

1.3.5.1. Phosphatidylcholine	16
1.3.5.2. Phosphatidylglycerol	17
1.3.5.3. Minor phospholipids and neutral lipids	18
1.3.5.4. Lamellar bodies	18
1.3.6. Synthesis, secretion and turnover of surfactant	18
1.3.7. Catabolism of surfactant	21
1.3.8. Pulmonary surfactant in lung disease	21
1.4. The immune system in health and disease—a brief overview	23
1.4.1. Lymphoid organs	25
1.4.2. Spleen	25
1.4.3. Thymus	27
1.4.3.1. Function of the Thymus	27
1.5. Role of fatty acids in immune cells	27
1.6. PL metabolism on spleen and thymus	29
Aim and scope of the study	31

CHAPTER – II

Impact of LPS on lung phospholipid metabolism

Synopsis	32
2.1. Introduction	33
2.2. Materials and Methods	34
2.2.1. Materials	34
2.2.2. Animals	35
2.2.3. Myeloperoxidase (MPO) assay	35
2.2.4. Bronchoalveolar lavage fluid collection and BALF cells analysis	36
2.2.5. Protein concentration of BALF (PCBALF)	36
2.2.6. Determination of serum marker enzymes for organ function	36
2.2.7. Biochemical parameters	37
2.2.7.1. Determination of lipid peroxidation	37
2.2.7.2. Estimation of reduced glutathione (GSH)	38
2.2.7.3. Estimation of ascorbic acid	38
2.2.8. Quantitative analysis of antioxidant enzyme activities	39

2.2.8.1. Superoxide dismutase (SOD: E.C. 1.15.1.1) assay	39
2.2.8.2. Catalase (CAT: 1.11.1.6) assay	39
2.2.8.3. Glutathione peroxidase (GPx: EC 1.11.1.9) assay	40
2.2.9. Histopathological examination	41
2.2.10. Lipid extraction and separation by thin layer chromatography	41
2.2.10.1. Phospholipid determination by phosphorus assay	42
2.2.10.2. Perfusion of lung	42
2.2.10.3. Isolation and purification of alveolar type II cells	43
2.2.10.4. Isolation of alveolar type-II cells	43
2.2.10.5. Purification of alveolar type- II cells	44
2.2.10.6. Trypan Blue Staining	44
2.2.10.7. Papanicolaou staining	45
2.2.11. <i>In vitro</i> [³² P]orthophosphate labelling in lung tissue	45
2.2.11.1. <i>In vitro</i> [³² P] labelling of lung tissue pre-incubated with LPS	46
2.2.11.2. <i>In vitro</i> [³² P]orthophosphate post-labelling of lung tissue	46
2.2.12. Metabolic labeling of alveolar type II cells	46
2.2.13. Lipid extraction and separation by thin layer chromatography	47
2.2.14. Fatty acid analysis	47
2.2.15. Extraction of total RNA and cDNA synthesis	48
2.2.15.1. Reverse Transcriptase-Polymerase Chain Reaction	49
2.2.16. Statistical analysis	49
2.3 Results	50
2.3.1. <i>In vitro</i> metabolic labelling of lung phospholipids by [³² P]orthophosphate.	50
2.3.2. Labelling of phospholipids along with LPS	50
2.3.3. Pre-incubation of tissue with compound before phospholipid labelling	52
2.3.4. Post-incubation of LPS with labelled lung phospholipids	54
2.3.5. Isolation and purity characterization of AEC2 cells.	54
2.3.6. Metabolic labelling of AEC2 phospholipid in the presence of LPS	55
2.3.7. Effect of LPS on phospholipid molecular species	57
2.3.8. LPS administration leads to lung neutrophil sequestration	59

2.3.9.	Effect of LPS on BALF neutrophil content and protein concentration of BALF	59
2.3.10.	LPS exposure increases the serum marker enzymes and depicted multiple organ failure	59
2.3.11.	Levels of lipid peroxidation and non-enzymatic antioxidants	61
2.3.12.	Effect of enzymatic antioxidants SOD, CAT and GPx	61
2.3.13.	Lung histology of LPS induced ARDS rats	62
2.3.14.	LPS administration impairs lung surfactant phospholipids	63
2.3.15.	Influence of LPS on lung phospholipid molecular species alteration	64
2.3.16.	Expression of PL remodeling enzymes	68
2.4.	Discussion	71
2.4.1.	<i>In vitro</i> metabolic labelling of whole lung and AEC2 phospholipids with LPS shows alteration in major surfactant PL	71
2.4.2.	Effect of LPS on Lung fatty acid composition	73
2.4.3.	LPS administration leads to lung neutrophil sequestration	74
2.4.4.	Protein concentration of BALF confirms vascular leakage	74
2.4.5.	Elevated serum marker enzymes depicted multiple organ failure	75
2.4.6.	LPS induced oxidative stress	75
2.4.7.	LPS exposure leads to deficiency in lung antioxidants	76
2.4.8.	LPS impairs surfactant phospholipid metabolism	77
2.4.9.	Impact of LPS on fatty acid composition of lung phospholipids	78
2.4.10.	Impact of LPS on the gene expression of PL remodeling enzymes	80

CHAPTER – III

Role of phospholipids, fatty acid changes and immune impairment in spleen and thymus during LPS endotoxemia

	Synopsis	83
3.1.	Introduction	84
3.2.	Materials and Methods	85
3.2.1.	Material	85
3.2.2.	Animals	85
3.2.3.	In vitro metabolic labelling of spleen and thymus tissues with LPS	85
3.2.4.	In vivo model of ALI/ARDS	85