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Development of methodologies to separate proteins from *Daboia russelii* (Russell's Viper) venom from human plasma proteins

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Objective: Russell's viper (*Daboia russelii russelii)* envenoming is frequent and significantly important in a number of South-East Asian countries, including Sri Lanka, India, Pakistan, Thailand, Taiwan, China, Myanmar and some regions of Indonesia. Clinical observations in victims comprise venom-induced consumption coagulopathy, acute kidney damage and mild local effects, and these effects vary according to geographical location. Current diagnostic tests for Russell's viper envenoming lack sensitivity and specificity and do not account for geographical variability of venom composition. Furthermore, there are substantial adverse effects from the administration of anti-venom, so improved diagnostic tests will improve outcomes from envenoming.

Methods: The current study describes the development of a methodology to separate Russell's viper venom components from the most prominent human plasma proteins such that the amount of individual venom components can be quantified following envenoming.

Results: Initially, Russell's viper venom and human plasma were fractionated via cation exchange chromatography at pH=4.0 using a Resource S 6 mL column such that the major venom components were eluted at 0.3M NaCl and plasma proteins were mostly retained on the column. The eluent was then concentrated using an Amicon Ultra-15 3 kDa molecular weight cut-off centrifugal filter and further separated via RP-HPLC using an Agilent AdvanceBio PeptidePlus HPLC column (150 X 2.1 mm) with the eluent being monitored via UV detection at 280 nM. Fractions were collected and subject to trypsin digest followed by tandem mass spectrometry (MS/MS) and identified fragments were matched to proteins within the UniProt database.

Conclusion: This work has demonstrated that it is feasible to purify Russell's viper venom proteins from the major plasma proteins and is a necessary prelude to the development of methods that will allow for quantitation of multiple venom proteins from the plasma of subjects following envenoming.