

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION	1
1.1 Neonatal anoxia	2
1.2 Causes of anoxia	2
1.3 Epidemiology	3
1.4 Clinical diagnosis	5
1.4.1 APGAR score	5
1.4.2 Sarnat score	5
1.4.3 Pulse Oximetry	5
1.4.4 Analysis of blood lactate & pH	5
1.5 Models for neonatal anoxia	6
1.5.1 Hypoxia–ischemia	6
1.5.2 Perinatal asphyxia	6
1.5.3 Neonatal anoxia	6
1.6 Current treatment for anoxia	7
1.7 Pathophysiology of neonatal anoxia	8
1.8 Apoptosis	10
1.8.1 Extrinsic pathway of apoptosis	10
1.8.2 Intrinsic pathway of apoptosis	10
1.9 Role of pro-apoptotic and apoptotic mediators in mitochondrial-linked cell death	11
1.9.1 Caspases	11
1.9.2 Bcl-2 family proteins	11
1.10 Pathophysiological changes in mitochondrial molecular machinery after anoxia	12
1.11 Hypothesis	14
1.12 Proposed treatment strategies for neonatal anoxia	15
1.13 Key interrogations	17
1.14 Objectives of thesis	18
CHAPTER 2: TEMPORAL PATHOLOGICAL CHANGES IN MITOCHONDRIAL BIOENERGETICS IN TWO EPISODIC ANOXIA MODEL IN RATS	19
2.1 Abstract	20
2.2 Introduction	21
2.3 Materials and methods	22
2.3.1 Animals	22
2.3.2 Anoxia model	22
2.3.3 Reagents	23
2.3.4 Behavioral parameter assessment	24
2.3.5 Mitochondrial Isolation	24
2.3.6 Measurement of mitochondrial function	24
2.3.7 Assessment of brain mitochondrial nitrite level	25
2.3.8 Experimental design	25
2.3.9 Statistical analysis	26
2.4 Results	26
2.5 Discussion	30

CHAPTER 3: MITOCHONDRIAL MEDIATED APOPTOSIS AND INSULT PROGRESSION POST ANOXIA	33
3.1 Abstract	34
3.2 Introduction	35
3.3 Materials and Methods	37
3.3.1 Animals and groups	37
3.3.2 Anoxia procedure	38
3.3.3 Materials	38
3.3.4 Behavioral parameter assessment	38
3.3.5 Measurement of peripheral oxygen saturation	39
3.3.6 Laser speckle blood-flow imaging	39
3.3.7 Mitochondrial Isolation	40
3.3.8 Measurement of Mitochondrial Function	40
3.3.9 Evaluation of mitochondrial membrane potential (MMP) in cortical brain region	40
3.3.10 Estimation of mitochondrial permeability transition pore (MPT) opening	40
3.3.11 Estimation of LPO and NO levels	41
3.3.12 Assessment of mitochondrial SOD and CAT activity	41
3.3.13 Western blot analysis for cytoplasmic cytochrome-C, caspase-9, caspase-3, Bax, Bcl-2 and mitochondrial Bax and Bcl-2	41
3.3.14 Flow cytometric measurement of cell death using AnnexinV/PI	42
3.3.15 Statistical analysis	43
3.4 Results	43
3.5 Discussion	61
CHAPTE 4: MITOCHNDRIAL-LINKED APOPTOTIC MARKERS IN ANOXIC NEONATES	66
4.1 Abstract	67
4.2 Introduction	68
4.3 Material and methods	69
4.3.1 Patients	69
4.3.2 Inclusion Criteria	69
4.3.3 Exclusion Criteria	69
4.3.4 CSF Sampling	70
4.3.5 Cytochrome-C, caspase-9 and caspase-3 measurement	70
4.3.6 Statistical Analysis	70
4.4 Results	70
4.5 Discussion	71
CHAPTER 5: EVALUATION OF MITOCHONDRIAL UNCOUPLER 2,4 DNP IN ANOXIC RATS	74
5.1 Abstract	75
5.2 Introduction	76
5.3 Materials and methods	78
5.3.1 Animals	78
5.3.2 Anoxia model	79
5.3.3 2, 4-DNP preparation and dosing	79

