Supplementary Materials: Identification of Immunoreactive Peptides of Toxins to Simultaneously Assess the Neutralization Potency of Antivenoms against Neurotoxicity and Cytotoxicity of *Naja atra* Venom

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Table S1. Identification of *N. atra* venom components within the HPLC chromatographic fractions using LC-MS/MS.

[a]Peak	Protein identity	[b]AC no.	Significant peptide	Ion, m/z	MS score	Toxin family	[c]Toxicity score
1	Cobrotoxin -	P60770	K.NGIEINCCTTDR.C R.GCGCPSVK.N	726.7767 (2+) 432.6666 (2+)	88	3FTX	60.3
2	Cobrotoxin-b	P80958	K.VKPGVNLNCCTTDR.C K.TCSGETNCYK.K K.TCSGETNCYKK.W	545.2432 (3+) 610.2364 (2+) 674.2805 (2+)	170	3FTX	5.0
3	Phospholipase A_2	P00598	R.SWWDFADYGCYCGR.G R.LAAICFAGAPYNNNNYNID LK.AR.CCQVHDNCYNEAEK.I K.TYSYECSQGTLTCK.G K.GGNNACAAAVCDCDR.L K.NMIQCTVPSR.S R.GGSGTPVDDLDR.C K.ISGCWPYFK.T	921.8533 (2+) 1178.5278 (2+) 609.5383 (2+) 849.3058 (2+) 805.7562 (2+) 603.2667 (2+) 594.7442 (2+) 579.2611(2+)	859	PLA_2	N.D.
4	Cardiotoxin A5	P62375	K.CHNTQLPFIYK.T K.YVCCSTDKCN.- R.GCADNCPK.N K.FPLKFPVK.R K.YVCCSTDK.C	710.8403 (2+) 653.7474 (2+) 461.1946 (2+) 488.3006 (2+) 516.6811 (2+)	163	3FTX	25.95
5	Cardiotoxin A1	P60304	K.MFMMSDLTIPVK.R K.LIPIASK.T R.GCIDVCPK.N K.YVCCNTDR.C K.RGCIDVCPK.N K.MFMMSDLTIPVKR.G	706.8523 (2+) 371.2426 (2+) 474.6818 (2+) 544.2131(2+) 552.7282 (2+) 523.5909 (3+)	308	3FTX	
6	Cardiotoxin A3	P60301	K.LVPLFYK.T K.MFMVATPK.V K.MFMVATPK.V	440.2615 (2+) 462.7289 (2+) 478.7285 (2+)	274	3FTX	
7	Cardiotoxin A6	P80245	K.MFMVAAPK.V K.CNQLIPPFYK.A	447.7211 (2+) 640.3278 (2+)	284	3FTX	
8	Cysteine-rich secretory protein	Q7T1K6	R.WANTCSLNHSPDNLR.V R.AGCAVSYCPSSAWSYFYVCQ YCPSGNFQGK.T R.VSPTASNMLK.M K.LTNCDSLLK.Q K.EIVDLHNSLR.R K.SNCPASCFCR.N K.QSSCQDDWIK.S	595.5896 (3+) 1167.8121 (3+) 524.2760 (2+) 532.2622 (2+) 598.3191 (2+) 629.7216 (2+) 633.7682 (2+)	223	CRISP	N.D.
9	Zinc metalloproteinase-disintegrin-like kaouthiagin-like	D3TTC1	R.VAKDDCDLPELCTGQSAECP TDSLQR.N R.NDNAQLLTGIDFNGNTVGR. A K.FEVKPAASVTLK.S R.TAPAFQFSSCSIR.E K.DKFEVKPAASVTLK.S	989.0843 (3+) 1009.9933 (2+) 430.5788 (3+) 736.3360 (2+) 511.6191 (3+)	192	SVMP	N.D.

