Immunological properties of *Hypnale hypnale* (hump-nosed pit viper) venom: Antibody production with diagnostic and therapeutic potentials

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**Abstract**

Envenomation by hump-nosed pit viper (*Hypnale hypnale*, Hh) in Sri Lanka has caused significant morbidity and mortality, attributed to 35% of total venomous snakebites. In Southwestern India (Kerala), *H. hypnale* was increasingly identified as a dangerous and common source of envenomation, second to the Russell’s viper but ahead of the cobra bites. Unfortunately, there is still no specific antivenom to date. This study aims to investigate the immunological properties of the venom and to assess the feasibility of specific Hh antivenom production as well as the development of a diagnostic assay. Hh venom elicited satisfactory titers of anti-Hh IgG in rabbits after 3rd immunization. The anti-Hh IgG, isolated with caprylic acid precipitation method, was effective in neutralizing the venom lethality (potency = 48 LD\(_{50}\) per ml IgG) as well as its procoagulant, hemorrhagic and necrotic effects, indicating the possibility to produce the specific antivenom using the common immunization regime. Cross-reactivity studies using indirect ELISA showed that anti-Hh IgG cross-reacted extensively with several Asiatic crotalid venoms, particularly that of *Calloselasma rhodostoma* (73.6%), presumably due to the presence of venom antigens common to both snakes. Levels of immunological cross-reactivity were vastly reduced with double-sandwich ELISA. Further work demonstrated that the assay was able to distinguish and quantify venoms of *H. hypnale*, *Daboia russelli* and *Echis carinatus sinhaleus* (three common local vipers) used to spike human sera at various concentrations. The assay hence may be a useful investigating tool for diagnosing biting species and studying the time course profile of venom concentrations in blood.

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1. Introduction

The Merrem’s hump-nosed pit viper, *Hypnale hypnale* is a medically important venomous snake in Sri Lanka and southwestern coast of India (de Silva et al., 1994; Warrell, 1995; Joseph et al., 2007; Ariaratnam et al., 2008). The hump-nosed pit viper bite has been recorded as the major cause of venomous snake bites in Sri Lanka (35% of total venomous bites, Ariaratnam et al., 2008), and being second to the Russell's viper but ahead of the cobra bite as a major cause of envenoming in central Kerala, India (Joseph et al., 2007). Envenomed victims often develop debilitating localized features, e.g. pain, edema, hemorrhagic blistering and necrosis; while in worse scenarios, potentially fatal systemic envenoming ensues with predominant features of hemostatic dysfunction that includes intravascular coagulopathy and spontaneous systemic bleeding. An infrequent but fatal complication, acute kidney injury, had been reported as well (Joseph et al., 2007; Ariaratnam et al., 2008), and as described by Sitprija (2008), such complication is likely secondary to consumptive coagulopathy, mediated through the venom procoagulant and fibrinolytic actions. Despite its high prevalence and severe sequelae, there is still no specific antivenom to date for the treatment of *H. hypnale* envenomation (Ariaratnam et al., 2008). Nevertheless, Tan et al. (2011a) showed that two commercial antivenoms (monovalent and polyvalent) raised against Malayan pit viper (*Calloselasma rhodostoma*) venom effectively neutralized the venom lethality as well as its procoagulant, hemorrhagic and necrotic toxicities in mice. Such paraspecific venom neutralization was possible due to the presence of similar antigenic properties between the venoms of *H. hypnale* and *C. rhodostoma*, between which close phylogenetic relationships had been established (Parkinson et al., 1997; Vidal and Lecointre, 1998).

Snake envenomation, known for its neglected status, faces various global pertinent challenges including that for antivenom production and supply (Gutiérrez et al., 2006; WHO, 2009). In snake envenomation, serotherapy, i.e. administration of antivenom is the only definitive therapeutic treatment (Chippaux and Goyffon, 1998). In our previous study, the Malayan pit viper antivenom has been suggested for therapeutic use in endemic areas where antidotes...