Appendix I

Alignment of peptide fragments obtained from tandem mass spectrometry of crude *Daboia russelii* venom

Appendix I:

Alignment of peptide fragments obtained from tandem mass spectrometry of crude venom of *Daboia russelii* with the snake venom protein families in the database (A-M). The peptide fragments obtained are highlighted with different colours (except black) while the identical peptide fragments are highlighted with the same colour. The amino acid substitutions in unique peptides fragments are underlined.

(A): Phosphodiesterase Family (02)

```
586829527 LKESVEPQVSCRYRCNETFSKMASGCSCDDKCTERQACCQDYEDTCVLPTQSWSCSKLRCSEKRMANVLCSCSEDCLEKKDCCTDYKSICKGETSWLKDQCASSSA
538259853 LKKSVQPQVSCRYRCNETFNKMTSGCSCDDKCTERQACCSDYEDTCVLPTQSWSCSKLRCGEKRIANVLCSCSEDCLEKKDCCTDYKSICKGETSWLKDKCASPSA

586829527 AQCPSGFEQSPLILFSMDGFRAGYLETWDSLMPNINKLKTCGTHAKYMRAVYPTKTFVNHYTIVTGLYPESHGIIDNNIYDVTLNLNFSLSAPTMTNPAWWGGQPI
538259853 TQCPAGFEQSPLILFSMDGFRAGYLETWDSLMPNINKLKTCGTHAKYMRAVYPTKTFVNHYTIATGLYPESHGIIDNNIYDVNLNLNFSLSASTAKNPAWWGGQPI

586829527 WHTVTYQGLKAATYFWPGSEVKINGSYPTIYKVYNKSIPFEARVTEVLKWLDLPKAERPDFVTLYIEEPDTTGHKFGPVSGEIIMALQMADRTLGMLMEGLKQRNL
538259853 WHTATYQGLKAATYFWPGSEVKINGSYPTIFKNYNKSVPFEARVTELLKWLDLPKAERPDFYTLYIEEPDTTGHKYGPVSGEIIKALEMADRTLGMLMEGLKQRNL

586829527 LHNCVNLILLADHGMEQISCNRLEYMTDYFDKVDFFMYEGPAPRIRSKNVPKDFYTFDSEGIVRNLTCQKPKQYFKAYLAKDLPKRLHYVNNIRIDKVNLMVDQQW
538259853 LHNCVNLILLADHGMEEISCDRLEYMANYFNNVDFYMYEGPAPRIRSKNVPKDFYTFDSEGIVKNLTCRKPKQYFKAYMSKDLPKRLHYANNIRIDKVNLMVDQQW

586829527 MAVRNKNYNRCNGGTHGYDNEFKSMQAIFLAHGPGFKGKNEVTSFENIEVYNLMCDLLKLKPAPNNGTHGSLNHLLKNPFYNPSPAKEQTSPLSCPFGPVPSPDVS
538259853 MAVRDKKFTRCKGGTHGYDNEFKSMQAIFLAHGPGFNEKNEVTSFENIEVYNLMCDLLKLKPAPNNGTHGSLNHLLKNPFYTPSPAKEQSSPLSCPFGPVPSPDVS

586829527 GCKCSSITDLGKVNERLNLNNQAKTESEAHNLPYGRPQVLQNHSKYCLLHQAKYISAYSQDVLMPLWSSYTINKSPPTSVPPSASDCLRLDVRIPAAQSQTCSNYQ
538259853 GCRCSSITELEKVNQRLNLNNQAKTESEAHNLPYGRPQVLQNHSKYCLLHQAKYISAYSQDILMPLWSSYTIYRSTPTSVPPSASDCLRLDVRIPEAQSQTCSNYQ

586829527 PDLTITPGFLYPPNFGSSNFEQYDALITSNLVPMFKGFTRLWNYFHGTLLPKYARERNGLNVISGPIFDYNYDGHFDSYDTIKEYVNDTKIPIPTHFFVVLTSCEN
538259853 LDLTITPSFLYPPNFNSSNFEQYDALITSNIVPMFKGFTRLWNYFHTTLIPKYARERNGLNVISGPIFDYNSDGHFDSYDTIKQYVKNTKIPIPTHYFVVLTSCEN

586829527 QINTPLNCPGSLKVLSFILPHRPDNSESCADTSPDNLWVEERIQTHTARVRDVELLTGLNFYSGLKQPLPETLQLKTFLPIFVNPVN
538259853 QINTPLNCLGPLKVLSFILPHRPDNSESCADTSPENLWVEERIQIHTARVRDVELLTGLNFYSGLKQPLPETLQLKTFLPIFVNPVN
```

(B): PLA_2 enzyme Family (15)

```
13936543  HLYQFENMIYQKTGKFAIIAYSNYGCYCGWGGKGKPQDATDRCCFVHDCCYGRVNGCDPKMGTYSYSFQNGDIVCGGDDPCLRAVCECDRVAANCFAENLKTYNKKY
50874332  HLSQFGDMINKKTGIFGIMSYIYYGCYCGWGGKGKPLDATDRCCFVHDCCYGRVNGCDPKLSTYSYSFENGDIVCGGDDPCLRAVCECDRVAAICFGENMNTYDKKY
223635543 HLLQFNKMIKFETRKNAIPFYAFYGCYCGWGGRGRPKDATDRCCFVHDCCYGKLAKCNTKWDIYPYSLKSGYITCGKGTWCEEQICECDRVAAECLRRSLSTYKYGY
1408314   NLFQFGDMILQKTGKEAVHSYAIYGCYCGWGGQGRAQDATDRCCFAQDCCYGRVNDCNPKMATYTYSFENGDIVCGDNDLCLRAVCECDRAAAICLGENVNTYDKNY
37927199  NLFQFGEMILQKTGKEVVHSYAIYGCYCGWGGQGRAQDATDRCCFVHDCCYGTVNDCNPKTATYSYSFENGDIVCGDNDLCLRTVCECDRAAAICLGQNVNTYDKNY
400714    NLFQFGEMILEKTGKEVVHSYAIYGCYCGWGGQGRAQDATDRCCFVHDCCYGTVNDCNPKTATYSYSFENGDIVCGDNDLCLRTVCECDRAAAICLGQNVNTYDKNY
3914259   NLFQFAEMIVKMTGKNPLSSYSDYGCYCGWGGKGKPQDATDRCCFVHDCCYEKVKSCKPKLSLYSYSFQNGGIVCGDNHSCKRAVCECDRVAATCFRDNLNTYDKKY
40889259  NLYQFGRMIWNRTGKLPILSYGSYGCYCGWGGQGPPKDATDRCCLVHDCCYTRVGDCSPKMTLYSYRFENGDIICDNKDPCKRAVCECDREAAICLGENVNTYDKKY
129506    NLYQFGKMIFKMTGKSPIFSYGDYGCYCGWGGKGTPVDATDRCCFVHDCCYGRVNSCNPKRSTYSYSFQNGGIVCDDQNLCKRAVCECDRVAAICFGENVNTYDKKY
3914268   NLLQFENMIRNVAGRSGIWWYSDYGCYCGKGGHGRPQDASDRCCFVHDCCYGKVNGCNPKKAVYIYSLENGDIVCGGDDPCRKEVCECDKAAAICFRDNKDTYDNKY
408407675 SLLEFGKMILEETGKLAIPSYSSYGCYCGWGGKGTPKDATDRCCFVHDCCYGNLPDCNPKSDRYKYKRVNGAIVCEKGTSCENRICECDKAAAICFRQNLNTYSKKY
24638087  SLLEFGKMILEETGKLAIPSYSSYGCYCGWGGKGTPKDATDRCCFVHDCCYGNLPDCNPKSDRYKYKRVNGAIVCEKGTSCENRICECDKAAAICFRQNLNTYSKKY
298351762 SLLEFGMMILEETGKLAVPFYSSYGCYCGWGGKATPKDATDRCCFVHDCCYGNLPDCNPKSDRYKYKRVNGAIVCEQGTSCENRICECDKAAAICFRRNLNTYSKIY
403399517 SLMQFEMLIMKLAKSSGMFWYSAYGCYCGWGGQGRPQDATDRCCFVHDCCYGKATGCDPKKDVYTYSEENGDIVCGGDDPCRKEVCECDKAAAICFRDNMDTYNSKT
123907686 SLLEFGRMIKEETGKNPLFSYISYGCYCGWGGQGQPKDATDRCCFVHDCCYGKLWSCSPKTDIYFYYRKNGAIVCARGTWCEKQICECDKAAAICFRENLGTYKAEY
```

```
13936543  WLSSIID-CKEESEKC
50874332  MLYSLLD-CGEESEQC
223635543 MFYPDSR-CRGPSETC
1408314   EYYSISH-CTEESEQC
37927199  EYYSISH-CTEESEQC
400714    EYYSISH-CTEESEQC
3914259   HNYPPSQ-CTGT-EQC
40889259  KSYED---CTEEVQEC
129506    KDYPTSQ-CTETEQ--
3914268   WNIPSEN-CQEESEPC
408407675 MLYPDFL-C-KGELRC
24638087  MLYPDFL-C-KGELKC
298351762 MLYPDFL-C-KGELKC
403399517 YWMFPAKNCQEESEPC
123907686 ESYGKSR-CTEKSLKC
```

