

## Contents

Abstract	i-iv
Acknowledgement	viii-ix
Table of contents	x-xiii
List of tables	xiv
List of figures	xv-xvii
List of abbreviations	xviii-xxi

### Chapter 1: General introduction and review of literature 1-39

1.1 Introduction	1-2
1.2 Snakes	2-3
1.3 General distribution	3-4
1.4 Classification and families of snakes	4-5
1.5 Cobras	5-7
1.6 <i>N. kaouthia</i> (Monocled cobra)	7-8
1.7 <i>N. kaouthia</i> of North East India, Assam	8-9
1.8 Venom, poison and toxins	9-10
1.9 Snake venom gland and delivery apparatus	10-11
1.10 Snake venom	11-12
1.11 Snake venom protein families	12-14
1.11.1 Phospholipase A <sub>2</sub> (PLA <sub>2</sub> )	14-16
1.11.2 L-amino acid oxidase (LAAO)	16-17
1.11.3 Proteases Snake venom Metalloproteinase (SVMP)	17
1.11.4 Hyaluronidase	17-18
1.11.5 Acetylcholinesterases (AChE)	18
1.11.6 Snake venom nerve growth factors	18-19
1.11.7 Cysteine rich secretory proteins (CRISP)	19-20
1.11.8 Snaclecs	20
1.11.9 Kunitz-type serine protease inhibitor (KTSPI)	20
1.11.10 Disintegrins	20-21
1.11.11 Three finger toxins (3FTxs)	21-23
1.11.12 Accelerated evolution of 3FTxs	23-24
1.11.13 Structural variations in 3FTxs	25-28
1.11.14 Classification of 3FTxs and their functional properties	29
1.11.14.1 Neurotoxins	29
1.11.14.1.1 α-neurotoxins	29-30
1.11.14.1.2 κ-neurotoxins	30
1.11.14.2 Cardiotoxins	31
1.11.14.3 Anticoagulant 3FTxs	32
1.11.14.4 Fasciculins	32-33
1.11.14.5 Muscarinic toxins	33
1.11.14.6 Non-conventional 3FTxs	34-35
1.11.14.7 L-type calcium channel blockers	35-36
1.11.14.8 β-cardiotoxin	36-37
1.11.14.9 Platelet aggregation inhibitors	37-38
1.11.14.10 Hannalgesin	38
1.11.14.11 Orphan toxins	38
1.12 Aim and scope of the thesis	38-39

<b>Chapter 2: Materials and Methods</b>	<b>40-59</b>
2.1 Materials	40-41
2.1.1 Collection of snake venom	41
2.1.2 Preparation and storage of venom samples	41
2.1.3 Animals	41-42
2.2 Methods	42
2.2.1 Determination of protein content	42-43
2.2.2 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)	43
2.2.3 Phospholipase A <sub>2</sub> (PLA <sub>2</sub> ) activity	44
2.2.3.1 Colorimetric method	44
2.2.3.2 Turbidometric method	44-45
2.2.4 LD <sub>50</sub> determination	45
2.2.5 Behavioral study of the experimental animals	45
2.2.6 Edema inducing activity assay	45-46
2.2.7 Hemorrhagic activity	46
2.2.8 Myotoxicity studies	46
2.2.8.1 Lactate dehydrogenase assay (LDH)	47
2.2.8.2 Creatine Kinase activity assay (CK)	47-48
2.2.9 Direct and indirect hemolytic activity assay	48-49
2.2.10 Caseinolytic assay	49
2.2.10.1 Tyrosine standard curve	49
2.2.10.2 Digestion of casein	49
2.2.11 Fibrinogenolytic activity	49-50
2.2.12 <i>In-vitro</i> coagulant assays	50
2.2.12.1 Recalcification time	50
2.2.12.2 Prothrombin time (PT) test	50-51
2.2.12.3 Activated partial thromboplastin time (APTT) test	51
2.2.13 Whole citrated blood analysis	51
2.2.13.1 Thromboelastometry analysis	51
2.2.13.2 Sonoclot analysis	52
2.2.14 Bactericidal activity assay	52
2.2.14.1 Well diffusion	52
2.2.14.2 Disc diffusion	52-53
2.2.15 Neutralization studies	53
2.2.16 Western blotting	53-54
2.2.17 Cytotoxicity	54
2.2.18 Neurotoxicity	54
2.2.18.1 Dissection and isolation of sciatic nerve from common Asian toads	54-55
2.2.18.2 Recording of compound action potential (CAP) and determination NCV (Nerve conduction velocity)	55-56
2.2.19 Scanning electron microscopy	56
2.2.20 Liquid chromatography-mass Spectrometry (LC/MS) of crude venom	56
2.2.21 Fractionation of crude venom	57
2.2.22 Purification of the 3FTx (Nk-3FTx)	57
2.2.23 Molecular weight determination	58

