

Preparation of a novel antivenom against *Atractaspis* and *Walterinnesia* venoms[☆]

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Abstract

The two deadly snakes, *Walterinnesia aegyptia* (black desert cobra) and *Atractaspis microlepidota* (mole viper) share a common habitat in the central, eastern and western provinces of Saudi Arabia. Bites by either snake were characterized by rapid death, sometimes before reaching any medical facility. Confusing reports of “a black snake bite” are frequently found. The NAVPC had succeeded in preparing a highly effective antivenom against *W. aegyptia* venom which is now available in the market, but no antivenom against *Atractaspis* venom is found worldwide. This is probably because of the low molecular weight of sarafotoxins in the venom and hence their poor antigenic properties. At the NAVPC, sarafotoxins were separated by sequential gel filtration of *A. microlepidota* venom, while toxin T_{III} of *W. aegyptia* venom obtained by cation exchange chromatography and gel filtration. Conjugation of the two toxins was carried out using glutaraldehyde in a two-step procedure followed by exhaustive dialysis. The conjugate was utilized to hyperimmunize 3-years old horses for 10 months, applying a low-dosage protocol and immunostimulants; the crude venoms of both snakes being added during the last 2 months. The $F(ab')_2$ fraction of the antivenom was obtained by pH-guided salt precipitation, enzyme digestion and tangential desalting and filtration. The bivalent antivenom obtained protected mice and rats against the lethal effects of both venoms and rescued the rats challenged with lethal doses of the venoms in recovery experiments. It also neutralized the haemorrhagic, necrotizing and the cardiotoxic effects of *A. microlepidota* venom and the neuromuscular blocking effect of *W. aegyptia* venom. The antivenom offers a good rescue potential to those who are bitten by “a black snake” in Saudi Arabia.

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1. Introduction

The burrowing asps of the genus *Atractaspis* comprise about 15 species (Laurent, 1950), that are widely distributed throughout almost the entire

African continent. They are also found in Saudi Arabia, Palestine, Sinai peninsula and Jourdan (Joger, 1984). In Saudi Arabia, the snakes inhabit the eastern coast of the red sea and the middle and western provinces (Gasperti, 1988), sharing *Walterinnesia aegyptia* the same habitat. There are two species of *Atractaspis* in Saudi Arabia, *Atractaspis microlepidota andersonii* and *Atractaspis microlepidota engaddensis*. It is too difficult to differentiate between them morphologically. These snakes have a peculiar mode of striking with only one fang, which

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