(C): SvSPs Family (11)

```
380875417 QKSSELVIGGDECNINEHRSLVYLYNDSNFQ--CGGTLINQEWVLSAAHCDMENMEIYLGVHNLSLPNKDQKRRDPKEKFFCLSSKNYTKWDKDIMLIKLNRPV
406609998 QKSSELVIGGDECNINEHRSLVVLFNSSSFL--CGGTLINQEWVLTAAHCDSKNFQMLFGVHSKKILNEDEQTRDPKEKFICPNKKKDDENDKDIMLIRLDSPV
13959617  QKSSELVVGGDECNINEHRSLVAIFNSTGFF--CSGTLINQEWVVTAAHCDSNNFKMKFGAHSQKVLNEDEQIRNPKEKFICPNKKNNEVLDKDIMLIKLDSSV
13959655  QKSSELVIGGDECNINEHPFPVALHTARSKRFYCAGTLINQEWVLTAARCDRKNIRIILGVHSKNVPNEDQQIRVPKEKFFCLSSKTYTRWDKDIMLIRLKKPV
381141431 QKSSELVIGGDECNINEHPFLALMYNSTSMKFHCSGTLLNEEWVLTAAHCDMENMQIYLGVHDKKNPNKDQQTRVPKEMFFCLSNKSYTPWDKDIMLIRLNSPV
311223824 QKSSELVVGGDECNINEHRSLVFLYNNS---FGCSGTLINQQWVLSAVHCDMENVRIYLGVHNLTLRNNAEIRLPEER-FFCLSNKNYTKWDKDIMLIKLDRPV
297593764 QKSSELVTGGDECNINEHPFLVALHTARSKRFHCTGTLINEQWVLTAARCNRKNIRIKLGVHNKNVRNENEEMRVPAEKVFCVSSKTYTRWDKDIMLIKMKRPV
380875421 QKSSELVVGGDECNINEHRSLVFLYNSSFG---CGGTLINQEWVLSAAHCDMENMRIYLGWHNFSLPNMNQKRRVAKEKFFCLSSKNYTEWDKDIMLIKMNRPV
90116798  ------VIGGDECNINEHPFLVLVYYDDYQ---CGGTLINEEWVLTAAHCNGKNMEIYLGVHSKKVPNKDVQRRVPKEKFFCDSSKTYTKWNKDIMLIRLDRPV
134129    ------VVGGDECNINEHPFLVALYTSTSSTIHCGGALINREWVLTAAHCDRRNIRIKLGMHSKNIRNEDEQIRVPRGKYFCLNTKFPNGLDKDIMLIRLRRPV
82117246  ------VIGGDECNINEHRFLALLYSERFQ---CGGTLINEEWVLTAAHCDMGNMYIYLGVHNVSVQYDDEQRRYPKKKYFCLSSRNYNQWDNDIMLIRLNRPV

380875417 K-STHIAPLSLPSSPPSVGSVCRIMGWGTVTSPNETLLDVPHCANINILNYTVCRAASPRLPTQSRTLCAGILQGGIDACKGDSGGPLICNGQIQGIVSWGNHP
406609998 SNSEHIAPLSLPSSSPTVDSVCRIMGWGTIKPTEETYPDVPHCANINILDHTVCRAAYPVLLAESSTLCAGILEGGKDTCVGDSGGPLICNGQIQGIVSWGGHP
13959617  SNSEHIAPLSLPSSPPSVGSVCRIMGWGSITPTKVTYPDVPYCANINLLDDAECKPGYPELLPEYRTLCAGIVQGGKDTCGGDSGGPLICNGQFHGIVSYGAHP
13959655  NDSTHIVPLSLPSSPPSVGSVCRIMGWGTITTTKVTYPDVPHCANINMFDYSVCRKVYRKLPEKSRTLCAGILQGGIDSCKVDNGGPLICNGQIQGIVSWGGCG
381141431 TYSTHIAPFSLPSSPPTVGSVCRIMGWGAITSPNETYPDVPHCANIEIYDYSVCRKAYGGLPEKSRTLCAGVLQGGIDTCLADSGGPLICNGQFQGIVAWGRHP
311223824 KTSTYIAPLSLPSSPPRVGSVCRIMGWGAITSPNETFPGVTHCANINILPYSVCRAAYKGLPAQSRTLCGGILEGGIGSCMGDSGGPLICNGEMHGIVAWGDDT
297593764 NNSTHIAPLSLPSNPASVGSVCRIMGWGTITTTKVTYPDVPHCANIKIFDYSVCRGAYRKLPEKSRTLCAGVLEGGIDSCKADTGGPLICNGQFQGIASWGGQP
380875421 TYSTHVAPLSLPSSPPSVGSVCRIMGWGAITSPNETYPDVPHCANINILNYTVCRAAHPWLPAQSRTLCAGILQGGIDTCKGDSGGPLICNGQIQGIVSWGDNP
90116798  RKSAHIAPLSLPSSPPSVGSVCRVMGWGTITSPQETYPDVPHCAKINLLDYSECRAAYPGLPPKSRTLCAGVLEGGKDTCGGDSGGPLICNGQFQGIVSWGGDP
134129    TYSTHIAPVSLPSRSRGVGSRCRIMGWGKISTTEDTYPDVPHCTNIFIVKHKWCEPLYPWVPADSRTLCAGILKGGRDTCHGDSGGPLICNGQIQGIVAGGSEP
82117246  RNSAHIAPLSLPSGPPSVGSVCRVMGWGTITSPNETYPDVPHCANINILDYEVCRAAYAGLPATSRTLCAGILEGGKDSCRGDSGGPLICNGEIQGIVSWGGNI

380875417 CAQPLKPGHYTHVFDYTDWIQSIIAGNTTATCPP
406609998 CGQGSKPGVYTKVFDHLDWIKSIIAGNTAVTCPP
13959617  CGQSLKPGIYTTVFDYNDWIKSIIAGNTAATCPP
13959655  QAQPHKPALYTNVFDYTDWIQSIIAGNITATCPP
381141431 CAQPQLPAFYTKVFDYSDWIQSIIAGNTAATCPS
311223824 CAQPHKPVHYTKVYDYTDWIQSIIAGNTAATCPP
297593764 CAQPLKPALYT----------------------
380875421 CAQPLKPGHYTNVFDYTDWIQSIIAGNTTATCPP
90116798  CAQPHEPGSYTNVFDHLDWIKGIIAGNTDATCPL
134129    CGQHLKPAVYTKVFDYNNWIQNIIAGNRTVTCPP
82117246  CAQPREPGLYTKVFDYIDWIQSIIAGNTTVNCPP
```

(D): SvMP

RVV X light chain (α subunit) (03)

```
73621141  GLDCPPDSSPYRYFCYRVFKEQKNWADAERFCAERPNNGHLVSIESMEEAEFVAQLLSKITGKFITHFWIGLRIEDKKQQCRSEWSDGSSVSYDNLLKREFRKCF
300490458 GLDCPPDSSLYRYFCYRVFKEHKTWEAAERFCMEHPNNGHLVSVESMEEAEFVAKLLSNITEKFITHFWIGLMIKDKEQECSSEWSDGSSVSYDNLDKREFRKCF
300079896 GLDCPPDSSPYRYFCYRVFKLRKSWEAAERFCMEHPNNGHLVSIESMEEAEFVAKLLSNTTGKFITHFWIGLRIKDKEQECSSEWSDGSSVSYDNLGKEEFRKCF

73621141  GLEKGTGYRSWFNLNCEEPYPFVCKVPPNC
300490458 VLEKESGYRMWFNRNCEERYLFVCKVPPEC
300079896 VLQKESGYRMWFNHKCEEPYPFVCKVPPEC
```

RVV X light chain (β subunit) (03)

```
73620113  KQDCLSDWSFYEGYCYKVFNEKKTWEDAEKFCTEQHKGSHLLSLHNIAEADFVLKKTLAMLKDGVIWMGLNDVWNECNWGWTDGAKLDYKAWNEGTNCFVFKIAK
251205    VLDCPSGWLSYEQHCYKGFNDLKNWTDAEKFCTEQKKGSHLVSLHSREEEEFVVNLISENLEYPATWIGLGNMWKDCRMEWSDRGNVKYKALAEESYCLIMITHE
73620112  AFCCPSGWSAYDQNCYKVFTEEMNWADAEKFCTEQKKGSHLVSLHSREEEKFVVNLISENLEYPATWIGLGNMWKDCRMEWSDRGNVKYKALAEESYCLIMITHE

73620113  NHWSHMDCSSTHNFVCKFRV
251205    KEWKSMTCNFIAPVVCKF
73620112  KVWKSMTCNFIAPVVCKF
```

