### TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Contents</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title page</td>
<td>(i)</td>
</tr>
<tr>
<td>Certificate/s (Supervisor)</td>
<td>(ii)</td>
</tr>
<tr>
<td>Certificate/s (Co-supervisor)</td>
<td>(iii)</td>
</tr>
<tr>
<td>Declaration</td>
<td>(iv)</td>
</tr>
<tr>
<td>Copyright Transfer Certificate</td>
<td>(v)</td>
</tr>
<tr>
<td>Acknowledgement</td>
<td>(vi)-(vii)</td>
</tr>
<tr>
<td>List of Tables</td>
<td>(viii)</td>
</tr>
<tr>
<td>List of Figures</td>
<td>(ix)-(xi)</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>(xii)-(xiii)</td>
</tr>
<tr>
<td>Abstract</td>
<td>(xiv)-(xvii)</td>
</tr>
</tbody>
</table>

**Chapter 1: Introduction and Review of Literature**

1.1 Synthetic pyrethroids: Deltamethrin 1

1.2 Deltamethrin exposure 3

1.3 Deltamethrin: Toxic effects in non targets 4

1.3.1 Acute toxicity 4

1.3.2 Chronic toxicity 5

1.3.3 Reproductive effects 6

1.3.4 Carcinogenic effects 7

1.3.5 Organ toxicity 7
1.3.6 Hepatotoxicity 8
1.3.7 Neurotoxicity 8
1.3.8 Immunotoxicity 9
1.4 Ecological effects 9
1.5 Biological effects 10
1.5.1 Effect of Deltamethrin on body’s enzymatic pool 10
1.5.2 Oxidative stress and inflammation associated with deltamethrin 11
1.5.3 Endocrine effects 13
1.6 Biomarkers in Toxicology 14
1.7 Challenges in ascertaining biomarkers of Toxicology 16
1.8 Omics in toxicology 17
1.9 Xenobiotics induced hepatotoxicity: need of more colloquial markers 20

Chapter 2: Effect of Deltamethrin causes hepatotoxicity and immune dysregulation in Wistar rats upon oral exposure 23

2.1 Introduction 24
2.2 Material and Methods 24
2.2.1 Reagents 25
2.2.2 Rodent model, maintenance and treatment schedule 25
2.2.3 Body Weight Gain (%) 25
2.2.4 Determination of reactive oxygen species (ROS) 26
2.2.5 Oxidative stress parameters 26
2.2.6 Histopathogical assessment 26
2.2.7 Liver functional test and hematological analysis 27
2.2.8 Western blotting

2.2.9 Splenocyte culture

2.2.10 Lymphoproliferation assay

2.2.11 Statistical analysis

2.3 Results

2.3.1 Effects of DLM on body weight and relative organ weights

2.3.2 Effect of DLM on liver function and hematology

2.3.3 Effect of DLM on liver histology

2.3.4 DLM induces oxidative stress and antioxidant defense

2.3.5 DLM exposure induced inflammation in rat liver

2.3.6 Immunosuppressive potential of DLM

2.4 Discussion

2.5 Conclusion

Chapter 3: Proteomic identification of plasma signatures to delineate early responsive markers against deltamethrin intoxication in Wistar rats

3.1 Introduction

3.2 Material and Methods

3.2.1 Reagents

3.2.2 Rodent model, maintenance and treatment schedule

3.2.3 Xenobiotic metabolism (UPLC-MS/MS analysis)

3.2.4 2-DE profiling and protein identification by mass spectrometry

3.2.5 Protein staining, image acquisition and data analysis

3.2.6 Trypsin digestion and protein identification by mass spectrometry

3.3.7 Construction and analysis of the network of identified proteins
### 3.3 Results

#### 3.3.1 Pharmacokinetics study of DLM and its metabolite

#### 3.3.2 2-DE profiling and Mass spectroscopic (MS) identification of modulated proteins in DLM treated groups

### 3.4 Discussion

### 3.5 Conclusion

---

**Chapter 4: Evaluation and physiological correlation of plasma proteomic fingerprints for Deltamethrin-induced hepatotoxicity in Wistar rats**

#### 4.1 Introduction

#### 4.2 Material and Methods

##### 4.2.1 Reagents

##### 4.2.2 Rodent model, maintenance and treatment schedule

##### 4.2.3 Body weight gain

##### 4.2.4 Determination of reactive oxygen species (ROS)

##### 4.2.5 Oxidative stress parameters

##### 4.2.6 Liver functional test and serum analysis

##### 4.2.7 Histopathogical assessment

##### 4.2.8 *Ex-vivo* phagocytosis in peritoneal macrophages

##### 4.2.9 Western blotting

##### 4.2.10 2-DE profiling and protein identification by mass spectrometry

##### 4.2.11 RNA extraction and cDNA synthesis

##### 4.2.12 Quantification of mRNA expression by qRT-PCR

##### 4.2.13 Statistical analysis

#### 4.3 Results

##### 4.3.1 NAC supplementation restored the loss of body weight and relative organ weight

##### 4.3.2 Effect of NAC on hepatic functions and biochemical parameters
4.3.3 NAC supplementation also suppressed DLM induced inflammation response

