

Table of Contents

Contents	Page No.
LIST OF FIGURES	xxi
LIST OF TABLES	xxviii
LIST OF ABBREVIATIONS AND SYMBOLS	xxxii
PREFACE	xxxiv
Chapter 1	
Introduction	1
Chapter 2	
Hypothesis & Objectives	10
2.1. Major Objectives	11
2.2. Specific Objectives	14
Chapter 3	
Effects of ambroxol and rebamipide on GCase activity <i>in vitro</i>	15
3.1. Introduction	16
3.2. Materials and Methods	17
3.2.1. Animals.....	17
3.2.2. Materials	18
3.2.3. Isolation of endoplasmic reticulum (ER) from the brain region of rats	18
3.2.4. Effect of drugs on GCase activity <i>in vitro</i>	19
3.2.5. Statistical Analysis	20
3.3. Results & Discussion	21
3.4. Conclusions.....	21
Chapter 4	
(a) Validation of GCase as a target in 6-OHDA-induced model of PD in rats	23
(b) Assessing the effects of sub-acute administration of ambroxol in 6-OHDA-induced model of PD in rats	23
4.1. Introduction.....	24
4.2. Materials and Methods	28

4.2.1. Animals.....	28
4.2.2. Materials	29
4.2.3. Surgery and Microinjection.....	30
4.2.4. Experimental Design	31
4.2.5. Behavior Parameters.....	34
4.2.5.1. Apomorphine-induced rotational behavior	34
4.2.5.2. Open field test.....	34
4.2.5.3. Rotarod test	34
4.2.5.4. Grip Strength Test	35
4.2.5.5. Bar Catalepsy Test.....	35
4.2.6. Estimation of striatal monoamines and their metabolites	36
4.2.7. Measurement of GCCase enzymatic activity	36
4.2.8. Estimation of mitochondrial function	37
4.2.9. Rat α -synuclein measurement	37
4.2.10. Nissl's staining	38
4.2.11. Western blot for cytochrome-C, caspase-9, and caspase-3 protein expressions	39
4.2.12. Statistical Analysis	40
4.3. Results	40
4.3.1. Behavior Parameters.....	40
4.3.1.1. Ambroxol decreased catalepsy and apomorphine-induced changes in rotational behavior in 6-OHDA-infused rats	40
4.3.1.2. Ambroxol increased rotarod retention time and grip strength scores in 6-OHDA-infused rats	41
4.3.1.3. Ambroxol improved spontaneous locomotor activity in open field test in 6-OHDA-infused rats	42
4.3.2. Ambroxol increased DA and its metabolites DOPAC and HVA in striatal tissues of 6-OHDA-infused rats	43
4.3.3. 6-OHDA decreased and treatment with ambroxol increased GCCase activity and mitochondrial function in terms of MTT reduction in rat striatal and nigral tissues.....	47
4.3.4. Ambroxol increased soluble α -synuclein concentration in nigral tissues of 6-OHDA-infused rats	51
4.3.5. Ambroxol increased nigral cells in 6-OHDA-infused rats	52
4.3.6. Ambroxol decreased the expressions of cytochrome-C, caspase-9 and caspase-3 proteins in nigral tissues of 6-OHDA-infused rats.....	52
4.4. Discussion	54

4.5. Conclusions.....	61
-----------------------	----

Chapter 5

Evaluation of rebamipide in sub-acute doses for its action against 6-OHDA-induced toxicity in rats.....64

5.1. Introduction.....	65
5.2. Materials and Methods	67
5.2.1. Animals.....	67
5.2.2. Materials	68
5.2.3. Surgery and Microinjection.....	68
5.2.4. Experimental Design	68
5.2.5. Behavioral Parameters.....	71
5.2.5.1. Apomorphine-induced head rotation	71
5.2.5.2. Open field test.....	71
5.2.5.3. Rotarod Test.....	71
5.2.5.4. Grip Strength test.....	72
5.2.5.5. Bar Catalepsy Test.....	72
5.2.6. Estimation of striatal DA and its metabolites	72
5.2.7. Estimation of mitochondrial function, oxidative stress and bioenergetics	72
5.2.7.1. Isolation of mitochondria.....	72
5.2.7.2. Measurement of Mitochondrial respiratory complex-I, II, IV and V activity	72
5.2.7.3. Mitochondrial lipid peroxidation (LPO) measurement	73
5.2.7.4. Evaluation of mitochondrial bioenergetics.....	73
5.2.8. GCase activity measurement	74
5.2.9. α -synuclein measurement	74
5.2.10. Western blot analysis for cytochrome-C, caspase-9, and caspase-3 protein expressions.....	74
5.2.11. Statistical Analysis	74
5.3. Results	75
5.3.1. Behavior Parameters.....	75
5.3.1.1. Rebamipide attenuated 6-OHDA-induced changes in apomorphine-induced rotation and cataleptic behavior in rats.....	75
5.3.1.2. Rebamipide decreased 6-OHDA-induced changes in rotarod retention time and grip strength score.....	76