RVV X heavy chain (06)

```
300079900 KYENIEKEDETPKMCGVTQTNWESDKPIKKASQLVSTSAQFNKA--FIELIIIVDHSMAKKC--NSTATNT--KIYEIVNSANEIFNPLNIHVTLIGVEFWCDRDLINV
162329887 ---------------------------------LVSTSAQFNKI--FIELVIIVDHSMAKKC--NSTATNT--KIYEIVNSANEIFNPLNIHVTLIGVEFWCDRDLINV
73621852  KYENIEKEDEAPKMCGVTQTNWESDEPIKKASQLVATSAKRKFHKTFIELVIVVDHRVVKKY--DSAATNT--KIYEIVNTVNEIFIPLNIRLTLIGVEFWCNRDLINV
123896981 KYENIEEEDEAPKMCGVKHTNRESDKSIKKASQLNLTPEQQRYLNTPKHIKVAIVADYLIFRKYGRNLFTIRAKIYEILNILNEIYKAFNIHVALVFLEIWSNGDKINL
297593790 KYENIEKEDEAPKICGVKKTNWESDKSIQEASQLNLTPEQQRYLNSEKHIKVAIIADYLIYRKYGRNLFTIRTRIYEIINILNAIYRAFHMHVALVFLEIWSNGDKINV
83523646  KYENIEEEDEAPKMCGVKQSNRESDEPIKKASGLIVPSQKRRLDQKFIELVMVVDHSMVTKY--NNDSTAVRTWIYEMVNTVNEIYLPLNIRVPLVGIVFWSNRDLINV


300079900 TSSADETLDSFGEWRASDLMTRKSHDNALLFTDMRFDLNTLGITFLAGMCQAYRSVGIVQVQGNRNFKTAVIMAHELSHNLGMYHDGKNCICNDSSCVMSPVLSDQPSK
162329887 TSSA-DTLNSFGEWRASDLMTRKSHDNALLFTDMRFDLNTLGITFLAGMCQAYRSVGIVQEQGNRNFKTAVIMAHELSHNLGMYHDGKNCICNDSSCVMSPVLSDQPSK
73621852  TSSADDTLDSFGEWRGSDLLNRKRHDNAQLFTDMKFDLSTLGITFLDGMCQAYRSVGIVQEHGNKNFKTAVIMAHELGHNLGMYHDRKNCICNDSSCIMSAVLSSQPSK
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```
123896981 FPAANVTLDLFGKWRERDLMNRKNHDNTQLLTGMNFDGPTAGLGYVGTMCHPQFSAAVVQDHNKINFLVALAMAHELGHNLGMTHDEQFCTCGAKSCIMSATLSCEGSY
297593790 LPAANVTLDLFGKWRLSDLLNRREHDNAQLLTGINFDGPTAGLGYVGSMCEPQYSAAIVQDHNKINILVAMAMAHELGHNLGMNHDEKFCTCGAKSCIMSGTLSCEGSF
83523646  TFTADDTMDSFGEWRASYLLNRKRHDYAQLLTNITLDFDSLGMAFIDGMCKSDRSVGLIRDDSSTTFRTAVIMAHEMGHSLGMEHDSRSCKCAASPCIMSKALGKQPTK

300079900 LFSNCSIHDYQRYLTRYKPKCILYPPLRKDIVSPPVCGNEIWEEGEECDCGSPADCQNPCCDAATCKLKPGAECGNGLCCYQCKIKTAGTVCRRARNECDVPEHCTGQS
162329887 LFSNCSIHDYQRYLTRYKPKCIFNPPLRKDIVSPPVCGNEIWEEGEECDCGSPANCQNPCCDAATCKLKPGAECGNGLCCYQCKIKTAGTVCRRARDECDVPEHCTGQS
73621852  LFSNCSNHDYRRYLTTYKPKCILNPPLRKDIASPPICGNEIWEEGEECDCGSPKDCQNPCCDAATCKLTPGAECGNGLCCEKCKIKTAGTVCRRARDECDVPEHCTGQS
123896981 RFSNCSREENRRYLINKMPQCILIKPSRTDIVSPPVCGNSLVEVGEDCDCGSPGYCRNPCCNAATCKLTPGSQCADGECCDQCRFTRAGTECRPARDECDKADLCTGQS
297593790 RFSNCSQEENRKYLIRKMPQCILKKPLKTDIVSPPVCGNYLVELGEDCDCGTPTFCQNPCCNAATCKLTPGSQCADGECCDQCRFRRAGTECRPAKDECDMADLCNGQS
83523646  VFSSCSYDDYRMYLAKYKPKCILDPPLRKDIASPAVCGNKIWEEGEECDCGSPEDCRNPCCDAETCELFPAAECADGPCCHKCKIRTAGTICRPARDECDVTEHCTGQS

300079900 AECPRDQLQQNGQPCQNNRGYCYNGDCPIMRNQCISLFGSRATVAKDSCFQENLKGSYYGYCRKENGRKIPCAPQDVKCGRLFCLNNSPRNKNPCNMHYSCMDQHKGMV
162329887 AECPRDQLQQNGKPCQNNRGYCYNGDCPIMRNQCISLFGSRANVAKDSCFQENLKGSYYGYCRKENGRKIPCAPQDVKCGRLFCLNNSPRNKNPCNMHYSCMDQHKGMV
73621852  AECPADGFHANGQPCQNNNGYCYNGDCPIMTKQCISLFGSRATVAEDSCFQENQKGSYYGYCRKENGRKIPCAPQDIKCGRLYCLDNSPGNKNPCKMHYRCRDQHKGMV
123896981 AECPADQFQRNGQPCQNNSGYCYNGICPVMRNQCISLFGSRAIVAEDACFQFNSLGIDYGYCRKENGRKIPCAPEDVKCGRLYCFDNLPEHKNPCQIFYTPRDEDKGMV
297593790 DECPKDQFQRNGHPCQNNNGYCYNGKCPVMGNQCISLFGSRATVAEDACFQFNRLGSDYGYCRKENGIKIPCAPEDVKCGRLYCFDNLPEHKNPCQIYYTLRDENKGMV
83523646  AECPRNELQRNGEPCLDKLGYCYNGDCPIMRNQCISLFGSRATVAEDSCFQQNLNGSEHGYCAKENGRKIPCAPQDVKCGRLYCLDNSSRKKNPCKMHYLNADQHKGMV

300079900 DPGTKCEDGKVCNNKRQCVDVNTAYQSTTGFSQI
162329887 DPGTKCEDGKVCNNKRQCVDVNTAYQSTTG----
73621852  EPGTKCEDGKVCNNKRQCVDVNTAY---------
123896981 DPGTKCENGKVCINGK-CVDVNTAY---------
297593790 EPGTKCENGKVCINGK-CVDVNTAY---------
83523646  EPGTKCEDGKVCINRK-CVDVKTAYYSTTGFSQI
```

(E): KSPI Family (03)

```
159883524 HDRPKFCYLPADPGECMAYIRSFYYDSESKKCKEFIYGGCHGNANNFPTRDKCRQTCRAPRKGRHT
159883540 HDRPKFCYLPADPGECLAHMRSFYYDSESKKCKEFIYGGCHGNANKFPSRDKCRQTCGASAKGRPT
123913154 QDRPKFCHLPVDSGICRAHIPRFYYNPASNQCQGFIYGGCGGNANNFETRDQCRHTCGGK------
```

(F): Disintegrin (02)

```
123916448 ----------------------------------------------CTTGPCCRQCKLKPAGTTCWRTSVSSH-YCTGRSCECPSYPGNG
50365991  ---------------MHMEAGEECDCGSPGNPCCDAATCKLRQGAQCAEGLCCDQCRFMKKGTVCRIARGDDMDDYCNGISAGCPRNPFHAKLAAALEH
```

(G): LAAO Family (05)

```
347602327 ADDKNPLEECFREADYEEFLEIAKNGLKKTSNPKDIVVVGAGMSGLSAAYVLAGAGHKVTVLEASQLVGGRVRTHRNAKEGWYANLGPMRIPEKHRIVREYIRKF
395406796 ADDKNPLEECFREDDYEEFLEIAKNGLKKTSNPKHIVIVGAGMSGLSAAYVLAGAGHKVTVLEASERPGGRVRTHRNVKEGWYANLGPMRVPEKHRIIREYIRKF
10120762  ADDRNPLAECFQENDYEEFLEIARNGLKATSNPKHVVIVGAGMAGLSAAYVLAGAGHQVTVLEASERPGGRVRTYRNEEAGWYANLGPMRLPEKHRIVREYIRKF
75570145  ADDRNPLEECFRETDYEEFLEIARNGLKATSNPKHVVIVGAGMSGLSAAYVLSGAGHQVTVLEASERAGGRVRTYRNDKEGWYANLGPMRLPEKHRIVREYIRKF
538259837 ADDRNPLEECFRETDYEEFLEIARNGLKKTSNPKHVVIVGAGMSGLSAAYVLAGAGHQVTVLEASERAGGRVRTYRNDKEGWYANLGPMRLPEKHRIVREYIRKF

347602327 GLELNEFVQETDNGWYFVKNIRKRVGEVKKDPGLLKYPVKPSEAGKSAGQLYQEALGKAVEELKRTNCSYMLNKYDTYSTKEYLIKEGNLSTGAVDMIGDLMNED
395406796 GLKLNEFVQETENGWYFIKNIRKRVGEVKKDPGLLKYPVKPSEAGKSAGQLYQESLGKAVEELKRTNCSYILNKYDTYSTKEYLIKEGNLSPGAVDMIGDLLNED
10120762  DLRLNEFSQENDNAWYFIKNIRKKVGEVKKDPGLLKYPVKPSEAGKSAGQLYEESLGKVVEELKRTNCSYILNKYDTYSTKEYLIKEGDLSPGAVDMIGDLLNED
75570145  GLQLNEFSQENDNAWYFIKNIRKRVGEVKKDPGVLKYPVKPSEEGKSAGQLYEESLGKVVEELKRTNCSYILNKYDTYSTKEYLLKEGNLSPGAVDMIGDLMNED
538259837 GLQLNEFSQENDNAWHFIKNIRKRVGEVKKDPGVLKYPVKPSEEGKSAGQLYEESLRKVEKELKRTNCSYILNKYDTYSTKEYLIKEGNLSPGAVDMIGDLLNED

347602327 SGYYVSFVESMKHDDIFAYEKRFDEIVGGMDQLPTSMYRAIEKSVLFKARVTKIQQNAEKVRVTYQTAAKTLSDVTADYVIVCTTSRAARRINFKPPLPPKKAHA
395406796 SGYYVSFIESLKHDDIFAYEKRFDEIVGGMDQLPTSMYRAIEESVHFKARVIKIQQNAEKVTVTYQTTQKNLLLETADYVIVCTTSRAARRITFKPPLPPKKAHA
10120762  SGYYVSFIESLKHDDIFAYEKRFDEIVDGMDKLPTAMYRDIQDKVHFNAQVIKIQQNDQKVTVVYETLSKETPSVTADYVIVCTTSRAVRLIKFNPPLLPKKAHA
75570145  SGYYVSFPESLRHDDIFAYEKRFDEIVGGMDKLPTSMYRAIEEKVHLNAQVIKIQKNAEKVTVVYQTPAKEMASVTADYVIVCTTSRATRRIKFEPPLPPKKAHA
538259837 SGYYVSFIESMKHDDIFAYEKRFDEIVGGMDQLPTSMYQAIEEKVRFNTRVIKIQQNAKKVTVTYQTPAKDTSLVTADYVIVCTTSRAARRINFRPPLPPKKAHA

347602327 LRSVHYRSATKIFLTCTKKFWEDDGIQGGKSTTDLPSRFIYYPNHNFTSGVGVIIAYGIGDDSNFFLSLTLNECADIVFSDLSSIHQLPKNDIQKFCNPSVIQKW
395406796 LRSVHYRSGTKIFLTCTKKFWEDDGIQGGKSTTDLPSRFIYYPNHNFTTGVGVIIAYGIGDDANFFQALNLNECADIVFNDLSSIHQLPKKDLQTFCYPSIIQKW
10120762  LRSVHYRSGTKIFLTCTTKFWEDDGIHGGKSTTDLPSRFIYYPNHNFTNGVGVIIAYGIGDDANFFQALDFKDCADIVFNDLSLIHQLPKKDIQSFCYPSVIQKW
75570145  LRSVHYRSGTKIFLTCTKKFWEDEGIHGGKSTTDLPSRFIYYPNHNFTSGVGVIIAYGIGDDANFFQALDFKDCADIVINDLSLIHQLPREEIQTFCYPSMIQKW
538259837 LRSVHYRSGTKIFLTCTKKFWEDDGIHGGKSTTDLPSRFIYYPNHNFTSGVGVIIAYGIGDDANFFQALDFKSCADIVMNDLSLIHQLPKKDIQAFCYPSMIQKW

347602327 SLDRYAMGAITTFTPYQFQDYSKALTAPAGRVYFAGEYTANAHGWIDSTIKSGLTAARDVNQASEL------------
395406796 SLDKYAMGAITTFTPYQFQHFSEALTAPVGRIFFAGEYTANAHGWIDSTIKSGLTAARDVNRASEL------------
10120762  SLDKYAMGGITTFTPYQFQHFSDPLTASQGRIYFAGEYTAQAHGWIDSTIKSGLRAARDVNLASENPSGIHLSNDNEL
75570145  SLDKYAMGGITTFTPYQFQHFSEPLTASVDRIYFAGEHTAEAHGWIDSTIKSGLRAARDVNRASEQ------------
538259837 SLDKYAMGGITTFTPYQFQHFSEALTAPVGRIFFAGEYTAHAHGWIDSTIKSGLTAARDVNRASENP-----------
```

