

Table of contents

List of Figuers	xiii
List of Tabels	xviii
List of abbrivations	xix
List of Symbols	xxv
Preface	xxvii
1 Chapter 1: Introduction	1
2 Chapter 2: Literature review	9
2.1 Skin cancer and melanoma.....	9
2.1.1 Risk factors	11
2.1.2 Diagnosis.....	11
2.1.3 Progression of melanoma.....	12
2.1.4 Types of melanomas	13
2.1.5 Pathogenesis.....	14
2.1.6 Current therapeutic options for melanoma	16
2.1.7 Various <i>in-vivo</i> tumor models with special reference to the syngeneic tumor model	19
2.2 Role of drug repurposing and flavonoids in melanoma	20
2.2.1 Drug repurposing	20
2.2.2 Flavonoids.....	22
2.3 Dihydroartemisinin.....	25
2.3.1 Physicochemical properties	26
2.3.2 Mechanism of actions in the cancer.....	26
2.3.3 Role of dihydroartemisinin in melanoma	28
2.4 Hesperidin	29
2.4.1 Physicochemical properties	30
2.4.2 Mechanism of actions	31
2.4.3 Role of hesperidin in melanoma	32
2.5 Exosomes as a drug delivery vehicle	33
2.5.1 Advantages of exosomes as a drug delivery vehicle	34
2.5.2 Biogenesis of exosomes.....	36
2.5.3 Isolation of exosomes	37

2.5.4	Drug loading methods.....	40
3	Chapter 3: Rational and Objectives.....	44
3.1	Rational	44
3.2	Objectives.....	45
4	Chapter 4: Material, instruments and software used in the experiments.....	47
5	Chapter 5: Isolation, characterization, <i>in vitro</i> and <i>in vivo</i> investigation of dihydroartemisinin-loaded bovine milk exosomes.....	55
5.1	Background	55
5.2	Objectives.....	56
5.3	Methodology	56
5.3.1	HPLC analysis	56
5.3.2	Isolation of exosomes	61
5.3.3	Immunoblotting analysis.....	62
5.3.4	Preparation of drug-loaded exosomes.....	62
5.3.5	Drug loading analysis	64
5.3.6	Characterization	64
5.3.7	X-Ray diffraction	65
5.3.8	Stability study	65
5.3.9	Drug release	65
5.3.10	Cell culture and conditions	65
5.3.11	Cytotoxicity study.....	66
5.3.12	Qualitative cell uptake assay.....	66
5.3.13	Apoptosis assay by 4,6-Diamidino-2-phenylindole (DAPI) staining.....	67
5.3.14	Apoptosis assay.....	67
5.3.15	Reactive oxygen species assay.....	67
5.3.16	Mitochondrial membrane potential assay	68
5.3.17	Colony formation assay	68
5.3.18	Cell migration assay.....	69
5.3.19	Wound healing assay.....	70
5.3.20	Immunoblotting.....	71
5.3.21	Animal studies	71
5.3.22	Pharmacokinetics	72
5.3.23	Tissue distribution.....	72
5.3.24	<i>In vivo</i> anti-cancer efficacy study	73
5.3.25	Evaluation of toxicity via biochemical and hematological parameters	74

5.3.26	Histopathology of organs from tumor-bearing mice.....	74
5.3.27	<i>In vivo</i> pulmonary metastasis study	75
5.3.28	Statistical analysis.....	75
5.4	Results and discussion.....	76
5.4.1	HPLC Analysis.....	76
5.4.2	Isolation and immunoblotting analysis	82
5.4.3	Isolation and characterization of exosomes and DHA-loaded exosomes.....	83
5.4.4	Optimization of DHA-loaded exosomes.....	84
5.4.5	X-Ray diffraction	88
5.4.6	Stability study	88
5.4.7	Drug release	89
5.4.8	Cytotoxicity study.....	90
5.4.9	Qualitative cell uptake assay.....	91
5.4.10	Apoptosis assay by 4,6-Diamidino-2-phenylindole (DAPI) staining.....	92
5.4.11	Apoptosis assay.....	93
5.4.12	Reactive oxygen species assay.....	94
5.4.13	Mitochondrial membrane potential assay	95
5.4.14	Colony formation assay	96
5.4.15	Cell migration assay.....	97
5.4.16	Wound healing assay.....	98
5.4.17	Immunoblotting.....	100
5.4.18	Pharmacokinetics	101
5.4.19	Tissue distribution.....	102
5.4.20	<i>In vivo</i> anti-cancer efficacy study	103
5.4.21	Toxicity estimation via biochemical parameters and blood parameters	105
5.4.22	Histopathology of organs from tumor-bearing mice.....	107
5.4.23	<i>In vivo</i> tumor migration study.....	108
5.5	Conclusion.....	110
5.6	Summary points.....	110
6	Chapter 6: Isolation, characterization, <i>in vitro</i> and <i>in vivo</i> investigation of hesperidin-loaded bovine milk exosomes.....	113
6.1	Background	113
6.2	Objectives.....	114
6.3	Methodology	114
6.3.1	HPLC analysis	114

6.3.2	Isolation of exosomes	119
6.3.3	Drug loading	119
6.3.4	Characterization	120
6.3.5	X-Ray diffraction	120
6.3.6	Drug release studies	121
6.3.7	Cells and culture conditions	121
6.3.8	Cell cytotoxicity and biocompatibility.....	122
6.3.9	Qualitative cell uptake assay.....	122
6.3.10	DNA-fragmentation assay with DAPI.....	122
6.3.11	Reactive oxygen species assay.....	123
6.3.12	Mitochondrial membrane potential assay	123
6.3.13	Colony formation assay	124
6.3.14	Wound healing assay.....	124
6.3.15	Transwell migration assay	124
6.3.16	Animal studies	125
6.3.17	Pharmacokinetics	125
6.3.18	<i>In vivo</i> anti-cancer efficacy	126
6.3.19	Histopathology	126
6.3.20	Biochemical parameters.....	127
6.3.21	Statistical analysis.....	127
6.4	Results and discussion.....	127
6.4.1	HPLC Analysis.....	127
6.4.2	Isolation and characterization of bovine milk exosomes and hesperidin-loaded exosomes	134
6.4.3	X-ray diffraction	136
6.4.4	Drug release	137
6.4.5	Cytotoxicity.....	137
6.4.6	Qualitative cell uptake assay.....	139
6.4.7	DNA-Fragmentation assay.....	140
6.4.8	Reactive oxygen species assay.....	141
6.4.9	Mitochondrial membrane potential assay	142
6.4.10	Colony formation assay	143
6.4.11	Transwell migration assay	144
6.4.12	Wound healing assay.....	145
6.4.13	Pharmacokinetics	147

6.4.14	Anti-cancer efficacy study	148
6.4.15	Histopathology	150
6.4.16	Biochemical assay	151
6.5	Conclusion.....	152
6.6	Summary points.....	153
7	Chapter 7: Summary and Conclusion	155
7.1	Summary	155
7.2	Conclusion.....	156
	References	159
8	Appendices	172
8.1	Appendix A: Institutional Animal Ethics Committee (IAEC) certificates	172
8.2	Appendix B: Publications.....	174
8.2.1	List of publications from thesis works.....	174
8.2.2	List of other publications during Ph.D.....	174