			K.ASCICIPGPCIMLK.K	810.3913 (2+)			
10	Zinc metalloproteinase-disintegrin-like atragin	D3TTC2	R.AAKDDCDLPELCTGQSAECP TDVFQR.N	995.0845 (3+)	281	SVMP	N.D.
			K.TSAAVVQDYSSR.T	642.3005 (2+)			
			R.KIPCAAK.D	394.2237 (2+)			
			R.GFCTCGFNK.C	545.7274 (2+)			
			R.DSCFTLNQR.T	570.7505 (2+)			
			R.ATLNLFGEWR.E	603.8043 (2+)			
			R.TKPAYQFSSCSVR.E	765.8667 (2+)			
			K.LQHEAQCDSEECCEK.C	641.5699 (3+)			
			K.DDCDLPELCTGQSAECPTDV FQR.N	905.0342 (3+)			

[a] The peak refers to the peak number highlighted in Figure S1. [b] AC is the abbreviation of accession number in Uniprot database. [c] Toxicity score was calculated according to Laustsen, A et al. [25] by the ratio of protein abundance (%) estimated from the reverse phase HPLC chromatography to its medium lethal dose (LD_{50}). The toxicity score of the crude venom, which abundance was defined as 100%, was 149.3. N.D. represented LD_{50} was undetectable at a cut-off value of 50 μg per mouse. Abbreviations: SVMP indicates snake venom metalloproteinase. CRISP is cysteine-rich secretory protein. 3FTX means three finger toxins.

Table S2. List of synthetic peptides used for the immunoreactive peptide mapping study.

Index	Peptide sequence
$CTXA3_{1-15}$	LKCNKLVPLFYKTCP
$CTXA3_{5-19}$	KLVPLFYKTCPAGKN
$CTXA3_{11-25}$	YKTCPAGKNLCYKMF
$CTXA3_{15-29}$	PAGKNLCYKMFMVAT
$CTXA3_{21-35}$	CYKMFMVATPKVPVK
$CTXA3_{26-40}$	MVATPKVPVKRGCID
$CTXA3_{31-45}$	KVPVKRGCIDVCPKS
$CTXA3_{36-50}$	RGCIDVCPKSSLLVK
$CTXA3_{43-57}$	PKSSLLVKYVCCNTD
$CTXA3_{46-60}$	SLLVKYVCCNTDRCN
$sNTX_{1-15}$	LECHNQQSSQTPTTT
$sNTX_{4-18}$	HNQQSSQTPTTTGCS
$sNTX_{11-25}$	TPTTTGCSGGETNCY
$sNTX_{16-30}$	GCSGGETNCYKKRWR
$sNTX_{21-35}$	ETNCYKKRWRDHRGY
$sNTX_{26-40}$	KKRWRDHRGYRTERG
$sNTX_{31-45}$	DHRGYRTERGCGCPS
$sNTX_{36-50}$	RTERGCGCPSVKNGI
$sNTX_{39-53}$	RGCGCPSVKNGIEIN
$sNTX_{45-59}$	SVKNGIEINCCTTDR
$sNTX_{48-62}$	NGIEINCCTTDRCNN
[a] TFF	TFFLTQGALLNDK
[b] GIL	GILGFVFTLTVPSER

[a] TFF is the abbreviation of TFFLTQGALLNDK which is a partial sequence of neuraminidase of H1N1 influenza virus. [b] GIL is the abbreviation of GILGFVFTLTVPSER which is a partial sequence of M1 protein of H5N1 influenza virus.

