TABLE OF CONTENTS

Contents	Page No.
Abstract	**i-v**
Declaration	
Certificate	
Acknowledgements	**vi-vii**
Table of Contents	**viii-xiv**
List of Tables	**xv-xvi**
List of Figures	**xvii-xxvi**
Abbreviations	**xxvii-xxx**
CHAPTER-I	
INTRODUCTION	**1-38**
1.1 Scorpions: evolution, diversity and geographical distribution of medically significant scorpions of the world	1
1.1.1 The titans of Paleozoic era: the Silurian ancestral scorpions	1
1.1.2 Parioscorpiovenator gen. et sp. Nov': the earliest scorpion through evolutionary routes	1
1.1.3 The origin and diversity of scorpion toxin peptide scaffolds: evolution stings	3
1.1.4 Geographical distribution of venomous scorpions	4
1.1.5 Scorpions: taxonomic classification, anatomy, morphology and reproduction	6
1.1.6 Anatomy and Morphology	7
1.1.6.1 Prosoma or cephalothorax	8
1.1.6.2 Preabdomen or mesosoma	9
1.1.6.3 Tail or metasoma	11
1.1.6.4 Habitat and food of scorpions	11
1.1.6.5 Reproduction in scorpions	13
1.2 Scorpions venom: origin, evolution, composition, and functions	14
1.2.1 The origin and diversity of scorpion toxin peptide scaffolds: evolution stings	14
1.2.2 Structure-Function analysis of scorpion toxins	17
1.3 Scorpionism: epidemiology, pathophysiology and clinical manifestations	18
1.3.1 Origin of scorpionism	18
1.3.2 Importance of understanding the epidemiology of scorpionism	18
1.3.3 Scorpion envenomation: Prevention and treatment against MT stings	20
Bibliography	25

Contents	TABLE OF CONTENTS	Page no.

CHAPTER II

REVIEW OF LITERATURE **39-72**

2.1 Biochemical and proteomic characterization of some medically essential scorpions around the world 39

42

2.2 Pharmacological targets of scorpion venom toxins 42

2.2.1 Na^+ and K^+channel toxins: pathophysiology and mechanism of action 44

2.2.1.1 K^+ channel blockers and mechanism of action 46

2.2.1.2 Na^+ channel blockers and mechanism of neurotoxin binding with Na^+ channel 48

2.2.2 Glycaemic response of scorpion venom and administration of insulin 48

2.2.3 Scorpion venom-induced inflammatory response 50

2.2.4 Erectile dysfunction by scorpion stings 51

2.3 Epidemiological study of scorpion stings 51

2.3.1 Epidemiology of scorpionism in America 52

2.3.2 Epidemiology of scorpionism in Asia 54

2.3.3 Epidemiology of scorpionism in Africa 55

2.3.4 Epidemiology in Mexico 55

2.4 Limitation of antivenom therapy and their improvement protocol for better treatment of scorpion stings 57

Bibliography

Contents	TABLE OF CONTENTS	Page no.

CHAPTER III
MATERIALS AND METHODS **73-102**
3.1 Materials 73
3.1.1 Venoms, antivenoms and drugs 73
3.1.2 Chromatographic columns, matrices, and other fine chemicals 73
3.2 Methods 74
3.2.1. Proteomic analysis of Indian red scorpion (*Mesobuthus tamulus*) venom 74
3.2.1.1 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of MTV 74
3.2.1.2. Tandem Mass Spectrometry Analysis of Tryptic Peptides 76
3.2.2. Determination of enzymatic activities and some pharmacological properties of MTV 77
3.2.2.1. Assay of enzymatic activities 77
3.2.2.1.1 MTV metalloproteinase (SVMP) activity 77
3.2.2.1.2 L-amino acid oxidase (LAAO) activity 77
3.2.2.1.3 Phospholipase A_2 (PLA_2) activity 77
3.2.2.1.4 Fibrino(geno)lytic activity 78
3.2.2.1.5 Nucleotidase activities 78
3.2.2.1.6 Hyaluronidase activity 79
3.2.2.2. Assay of pharmacological properties 79
3.2.2.2.1 Effect on plasma clotting time 79
3.2.2.2.2 Effect of haemolytic activity 79
3.2.2.2.3 Effect on platelet count 80
3.2.2.2.4 Effect of amidolytic activity 80
3.2.3. Assessment of immunological cross-reactivity between MTV and commercial anti-scorpion antivenom by ELISA and immunoblot analysis 80
3.2.4. *In vitro* laboratory analyses of commercial anti-scorpion (*Mesobuthus tamulus*) antivenoms 81
3.2.4.1. Physiochemical characterization 81
3.2.4.2. Electron microscopic characterization 82
3.2.4.3. Mass spectrometry analysis 82
3.2.4.4. FPLC-size exclusion chromatography and SDS-PAGE analyses to determine the purity of the active substance 83
3.2.4.5. Determination of Fc content
3.2.4.6. Determination of particle size distribution (protein aggregation) by dynamic light scattering (DLS) analysis 84 84

