COMPARISON OF *IN-VIVO* NEPHROTOXICITY CAUSED BY THE VENOMS OF SOUTH ASIAN HUMP-NOSED PIT VIPERS (VIPERIDAE: CROTALINAE: *HYPNALE*)

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INTRODUCTION

Hump-nosed pit vipers (Genus: *Hypnale*) are the smallest of the known pit vipers and range in Sri Lanka and Western Ghats of India. Taxonomy of this genus was recently revised and identity of three species namely, *Hypnale hypnale* (HH), *Hypnale nepa* (HN), and *Hypnale zara* (HZ) were established by Maduwage *et al.* (2009). These vipers are responsible for the highest number of snakebites in Sri Lanka, but most result in local effects only. Bilateral renal cortical necrosis and acute and chronic renal failure have been recorded in HH bite victims. Frequency of acute renal failure however, was reported to vary from 1-10% in HH bite victims (See Ariyaratnam *et al.* 2008). *In-vitro* nephrotoxic properties of HH venom was reported by Gunatilake (2000). Nephrotoxic properties of HN are yet unknown and one case report showed such activity of HZ venom. Studies of earlier workers suggest that renal effects of HH venom are highly variable and unpredictable. Hence, this study was undertaken to comparatively explore the nephrotoxic effects of the three *Hypnale* venoms.

MATERIALS AND METHODS

BALB/c mice, males and females, 10–12 weeks of age, and weighting between 18–23 g were used. These were reared in standard cages under laboratory conditions. Desiccated, pooled venoms from specimens (HH: n=6; HN: n=3;

HZ: n=2) of the three *Hypnale* species dissolved in sterile 0.9% NaCl solution were used. Protein assay of the venoms was done using Bradford method, measuring the absorbance at 595nm using a microplate reader. Varying doses of venoms of HH, HN and HZ ranging from 0.1to 11.5 µg/g in solutions of 300 µl were intraperitoneally injected to 28, 22 and 22 mice respectively. Four groups of control mice (2 in each group) were injected with 300 µl sterile 0.9% NaCl solution and were sacrificed at 2, 12, 24 hours and 7 days. Mice were observed closely and kidneys of all dead mice were examined and preserved in 0.9% formol saline within 30 minutes of death. Mice that survived were sacrificed after 7 days and kidneys were similarly removed, examined and preserved. All kidney tissues were sectioned and stained with Haematoxilin and Eosin (H&E). Ethical clearance was obtained from the Research, Publications and Ethics review Committee of the Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka.

RESULTS AND DISCUSSION

All three venoms caused renal tubular and glomerular congestion (Figure 1a) at all doses tested. Petechial haemorrhages in renal parenchyma (Figure 1b) were seen in kidneys of mice envenomed with all three venoms. Hydrophic degeneration of renal tubules (Figure 1c) was the most consistent degenerative feature in kidney tubular cells. All three venoms, in addition caused dilatation of renal tubules (Figure 1d) and flattening of tubular cells with interrupted tubular brush border. All three venoms caused renal tubular necrosis (Figure 1d) showing diffuse nuclear pyknosis of most of the tubular cells and shedding of the tubular cells into lumen. Degeneration and necrosis of tubules were mostly seen in proximal tubules. Similar observations were made by Gunatilake (2000). Tubular necrosis was extensive in mice that died 18 to 72 hours of envenoming. All necrosed tubules were evenly distributed within cortices, and their basement membranes were found intact. This indicates that the tubular necrosis caused by *Hypnale* is due to direct venom mediated tubular damage. Glomerular changes were mild and were restricted to congestion.

Tubular necrosis is the commonest cause of acute renal failure in snakebite (Sitprija 2006). In control mice and the mice that survived for 7 days following envenoming by the venoms all *Hypnale* species, the histological appearances were normal (Figure 1e and 1f). Figure 2 shows the minimum venom doses of the three *Hypnale* species that caused each observed histopathological change. This shows that HH venom is able to produce petichial haemorrhages, tubular degeneration and tubular necrosis at much lower doses as compared to HN and HZ venoms. HZ venom caused all the above lesions at lower doses

than that of HN venom. Therefore HH venom is the most nephrotoxic of all *Hypnale* venoms.

