

**CHAPTER-1**

1.	Introduction .....	1
1.1	Peptide Therapeutics .....	1
1.1.1	Peptide therapeutics for addressing unmet needs in healthcare .....	2
1.2	Oxytocin.....	6
1.3	Vasopressin.....	7
1.4	Renin Angiotensin System (RAS) .....	8
1.5	Ready to Infuse (RTI) Drug Delivery Systems.....	8
1.6	Nanocarriers-Based Delivery Approach .....	9
1.6.1	Nanocarriers mediated delivery of peptides to brain .....	10
1.7	Conjugation of Peptide Molecules to Enhance the Pharmacokinetics and Pharmacodynamic Attributes.....	12
1.8.	Aims & Objectives.....	13
1.8.1	Aims.....	13
1.8.2	Ready to infuse (RTI) formulation .....	13
1.8.3	Nano-formulations for nose to brain delivery.....	13
1.8.4	Chemical modification to improve half-life and bioavailability of oxytocin ....	14
1.9	Objectives .....	14
1.9.1	Ready to infuse (RTI) drug delivery system.....	14
1.9.2	Nano-formulations for nose to brain delivery.....	14
1.9.3	Oxytocin-conjugates for nose to brain delivery.....	14
1.10.	Plan of Work.....	15
1.10.1	Development and Characterization of Ready to Infuse Formulations.....	15
1.10.2	Development and Characterization of Nano-formulations for Nose to Brain Delivery.....	15
1.10.3	Synthesis and Characterization of Oxytocin Conjugate for Nose to Brain Delivery.....	15

**CHAPTER-2**

2. Literature Review.....	20
2.1 Peptide therapeutics .....	20
2.2 Challenges of peptides as therapeutic agents.....	21
2.3 Drug profile.....	23
2.3.1 Oxytocin.....	23
2.3.1.1 Functions of OXT .....	23
2.3.1.2 Pharmacology and Biochemistry .....	24
2.3.1.3 Clinical usage of OXT in Gynaecology and obstetrics: a life-saving drug in women.....	25
2.3.1.4 Cardiovascular protective properties .....	31
2.3.1.5 Oxytocin in Brain Disorders .....	31
2.3.1.6 Neuropsychiatric disorders .....	33
2.3.1.7 Neurodegenerative disorders .....	37
2.3.1.8 Clinical Studies in Neurological Disorders.....	40
2.3.2 Vasopressin.....	41
2.3.2.1 Chemistry, biogenesis and pharmacology .....	45
2.3.2.2 Pharmacokinetics of Vasopressin .....	47
2.3.2.3 Role of V1 and V2 receptors .....	47
2.3.2.4 Cerebrospinal fluid and Vasopressin .....	51
2.3.2.5 Vasopressin in neurological disorders: Clinical and preclinical studies of Vasopressin.....	54
2.3.3 Angiotensin.....	64
2.3.3.1 Pharmacology of Angiotensin.....	65
2.3.3.2 Pharmacokinetics of Angiotensin-II .....	69
2.3.3.3 Preclinical applications of RAS in neurological disorders .....	69
2.3.3.4 Clinical applications of RAS in neurological disorders.....	76
2.4 Recent advancement for enhanced neuronal delivery using various carrier systems .....	84
2.4.1 Recent therapeutic strategies for peptide delivery .....	85
2.4.1.1 Nanocarriers-based delivery approach.....	86
2.4.1.2 Nanocarriers mediated delivery of peptide to brain.....	91
2.4.2 Ready to Infuse (RTI) formulation .....	101
2.4.3 Conjugation of peptide molecules to enhance their pharmacokinetics and pharmacodynamic attributes .....	102

## **TABLE OF CONTENT**

---

### **CHAPTER-3**

3. Analytical methods .....	132
3.1 Materials and Instruments.....	132
3.2 Instruments.....	139
3.3 High Performance Liquid Chromatographic Method for Oxytocin .....	139
3.3.1. Instrumentation and chromatographic conditions.....	139
3.3.2. HPLC method validation .....	141
3.3.3. Results and Discussion .....	143
3.3.3.1 HPLC method development.....	143
3.3.3.2 HPLC method validation .....	143
3.4 High Performance Liquid Chromatographic Method for Vasopressin.....	147
3.4.1 Instrumentation and chromatographic conditions.....	147
3.4.2 HPLC method validation .....	149
3.4.3 Results and Discussion .....	150
3.4.3.1 HPLC method Development.....	150
3.4.3.2 HPLC method validation .....	150
3.5 High performance Liquid chromatographic method for Angiotensin II.....	154
3.5.1 Instrumentation and chromatographic conditions.....	154
3.5.2 HPLC method validation .....	154
3.5.3 Results and Discussion .....	156
3.5.3.1 HPLC method Development.....	156
3.5.3.2 HPLC method validation .....	156