(H): Nucleotidase Family (03)

```
338855300 SGKCTGQDCYGGVARRATKIRELRAKHRHVLLLDAGDQYQGTVWFNFFKGREVVKFMNSLRYDAMALGNHEFDNGLAGLLDPLLKHANFPILSANIRPKGSIASN
538259847 ---------VHGVARRATKIRELRAKHRHVLLLDAGDQYQGTIWFSFFKGREVVKFMNSLRYDAMALGNHEFDNGLAGLLDPLLKHANFPILSANIRPKGSIASN
586829529 ----------------------------------------------------------------------------------------------------

338855300 ISGYILPYKIINVGSEKVGIIGYTTKETPVLSNPGPYLEFRDEVEELQNHANKLTTLGVNKIIALGHSGFSEDQRIARKVKGVDVVVGGHTNTFLYTGSPPSTEV
538259847 ISGYILPYKIINVGSEKVGIIGYTTKETPVLSNPGPYLEFRDEVEELQNHANKLTTLGVNKIIALGHSGFLEDQRIARKVKGVDVVVGGHTNTFLYTGSPPSTEV
586829529 -------------AREKVGIIGYTTKETPVLSNPGPYLEFRDEVEELQIHANKLTTLGVNKIIALGHSGFFEDQRIARKVKGVDVVVGGHTNTFLYTGSPPSTEV

338855300 AAGNYPFMVQSDDGRQVPVVQAYAFGKYLGYLNVIFDDKGNVIKSSGNPILLNKDISEDQDIKAEVNKMKIQLHNYSSQEIGKTIVYLNGTTQACRFHECNLGNL
538259847 AAGNYPFMVQSDDGRQVPVVQAYAFGKYLGYLNVIFDDKGNVIKASGNPILLNKDISEDQDVKAEVNKMKIQLRNYSSQEIGKTIVYLNGTTQACRFHECNLGNL
586829529 PAGNYPFMVQSDDGRQVPVVQAYAFGKYLGYLNVVFNDKGNVIKASGNPILLNKDIPEDQVVKAQVNKMKIQLQNYYSQEIGKTIVYLNGTTQACRFHECNLGNL

338855300 ICDAVIYNNVRHPDDNEWNHVSMCIVNGGGIRSPIDERTNNGTITLEELTAVLPFGGTFDLLQIKGSALKQAFEHSVHRHGEGMGELLQVSGIKVVYDLSRKPGS
538259847 ICDAVIYNNVRHPDDNEWNHVSMCIVNGGGIRSPIDERTNNGTITLEELTAVLPFGGTFDLLQIKGCALKEAFEHSVHRHGQGMGELLQVSGIKVVYDLSRKPGN
586829529 ICDAVIYNNLRHPDDNEWNHVSMCIVNGGGIRSPIDERANNGIITLEELTSVLPFGGTFDLLQIKGSALKQAFEHSVHRHGQGTGELLQVSGIKVVYDLSQKPGS

338855300 RVLSLNVLCTECRVPTYVPLEKEKTYKLLLPSFLAAGGDGYHMLKGDSSNHSSGNLDISIVGDYIKRMGKVFPAVEGRMIFSAGTLFQAQLFLTWGLCVSLLYFIL
538259847 RVVSLNVLCTECRVPTYVPLEKEKTYKLLLPSFLAAGGDGYHMLKGDSSNHSSGNLDISIVGDYIKRMGKVYPAVEGRVIFSPGTLFQAQLFLTWGLCISLLYFIL
586829529 RVVSLNVLCTKCRVPTYVPLEMEKTYKVLLPSFLATGGDGYHMLKGDSSNHNSGDLDISIVGDYIKRMEKVFPAVEGRVTFLDGTLFQAQLFLTWGLCISLLFFIL
```

(I): CRISPs (Helveprins) Family (07)

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190195337 SVDFDSESPRKPEIQNEIVDLHNSLRRSVNPTASNMLKMEWYPEAAANAERWAYRCIEEHSSRDSRVLEGIKCGENIYMSPNPMKWTEITHAWHGEYKDFKYGVGA
190195307 SVDFDSESPRKPEIQNEIVDLHNSLRRSVNPTASNMLKMEWYPEAAANAERWAYRCIESHSPRDSRVLEGIKCGENIYMSPVPIKWTEIIHGWHGENKNFKYGIGA
190195323 SVDFDSESPRKPEIQNEIVEFHNSLRRSVNPTASNMLKMEWYPEAAANAERWAFRCILDHSPYNSRVIGGIKCGENIYMSSNPIKWIEIIRKWHDEKKNFIYGKGA
190195319 SVDFDSESPRKPEIQNKIVDLHNFLRRSVNPTASNMLKMEWYPEAAANAERWAYRCIESHSPRDSRVLEGIKCGENIYMSPVPMKWTEIIHAWHGENKDFKYGIGA
190195321 SVDFDSESPRRPEIQNEIVDLHNSLRRSVTPTASNMLKMEWYPEAAANAERWAFRCILNHSPYNSRVIGGIKCGENIYMSPYPMKWTAIIHEWHKEKKDFVYGQGA
190195329 -VDFDSESPRKPEIQNKIVDLHNSLRRSVNPTASNMLKMEWYPEAAANAERWAYRCIESHSPRNSRVLGGIKCGENIYMSSIPITWNEIIHAWHGEYKDFIFGVGA
1778013   ----------------------------------------MEWYPEAAANAERWAYRCIESHSSRDSRVIGGIKCGENIYMSPYPAKWTDIIHAWHGEYKDFKYGVGA

190195337 DPPNAVTGHYTQIVWYKSHHLVCC-CLCPLSKYSYFYVCQYCPAGNIIGKIATPYTSGPPCGDCPSACDNGLCTNPCTQEDKYTNCKSLLQQDSCQDAGMQSKCSA
190195307 EPSNAVTGHFTQIVWYKSYRVGCAAAYCPSSKYSYFYVCQYCPAGNIRGKTATPYKSGPPCGDCPSACDNGLCTNPCTKEDKYTNCKSLVQQAGCEDKQIQSDCSA
190195323 NPSNAVVGHYTQVVWYKSYRIGCAAAYCPSSAYKYFYVCQYCPAGNIVGRTATPYKSGQPCGDCPSACDNGLCTNPCRREDVFTNCIDMAKGRSCQDNYMKLNCPA
190195319 DPPNAVIGHYTQVVWYKSYRIGCAAAYCPSSEYSYFYVCQYCPAGNIIGKTATPYKSGPPCGDCPSACDNGLCTNPCTKEDKYTNCKSLVQQAGCQNERMQSDCSA
190195321 SPANAVVGHYTQIVWYKSYRSGCAAAYCPSSEYNYFYVCQYCPAGNIIGKIATPYTSGPPCGDCPSACDNGLCTNPCSHHDEFTNCKDLV-KQGCHSNYLKTKCPA
190195329 NPPNAVTGHYTQIVWYKSYRIGCAAAYCPSSEYSYFYVCQYCPAGNIRGKTATPYKSGPPCGDCPSACDDGLCTNPCTREDKYTNCNSLVQKSGCQDTWMQSNCPA
1778013   VPSNAATGHYTQIVWYKSYRGGCAAAYCPSSKYRYFYVCQYCPAGNMIGKTATPYTSGPPCGDCPSDCDNGLCTNPCTQENTYSNCNSLVQQSSCQDNNMKTKCPA