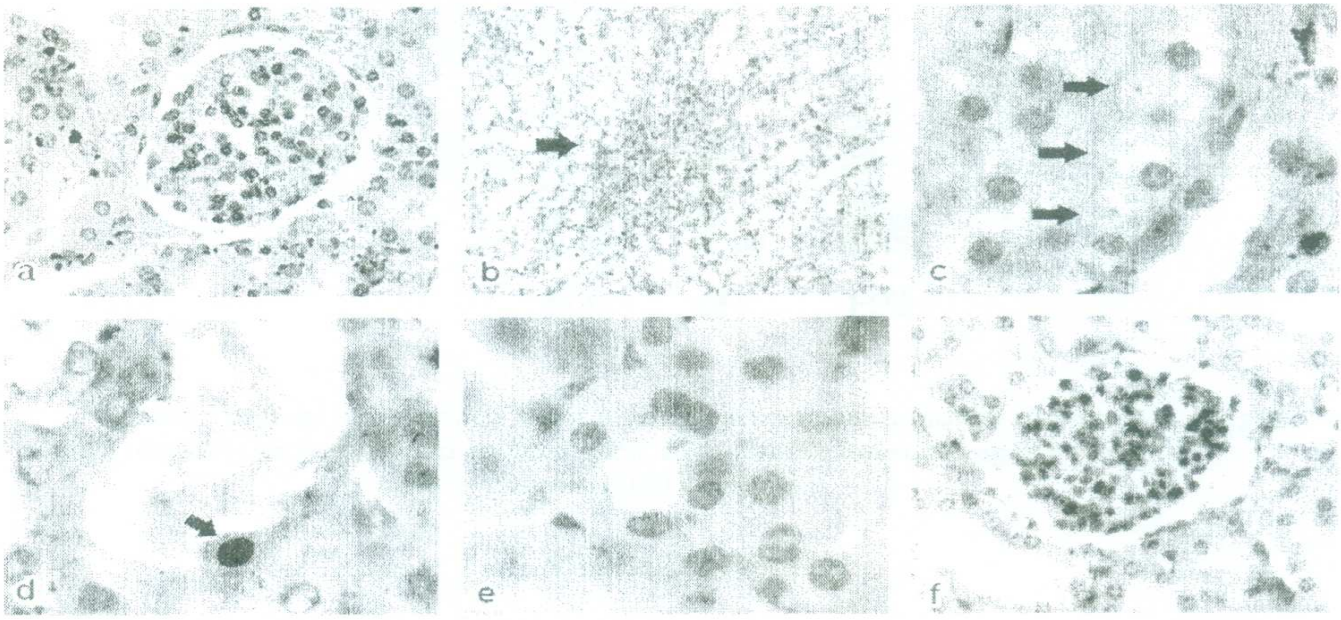

Figure 1- Renal histopathology caused by three *Hypnale* venoms (H&E; a,f:X800; b: X200; c,d,e: X2000)

(a), Highly congested glomerulus and peritubular vasculature; (b), peticheal haemorrhage in renal parenchyma (arrow);(c), hydrophic degeneration of proximal convoluted tubules (arrow); (d), necrosed proximal tubule with dilated lumen (pyknotic nucleus is indicated by black arrow and a vesicular nucleus is indicated by white arrow) seen in mice envenomed with *Hypnale* venoms. Proximal tubule (e) and glomerulus (f) of a control mouse are with normal appearence.

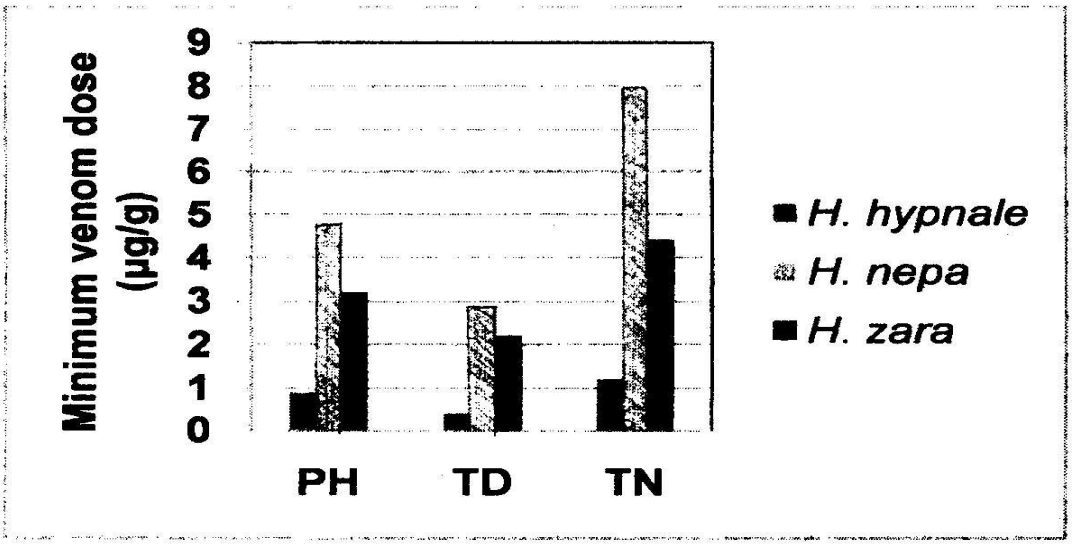

Figure 2- Minimum venom doses of the three *Hypnale* species that lead to petechial haemorrhages in renal parenchyma (PH), tubular degeneration (TD) and tubular necrosis (TN) in mouse kidneys.

CONCLUSION

All three *Hypnale* venoms have *in-vivo* nephrotoxic properties. HH venom has more nephrotoxic properties than HN and HZ venoms. Since HN and HZ venoms also lead to tubular necrosis, these findings suggest a possibility of acute renal failure occurring in patients bitten by HN and HZ.

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