### **CHAPTER-4**

4. Design and development of RTI injection for oxytocin, vasopressin and angiotensin .....	162
4.1 Introduction.....	162
4.2 Materials and Instruments.....	162
4.3 Formulation Development .....	164
4.3.1 Development and Optimization of Ready to Infuse formulation of Oxytocin	164
4.3.1.1 Manufacturing procedure.....	164
4.3.1.2 Preliminary Feasibility and Developmental trials.....	165
4.3.1.3 Selection of Osmogens .....	167
4.3.1.4 Optimization of selected excipients .....	169
4.3.1.5 pH Stability Study.....	171
4.3.1.6 Sterilization method selection for Oxytocin RTI.....	174
4.3.1.7 Evaluation of optimized formulation prepared by aseptic filtration method...	176

## TABLE OF CONTENT

4.3.1.8 Temperature cycling study.....	179
4.3.1.9 Oxygen Sensitivity Study .....	180
4.3.1.10 Photostability study for Oxytocin RTI.....	182
4.3.1.11 Hold time/Manufacturing vessel compatibility .....	183
4.3.1.12 Filter membrane compatibility study.....	185
4.3.1.13 Stability study of optimized RTI formulation of Oxytocin.....	186
4.3.1.14 Stability study with higher strength.....	190
4.3.1.15 Stability Study Report at $5 \pm 3^\circ\text{C}$ & $25^\circ\text{C}/40\%\text{RH}$ .....	193
4.3.1.16 Stability Study: Assay vs Months .....	194
4.3.2 Development and Optimization of Ready to Infuse formulation of Vasopressin	203
4.3.2.1. Formulation development for Selection of Osmogens .....	203
4.3.2.2. pH Stability Study .....	205
4.3.2.3. Manufacturing Process for RTI formulation of Vasopressin.....	206
4.3.2.4. Sterilization method selection for Vasopressin RTI .....	207
4.3.2.5. Photostability study for Vasopressin RTI.....	207
4.3.2.6. Stability study of Vasopressin RTI formulation .....	208
4.3.2.7. Stability Study Report at $5 \pm 3^\circ\text{C}$ & $25^\circ\text{C}/40\%\text{RH}$ for final formulation as per ICH Q1E .....	214
4.3.2.8. Stability Report at $5 \pm 3^\circ\text{C}$ & $25^\circ\text{C}/40\%\text{RH}$ .....	224
2.3.3 Development and Optimization of Ready to Infuse formulation of Angiotensin-II .....	224
4.3.3.1 Formulation development .....	224
4.3.3.3 pH Stability Study .....	226
4.3.3.4 Sterilization method selection for Angiotensin-II RTI .....	226
4.3.3.5 Photostability study for Angiotensin-II RTI.....	226
4.3.3.6 Manufacturing process and Stability study for RTI formulation of Angiotensin-II .....	228
4.3.3.7 Stability report at $5 \pm 3^\circ\text{C}$ & $25^\circ\text{C}/40\%\text{RH}$ .....	242
4.4 <i>In vitro</i> Biological Reactivity of Final RTI formulations of Oxytocin, Vasopressin and Angiotensin-II by Agarose Diffusion Assay .....	242
4.5 Hemolytic Toxicity Study .....	245
<b>CHAPTER-5</b>	
5. Development, characterization and evaluation of liposomes for nose to brain delivery of oxytocin and vasopressin .....	249
5.1 Background .....	249
5.2 Materials and Instruments .....	251
5.3 Preparation, characterization and evaluation of drug loaded liposomes.....	253

## TABLE OF CONTENT

5.3.1 Fabrication of Liposomes for Oxytocin and Vasopressin.....	253
5.3.2 Methodology for characterization of liposomes .....	254
5.3.2.1 Micromeritics of liposomes .....	254
5.3.2.2 Percentage drug entrapment (%EE).....	255
5.3.3 Optimization of liposomes for Oxytocin .....	255
5.3.3.1 Optimization of lipid ratio .....	255
5.3.3.2 Optimization of process variables.....	255
5.3.4 Optimization of liposomes for vasopressin.....	257
5.3.4.1 Optimization of lipids ratio.....	257
5.3.4.2 Optimization of process variables.....	257
5.3.5 Stability of liposomes formulations .....	257
5.3.6 <i>In vitro</i> cell line study .....	258
5.3.6.1 % Cell viability and Cell uptake study on SH-SY5Y cells.....	258
5.3.6.2 Evaluation on monocytic cells (THP-I) .....	261
5.3.7 Neurological activities of developed formulations in experimental animals... 263	
5.3.7.1 Rationale for <i>In vivo</i> studies .....	263
5.3.7.2 Morris water maze test (MWM) .....	264
5.3.7.3 Brain Hippocampus histopathological evaluation .....	265
5.3.7.4 Gene Expression Studies.....	266
5.3.7.5 Biochemical analysis .....	267
5.4. Results and discussion .....	268
5.4.1. Fabrication of Liposomes for Oxytocin and Vasopressin.....	268
5.4.2. Optimization of liposomes for oxytocin and vasopressin.....	268
5.4.2.1. Oxytocin loaded liposomes.....	268
5.4.2.2. Vasopressin loaded liposomes .....	270
5.4.3. Physicochemical attributes of optimized formulations.....	271
5.4.4. Stability study of liposomes formulations .....	273
5.4.4.1. Oxytocin-Loaded Liposomes.....	273