2.2.24 N-terminal sequencing of the purified protein	58
2.2.25 ESI LC-MS/MS (Electrospray ionization Liquid chromatography/tandem mass spectrometry)	58-59
2.2.26 Statistical analysis	59
<b>Chapter 3: Biochemical and biological characterization of crude <i>Naja kaouthia</i> venom</b>	<b>60- 95</b>
3.1 Introduction	60
3.2 Results	61
3.2.1 Collection of venom, preparation and storage	61-62
3.2.2 Determination of total protein content	63-64
3.2.3 SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis)	64-65
3.2.4 Lethal dose ( $LD_{50}$ ) determination of crude <i>N. kaouthia</i> venom	65-67
3.2.5 Behavioral study	67-68
3.2.6 Hemorrhagic activity assay	68
3.2.7 Edema inducing activity assay	68 -70
3.2.8 Phospholipase A <sub>2</sub> (PLA <sub>2</sub> ) activity assay	70
3.2.8.1 Colorimetric method	70-71
3.2.8.2 Turbidometric method	71
3.2.9 Indirect hemolytic activity assay	72
3.2.10 Direct hemolytic activity assay	72-73
3.2.11 Caseinolytic activity assay	73
3.2.12 <i>In-vitro</i> coagulation activities	74
3.2.13 Whole blood coagulation analysis	75
3.2.13.1 Analysis by Rotem® Analyzer	75-76
3.2.13.2 Sonoclot analysis	77
3.2.14 Fibrinogenolytic activity	78
3.2.15 Bacteriacidal activity	78-79
3.2.16 Myotoxicity studies	79-80
3.2.17 Cytotoxicity	80-82
3.2.18 Neurotoxicity	83-84
3.2.19 Scanning electron microscopy (SEM)	84-86
3.2.20 Neutralization studies	86-88
3.2.21 Western blotting	88-89
3.3 Discussion	89-95
<b>Chapter 4: Partial compositional analysis of crude <i>Naja kaouthia</i> venom</b>	<b>96-108</b>
4.1 Introduction	96
4.2 Results	97
4.2.1 Fractionation of crude venom	97-101
4.2.2 Liquid chromatography mass spectrometry (ESI-LC/MS) of crude venom	101-104
4.2.3 Relative abundance of various proteins	105
4.3 Discussion	106-108

<b>Chapter 5: Purification of Nk-3FTx, a three finger toxin from <i>Naja kaouthia</i> venom</b>	<b>109-122</b>
5.1 Introduction	109
5.2 Results	110
5.2.1 Fractionation of crude venom	110-111
5.2.2 Re-chromatography of peak	111-112
5.2.3 Molecular weight determination of Nk-3FTx	113
5.2.4 N-terminal sequencing of Nk-3FTx	113-114
5.2.5 ESI-LC MS/MS of Nk-3FTx	114-116
5.2.6 Sequence analysis	116-119
5.3 Discussion	120-122
<b>Chapter 6: Functional characterization of purified Nk-3FTx</b>	<b>123-146</b>
6.1 Introduction	123-125
6.2 Results	126
6.2.1 Biochemical and biological activities	126-133
6.2.2 Behavioral study	133-134
6.2.3 Sequence Analysis	134-136
6.2.4 Neurotoxicity studies	137-141
6.3 Discussion	141-146
<b>Chapter 7: Conclusion and future prospects</b>	<b>147-150</b>
7.1 Conclusion	147-149
7.2 Future prospective	149-150
<b>Bibliography</b>	<b>151-177</b>
Appendix I: List of Publications	xxii
Appendix II: Presentations in conferences and seminar	xxiii
Appendix III: Reprints of published papers	xxiv
Appendix IV: Reprints of poster presented	xxv-xxvi
Appendix V: Permissions and ethical approvals	xxvii