

Table of Contents

CERTIFICATE.....	i
DECLARATION BY THE CANDIDATE	ii
COPYRIGHT TRANSFER CERTIFICATE.....	iii
Acknowledgements	iv
Table of Contents	viii
List of Figures.....	xii
List of Tables	xiv
List of Abbreviations	xvi
Preface.....	xvii
1 Chapter 1: General Introduction	1
1.1 Introduction.....	2
1.2 Diabetes comorbid depression	4
1.2.1 Diagnostic features	4
1.2.2 Prevalence	5
1.2.2.1 Diabetes comorbid depression in India	6
1.2.3 Pathophysiology	8
1.2.3.1 Oxidative stress	9
1.2.3.2 HPA axis alteration	12
1.2.3.3 Neuronal damage.....	15
1.2.3.4 Monoamine dysregulation.....	18
1.2.3.5 Mitochondrial dysfunction	19
1.2.3.6 Inflammation	21
1.2.3.7 Epigenetic modification	24
1.3 Management and treatment.....	27
1.4 Metformin.....	29
1.4.1 Chemistry of metformin	30
1.4.2 Pharmacokinetics of Metformin.....	31
1.4.2.1 Absorption and bioavailability	31
1.4.2.2 Distribution.....	32
1.4.2.3 Metabolism.....	32
1.4.2.4 Elimination	32

1.4.3	Role of metformin in diabetes	33
1.4.4	Mechanism of metformin in diabetes	34
1.4.5	Central nervous system regulation	36
1.4.6	Regulation of gut-mediated satiety signals	38
1.5	Ascorbic Acid	40
1.6	Aim	43
2	Chapter 2: Ascorbic acid monotherapy against diabetes comorbid depression in rats	45
2.1	Introduction.....	47
2.2	Materials and methods	49
2.2.1	Animals	49
2.2.2	Materials and reagents.....	50
2.2.3	Induction of diabetes mellitus in rats	50
2.2.4	Induction of comorbid depression in diabetic rats	51
2.2.5	Experimental design	51
2.2.6	Forced swim test.....	53
2.2.7	Blood and organ collection.....	53
2.2.8	Assessment of plasma glucose, insulin, and corticosterone	53
2.2.9	Preparation of prefrontal cortex homogenate	54
2.2.10	Assessment of oxidative stress markers in the prefrontal cortex	54
2.2.11	Assessment of IL-10 levels	56
2.2.12	Statistical analysis	56
2.3	Results	57
2.3.1	Ascorbic acid against comorbid depression	57
2.3.2	Ascorbic acid against hyperglycemia and hypoinsulinemia	58
2.3.3	Ascorbic acid against oxidative stress.....	60
2.3.4	Ascorbic acid against inflammation	62
2.4	Discussion.....	63
2.5	Conclusion	68
3	Chapter 3 Metformin monotherapy: a potential strategy against diabetes comorbid depression in rats.....	69
3.1	Introduction.....	71
3.2	Materials and methods	72

3.2.1	Materials and reagents.....	72
3.2.2	Induction of diabetes and comorbid depressive-like behavior.....	72
3.2.3	Experimental design.....	72
3.2.4	Forced swim test.....	74
3.2.5	Blood and brain sample collection.....	74
3.2.6	Estimation of plasma glucose, insulin, and corticosterone levels.....	74
3.2.7	Estimation of monoamines and inflammatory cytokines.....	74
3.2.8	Estimation of oxidative stress markers.....	75
3.2.9	Statistical analysis.....	75
3.3	Results.....	76
3.3.1	Metformin against comorbid depression.....	76
3.3.2	Metformin against hyperglycemia and hypoinsulinemia.....	77
3.3.3	Metformin against reduction in monoamine levels.....	78
3.3.4	Metformin against inflammatory cytokines in prefrontal cortex.....	79
3.3.5	Metformin against oxidative stress markers in prefrontal cortex.....	80
3.4	Discussion.....	81
3.5	Conclusions.....	83
4	Chapter 4: Metformin and ascorbic acid combination therapy: a potential strategy against diabetes comorbid depression in rats.....	85
4.1	Introduction.....	87
4.2	Materials and methods.....	89
4.2.1	Induction of type 2 diabetes and comorbid depressive-like behavior.....	89
4.2.2	Experimental design.....	89
4.2.3	Forced swim test.....	91
4.2.4	Collection of blood, brain, and adrenal glands.....	91
4.2.5	Estimation of plasma glucose and insulin.....	91
4.2.6	Estimation of plasma corticosterone.....	91
4.2.7	Preparation of brain homogenate.....	92
4.2.8	Estimation of monoamines in the prefrontal cortex.....	92
4.2.9	Estimation of oxidative stress in the prefrontal cortex.....	92
4.2.10	Estimation of proinflammatory cytokines in the prefrontal cortex.....	92
4.2.11	Statistical analysis.....	93
4.3	Results.....	93

4.3.1	Effects of combination therapy on comorbid depression	93
4.3.2	Effects of combination therapy on hyperglycemia and hypoinsulinemia ..	95
4.3.3	Effects of combination therapy on brain monoamine levels	97
4.3.4	Effects of combination therapy on oxidative stress.....	98
4.3.5	Effects of combination therapy on proinflammatory cytokine levels	100
4.4	Discussion.....	102
4.5	Conclusion	108
5	Chapter 5: Combination therapy of metformin and ascorbic acid modulates BDNF, caspase, NF-κB, and mitochondrial membrane potential in diabetes comorbid depressed rats	109
5.1	Introduction.....	111
5.2	Materials and methods	113
5.2.1	Experimental design	113
5.2.2	Estimation of mitochondrial membrane potential	114
5.2.3	Western blotting of BDNF, caspase-9, and caspase-3	115
5.2.4	Real-time PCR (Quantitative Polymerase Chain Reaction, qPCR)	116
5.2.5	Immunohistochemistry	116
5.2.6	Statistical analysis	117
5.3	Results	117
5.3.1	Confirmation of activity against depressive-like markers.....	117
5.3.2	Effect on mitochondrial membrane potential and ROS	119
5.3.3	Effect on caspase -9 and caspase-3 expression in the prefrontal cortex...	120
5.3.4	Effect on BDNF expression in the prefrontal cortex.....	121
5.3.5	Effect on NF- κ B expression in the prefrontal cortex	122
5.4	Discussion.....	123
5.5	Conclusion	125
6	Chapter 6: General discussion and conclusions.....	127
6.1	Summary of major findings.....	132
6.2	Scope for further work	134
7	Chapter 7: References	135
8	Chapter 8: Publications.....	173
8.1	Peer reviewed papers.....	175
8.2	Conference abstracts.....	175