5.3.1.3. Rebamipide decreased 6-OHDA-induced changes in number of central squares crossed, ambulation, grooming and rearing in open field test	76
5.3.2. Rebamipide ameliorated 6-OHDA-induced changes in striatal dopaminergic system	80
5.3.3. Rebamipide attenuated 6-OHDA-induced decrease in mitochondrial respiratory complex activities in rat striatal tissues	82
5.3.4. Rebamipide decreased 6-OHDA-induced increase in Mitochondrial LPO activity in rat striatal tissues	82
5.3.5. Rebamipide attenuated 6-OHDA-induced changes in different states of mitochondrial respiration and mitochondrial RCR in rat striatal tissues.....	84
5.3.6. Rebamipide decreased 6-OHDA-induced changes in GCase enzymatic activity and soluble α -synuclein concentration in rat nigral tissues	85
5.3.7. Rebamipide decreased 6-OHDA-induced increase in protein expressions of cytochrome-C, caspase-9 and caspase-3 in rat nigral tissues.....	86
5.4. Discussion	90
5.5. Conclusions.....	96

Chapter 6

To observe the role of nuclear factor erythroid 2-related factor 2 (Nrf2) activity in rebamipide-mediated changes against 6-OHDA toxicity in rats

6.1. Introduction.....	99
6.2. Materials and Methods	101
6.2.1. Animals.....	101
6.2.2. Materials	102
6.2.3. Stereotaxic Surgery	102
6.2.4. Experimental Design	103
6.2.5. Behavioral Parameters.....	105
6.2.5.1. Apomorphine-induced head rotation	105
6.2.5.2. Open field test.....	105
6.2.5.3. Rotarod Test.....	105
6.2.5.4. Grip Strength test.....	105
6.2.5.5. Bar Catalepsy Test.....	106
6.2.6. Estimation of TH, DA and DAT levels	106
6.2.7. Measurement of nuclear factor erythroid 2-related factor 2 (Nrf2)	106
6.2.8. Measurement of SOD and CAT activity.....	106
6.2.9. Assessment of GSH levels	107

6.2.10. Estimation of GCase activity and α -synuclein concentration	107
6.2.11. Measurement of mitochondrial complex-I activity	107
6.2.12. Nissl's staining	108
6.2.13. Statistical Analysis	108
6.3. Results	108
6.3.1. Behavior Parameters.....	108
6.3.1.1. The combination of rebamipide with Nrf2i partly blocked attenuation of 6-OHDA-induced motor deficits by rebamipide in apomorphine-induced rotation, cataleptic behavior and grip strength score	108
6.3.1.2. The combination of rebamipide with Nrf2i abolished the attenuation of 6-OHDA-induced motor deficits by rebamipide in rotarod retention time	109
6.3.1.3. The combination of rebamipide with Nrf2i partly blocked the attenuation of 6-OHDA-induced motor deficits by rebamipide in number of central squares crossed, and abolished the reduction of 6-OHDA-induced changes by rebamipide in ambulation, grooming and rearing of open field test	110
6.3.2. The combination of rebamipide with Nrf2i partly blocked the elevation of nigral TH levels by rebamipide against 6-OHDA-infused rats	114
6.3.3. The combination of rebamipide with Nrf2i abolished the elevation of striatal DA and DAT levels by rebamipide against 6-OHDA-infused rats.....	114
6.3.4. The combination of rebamipide with Nrf2i abolished the elevation of nuclear Nrf2 by rebamipide against 6-OHDA-infused rats	117
6.3.5. The combination of rebamipide with Nrf2i partly blocked the elevation of nigral SOD and CAT activity as well as GSH level by rebamipide against 6-OHDA-infused rats	118
6.3.6. The combination of rebamipide with Nrf2i abolished the elevation of mitochondrial complex-I activity, GCase activity and soluble α -synuclein concentration in nigral tissues by rebamipide against 6-OHDA-infused rats.....	120
6.3.7. The combination of rebamipide with Nrf2i abolished the elevation in number of nigral cells by rebamipide against 6-OHDA-infused rats	122
6.3.8. Correlation analysis between the Nuclear Nrf2 levels and different PD parameters.....	124
6.4. Discussion	127
6.5. Conclusions.....	134

