IPD Project Details

Project ID: IPD1168

Project Title: To the islands their own: Venomics of the enigmatic Andaman cobra (N. sagittifera) and the preclinical failure of Indian antivenoms in Andaman and Nicobar Islands

Description: In this study, we unveil the venom composition, biochemistry, pharmacological activity, and potency of the Andaman cobra (N. sagittifera).

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Sample Preparation: Naja naja and Naja sagittifera venom HPLC fractions (40 µg each) were dissolved in 25 mM ammonium bicarbonate and subjected to mass spectrometric analyses. A final concentration of 10 mM was achieved by adding 100 mM of DTT (dithiothreitol), and the mixture was incubated at 37o C for 30 mins. Following the incubation period, iodoacetamide (100 mM) was added to the solution, and the mixture was subjected to another round of incubation in the dark (37o C for 30 minutes). The pH of the solution was adjusted to 8 using 25 mM ammonium bicarbonate.

Peptide Separation: The process followed by overnight digestion of the sample with trypsin (0.2 µg/ml; 1:50) at 37o C. The reaction was stopped using 0.1% formic acid, and the sample was desalted with ZipTip containing acetonitrile and 0.1% formic acid. Peptides were then separated using Thermo EASY nLC 1200 series system (Thermo Fisher Scientific, MA, USA) with a 50 cm x 75 µm, C18 (3 µm, 100 Å) nano-LC column at a flow rate of 300 nL/min. Here, the mobile phase was constituted by 0.1% formic acid in HPLC grade water (buffer A) and elution buffer of 0.1% formic acid in 80% Acetonitrile (buffer B). The concentration of the buffer was altered as follows: 10-45% over 98 min, 45-95% over 4 min and 95% over 18 min.

Protein Characterization: The mass spectrometry data were acquired with an online Thermo Orbitrap FusionTM Mass Spectrometer (Thermo Fisher Scientific, MA, USA) that was coupled with the Thermo EASY nLC 1200 series system. MS scans were performed in an Orbitrap detector using the following parameters: positive ion polarity, resolution 120000, scan range (m/z) of 375-1700, and maximum injection time of 50 ms.

The ion trap detector was used for MS/MS scans with successive parameters: high collision energy fragmentation (30%), scan range (m/z) of 100-2000 and maximum injection time of 35 ms. PEAKS Studio X+ (Bioinformatics Solutions Inc., ON, Canada) was used to search the MS/MS spectra against the UniProt/SwissProt and NCBI-NR Serpentes databases (taxid: 8570; November 2020) with peptide mass tolerance of 10 ppm, fragment mass tolerance of 0.6 Da, and False Discovery Rate (FDR) of 0.1. Hits with at least one unique matching peptide were considered for downstream analyses.

Experiment Type: Shotgun proteomics

Species: Naja sagittifera-195058, Naja naja-35670

Tissue: Venom (bto:0001439)

Cell Type:

Disease: Unknown

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue

PubMed ID: 34759827