190195337 SCFCQNKII
190195307 ICFCQNKII
190195323 ACFCRNEIK
190195319 ICFCQNKII
190195321 SCFCHNEII
190195329 ICFCQNKII
1778013   SCFCQNKII
```

(J): Snaclec Family (06)

Debocetin α subunit
123899657 DCPSEWSSHEGHCYKVFKLLKTWEDAEKFCTQQANGWHLASIESVEEANFVAQLASETLTKSKYHAWIGLRDQSKRQQCSSHWTDGSAVSYETVTKYTKCFGL
123899657 NKETKYHEWITLPCGDKNPFICKSWVLH

Debocetin β subunit
300490464 KQDCLSDWSFYEGYCYKVFNEKKTWEDAEKFCNEQVNGGYLVSFRSSEEMDFVIRMTFPIFRFDFFWIGLRDFWRDCYWRWSDGVNLDYKAWSREPNCFVSKTT
300490464 DNQWLRWNCNDPRYFVCKSRVSC

P31 α subunit
300490478 DLDCPSGWSAYDQHCYQAVDEPKSWADAEKFCTEQANSGHLVSIKSVGEANFVAQLASGFMQKDGIYVWIGLRDRRKEQQCRSEWTDGSKIIYVNWKEGESKMC
300490478 QGLAKWTYFHKWDYVNCAEHYRFVCKFPPQY

P31 β subunit
300490484 GFSCPNGWSSFGQHCYKVIEPLKNWTDAEKFCREQHKGSHLASIHSSEEEAFVSKVASKVLKFGSVWIGLNDPWHNCNWEWSDNARFDYKAMTRRPYCTVMVLK
300490484 PDRIFWFNRGCEKFVSFVCKFLA

P68 α subunit
300490470 DFDCPSGWSAHDQHCYKAFDEPKRSGDAETFCTEQANSGHLVSIESVEEAEFVAQLISENIKTPADYVWIGLRNQRKAQYCISKWTDGSSVIYKNVIERFIKNC
300490470 FGLEKESDYRTWFNLSCGDDYPFVCKFPPRC

Alboaggregin A
3023231 GFDCPFGWSSYEGYCYKVYNKKMNWEDAESFCREQHKRSHLVSFHSSGEVDFVVSKTFPILRYDFVWMGLSDIWKECTKEWSDGARLDYKAWSGKSYCLVSKTT
3023231 NNEWLSMDCSRTRYPVCKFCG

Crotocetin
82129809 DFDCPSGWSAYDQYCYRVIKQLKTWEDAEWFCTKQAKGAHLVSVESAGEADFVAQLVAENIKQNKYYVWIGLRIQNKGQQCSTKWSDGSSVNYENLLKSYSKKCF
82129809 GLKKETEFLQWYNTDCEEKNLFVCKFPPQR

Snaclec A14
218526485 DFDCPPDWSAYDQHCYKAFDEPKRSGDAEKFCTQQANGGHLVSIESVEEAEFVAQLISENIKTSADYVWIGLWNQRKAPYCVSKWTDGSSVIYKNVIERFIKNCFG
218526485 LEKETNYRTWFNLSCGDDYPFVCKSPA

(K): VNGF (01)

335892642 SEDNVSLRSPATPDLSDTSCAKTHEALKTSRNTDQHYPAPNKAEDQEFGSAANIIVDPKLFQKRRFQSPRVLFSTQPPPLSRDEQSVEFLDNADSLNRNIRAKR
335892642 ATHPVHNQGEFSVCDSVSVWVANKTTATDMRGNVVTVMVDVNLNNNVYKQYFFETKCKNPNPVPSGCRGIDAKHWNSYCTTTDTFVRALTMERNQASWRFIRIN
335892642 TACVCVISRKNDNF

(L): VEGF (02)

327478537 ------------QVRPFLDVYERSACQTRETLVSILQEHPDEISDIFRPSCVAVLRCSGCCTDESMKCTPVGKHTADIQIMRMNPRTHSSKMEVMKFMEHTA
327488518 APAQGDGDRQQGEVISFLTVYERSACRPVETMVDIFQEYPDEVEYIFKPSCVALMRCGGCCNDEALECVPTEVYNVTMEIMKLKPFQSQHIHP-MSFQQHSK

327478537 CECRPRWKQGEPEGPKEPRRGGVRAKFPFD
327488518 CECRPKKEVRIRQENHCEPCSERRKHLYKQDPLTCKCSCKFTDSRCKSKQLELNERTCRCEKPRR

(M): Hypothetical like protein (01)

387016758 MACSRACSGENGEQATSQNNSGDNERQWQQERLNREEAYYQFINALSDEDYRLMRDRNLLGTPGEITADELQQRLQSAKENQASQSEPENREWEDSETLGENI
387016758 TSNSLLEWLNTFHHTENSTHSGQSGNQTWRAVSQANPSSGEFRFSLEININHEQNNDNTPGEQLNEFLYGHSSRMHMENRPVIANSPVASRTRSRTLANSVGP

387016758 GFVSSGIGNVGGLLTQNAEENSRFFSGRLGARNRSSASSTPNSLLDDNEHNIIRQRRTQRVTPVRYYRGRARTRRNSRQRTDILRLRSTFRGQFQSLLENGQP
387016758 VNMQQIHAGTNRAHTTQPSPEQTEEQASSLGITLEEEESVRAASASRRHPSITLDLQVRRIRPRENRDRDSIASRTRSRVGMADNLVTPESDNEGFIQNVSRS
387016758 EYAGIRTYVNTIRIPLHRGSDTGLGESSSVAVRSILRQIMTGFGELSSLMDTETSSETESNSQHLPDIPPSMPSFRTLNSEFLTTSPRDRLTDQDSTEGQGET
387016758 NSIQHHQNNNTPNSRASFVENGTLPILRLVPYLLLEEDSSDNLRGLTKDQIDNLSTRNYENPHSEDDEISKTCSVCINEYVVGNKLRQLPCMHEFHFHCIDRW
387016758 LSENSTCPICRQPVVT

Appendix II

List of Research Publications

Publications related to PhD work

1. **Maitreyee Sharma**, Neeharika Gogoi, B. L. Dhananjaya, Jaideep C. Menon, and Robin Doley, "**Geographical variation of Indian Russell's viper venom and neutralization of its coagulopathy by polyvalent antivenom"**. *J. Toxicol. Toxin. Rev*., **33**, (1-2), 7-15, 2014.

2. **Maitreyee Sharma**, Diganta Das, Janaki Krishnamoorthy Iyer, R. Manjunatha Kini and Robin Doley, **"Unveiling the complexities of *Daboia russelii* venom, a medically important snake of India, by tandem mass spectrometry",** *Toxicon*, **107**, 266-281, 2015.

3. **Maitreyee Sharma**, Janaki Krishnamurthy Iyer, Norrapat Shih, Munmi Majumder, Venkata Satish Kumar Mattaparthi, Rupak Mukhopadhyay, Robin Doley, **"Daboxin P, a major phospholipase A_2 enzyme from the Indian *Daboia russelii russelii* venom targets Factor X and Factor Xa for its anticoagulant activity."** *PLoS One***, 11** (4), 2016**.**

Other Publications:

1. Diganta Das, **Maitreyee Sharma**, Hemanga Kumar Das, Partha Pratim Sahu & Robin Doley, **"Purification and characterization of Nk-3FTx: A three finger toxin from the venom of North East Indian Monocled cobra."** *J. Biochem Molecular Toxicology*, **30** (2), 59-70, (2015).

2. Maitreyee Sharma and Robin Doley, **"Snake venom variation and neutralization by Polyvalent Antivenom."** Newsletter, Toxinological Society of India, **2**, (2), 2012.

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 DOI: 10.3109/15569543.2013.855789

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REVIEW ARTICLE

Geographical variation of Indian Russell's viper venom and neutralization of its coagulopathy by polyvalent antivenom

Maitreyee Sharma[1], Neeharika Gogoi[1], B. L. Dhananjaya[2], Jaideep C. Menon[3], and Robin Doley[1]

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Abstract

Indian Russell's viper venoms of four different geographical locations were found to vary in composition, coagulopathy and phospholipase A_2 (PLA_2) activity. Venom from Kerala showed highest procoagulant activity followed by Tamil Nadu, West Bengal and Karnataka whereas PLA_2 activity was highest in venom from West Bengal. The commercial polyvalent antivenom differentially neutralized the aforesaid activities of the crude venoms. Antivenomics study showed the presence of non-immunodepleted and partially immunodepleted proteins in the crude venoms. Thus, Indian Russell's viper venom from different region varies in composition and accentuates the need to design regiospecific antivenoms to confront the problem of envenomation more effectively.

Keywords

Antivenomics, coagulopathy, geographical variation, Indian Russell's viper, *in vitro* neutralization

History

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Introduction

Snake venom is a complex mixture of proteins and polypeptides which varies from species to species and also within the same species. This variation has been mainly attributed to difference in diet, gender, age, season and geographical locations of the snakes (Alape-Giron et al., 2008; Chippaux et al., 1991; Daltry et al., 1996a,b; Jayanthi & Gowda, 1988; Menezes et al., 2006; Minton & Weinstein, 1986; Williams & White, 1992). Clinical symptoms of envenomation like neurotoxicity, myotoxicity, hemotoxicity, anticoagulant, procoagulant, haemorraghic, necrosis, renal damage and muscular paralysis in prey/victims might also vary within the same species due to this variation in venom composition (Hung et al., 2002a; Markland, 1998; White, 2005). The haemostatic system of prey/victim is a common target of all the snakes for capture of prey. The anticoagulant components of venom cause defective coagulation of blood leading to excess blood loss from the bite site and also from gums and internal organs. This in turn causes hypovolemic shock to vital organs like brain, kidney and pituitary glands leading to death (White, 2005). The procoagulant components of venom proteins cause excess clot formation leading to thrombosis in the blood vessels. This is often followed by consumptive coagulopathy which leads to heavy bleeding at later stages and thus compromising the functioning of vital organs like kidney, heart and brain (White, 2005). Some of the venom protein families act as both procoagulant and anticoagulant. For example, snake venom phospholipase A_2 (PLA_2) enzymes hydrolyze the membrane phospholipids of platelets leading to the release of arachidonic acid and platelet aggregation factors which cause platelet aggregation during the process of primary haemostasis (Braud et al., 2000). Some of them act as inhibitors of secondary haemostasis by enzymatically hydrolyzing the membrane phospholipids that are required to form complexes like the prothrombinase, extrinsic tenase and intrinsic tenase (Kini & Evans, 1989). Further, some PLA_2 enzymes interact non-covalently with some clotting factors of these complexes and exhibits anticoagulant activity (Kini, 2006, 2011). Snake venom proteases like metalloproteases and serine protease have been reported to inhibit or activate the components of haemostasis. They cause severe vascular damage by interacting with the extracellular matrix (White, 2005). This in turn interfere the regulation of the coagulation cascade. A schematic representation of the various protein families of snake venom acting as agonist and antagonist to the secondary haemostatic system is shown in Figure 1(a and b).