Chapter 7

To assess the sub-chronic dose of ambroxol for neurorestorative effects against 6-OHDA-induced model of PD in rats.....	137
7.1. Introduction.....	138

7.2. Material and Methods.....	140
7.2.1. Animals.....	140
7.2.2. Materials	141
7.2.3. Intrastratial administration of 6-OHDA.....	141
7.2.4. Experimental Procedure	141
7.2.5. Behavioral Parameters.....	143
7.2.5.1. Open field test.....	143
7.2.5.2. Grip strength test.....	144
7.2.5.3. Rotarod test	144
7.2.5.4. Apomorphine-induced rotational behavior	144
7.2.5.5. Bar Catalepsy Test.....	144
7.2.5.6. Narrow Beam Walk Test	144
7.2.6. Measurement of TH and DAT levels	145
7.2.7. Estimation of GCase activity and soluble α -synuclein concentration.....	145
7.2.8. Measurement of mitochondrial complex-I activity	145
7.2.9. Nissl's staining.....	145
7.2.10. Statistical Analysis.....	145
7.3. Results	146
7.3.1. Behavioral Parameters.....	146
7.3.1.1. Ambroxol attenuated 6-OHDA-induced increase in rotational behavior caused by apomorphine and cataleptic behavior in rats.....	146
7.3.1.2. Ambroxol inhibited 6-OHDA-induced changes in rotarod retention time and grip strength score	146
7.3.1.3. Ambroxol attenuated 6-OHDA-induced decrease in number of central squares crossed, ambulation, grooming and rearing in open field test	147
7.3.1.4. Ambroxol inhibited 6-OHDA-induced changes in beam performance in narrow beam walk test	148
7.3.2. Ambroxol has Restorative Effects on 6-OHDA-induced reduction in nigral TH levels	148
7.3.3. Ambroxol attenuated 6-OHDA-induced changes in mitochondrial complex-I, GCase enzymatic activity and soluble α -synuclein concentration in rat nigral tissues	152
7.3.4. Ambroxol has Restorative Effects on 6-OHDA-induced decrease in DAT levels in rat striatal tissues.....	152
7.3.5. Ambroxol has Restorative Effects on 6-OHDA-induced nigral cell loss	155
7.4. Discussion	157

7.5. Conclusions.....	163
-----------------------	-----

Chapter 8

Evaluation of sub-chronic administration of rebamipide for disease-modifying effects against 6-OHDA-induced model of PD in rats166

8.1. Introduction.....	167
8.2. Materials and Methods	169
8.2.1. Animals.....	169
8.2.2. Materials	170
8.2.3. Stereotaxic surgery	170
8.2.4. Experimental Design	170
8.2.5. Behavioral Parameters.....	172
8.2.5.1. Apomorphine-induced rotations	172
8.2.5.2. Cataleptic behavior	172
8.2.5.3. Rotarod test	172
8.2.5.4. Grip strength	173
8.2.5.5. Open field behavior.....	173
8.2.5.6. Narrow beam walk	173
8.2.6. Estimation of TH levels, soluble α -synuclein concentration and DAT levels	173
8.2.7. Estimation of mitochondrial respiratory complex-I and GCase activities in nigral tissues	173
8.2.8. Nissl's staining.....	173
8.2.9. Statistical Analysis.....	173
8.3. Results	174
8.3.1. Behavior Parameters.....	174
8.3.1.1. Behavioral recovery following rebamipide administration against 6-OHDA-induced alterations in bar catalepsy and apomorphine-induced rotation test	174
8.3.1.2. Rebamipide attenuated 6-OHDA-induced changes in rotarod retention time and grip strength score.....	175
8.3.1.3. Rebamipide inhibited 6-OHDA-induced changes in the parameters of open field test	175
8.3.1.4. Rebamipide improved performance against 6-OHDA-induced deficits in narrow beam walk test	176
8.3.2. Rebamipide attenuated 6-OHDA-induced loss of nigral TH levels	179

