VENOMS OF SOUTH ASIAN HUMP-NOSED PIT VIPERS (GENUS: *Hypnale*) CAUSE MUSCARINIC EFFECTS IN BALB/c MICE

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

Neurotoxic effects of *Hypnale* bites have only been observed *in-vitro*[2] in which, authors demonstrated weak neurotoxicity in all three *Hypnale* venoms suggesting a post-synaptic site of action in neuromuscular junction. 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. Methods followed for mice handling, venom collection, venom storage, venom dissolving and venom protein assay followed[3]. BALB/c mice (18-23 g, both sexes), in three test groups (n=22) envenomed with 0.1 to 11.5 µg/g doses of the three *Hypnale* venoms in 300 µl volumes, intraperitoneally for lethality (LD_{50}) calculations in Silva *et al.*[3] were used. A control group of similar mice (n=5) received 300 µl volumes of 0.9% NaCl solution intra-peritoneally. 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 until 48 hours or until death. Close observations on the behavior of mice were done at 5 minute intervals in the 1st hour; at 15 minute intervals in 2nd and 3rd hours; hourly in the 4th, 5th and 6th hours and at 6 hourly intervals thereafter, until 48 hours. Changes in the behavior and appearance of mice were noted. Hypotonia was elicited by subjecting mice to wire hanging task. Reduced activity, hypotonia, myoclonic jerks excessive thirst, urinary incontinence, anal incontinence, mucous diarrhoea, excessive ocular secretions, excessive salivation and fur wetting were seen in the survived and the mice that succumbed, following envenoming with three *Hypnale* venoms.

All three venoms caused reduced activity during the 1st hour and hypotonia during 20 -75 minutes post envenoming in mice. This supports the previous observation of partial blockade of indirect and direct twitches of chick biventer cervicis preparations, caused by all three *Hypnale* venoms described[2]. 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 post-envenoming, separated from the time period of hypotonia and myoclonic jerks and indicated para-sympatheticomimetic effects of the three *Hypnale* venoms probably exerting via muscarinic acetylcholine receptors. Absence of flaccid paralysis during the period of cholinergic hyperactivity in mice indicates that *Hypnale* venom toxins exert para-sympathomimetic actions via selective agonism on muscarinic receptors in para-sympathetic system or by selectively blocking acetylcholinesterase activity in para-sympathetic system.

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

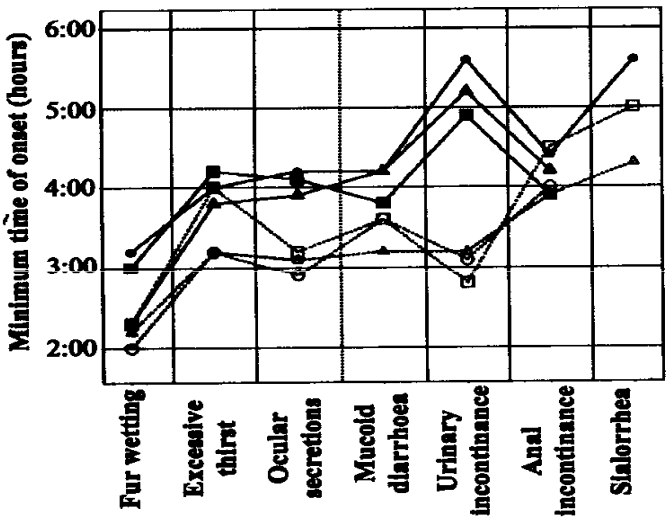

Figure1:Time sequance of cholinergic signs
(▲, *H. hypnale*; ■, *H. zara*;●, *H. nepa*; open icons indicate mice that subsequently succumbed; solid icons indicate mice that survived) .

Fur wetting behavior was commonly observed in this study, among envenomed mice and is likely to be a behavioral adaptation to negate hyperthermia. Clinically, signs of damage to the autonomic nervous system are extremely rare in humans with snake bite; The minimum venom doses that led to these signs indicated 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 indicated an interesting, yet unexplored area of neurotoxicity of *Hypnale* venoms, which needs to be further explored.

REFERENCES

1. Ariyaratnam, C.A., Thuraisingam, V., Kularatne, S.A.M., Sheriff, M.H.R., Theakston, R.D.G., de Silva, A. and Warrell, D.A.(2008)Frequent and potentially fatal envenoming by humpnosed pit vipers (*Hypnale hypnale* and *H. nepa*) in Sri Lanka: lack of effective antivenom. *Trans R Soc Trop Med Hyg* 2008; *102*: 112026 pp.
2. Maduwage, K,, Hodgson, W.C., Konstantakopoulos, N., O'Leary, M.A., Gawarammana, I. and Isbister, G.K.(2011) The *in vitro* toxicity of venoms from South Asian Humpnosed pit vipers (Viperidae: *Hypnale*). *J Venom Res* 2011; 2: 1723pp.
3. Silva, A., Gunawardena, P., Weilgama, D., Maduwage, K., and Gawarammana, I.(2012) Comparative *in-vivo* toxicity of venoms from South Asian hump-nosed pit vipers (Viperidae: Crotalinae: *Hypnale*). *BMC Res Notes* 2012; 5:471pp.