5.3.4	Behavioral parameter assessment	79
5.3.5	Mitochondrial Isolation	79
5.3.6	Measurement of Mitochondrial Function	79
5.3.7	Estimation of NADH dehydrogenase (Complex-I) activity	80
5.3.8	Estimation of mitochondrial succinate dehydrogenase (Complex-II) activity	80
5.3.9	Estimation of cytochrome-C oxidase (Complex-IV) activity	80
5.3.10	Estimation of mitochondrial F1F0 ATP synthase (Complex-V) activity	80
5.3.11	Evaluating mitochondrial membrane potential (MMP)	81
5.3.12	Mitochondrial permeability transition (MPT)	81
5.3.13	Mitochondrial oxidative stress	81
5.3.14	Western blot analysis for cytoplasmic cytochrome-C, caspase-9, caspase-3, Bax, Bcl-2 and mitochondrial Bax and Bcl-2	81
5.3.15	Statistical analysis	82
5.4	Results	94
5.5	Discussion	94

CHAPTER 6: EVALUATION OF ANTIOXIDANT TEMPOL IN ANOXIC MODEL 98

6.1	Abstract	99
6.2	Introduction	100
6.3	Materials and methods	101
6.3.1	Animals	101
6.3.2	Anoxia model	101
6.3.3	Tempol preparation and dosing	101
6.3.4	Chemicals	101
6.3.5	Behavioral parameter assessment	101
6.3.5.1	Righting reflex	101
6.3.5.2	Wire hanging maneuver	101
6.3.6	Mitochondrial Isolation	101
6.3.7	Measurement of Mitochondrial Function	101
6.3.8	Estimation of NADH dehydrogenase (Complex-I) activity	102
6.3.9	Estimation of mitochondrial succinate dehydrogenase (Complex-II) activity	103
6.3.10	Estimation of cytochrome-C oxidase (Complex-IV) activity	103
6.3.11	Estimation of mitochondrial F1F0 ATP synthase (Complex-V) activity	103
6.3.12	Evaluating mitochondrial membrane potential (MMP)	103
6.3.13	Mitochondrial permeability transition (MPT)	103
6.3.14	Mitochondrial oxidative stress	103
6.3.15	Western blot analysis for cytoplasmic cytochrome-C, caspase-9, caspase-3, Bax, Bcl- 2 and mitochondrial Bax and Bcl-2	104
6.3.16	Statistical analysis	104
6.4	Results	104
6.5	Discussion	117

CHAPTER 7: TO STUDY THE COMBINATION EFFECT OF MODULATORS IN TWO DIFFERENT CORTICAL MITOCHONDRIAL FRACTIONS AND LONG TERM NEUROBEHAVIORAL CHANGES POST ANOXIA INJURY	122
7.1 Abstract	123
7.2 Introduction	124
7.3 Materials and methods	126
7.3.1 Animals	126
7.3.2 Anoxia model	127
7.3.3 Drug preparation and dosing	127
7.3.4 Chemicals	127
7.3.5 Synaptic and non-synaptic mitochondria isolation	128
7.3.6 Measurement of Mitochondrial Function	128
7.3.7 Mitochondrial calcium measurements	129
7.3.8 Mitochondrial permeability transition (MPT)	129
7.3.9 Mitochondrial oxidative stress	129
7.3.9.1 Estimation of LPO and NO levels	129
7.3.9.2 Estimation of mitochondrial SOD and CAT activity	129
7.3.10 Western blot analysis for cytoplasmic cytochrome-C, caspase-9, caspase-3, Bax, Bel-2 and mitochondrial Bax and Bel-2	130
7.3.11 Behavioral Studies	130
7.3.12 Plasma Corticosterone	132
7.3.13 Statistical analysis	132
7.4 Results	133
7.5 Discussion	154
CHAPTER 8: SUMMARY AND CONCLUSION	159
REFERENCES	165