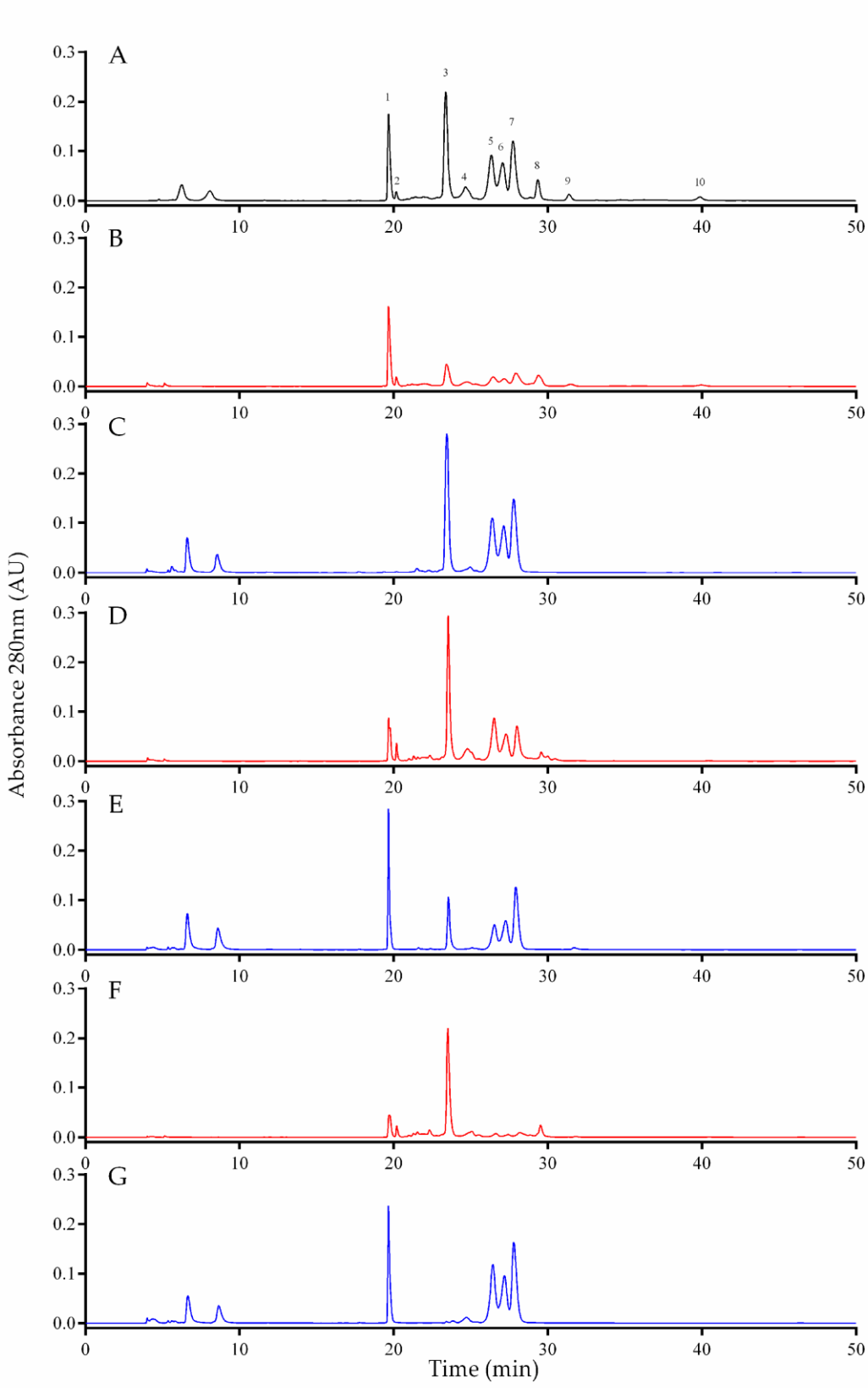

Figure S1. Evaluation of antivenom efficacy in capturing *N. atra* venom components. (**A**) HPLC chromatogram of 500 μg of *N. atra* crude venom. The component within the chromatographic peaks (no.1-10) were identified using LC MS/MS (Table S1). The elution (Elu) and flow through (FT) fractions of (**B**,**C**) BAV-, (**D**,**E**) SAV-Naja-, and (**F**,**G**) NPAV-immobilized affinity columns were collected and analyzed by reverse phase HPLC to determine the retained percentage of venom components.