Daboia russelii, commonly referred as Russell's viper, is one of the medically important snakes of the world (Warrell, 1989). In India, subspecies *Daboia russelii russelii* is found across the country and responsible for majority of the snakebites cases (Warrell, 1989). It is one of the members of the ''Big Four'' snakes of India (Simpson & Norris, 2007). Russell's viper envenomation mainly causes excess bleeding due to consumptive coagulopathy by haemostatically active

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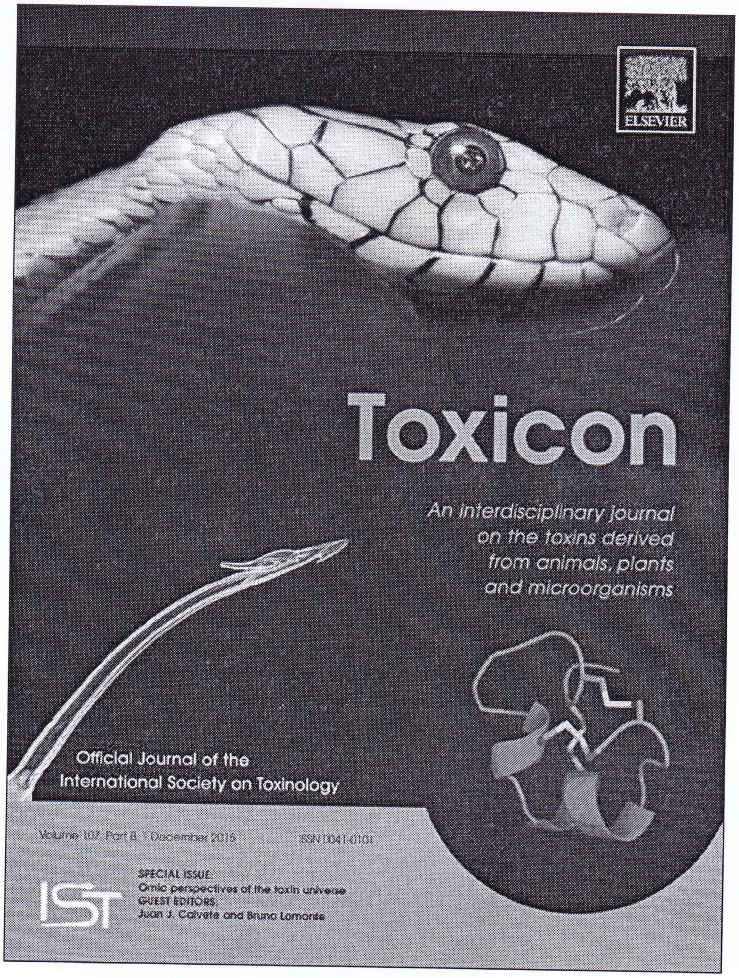
Maitreyee Sharma



Maitreyee Sharma

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Unveiling the complexities of *Daboia russelii* venom, a medically important snake of India, by tandem mass spectrometry

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ABSTRACT

Composition of Indian Russell's viper (*Daboia russelii russelii*) venom, a medically important snake and member of "Big Four" snakes of India was done by gel filtration chromatography followed by tandem mass spectrometry. The MS/MS analyses of tryptic digested gel filtration peaks divulged the presence of 63 different proteins belonging to 12 families. Phospholipase A_2 (PLA_2), serine proteases, metalloproteases, cysteine-rich secretory proteins, L-amino acid oxidase, C-type lectin-like proteins, kunitz-type serine protease inhibitor, disintegrin, nucleotidase, phosphodiesterase, vascular endothelial growth factor and vascular nerve growth factor families were identified. PLA_2 enzymes with isoforms of N-, S- and H-type based on their first N-terminal amino acid residue were observed. The venom is also found to be rich in RVV-X, RVV-V and thrombin-like enzymes. Homologues of disintegrins with RGD and RTS motifs were also observed. The high percentage of PLA_2 and proteases in the venom proteome could be responsible for the observed coagulopathy, haemorrhage and edema which can be correlated with the clinical manifestations of Russell's viper envenomation. This is the first proteomic analysis of Indian *D. russelii* venom which might assist in understanding the pathophysiological effects of viper envenomation. Such study will also be important for developing more effective antivenom for viper bite management.

1. Introduction

The incidences of snake envenomation in tropical countries, such as India, are among the most neglected health issues leading to thousands of mortality and morbidity cases every year. The management of the snakebites and awareness programs initiated by the government and the public health sector in India and elsewhere are insufficient and ineffective (Gutierrez et al., 2010; Warrell, 2011; Warrell et al., 2013; Bawaskar, 2014). It has been estimated that every year around 35,000 to 50,000 or more people die in India due to snakebites, majority of which are inflicted by cobras, kraits, saw scaled viper and Russell's viper (Warrell, 1999).

Russell's viper (*Daboia russelii russelii*) is widespread in Southeast Asia including India, Pakistan, Bangladesh, Sri Lanka, Myanmar, Thailand, Taiwan and Indonesia (Warrell, 1989). It is one of the most important venomous snakes of India which causes significant number of mortality and morbidity (Warrell, 1989). Pathophysiological manifestations of Russell's viper envenomation include coagulopathy, pain, swelling, myonecrosis, renal failure and neurotoxicity (Simpson and Norris, 2007). In India, polyvalent antivenom is raised against the "Big Four" snake venoms (namely *D. russelii*, *Naja naja*, *Echis carinatus* and *Bungarus caeruleus*) which is the only available treatment for viper envenomation. However, at times, administration of antivenom is accompanied by some anaphylactic reactions like nausea, vomiting, hypotension, respiratory discomfort and low body temperature (Singh et al., 2001; Deshpande et al., 2013). This could be due to the presence of large repertoire of non-specific antibodies (small portion of polyvalent antibodies are against specific snake venom). Alternatively, due to non-immunogenicity of venom toxins, some toxins might not be neutralized by antivenom (Pla et al., 2012; Gutierrez et al., 2013; Calvete et al., 2014). This is further complicated by intra-

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PLOS | ONE

RESEARCH ARTICLE

Daboxin P, a Major Phospholipase A2 Enzyme from the Indian *Daboia russelii russelii* Venom Targets Factor X and Factor Xa for Its Anticoagulant Activity

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Data Availability Statement: All relevant data are within the paper and its Supporting Information file.

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Abstract

In the present study a major protein has been purified from the venom of Indian *Daboia russelii russelii* using gel filtration, ion exchange and Rp-HPLC techniques. The purified protein, named daboxin P accounts for ~24% of the total protein of the crude venom and has a molecular mass of 13.597 kDa. It exhibits strong anticoagulant and phospholipase A_2 activity but is devoid of any cytotoxic effect on the tested normal or cancerous cell lines. Its primary structure was deduced by N-terminal sequencing and chemical cleavage using Edman degradation and tandem mass spectrometry. It is composed of 121 amino acids with 14 cysteine residues and catalytically active His48 -Asp49 pair. The secondary structure of daboxin P constitutes 42.73% of α-helix and 12.36% of β-sheet. It is found to be stable at acidic (pH 3.0) and neutral pH (pH 7.0) and has a Tm value of 71.59 ± 0.46°C. Daboxin P exhibits anticoagulant effect under *in-vitro* and *in-vivo* conditions. It does not inhibit the catalytic activity of the serine proteases but inhibits the activation of factor X to factor Xa by the tenase complexes both in the presence and absence of phospholipids. It also inhibits the tenase complexes when active site residue (His48) was alkylated suggesting its non-enzymatic mode of anticoagulant activity. Moreover, it also inhibits prothrombinase complex when pre-incubated with factor Xa prior to factor Va addition. Fluorescence emission spectroscopy and affinity chromatography suggest the probable interaction of daboxin P with factor X and factor Xa. Molecular docking analysis reveals the interaction of the Ca^{+2} binding loop; helix C; anticoagulant region and C-terminal region of daboxin P with the heavy chain of factor Xa. This is the first report of a phospholipase A_2 enzyme from Indian viper venom which targets both factor X and factor Xa for its anticoagulant activity.

Maitreyee Sharma

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Purification and Characterization of Nk-3FTx: A Three Finger Toxin from the Venom of North East Indian Monocled Cobra

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ABSTRACT: Snake venom three finger toxins (3FTxs) are a non-enzymatic family of venom proteins abundantly found in elapids. We have purified a 7579.5 ± 0.591 Da 3FTx named as Nk-3FTx from the venom of *Naja kaouthia* of North East India origin. The primary structure was determined by a combination of N-terminal sequencing and electrospray ionization liquid chromatography-mass spectrometry/mass spectrometry. Biochemical and biological characterization reveal that it is nontoxic to human cell lines and exhibit mild anticoagulant activity when tested on citrated human plasma. Nk-3FTx was found to affect the compound action potential (CAP) and nerve conduction velocity of isolated toad sciatic nerve. This is the first report of a non-conventional 3FTx from *Naja kaouthia* venom that reduces CAP for its neurotoxic effect. Further studies can be carried out to understand the mechanism of action and to explore its potential therapeutic application. © 2015 Wiley Periodicals, Inc. J. Biochem. Mol. Toxicol. 30:59–70, 2016; View this article online at wileyonlinelibrary.com. DOI 10.1002/jbt.21734

