## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure Number</th>
<th>Caption</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Schematic representation of different lifestyle elements leading to cardiovascular risk factors that ultimately results in CVD and chronic heart diseases (CHDs) including stroke, heart failure etc.</td>
<td>5</td>
</tr>
<tr>
<td>1.2</td>
<td>Pathological and physiological hypertrophy with possible causing factors and significant associated characteristics.</td>
<td>7</td>
</tr>
<tr>
<td>1.3</td>
<td>Different non cardiac mechanisms contributing in heart failure</td>
<td>9</td>
</tr>
<tr>
<td>1.4</td>
<td>Events associated with the toxicity of drugs.</td>
<td>11</td>
</tr>
<tr>
<td>1.5</td>
<td>Series of events taking place inside a cell upon drug induced oxidative stress generation and ultimately leading to programmed cell death.</td>
<td>18</td>
</tr>
<tr>
<td>1.6</td>
<td>Different mechanisms for mitochondria mediated drug-induced cardiotoxicity.</td>
<td>21</td>
</tr>
<tr>
<td>1.7</td>
<td>Structure of Norepinephrine</td>
<td>22</td>
</tr>
<tr>
<td>1.8</td>
<td>Structure of Doxorubicin</td>
<td>23</td>
</tr>
<tr>
<td>1.9</td>
<td>Doxorubicin induced activation of different pathways leading to cellular death by necrosis, apoptosis or autophagy and resulting in cardiomyopathy</td>
<td>24</td>
</tr>
<tr>
<td>1.10</td>
<td>Curcumin tautomers present at different pH of solvents. Keto form predominates in acidic and neutral solvents whereas, enol form is present majorly in alkaline solutions.</td>
<td>27</td>
</tr>
<tr>
<td>2.1</td>
<td>Schematic representation of methodology adopted for molecular docking</td>
<td>44</td>
</tr>
<tr>
<td>3.1</td>
<td>NE induced concentration and time dependent cell death</td>
<td>57</td>
</tr>
<tr>
<td>3.2</td>
<td>NE concentration induced morphological alterations in cardiomyoblasts</td>
<td>58</td>
</tr>
<tr>
<td>3.3</td>
<td>Quantitative growth inhibition analysis upon NE treatment</td>
<td>58</td>
</tr>
<tr>
<td>3.4</td>
<td>Alterations in cellular size analysis upon NE treatment</td>
<td>59</td>
</tr>
<tr>
<td>3.5</td>
<td>Cellular death characterization by TUNEL assay</td>
<td>59</td>
</tr>
</tbody>
</table>
3.6 Quantitative analysis of apoptotic damage by flow cytometer analysis using Annexin/PI stains

3.7 Graphical representation of Annexin-V/PI staining by flow cytometry data

3.8 MTT assay for lower and higher concentration ranges of NE

3.9 Trypan blue staining with derived hypertrophic, transition and apoptotic doses of NE

3.10 TUNEL assay for detection of apoptotic cell death in cardiomyoblasts upon NE treatment

3.11 NE induced morphological alterations in cardiomyoblasts at different concentrations

3.12 Haematoxylin-Eosin staining for altered cellular morphology upon NE treatment

3.13 Nuclear staining using DAPI stain in NE treated cells

3.14 Nuclear staining using PI stain in NE treated cells

3.15 Expression of marker genes for hypertrophy and cell death upon NE treatment

3.16 Rhodamine 123 staining of cardiac cells for alterations in mitochondrial membrane potential upon NE treatment

3.17 Intracellular ROS staining by DCFH-DA staining of H9C2 Cells

3.18 Caspase 8 and 9 staining of NE treated H9C2 cells

3.19 Analysis of ECM remodeling by Verhoeff van Gieson stain

3.20 Analysis of ECM remodeling by Picrosirius stain

3.21 Effect of Propranolol on NE induced cells to validate beta adrenergic receptor dependent activation of cell death

3.22 Dose optimization of Curcumin for NE induced cardiotoxicity at different doses

3.23 Confirmation of cell viability of Curcumin optimized doses with different NE treated groups in finalized experimental sets
3.24 Cellular and nuclear alterations upon Curcumin treatment in NE induced cells