4.3.4 The preventive effect of NAC on phagocytic activity of macrophages

4.3.5 Supplementation of NAC restored DLM exerted oxidative injury

4.3.6 NAC supplementation restored the expression of differentially regulated proteins in plasma of DLM treated animals

4.4. Discussion

4.5 Conclusion

Chapter 5: Deltamethrin induces caspase-independent cell death in liver cells

5.1 Introduction

5.2 Material and Methods

5.2.1 Reagents

5.2.2 Primary hepatocytes isolation, culture and In vivo treatment

5.2.3 HepG2 Cell culture

5.2.4 Cell viability assay

5.2.5 Measurement of intracellular reactive oxygen species (ROS) and Mitochondrial membrane potential (MMP)

5.2.6 Western blotting

5.2.7 Immunocytochemical quantifications

5.2.8 TUNEL and Annexin V binding assay

5.2.9 Caspase-3 activity assay

5.2.10 Cell cycle analysis

5.3 Results

5.3.1 Labeling of rat primary hepatocytes with Cytokeratin 19

5.3.2 DLM induces dose dependent cell death in rat primary hepatocytes

5.3.3 DLM exposure resulted in G2/M arrest in primary hepatocytes
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.3.4</td>
<td>DLM-induced cell death in primary hepatocytes was accompanied with ROS accumulation</td>
<td>94</td>
</tr>
<tr>
<td>5.3.5</td>
<td>DLM induces loss of mitochondrial membrane potential (MMP)</td>
<td>97</td>
</tr>
<tr>
<td>5.3.6</td>
<td>DLM-induced non-apoptotic cell death in primary hepatocytes</td>
<td>98</td>
</tr>
<tr>
<td>5.3.7</td>
<td>DLM treatment resulted in Caspase-3 independent cell death in primary hepatocytes</td>
<td>102</td>
</tr>
<tr>
<td>5.3.8</td>
<td>DLM induces necrotic damage and inflammatory response in primary hepatocytes</td>
<td>104</td>
</tr>
<tr>
<td>5.3.9</td>
<td>DLM-exposure alters the expression of TNF-α and Receptor interacting protein kinase (RIPK1, RIPK3)</td>
<td>107</td>
</tr>
<tr>
<td>5.3.10</td>
<td>Pharmacological inhibition of programmed necrosis resulted in protection of primary hepatocytes from DLM toxicity</td>
<td>109</td>
</tr>
<tr>
<td>5.3.11</td>
<td>DLM induces programmed necrosis in human hepatocellular carcinoma (HepG2) cells</td>
<td>110</td>
</tr>
<tr>
<td>5.4</td>
<td>Discussion</td>
<td>113</td>
</tr>
<tr>
<td>5.5</td>
<td>Conclusion</td>
<td>118</td>
</tr>
</tbody>
</table>

**Chapter 6: Assessment of endocrine disrupting potential of deltamethrin and its prime metabolite 3-phenoxy benzoic acid**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>Introduction</td>
<td>119</td>
</tr>
<tr>
<td>6.2</td>
<td>Material and Methods</td>
<td>120</td>
</tr>
<tr>
<td>6.2.1</td>
<td>Chemicals and reagents</td>
<td>120</td>
</tr>
<tr>
<td>6.2.2</td>
<td>Cell culture</td>
<td>120</td>
</tr>
<tr>
<td>6.2.3</td>
<td>Luciferase assay</td>
<td>121</td>
</tr>
<tr>
<td>6.2.4</td>
<td>Cell proliferation</td>
<td>122</td>
</tr>
<tr>
<td>6.2.5</td>
<td>Clonogenic assay</td>
<td>122</td>
</tr>
<tr>
<td>6.2.6</td>
<td><em>In Vitro</em> Wound Healing Assay</td>
<td>123</td>
</tr>
<tr>
<td>6.2.7</td>
<td>Western blotting</td>
<td>123</td>
</tr>
<tr>
<td>6.2.8</td>
<td>Quantification of pS2 mRNA expression by qRT-PCR</td>
<td>123</td>
</tr>
<tr>
<td>6.2.9</td>
<td>Receptor binding validation</td>
<td>124</td>
</tr>
</tbody>
</table>
6.2.10 Statistical analysis 124

6.3 Results 124

6.3.1 T47D-KBluc estrogen receptor transcriptional activation (ERTA) assay 124

6.3.2 Effect of DLM and 3-PBA on viability of MCF-7 and MDA-MB-231 cells 125

6.3.3 Effect of DLM and 3-PBA on clonogenicity of MCF-7 127

6.3.4 Effect of DLM and 3-PBA on wound healing (migration) potential of MCF-7 cells 128

6.3.5 DLM and 3PBA enhances the ER-α receptor expression and increase level of proliferation marker 130

6.3.6 DLM and 3-PBA enhances the pS2 mRNA expression 132

6.3.7 DLM has strong estrogenic potential over 3-PBA 133

6.4 Discussion 134

6.5 Conclusion 135

Chapter 7: Summary and conclusion 136-142

Bibliography 143-172

Appendices 173-184

List of publications 185-186