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Production and characterization of antibodies against proteins of venom of Sri Lanka Cobra (*Naja naja*)

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The commonest snakes in Sri Lanka are cobra (Naja *naja*) and its venom is not characterized at the molecular level. The available antivenom is produced against Indian snakes, common cobra, thel karawala, saw scaled viper and Russel's viper and is a mixture of polyvalent antibodies. Although it has been used to treat snake bitten patients in Sri Lanka for 20 years, it is not effective against complete control of symptoms and fatality of snake bitten patients. There are many reports of severe antivenom reactions, including severe anaphylactic reaction with hypotension following treatment. Therefore, it is very important to produce antibodies specific for toxins proteins of snakes in Sri Lanka.

Cobra Venom samples were collected and centrifuged at 12,000 rpm at 4^{o}C for 15 min. Proteins in the resulting supernatant were fractionated using Sephacryl S 200 gel filtration into five fractions. Rabbits were immunized with heat inactivated five separated fractions of venom of Cobra with Freund Adjuvant following a primary injection and two booster injection. Blood was collected after satisfactory booster injection. Antibodies were purified by ammonium sulphate precipitation followed by protein A-Sepharose chromatography. Antivenom vials manufactured by VNS Bioproducts Ltd.(AV1) and Bharat Serums and Vaccines Ltd (AV 2) in India were selected for the analysis.

Specific binding of antibodies with sephacryl S200 fractionated proteins of cobra venom were analysed by western blotting and dot blotting. Results suggest that all produced antibodies specifically bind with respective venom proteins used to raise antibodies. Further Indian antivenom preparations AV1 and AV2 bind with only phospholipase A_2 fraction and not bind with other toxic venom proteins. This might explain the less effectiveness of Indian antivenom for treatment of snake bitten patients in Sri Lanka.

Further studies are in progress to clarify the above.

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