) was examined for immunological cross-reactivity. GPVAV exhibited good immunoreactivity to *T. nebularis* venom and the antivenom effectively cross-neutralized the hemotoxic and lethal effects of *T. nebularis* (lethality neutralizing potency = 1.6 mg venom per mL antivenom). The findings supported GPVAV use in treating *T. nebularis* envenoming.

Keywords: *Trimeresurus nebularis*; *Popeia nebularis*; venom proteome; envenomation; proteomics; antivenom; neutralization

Key Contribution: This is the first reported venom proteome of *Trimeresurus (Popeia) nebularis*, a medically important montane pit viper in Malaysia (also known as Cameron Highlands pit viper). The qualitative composition and protein subtypes of the venom were revealed and correlated with toxicity study. The immunological profiling and cross-neutralization of the venom by a hetero-specific antivenom provide insights into clinical management of snakebite envenoming in the region.