KEYWORDS: ***Naja kaouthia*; Three finger toxin; Neurotoxicity; Compound action potential; Potassium channel**

INTRODUCTION

Snake venom is a complex mixture of proteins and polypeptides which are classified into various toxin families based on their structure and function. Three finger toxins (3FTxs) are one of the well characterized non-enzymatic families of snake venom proteins. They are abundantly found in elapids and hydrophiidae venom [1–3] and recently, transcripts are also reported in *Viperidae* family [4]. The amino acid sequence of 3FTx family ranges from 60 to 74 residues with eight or ten cysteine residues [5]. They are called "Three finger toxins" (3FTx) as the three loops (β stranded) project from the hydrophobic core which is connected by 4–5 disulfide bridges resembling three stretched fingers of our hand [5–7]. In non-conventional 3FTx, the fifth disulfide linkage is found in the first loop whereas in long chain α-neurotoxins and κ-neurotoxins it is present in the second loop [1, 8]. Functionally they exhibit various pharmacological effects on prey/victims. They are reported to be neurotoxic, cardiotoxic, cytotoxic, anticoagulant, myotoxic, platelet aggregation inhibition, etc. [6,9–13]. This family of protein constitutes the best example of a unique structural scaffold to support multiple biological functions as they are structurally conserved but functionally diverse. Mostly, 3FTxs exist as monomers, e.g. fulgimotoxin from green vine snake *Oxybelis fulgidus* [14], candoxin from Malayan krait *Bungarus candidus* [15], denmotoxin, from *Boiga dendrophila* (mangrove catsnake) [16], β-cardiotoxin from *Ophiophagus hannah* [17], hemachatoxin (P-type cardiotoxin) from *Hemachatus hemachatus* venom [18]. However, dimeric 3FTxs are also reported, e.g. κ-neurotoxins from *Bungarus sp.*, Hemextin AB from *hemachatus hemachatus* venom [12], haditoxin, from *Ophiophagus hannah* [19], irditoxin from *Boiga irregularis* (Brown tree snake) [20], etc.

Monocled cobra *(Naja kaouthia)* is the most common species of Asiatic cobra found in North East India [21, 22]. Phenotypically it can be distinguished by its

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Supporting Information is available in the online issue at www.wileyonlinelibrary.com

Maitreyee Sharma

Appendix III

List of Conferences/Seminars and Workshops attended

1. **Recent developments in Medical Biotechnology and Structure based drug designing (RDMBSBDD).** Organized by **Department of Biosciences and Bioengineering,** Indian Institute of Technology, Guwahati (IITG), December 6th & 7th, 2015.

2. **"International Conference on Disease Biology and Therapeutics 2014."** Organized by Institute of Advanced Study in Science & Technology, Guwahati, Assam. December 3rd-5th, 2014.

3. **"National Seminar on Recent advances in Biotechnology Research in North East India: Challenges and Prospects."** Organized by Department of Molecular Biology and Biotechnology, Tezpur University, Assam. November 27th -29th, 2014.

4. **"IV Annual Conference of Toxinological Society of India & International Colloquium."** Organized by Calcutta School of Tropical Medicine, Department of Clinical & Experimental Pharmacology. November 19th -22nd, 2014.

5. **2nd National Conference on Snakebite Management and Annual Conference of Toxinological Society of India.** Organized by Department of Studies in Biochemistry, University of Mysore and Karnataka Open University. December 10, 2012.

6. **Workshop on "Thrombosis and haemostasis Discovery and Development of tools and therapeutics."** Organized by Department of Studies in Biochemistry, University of Mysore and Karnataka Open University. December 8th & 9th, 2012.

Appendix IV

List of papers/posters presented in National and International seminar/conferences

1. **Sharma M**. & Doley, R. "**Proteome Mining for Anticoagulant Protein from Snake Venom with Therapeutic potential**". Recent developments in Medical Biotechnology and Structure based drug designing (RDMBSBDD). Organized by **Department of Biosciences and Bioengineering,** Indian Institute of Technology, Guwahati (IITG), December 6th & 7th, 2015. **(Poster)**

2. Sharma M & **Doley R**. "**Profiling of India *Daboia russelii* venom proteome: A step towards design and development of better antivenom".** TSICON 2015, Organized by LF Hospital Angamaly and SNIMS Chalakka. November 21st & 22nd, 2015. **(Invited lecture)**

3. Sharma, M. & **Doley, R.** "**Proteomics and Antivenomics in Combating Snakebite: A Neglected Tropical Disease." International Conference on Disease Biology and Therapeutics 2014.** Organized by Institute of Advanced Study in Science & Technology, Guwahati, Assam. December 3rd-5th, 2014. **(Oral)**

4. **Sharma**, M., & Doley, R. **"Unveiling the venom composition of Indian *Daboia russelii* by tandem mass spectrometry."** National Seminar on "Recent advances in Biotechnology Research in North East India: Challenges and Prospects". Organized by Department of Molecular Biology and Biotechnology, Tezpur University, Assam. November 27th -29th, 2014. **(Poster)**

5. **Sharma, M., &** Dr. Robin Doley. **"Indian Russell's viper venom analysis using proteomics tool and neutralization of its coagulopathy by polyvalent antivenom."** "IV Annual Conference of Toxinological Society of India & International Colloquium." Organized by Calcutta School of Tropical Medicine, Department of Clinical & Experimental Pharmacology. November 19th -22nd, 2014. (**Poster/Oral presentation**)

6. Sharma, M., Gogoi, N., Dhananjaya, B.L. & **Doley, R. "Geographic variation of Russell's viper venom and its relation to coagulopathy."** "5th International Conference on exogenous factors affecting Thrombosis and Haemostasis," Amsterdam, July 5th-6th 2013. **(Invited lecture)**

7. **Sharma, M**., Menon, J.C. and Doley, R. **"Compositional analysis of two Russell's viper venom of South India."** 2nd National Conference on "Snakebite Management and Annual Conference of Toxinological Society of India". Organized by Department of Studies in Biochemistry, University of Mysore and Karnataka Open University. December 10, 2012. **(Poster).**

Appendix V

Permissions and Approval from Ethical committee

DATE: 9.06.2012

CERTIFICATE

THIS IS TO CERTIFY THAT THIS SNAKE VENOM RELEASED ONLY FOR RESEARCH PURPOSE AND THIS PARCEL CONTAINS THE FOLLOWING SNAKE VENOMS.

1. COBRA SNAKE VENOM	1.000 gm
2. KRAIT SNAKE VENOM	1.000 gm
3. RUSSELL'S VIPER SNAKE VENOM	1.000 gm
4. SAW SCALED VIPER SNAKE VENOM	1.000 gm

SNAKE VENOMS WEIGHED BY

C.V. 9.6.12

Special Officer
IRULA SNAKE CATCHERS ICS LTD.,

SNAKE VENOM WEIGHMENT INSPECTED

9.6.12

FOREST RANGE OFFICER,
WILDLIFE ENFORCEMENT RANGE.
CHENNAI – 32.

FOREST RANGE OFFICER

SNAKE VENOM PARCEL DESPATCH Date 9.6.12

TO
Dr. Dr. D.Velmurugn,
Professor,
University of Madras,
Dept. of Crystallography and Biophysics,
Guindy Campus,
Chennai - 25.

TEZPUR UNIVERSITY
TEZPUR UNIVERSITY ANIMAL ETHICAL COMMITTEE
TEZPUR- 784 028, ASSAM, INDIA
(CPCSEA Regd. No 754/CPCSEA)

Approval No: DoRD-Pro/TUAEC/10-56/15/Res-02 Dated: 06/06/2015

Certificate

This is to certify that the project title "*Isolation, purification and characterization of novel three finger toxin from cobra venom*" has been approved by the IAEC.

Name of Chairman: *Prof. C. L Mahanta*
Dean Research and Development, TU

Name of CPCSEA link nominee: *Dr. P. Chakravarty*
Associate Professor
Silchar Medical College, Silchar, Assam.

Charu Lata Mahanta

(C. L Mahanta) Dean
Dean, R&D
&
Chairperson
IAEC

Research & Development
Tezpur University

Chairperson
IAEC, Tezpur University
Tezpur- 784 028, Assam, India

Pinaki

(Dr. P. Chakravarty)
CPCSEA Link nominee
IAEC

Dr. P. Chakravarty
M.B.B.S., D.M.C.H., M.D.
Associate Professor
Department of Pharmacology
SILCHAR MEDICAL COLLEGE & HOSPITAL

CPCSEA Nominee
Tezpur University
Assam, India