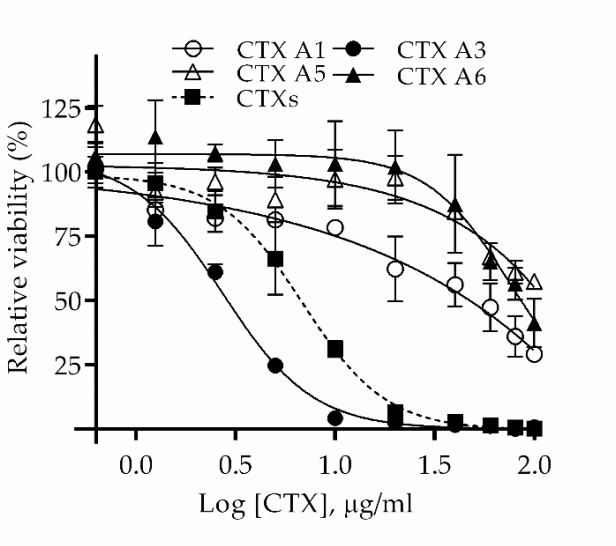

Figure S2. Analyzing the cytotoxicity of CTX analogs of *N. atra* venom using the cell-based assay.

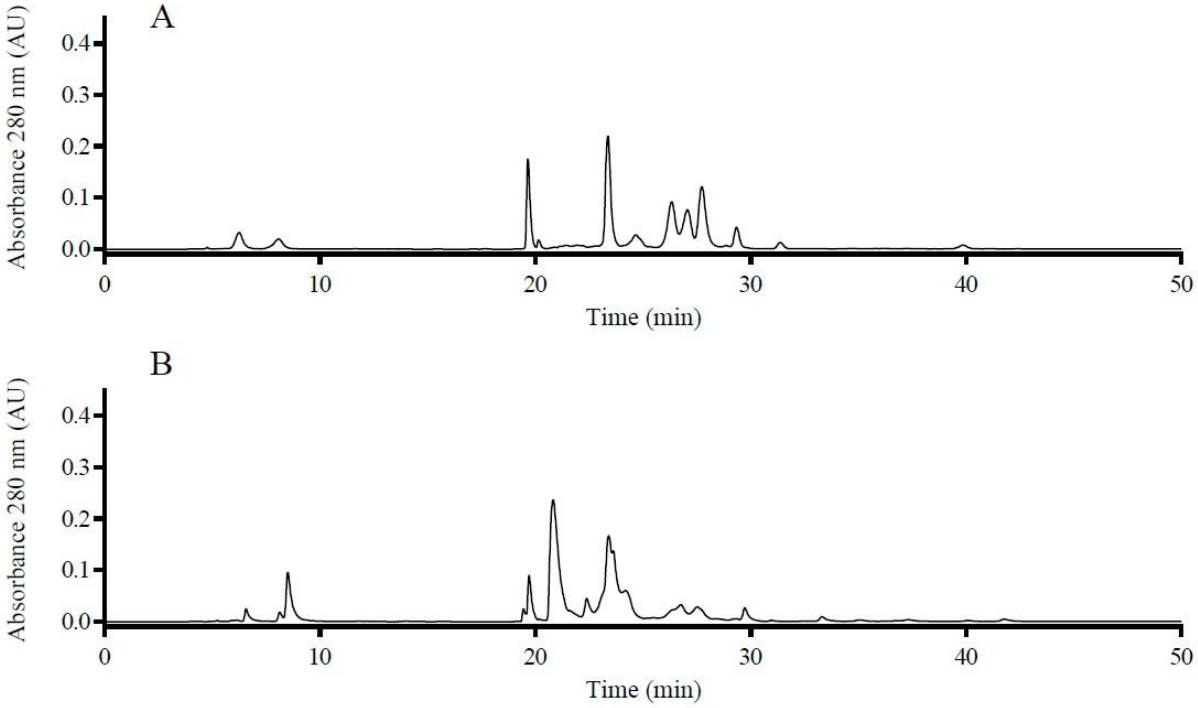

Figure S3. Reverse phase HPLC chromatograms of 300 µg of (**A**) *N. atra* and (**B**) *N. kaouthia* venom used in this study.