

INDEX

Chapter Number	Chapter Name	Page Number
1	INTRODUCTION AND LITERATURE REVIEW	1
	1.1 Parkinson's Disease - Global ageing related disorder and its prevalence	2
	1.2 Neuroanatomical changes in Parkinson's Disease	3
	1.3 Etiology of Parkinson's Disease	4
	1.4 Pathophysiology of Parkinson's Disease - genetic classification	4
	1.4.1 SNCA (α -synuclein) / PARK1/4	4
	1.4.2 LRRK2/PARK8	5
	1.4.3 PARKIN/PARK2	5
	1.4.4 PINK1/PARK6	5
	1.4.5 DJ-1/PARK7	6
	1.5 Spread of PD pathogenesis: beyond dopaminergic neurons	8
	1.6 Intercellular communication in PD – exosomes are all the hype	9
	1.7 Interorganellar crosstalk: role in PD	11
	1.8 Mitochondrial dysfunction in Parkinson's Disease	12
	1.9 Autophagy and lysosomal dysfunction in Parkinson's Disease	15
	1.10 Mitochondrial turnover – crosstalk with lysosomal functions	18
	1.11 Endolysosomal system: two interlinked pathways	21
	1.12 Crosstalk of autophagy and endolysosomal pathways determine the exosome release	22
	1.13 Exosomes – biogenesis	25
	1.13.1 Canonical pathway	25
	1.13.2 Non-canonical pathway	27
	1.14 Exosome biogenesis machinery: implications in PD	27
	1.15 Exosomal cargo and sorting	28
	1.16 Exosomal miRNA cargo: role in intercellular communication and PD pathogenesis	32
	1.17 Exosomal miRNAs – a promising biomarker and therapeutic potential	33
2	AIMS AND OBJECTIVES	36
	2.1 Rationale of hypothesis	37
	2.2 Specific objectives	39
3	MATERIALS AND METHODS	40
	3.1 Kits and reagents	41
	3.2 Cell culture and seeding density	41
	3.3 Isolation of primary neurons and seeding density	41
	3.4 Treatment of PD-stress inducers	42
	3.5 Rotenone rat model	42
	3.6 Exosome isolation: from cells, serum, and cerebrospinal fluid (CSF)	43
	3.7 Nanoparticle Tracking Analysis (NTA)	44

	3.8 Western Blotting	44
	3.9 Lysosomal acid phosphatase assay	45
	3.10 Fluorescence microscopy	45
	3.11 Confocal microscopy	45
	3.12 ATP assay	46
	3.13 Mitochondrial complex I assay by spectrophotometric method	46
	3.14 Exosome treatment	47
	3.15 miRNA loading into exosomes	47
	3.16 miRNA expression analysis by Next Generation Sequencing (NGS)	48
	3.17 RNA isolation and RT-qPCR	48
	3.18 Droplet Digital PCR	49
	3.19 Cell death assay by MTT	49
	3.20 Cell death detection by ELISA	49
	3.21 Caspase 3/7 activity	50
	3.22 ROS estimation and mitochondrial membrane potential	50
	3.23 Immunocytochemistry	51
	3.24 Details for primer sequences	51
	3.25 Statistical analysis	53
4	RESULTS AND DISCUSSION	54
	4.1 Exosome release is altered in Parkinson's Disease stress conditions and is modulated by the interorganellar crosstalk between mitochondria and lysosome	55
	4.1.1 Exosome release is enhanced in Parkinson's Disease stress conditions	56
	4.1.2 Parkinson's Disease stress conditions lead to mitochondrial dysfunctions	59
	4.1.3 Lysosomal functions are altered in Parkinson's Disease stress conditions	61
	4.1.4 Altered autophagic flux modulates exosome release in PD stress conditions	64
	4.1.5 Enhancement of autophagy flux by rapamycin decreases the release of exosomes in PD stress conditions	68
	4.1.6 Discussion	70
	4.2 PD-stressed neuronal exosomes alter cellular functions of bystander cells and exosomal miRNAs are altered in PD stress conditions	74
	4.2.1 PD-stressed exosomes are internalized by bystander neuronal cells and localize to mitochondria	75
	4.2.2 PD-stressed exosomes alter the mitochondrial functions and induces cell death	78
	4.2.3 PD stress conditions induce differential enrichment of miRNAs in neuronal exosomes	82
	4.2.4 Validation of identified exosomal miRNAs in neuronal cells	88
	4.2.5 Putative target analysis of miR-30a-5p and miR-181c-5p	93

	4.2.6 miRNAs are selectively sorted into exosomes	95
	4.2.7 miR-30a-5p and miR-181c-5p mimic-loaded exosomes rescue mitochondrial dysfunction and cell death in PD-stressed neuronal cells	98
	4.2.8 Discussion	103
	4.3 Rotenone induced exosomal miRNA alterations in rat cerebrospinal fluid and serum induces mitochondrial dysfunction and cell death	108
	4.3.1 Rotenone toxicity in primary neurons of rat embryos affects the exosome release	109
	4.3.2 Exosome release is altered in acute rotenone-treated rat models	111
	4.3.3 Specific miRNA levels are altered in both primary neurons and exosomes in PD stress conditions	114
	4.3.4 Exosomes from serum and cerebrospinal fluid of acute rotenone-treated rats have differential expressions of miRNAs	116
	4.3.5 Serum exosomes from acute rotenone-treated rats induce mitochondrial dysfunction and cell death in primary neurons from rat embryos	118
	4.3.6 Serum exosomes exacerbate rotenone-induced dopaminergic neuron cell death in primary midbrain neurons from rat embryos	121
	4.3.7 Discussion	122
5	SUMMARY AND CONCLUSION	127
	5.1 Summary	128
	5.1.1 Exosome release is modulated by the organellar crosstalk in PD stress conditions	128
	5.1.2 Neuronal exosomes are internalized by recipient cells and modulate the cellular functions	129
	5.1.3 Exosomal miRNAs are differentially sorted in PD stress conditions and alter mitochondrial functions and cell death in recipient cells	130
	5.1.4 Exosomal cargo from serum of PD-rats affects the cellular functions in primary midbrain neurons	131
	5.2 Conclusion	131
	5.3 Limitations of the study	133
	5.4 Future perspectives	134
6	BIBLIOGRAPHY	136
7	LIST OF PUBLICATIONS	158
	7.1 Publications from Ph.D. thesis work	159
	7.2 Publications from other associated projects during Ph.D. tenure	160
	7.3 Reprints of published articles	161