8.3.3. Rebamipide has restorative effects on 6-OHDA-induced loss of striatal DAT levels	180
8.3.4. Rebamipide attenuated 6-OHDA-induced changes in mitochondrial complex-I, GCase enzymatic activity and α -synuclein concentration in rat nigral tissues	181
8.3.5. Rebamipide inhibited 6-OHDA-induced nigral cell loss	182
8.4. Discussion	185
8.5. Conclusions	188

Chapter 9

Design, characterization and evaluation of transdermal patches of rebamipide in rodent model of PD190

9.1 Introduction	191
9.2. Materials and Methods	195
9.2.1. Animals	195
9.2.2. Materials	195
9.2.3. Preparation of backing membrane	196
9.2.4. Formation of patches over the backing membrane	196
9.2.5. Physicochemical evaluation of transdermal patches	199
9.2.5.1. Preformulation studies	199
9.2.5.2. Uniformity of weight	200
9.2.5.3. Patch Thickness	200
9.2.5.4. Folding endurance	200
9.2.5.5. Surface pH	200
9.2.5.6. Drug content uniformity	201
9.2.5.7. Swelling studies	201
9.2.5.8. Percentage moisture loss	202
9.2.6. <i>Ex vivo</i> drug permeation studies	202
9.2.7. Fourier transform infrared (FTIR) spectroscopy	203
9.2.8. Surface Morphology	203
9.2.9. Skin irritation studies	203
9.2.10. <i>In vivo</i> studies	204
9.2.10.1. Surgery and Microinjection	204
9.2.10.2. Experimental Design	204
9.2.10.3. Behavioral parameters	207
9.2.10.4. Measurement of DA, TH, DAT and soluble α -synuclein levels	207

9.2.10.5.	Measurement of GCase activity	208
9.2.10.6.	Quantification of rebamipide (HPLC analysis).....	208
9.2.10.7.	Nissl's staining	208
9.2.11.	Statistical Analysis	208
9.3.	Results	209
9.3.1.	Physicochemical characterization of Rebamipide	209
9.3.1.1.	Preformulation studies:	209
9.3.1.2.	Uniformity of thickness, folding endurance, surface pH and drug content 211	
9.3.1.3.	Weight and swelling behavior	213
9.3.1.4.	Moisture loss studies	213
9.3.2.	<i>Ex vivo</i> drug permeation studies.....	214
9.3.3.	FTIR spectroscopy	215
9.3.4.	Surface Morphology.....	218
9.3.5.	Skin irritation studies	219
9.3.6.	<i>In vivo</i> Studies	220
9.3.6.1.	Rebamipide containing transdermal patches attenuated 6-OHDA- induced motor deficits in rats	220
9.3.6.2.	Rebamipide-containing transdermal patches inhibited 6-OHDA-induced reduction in nigral TH and striatal DA levels in rats.....	221
9.3.6.3.	Transdermal patches of rebamipide inhibited 6-OHDA-induced alterations in GCase activity and soluble α -synuclein concentration in nigral tissues of rats 224	
9.3.6.4.	Rebamipide-containing transdermal patches inhibited 6-OHDA-induced reduction in striatal DAT levels in rats	224
9.3.6.5.	Quantification of rebamipide (HPLC analysis).....	226
9.3.6.6.	Transdermal patches of rebamipide attenuated 6-OHDA-induced reduction in the number of Nissl bodies in nigral tissues of rats.....	227
9.4.	Discussion	229
9.5.	Conclusions.....	232

Chapter 10

Summary & Scope for Further Work.....	235
10.1. Summary	236
10.2. Scope for Further Work.....	236

Chapter 11

Bibliography239

Permission from Central Animal Ethical Committee266

LIST OF PUBLICATIONS.....267