

## Proteomic profiling of FFPE mice kidney tissue under the effect of *Crotalus durissus terrificus* snake venom

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**Introduction:** According to the World Health Organization, snakebite envenoming is a neglected tropical disease that affects up to 2.7 million people each year, causing thousands of deaths and permanent disabilities. In Brazil, the *Crotalus durissus* species has one of the most lethal venoms. The major health complications of *C. d. terrificus* envenomation include Acute Kidney Injury (AKI), due to the venom's nephrotoxic effect, as well as its neurotoxic, myotoxic, and cardiotoxic activities. Although the venom's physiologic and biochemical toxic effects are well documented, few studies have explored its molecular proteomic profile. In this study, we have investigated the changes in the mouse kidney's proteomic profiles under the effect of *C. d. terrificus* snake venom.

**Methods:** Adult male Swiss mice were administered with an injection of 0.5 LD<sub>50</sub> of *C. d. terrificus* venom (0.36 µg/animal) in the gastrocnemius muscle, while the control group received a saline injection. After 1 h, 6 h, 12 h, and 24 h of injections, the kidneys were removed and fixed in 4% PFA for 24 h, then stored in 70% ethanol, and paraffin embedded. From each sample, 3x20 µm thickness tissue mounted on microscope glass slides were deparaffinized with xylene, rehydrated with a decreasing gradient of ethanol, and subjected to formalin-fixed paraffin-embedded (FFPE) protein extraction. The extraction involved adding the lysis buffer composed of 1,5% SDS and 0.1 M Tris-HCl pH 7.6, followed by high-intensity sonication, heating at 95° for 2 h, and repeated sonication. The samples were then centrifugated, and the supernatant was transferred to a new tube. The samples were reduced with TCEP, alkylated with CAA, digested with trypsin, and desalted using the single-pot, solid-phase-enhanced sample preparation (SP3) protocol. The obtained peptides were analyzed using the Vanquish Neo UHPLC system coupled to an Orbitrap Exploris 480 mass spectrometer with a label-free quantification (LFQ) approach and the Data Independent Acquisition (DIA) MS workflow. Proteins were identified and quantified using the DIA-NN software, and statistical analysis was performed in R. A phenotype analysis was carried out using the WebGestalt tool.

**Results:** We were able to identify over 4,000 proteins, from which more than 1,000 were present in all samples. By comparing venom treatment with the control group at different time points, we found several significantly upregulated and downregulated proteins. At 1 h, we highlight groups of proteins related to abnormal renal water transport, homeostasis and absorption, and kidney inner medulla morphology. At 6 h, we found proteins related to abnormal glomerular component morphology and kidney failure. At 12 h, we observed abnormal renal/urinary system morphology and phenotype, decreased urine osmolality, polyuria, and abnormal kidney collecting duct and medulla morphology. At 24 h, we found proteins related to abnormal cytokine and interleukin secretion.

**Conclusion:** Our finding helped to better understand the molecular mechanisms involved in *C. d.*

terrificus envenomation, highlighting potential proteins that can be used as targets for new therapeutic strategies to improve the current treatment of envenomation.

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