

TABLE OF CONTENTS

	Page
.....	
LIST OF FIGURES	IV
LIST OF TABLES	V
SUMMARY	VI
ACKNOWLEDGMENTS	IX
ABBREVIATION AND SYMBOLS	XI
 CHAPTER 1	
1 INTRODUCTION	1-6
 CHAPTER 2	
2 REVIEW OF LITERATURE	7-44
SECTION 1: AN INTRODUCTION TO STEM CELLS	8-21
2.1 AN INTRODUCTION TO STEM CELLS	9
2.1.1 Cell	9
2.1.2 Stem Cells	10
2.1.3 Types of stem cells	11
2.1.4 Classification of stem cells	13
2.1.4.1 Totipotent Stem Cells	13
2.1.4.2 Pluripotent Stem Cells	14
2.1.4.3 Multipotent Stem Cells.....	14
2.1.4.4 Unipotent Stem Cells.....	14
2.1.5 Stem Cells Niches	15
2.1.6 Adult Stem Cells	16
2.1.7 Types of Bone Marrow Stem Cells	17
2.1.7.1 Hematopoietic stem cells	17
2.1.7.2 Mesenchymal Stem Cells	19
2.1.7.3 Multipotent Adult Progenitor Cells	19
2.1.7.4 Endothelial Progenitor Cells.....	20
2.1.7.5 Local tissue stem cells.....	20
2.1.7.6 Side Population Cells	20
2.1.7.7 Cord blood Stem Cells.....	21
SECTION 2: Liver Cells and Liver Fibrosis	22-37
2.2 Liver	23
2.2.1 Liver Anatomy	24
2.2.2 Liver Cells	25
2.2.2.1 Hepatocytes	26

2.2.2.2	Liver sinusoidal cells.....	27
2.2.2.3	Endothelial Cells.....	28
2.2.2.4	Hepatic Stellate Cells.....	28
2.2.2.5	Kupffer cells	29
2.2.3	Liver Fibrosis	30
2.2.3.1	Carbon tetrachloride (CCl4) toxicity	33
2.2.3.2	Extracellular Matrix	34
2.2.3.3	Mechanism of Liver Fibrosis	36
	SECTION-III: STRATEGIES TO CURE LIVER FIBROSIS	38-48
2.3	Strategy for Anti-fibrosis	39
2.3.1	Liver Transplantation	40
2.3.2	Need for Stem Cell Therapy.....	41
2.3.3	Transplantation of Stem cell into fibrotic animal models	42
2.3.4	Cytokines	43
2.3.4.1	Hepatocyte Growth Factor.....	45
2.3.4.2	Fibroblast Growth Factor	47
	CHAPTER 3	
3	MATERIALS AND METHODS	49-65
3.1	In Vitro Studies	50-61
3.1.1	Animals	50
3.1.2	Isolation and Culturing of Mesenchymal Stem Cells	50
3.1.3	Fluorescent Activated Cell Sorting (FACS) analysis of MSCs.....	51
3.1.4	Cytokines pretreatment of MSCs	52
3.1.5	Morphological Analysis of Cells.....	52
3.1.6	RNA Extraction and cDNA synthesis.....	53
3.1.7	RT-PCR for Genes Expression Studies.....	54
3.1.8	Designing of Primers	54
3.1.9	Immunostaining Assay of Pretreated MSCs	55
3.1.10	Periodic Acid-Schiff (PAS) Stain for Glycogen Storage.....	56
3.1.11	Isolation and Culturing of Mouse Hepatocytes.....	57
3.1.12	Characterization of Cultured Mouse Hepatocytes.....	59
3.1.13	In Vitro Injury Model of Hepatocytes.....	59
3.1.14	Co-culture Model of Injured Hepatocytes with MSCs.....	60
3.1.15	Cell Viability Assay	60
3.1.16	Lactate Dehydrogenase Assay	60
3.1.17	RT-PCR Analysis of In Vitro Co-Culture Model.....	61

3.2	In Vivo studies	62-65
3.2.1	Preparation of Liver Fibrotic Mouse Model.....	62
3.2.2	Labeling and Transplantation of MSCs.....	62
3.2.3	Histological Analysis and Measurement of Collagen.....	63
3.2.4	Functional Analyses.....	63
3.2.5	TUNEL Assay Analysis.....	63
3.2.6	Gene expression Profiling of Liver after MSCs Transplantation.....	64
3.2.7	Statistical Analysis.....	65
 CHAPTER 4		
4	RESULTS	66-88
4.1	In Vitro Studies	67-80
4.1.1	Characterization of Cultured MSCs by FACS Analysis.....	67
4.1.2	Morphological Features of Cytokines Pretreated MSCs.....	68
4.1.3	Liver specific Genes and Protein Expression.....	69
4.1.4	PAS staining for Glycogen Storage of Pretreated MSCs.....	71
4.1.5	Culturing of Mouse Hepatocytes.....	72
4.1.6	Characterization of cultured Hepatocytes.....	74
4.1.7	Selection of CCl ₄ Dose in Hepatocytes Injury Model.....	76
4.1.8	Cyto-protective Effects of MSCs on Injured Hepatocytes.....	78
4.1.9	Gene Expression of Hepatocytes-MSCs Co-cultured Model.....	79
4.2	In Vivo Studies	81-88
4.2.1	Cytokines Pretreatment Enhanced Homing of MSCs in Liver.....	81
4.2.2	Reduction in Liver Fibrosis after Transplantation of MSCs.....	82
4.2.3	Gene expression analysis after pretreated MSCs transplantation.....	83
4.2.4	Liver Function Improves after Pretreated MSCs Transplantation.....	85
4.2.5	Restoration of Glycogen storage.....	86
4.2.6	Effect of MSCs Transplantation on Apoptosis.....	87
 CHAPTER 5		
5	DISCUSSION	89-96
 CHAPTER 6		
6	REFERENCES	97-121