PROCEEDINGS OF THE INSTITUTIONAL ANIMAL ETHICS COMMITTEE MEETING HELD ON 29th OCTOBER 2011 AT 11.30 AM IN THE CHAMBERS OF THE CHAIRMAN, DEPARTMENT OF STUDIES IN ZOOLOGY, MANASAGANGOTRI, MYSORE – 06

MEMBERS PRESENT

1. Prof. Mewa Singh — Chairman, IAEC
2. Dr. H. Krishnappa — Member & CPCSEA Nominee
3. Mr. D. R. Prahallada — Member
4. Prof. B. S. Vishwanath — Member
5. Prof. Shivabasavaiah — Member
6. Prof. H. N. Yajurvedi — Member & Convener IAEC

MEMBERS ABSENT :

1. Dr. Hari Krishna
2. Dr. Suresh Kumar

The Chairman welcomed the members. The committee noted that though some of the suggestions made in the earlier IAEC meeting regarding the preparation of the proposals were complied with, the Investigators have to prepare the proposals in still better manner, especially the protocols of experiments, requirement of animals in each experiment and restricting the number of animals per group only to requirement of the investigation. In addition names of all he individuals of the research group involved in proposed work have to be mentioned.

The committee unanimously agreed with the above suggestions and resolved to implement them.

The committee discussed at length about the proposals submitted by different Investigators and sought clarifications from Principal Investigators / Ph.D., guides / Ph.D., scholars about their animal requirements. In some of the proposals the animal number was reduced by suggesting modified protocols / alternatives. Following approvals were given.

a. The proposals submitted for class work by Chairpersons of different science Departments were accepted and animal requirement as shown in the table was approved (Nos. UOM/IAEC/11-16/2011).

Sl. No.	Name	Department	No. of animals indented	No. of auimals approved
1	Prof. Bharathi P. Salimath	Biotechnology, MGM	Mice – 180 Nos. Balb/c mice – 120Nos. Rabbit – 04 Nos.	Mice – 180 Nos. Balb/c mice – 120Nos. Rabbit – 04 Nos.
2	The Chairman	Biochemistry, MGM	Mice – 250 Nos. Rat – 150 Nos.	Mice – 250 Nos. Rat – 150 Nos.
3	The Chairman	Psychology, MGM	Rat – 20 Nos.	Rat – 20 Nos.
4	Dr. N. S. Devaki	Yuvaraja's College	Rat – 51 Nos.	Rat – 51 Nos.
5	The Chairman	Zoology, MGM, for Genetcs	Rat – 118 Nos.	Rat – 118 Nos.
6	The Chairman	Zoology, MGM	Rat – 493 Nos. Mice – 60 Nos.	Rat – 493 Nos. Mice – 60 Nos.

b. Dr. Shubha Gopal and Satisha K R (UOM/IAEC/17/2011) to utilize 128 mice for entire Ph.D. progrramme.

27/3/12

c. Prof. K. S. Rangappa and Rakesh K S (UOM/IAEC/18/2011) to reduce number of rats per group from 8 to 6 and total number from 440 to 330.

...2

d. Prof. V. A. Vijayan and Raghavendra B S (UOM/IAEC/19/2011): Requirement of 12 mice approved.
e. Prof. H. S. Prakash and Chandra Nayaka (UOM/IAEC/20/2011): Requirement of 4 rabbits approved and rabbits have to be procured from CPCSEA recognized breeders.
f. Dr. Kemparaju and Prathima R (UOM/IAEC/21/2011) : Requirement of 285 rats for entire Ph.D. programme approved.
g. Dr. Shailasree Sekhar and Ruma Karmakar (UOM/IAEC/22/2011) : Requirement of 96 rats approved.
h. Prof. H. S. Prakash and Ghffari (UOM/IAEC/23/2011) to reduce number of rats from 96 to 72 and to submit detailed plan of the work.
i. Prof. H. S. Prakash and Chethan J (UOM/IAEC/23-24/2011): To provide detailed plan of work showing experiment groups, treatments and animals in each group and requirement of 250 rats approved.
j. Prof. B. S. Vishwanath and Nanjaraj Urs A N (UOM/IAEC/25/2011): The proposal for 480 accepted in principle and individual experimental designs to be given in D-form.
k. Prof. B. S. Vishwanath and Yariswamy M (UOM/IAEC/26/2011) : Requirements of 200 mice approved.
l. Dr. M. Bhagya and Samson S (UOM/IAEC/27/2011) : Requirement of 22 lizards approved.
m. Dr. Asna Urooj and P. Vanitha Reddy (UOM/IAEC/28/2011) : Requirement of 124 rats approved.
n. Dr. Asna Urooj and Sudh Sairam (UOM/IAEC/29/2011) : Requirement of 150 rats approved.
o. Dr. M. Bhagya (UOM/IAEC/30/2011) :Utilization of unused different tissues of lizards approved earlier in other proposals permitted.
p. Prof. V. A. Vijayan and Prathibha K P (UOM/IAEC/31/2011) : Requirement of 12 mice approved.
q. Prof. Cletus J. M. D'Souza and Mamatha A M and Shubha M C (UOM/IAEC/32/2011) : Requirement of 02 rabbits approved. Rabbits to be procured from CPCSEA recognized breeders.
r. Dr. T. Shivanandappa and Mahsa Zarei (UOM/IAEC/33/2011) : Requirement of 270 mice approved.
s. Dr. T. Shivanandappa and Dileepkumar H V (UOM/IAEC/34/2011) : Requirement of 160 rats approved.
t. Dr. T. Shivanandappa and Niveditha (UOM/IAEC/35/2001) : Requirement of 120 rats approved.
u. Prof. B. S. Vishwanath and Zahra A (UOM/IAEC/36/2011) : Requirement of 46 rats approved.
v. Prof. B. S. Vishwanath and Vilas Hiremath (UOM/IAEC/37/2011) : Requirement of 75 rats approved
w. Prof. B. S. Vishwanath and Vikarm Joshi (UOM/IAEC/38/2011): Requirement of 456 Mice and 228 rats approved.
x. Prof. Cletus D. Souza (UOM/IAEC/39/2011) : Requirement of 02 hens approved.
y. Prof. Cletus D. Souza (UOM/IAEC/40/2011) : Requirement of 02 hens approved.
z. Prof. Cletus D. Souza (UOM/IAEC/41/2011) : Requirement of 02 Rabbits approved
aa. Prof. Cletus D. Souza (UOM/IAEC/42/2011) : Requirement of 48 rats approved.

Accepted

(H. N. YAJURVEDI)
CONVENER, IAEC

UNIVERSITY OF MYSORE

Communication of decision of the Institutional Human Ethical Committee (IHEC)

IHEC -UOM No. 62 /Ph.D/2011-12

Protocol title: Search for novel treatments for snake venom poisoning
Name of the Student: Mr Nanjaraj Urs A.N Research Guide: **Dr. B.S.Vishwanath**
Department: DOS in Biochemistry, Manasagangotri, Mysore, India
[√] New review [ ] revised review
Date of review (D/M/Y): 15/12/2011
Decision of the IHEC: [√] Recommended [ ] Recommended with suggestions [ ] Revision/Resubmission
Suggestions: To give a declaration stating that the subject's blood sample will be used only for the specified purpose Revise and submit the consent form
Recommended for a period of: Effective from the date of PhD enrolment / registration

Please note*

- Inform IEC in case of any change of study procedure and investigator.
- This permission is only for period mentioned above.
- Brief report to be submitted to IHEC.

Dr Asna Urooj
Member Secretary
Human Ethical Committee
University of Mysore
MYSORE-570 006

Dr N.M.Srinivas
Chairman
Human Ethical Committee
University of Mysore
MYSORE-570 006

NUS National University of Singapore

IACUC protocol 041/12

26 April 2012

Prof R Kini
DBS

Dear Prof Kini

Full Approval for an Application to use animals for research
New factor Xa inhibitors from tick saliva: Structure-function relationships

I am pleased to inform you that full approval is granted to your application to use animals for research for the project above. The IACUC protocol number ***041/12*** shall be used for future reference of this application.

Please ensure that :

(1) The protocol is strictly adhered to when carrying out the project;
(2) Approval from IACUC and OSHE (where applicable) is obtained for any modification of the protocol in the course of the project;
(3) Animals are transported by designated personnel with proper equipment via the appropriate route and appropriate transport vehicle;
(4) All personnel handling hazardous materials, including husbandry staff in departmental facilities and Comparative Medicine (CM) are informed of the risks and instructed on the handling methods.

In addition, please note that :

(1) You are required to submit the Annual Protocol Review (APR) one year from the IACUC approval date and yearly thereafter and to inform the IACUC when the protocol is completed or discontinued, using the APR form available from the IACUC website;
(2) If you intend to demonstrate and/or produce photographs or videos on your research findings or teaching / workshop procedures, using live animals, you are required to submit an application to IACUC for approval. Please contact the IACUC office for the form.

Yours sincerely

Prof Lam Toong Jin
IACUC Chair

cc Deputy President (Research & Technology)
Vice-Dean (Research), FoS
Head, DBS

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