3.2.4.7. Determination of IgA and IgE contamination 85
3.2.4.8. Determination of complement activation and endotoxin contamination 85
3.2.4.9. Determination of preservative content 86
3.2.4.10. Determination of kd value for scorpion venom-ASA interaction 86
3.2.4.10.1. Spectrofluorometric analysis 86
3.2.4.10.2. Atomic force microscopic (AFM) analysis 87
87
3.2.4.11. The spectrofluorometric titration to determine the presence of venom toxins-specific antibodies in commercial ASAs 88
3.2.5. Computational (*in sillico*) analysis to compare the binding efficiency of α1 adrenoreceptor antagonists with α1 adrenergic receptor (α1A, α1B and α1D) of human and mouse, and homologous receptor (SER6) in *Caenorhabditis elegans* 88
88
88
3.2.5.1. Preparation of the Ligand 3D structures for Docking 88
3.2.5.2. Protein-Ligand Docking 88
3.2.6. Determination of *in vivo* neutralisation potency of commercial ASAs, AAAs, and Ascorbic acid in *C. elegans* model 89
3.2.6.1. Cultivation and synchronization N2 *C. elegans* worms (N_2 eneration) 89
3.2.6.2. Determination of lethal concentration 50 (LC_{50}) of MTV in *C. elegans* 89
3.2.7. Determination of dose- and time-dependent neutralisation of MTV-induced toxicity in *C. elegans* by commercial ASAs, AAAs, and Ascorbic acid 89
3.2.8. *In vivo* neutralisation of MTV-induced generation of reactive oxygen species and alteration of mitochondrial transmembrane potential in *C. elegans* by ASA, AAAs, and Ascorbic acid 90
3.2.9. The *in vivo* neutralisation of MTV-induced lethality in *C. elegans* with individual components of the formulation and their combinations 91
3.2.10. *In vitro* DPPH free radical-scavenging activity of different concentrations of the formulated drug, individual components of the formulation, and their combinations 92

3.2.11. *In vivo* neutralisation of MTV-induced generation of reactive oxygen species (ROS) and alteration of mitochondrial transmembrane potential in *C. elegans* by different concentrations of the formulated drug and individual components of the formulation, and combination of thereof 93
3.2.12. *In vivo* neutralization of *M. tamulus* venom-induced biochemical changes by formulated drug [ASA (187.5 µg) : ascorbic acid (0.1 µg) : α1-adrenoreceptor antagonist (3 µM)] and combinations of commercial ASA and α1-adrenoreceptor antagonist as compared to components of the formulation at their optimum dose in albino Wistar strain rats 93
3.2.13. Validation of *in vivo* neutralisation of MTV-induced toxicity by formulated drug and combinations of commercial ASA and AAA in Wistar strain albino rats 94
3.2.13.1 Neutralisation of hyperglycemia and prolonged tail bleeding time 94
3.2.13.2 Neutralisation of changes in serum biochemical parameters 95
3.2.13.3 Neutralisation of morphological alterations in vital organs 95
3.2.14 Determination of MTV-induced inflammatory cytokines levels in Swiss albino mice 95
3.2.14. Statistical analysis 96
Bibliography 96