Brief report

Venoms of South Asian hump-nosed pit vipers (Genus: *Hypnale*) cause muscarinic effects in BALB/c mice.

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Abstract

Although clinical, in-vivo and in-vitro studies suggest the necrotic, haemorrhagic, pro-coagulant and nephrotoxic effects of South Asian Hump nosed pit vipers, reports on neurotoxic properties are limited to a single in-vitro study. Using BALB/c mice, for the first time, here we demonstrate the signs of envenoming suggestive of possible muscarinic effects of the venoms of all three *Hypnale* species. Further, we demonstrate that the muscarinic effects are occurred at lower venom doses by *H. hypnale* venom, compared to *H. nepa* and *H. zara*.

Key words: Hump-nosed vipers; Venom; Mouse; Cholinergic

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South Asian hump-nosed pit vipers of the genus *Hypnale* are the commonest cause of snakebite in Sri Lanka **(**1**)**. These snakes reportedly cause clinically significant envenomation also in South India. Bites by these snakes cause severe local envenoming and also frequently cause potentially fatal systemic envenoming due to coagulopathy and renal failure **(**2**).** The venoms of the three species of *Hypnale* (*H. hypnale*, *H. nepa* and *H. zara*) are known to possess necrotic, procoagulant, haemorrhagic and nephrotoxic effects in-vitro **(**3**)** and in-vivo **(**4**)**.

Neurotoxic effects of *Hypnale* bites have only been observed in-vitro and the weak neurotoxicity in all three *Hypnale* venoms was demonstrated suggesting a post-synaptic site of action in neuromuscular junction 3. Evidence for autonomic neurotoxic activity of the *Hypnale* venoms has not been described in any clinical or experimental study. We provide first evidences for such activity of the three *Hypnale* venoms by demonstrating autonomic signs in BALB/c mice following experimental envenoming.

All the experiments described here were carried out in the Animal House, Faculty of Medicine and Allied Sciences of the Rajarata University of Sri Lanka. Methods for mice handling, venom collection, venom storage, venom dissolving and venom protein assay was described earlier4. BALB/c mice (18-23g, both sexes), in three test groups (n=22 in each) envenomed with 0.1 to 11.5 µg/g doses of the three *Hypnale* venoms in 300 µl volumes, intraperitoneally for previous lethality (LD50) studies were used for this study. A control group of similar mice (n=5) received 300 µl volumes of 0.9% NaCl solution intraperitoneally. Each test and control mouse used were kept in separately tagged observational cages (6"X 6"X12") allowing access to food and water ad libitum and were observed for 48 hours or until death. Close observations on the behaviour of mice were done at 5 minute intervals in the 1st hour, 15 minute intervals in 2nd and 3rd hours, hourly in the 4th, 5th and 6th hours and thereafter, at 6 hourly intervals until 48 hours.

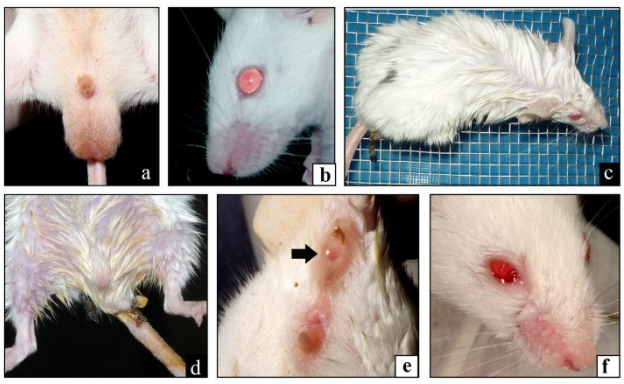

Figure 1: **Some cholinergic signs observed in envenomed mice (c, wet fur; d, mucoid diarrhea; e, anal incontinence; f, excessive ocular secretions) as opposed to the normal appearance of control mice (a & b)**.

Changes in the behavior and appearance of the mice were noted. Hypotonia was elicited by subjecting mice to wire hanging task.

Table 1 **Minimum doses of each venom that caused neurotoxic signs of envenoming**

Sign	Minimum venom dose (µg/g)		
	H. hypnale	***H. nepa***	***H. zara***
Reduced activity	0.97	2.82	1.44
Myoclonic jerks	1.13	2.92	1.65
Hypotonia	1.23	6.16	3.21
Fur wetting	1.23	3.92	2.66
Excessive thirst	0.88	2.92	1.94
Ocular secretions	0.92	3.32	2.88
Mucoid diarrhoea	0.92	3.18	2.32
Urinary incontinence	1.23	3.92	2.88
Anal incontinence	0.88	4.26	2.52
Sialorrhea	1.42	-	4.32

Reduced activity, hypotonia, myoclonic jerks excessive thirst, urinary incontinence, anal incontinence (Figure 1e), mucous diarrhoea (Figure 1d), excessive ocular secretions (Figure 1f), excessive salivation and fur wetting (Figure 1c) were seen in survived and succumbed mice as opposed to the controls (Figure 1 a&b). Table 1 shows the minimum venom dose for signs of envenoming for each Hypnale species. All three venoms caused reduced activity during the 1st hour and hypotonia during 20 -75 minutes after envenoming. This supports the previous observation of partial blockade of indirect and direct twitches of chick biventer cervicis preparations caused by all three Hypnale venoms.3 Urinary incontinence, anal incontinence, mucous diarrhoea, lacrimation and excessive salivation observed in mice at various frequencies represent a cholinergic syndrome. These features appeared 2-6 hours after envenoming. It's onset is separated from the time period of hypotonia and myoclonic jerks and indicate parasympatheticomimetic effects of the three Hypnale venoms probably exerting its actions via muscarinic acetylcholine receptors. Absence of flaccid paralysis during the period of cholinergic hyperactivity in mice indicates that Hypnale venom toxins exert parasympathomimetic actions via selective agonism on muscarinic receptors in parasympathetic system or by selectively blocking acetylcholinesterase activity in parasympathetic system. If such alteration occur in parasympathetic system, it is likely to be a partial alteration, as the cholinergic syndrome was resolved in 56.1% of all mice by 48 hours following envenoming. Fur wetting behaviour was commonly observed in this study, among envenomed mice and likely to be a behavioural adaptation to negate hyperthermia.

Clinically, signs of damage to the autonomic nervous system are extremely rare in humans with snake bite. However, mydriasis, tachycardia, constipation, and defective maturation that lasted for two years following

an envenoming by a Malayan krait indicating sympathetic hyperactivity (5).

The minimum venom doses that lead to the above signs indicates that H. hypnale venom has a higher neurotoxicity to mice, as compared to the other two. These neurological signs in mice caused by three Hypnale venoms indicate an interesting, yet unexplored area of neurotoxicity of Hypnale venoms, which needs to be further explored.

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Competing Interests
None

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