## ***TABLE OF CONTENT***

---

5.4.4.2. Vasopressin-Loaded Liposomes .....	274
5.4.5. <i>In vitro</i> cell line study .....	275
5.4.5.1. Evaluation on SH-SY5Y neuro cells .....	275
5.4.5.2. Evaluation on monocytic cells (THP-I) .....	279
5.4.5.3. Neurological effects of developed formulations in experimental animals .....	284

### **CHAPTER-6**

6. AEEA-Oxytocin conjugation for nose to brain delivery .....	301
6.1 Background .....	301
6.2 Materials and Instruments .....	301
6.3 Synthesis and Characterization of AEEA-Oxytocin .....	303
6.3.1 Synthesis of AEEA-Oxytocin .....	303
6.3.2 Characterization .....	305
6.4 <i>In vitro</i> cell line study .....	306
6.4.1 Evaluation on SH-SY5Y neuro cells .....	306
6.4.1.1 % Cell viability and biocompatibility .....	306
6.4.1.2 <i>In vitro</i> cell permeation study: Quantitative .....	307
6.4.1.3 Cell permeation study (Cell uptake) .....	307
6.4.1.4 Cell apoptosis assay .....	308
6.4.1.5 Reactive Oxygen Species (ROS) Assay .....	309
6.4.2 Evaluation on monocytic cells (THP-1) .....	309
6.4.2.1 Analysis using genomic microarrays (qRT-PCR) .....	309
6.4.2.2 Immunofluorescence assay (IFA) .....	309
6.5 Neurological activities in experimental animals .....	309
6.5.1 Methodology for Pharmacodynamics studies .....	309
6.5.2 Morris water maze (MWM) test .....	310
6.5.3 Brain Hippocampus histopathological evaluation .....	311
6.5.4 Gene Expression and Biochemical Studies .....	311

---

## ***TABLE OF CONTENT***

---

6.5.5	Biochemical analysis .....	312
6.5.6	Statistical Analysis .....	312
6.6	Results and discussion .....	312
6.6.1	Synthesis and characterization of AEEA-Oxytocin.....	312
6.6.2	<i>In vitro</i> cell line study .....	318
6.6.2.1	Evaluation on SH-SY5Y neuro cells .....	318
6.6.2.2	Evaluation on monocytic cells (THP-1) .....	325
6.6.3	Neurological effects in experimental animals.....	328
6.6.3.1	Morris water maze test.....	328
6.6.3.2	Gene expression .....	330
6.6.3.3	Biochemical estimations .....	333
6.6.3.4	Brain Histopathology examinations.....	335
<b><u>CHAPTER-7</u></b>		
7.	Summary and Conclusions .....	338
7.1	Summary .....	338
7.1.1	Analytical method development of oxytocin, vasopressin and angiotensin ....	339
7.1.2	Development of RTI dosage .....	341
7.1.2.1	Development of RTI dosage form for oxytocin.....	341
7.1.2.2	Development of RTI dosage form for vasopressin .....	344
7.1.2.3	Development of RTI dosage form for angiotensin .....	346
7.1.3	Development of lipidic liposomes for nose to brain delivery.....	348
7.1.3.1	Formulation and characterization .....	348
7.1.3.2	Evaluation on monocytic cells (THP-I) .....	349
7.1.3.3	<i>Ex vivo</i> evaluation on SH-SY5Y.....	350
7.1.3.4	Evaluation in AD induced animal model.....	351
7.1.4	AEEA-Oxytocin Conjugates for nose to brain delivery .....	354
7.1.4.1	Synthesis and Characterization of AEEA-Oxytocin.....	354
7.1.4.2	Evaluation on SH-SY5Y neuro cells .....	355

---

## ***TABLE OF CONTENT***

---

7.1.4.3 Evaluation of AEEA- OXT on monocytic cells (THP-I).....	357
7.1.4.4 Neurological effects in experimental animals.....	358
7.2 Conclusions.....	360
<b>ANNEXURE-I.....</b>	<b>363</b>