3.25 Effect of Curcumin on collagen content in NE treated cells

3.26 Expression of Hypertrophic and death markers upon Curcumin treatment

4.1 Concentration mediated deleterious effects of Doxorubicin by MTT assay

4.2 Trypan blue dye exclusion assay for increasing Doxorubicin concentrations

4.3 Concentration dependent Doxorubicin uptake FACS-Calibur

4.4 MTT cell viability assay increasing concentrations of Curcumin

4.5 Concentration and mode dependent effects of Doxorubicin and Curcumin on cardiomyoblasts

4.6 Trypan blue staining upon Doxorubicin and Curcumin treatment in finalized experimental set

4.7 Cellular proliferation and morphological alterations in presence of Curcumin

4.8 Cell-cycle analysis by sub-G1 inhibition upon different modes of Curcumin treatment

4.9 Graphical representation of number of cells present in G1/S/G2/M phases upon different time points of Curcumin treatment

4.10 NBT assay for cellular superoxides generation

4.11 Intracellular ROS production by DCFH-DA assay

4.12 SOD anti-oxidant enzymatic activity upon Doxorubicin and Curcumin treatment

4.13 Catalase anti-oxidant enzymatic activity upon Doxorubicin and Curcumin treatment

4.14 Rhodamine 123 staining for mitochondrial membrane permeability analysis

4.15 Effect of Curcumin treatment on the activity of different caspases

xx
4.16 Reverse Transcriptase PCR analysis of SOD, Catalase, pro and anti-apoptotic genes 110
4.17 MTT cell viability assay for deriving anti-cancer concentrations of Doxorubicin and Curcumin on MCF-7 human breast cancer cell line 111
4.18 Effect of selected anti-cancer concentrations on cardiomyocytes 112
4.19 Synergistic effects of Curcumin and Doxorubicin on cancer cells and cardiomyocytes 113
4.20 Cellular proliferation and morphological alterations in cancer cell lines 114
4.21 Analysis of intra-cellular ROS generation by NBT and DCFH-DA assay. 115
4.22 TUNEL assay for % dead cells upon different combination treatments of Doxorubicin and Curcumin 116
4.23 Annexin/PI staining for Curcumin mediated synergistic apoptosis by FACS-Calibur 117
4.24 Activity of different initiator and effector caspases for different combination treatments 118
4.25 Quantitative real time PCR for the mRNA expression of TNF-α, Bcl₂ and Bax genes 119
5.1 Schematic representation of different interlinked pathways regulating apoptosis in cardiac myocytes 128
5.2 Graphical representation of binding energies (kcal/mol) obtained by molecular docking using AutoDock Vina for 1ˢᵗ active site 132
5.3 Graphical representation of binding energies (kcal/mol) obtained by molecular docking using AutoDock Vina for 2ⁿᵈ active site 133
5.4 Representative picture of protein-ligand interactions of Caspase 2 with Curcumin, Doxorubicin and NE respectively as viewed in Pymol software for two selected active sites 134
6.1 Effect of mode dependent Curcumin effects on apoptotic and inflammatory marker expression in Doxorubicin induced cardiomyoblasts 142

xxi
6.2 Effect of Curcumin on protein expression analysis of apoptotic and hypertrophic marker in drug induced cardiotoxicity and Curcumin treatment

6.3 Expression analysis of Caspase 3 upon Curcumin treatment in NE and Doxorubicin induced cardiomyoblasts by Real-time PCR and SYBER green probe

6.4 Expression analysis of TNF-α upon Curcumin treatment in NE and Doxorubicin induced cardiomyoblasts by Real-time PCR and SYBER green probe

6.5 Expression analysis of SOD upon Curcumin treatment in NE and Doxorubicin induced cardiomyoblasts by Real-time PCR and SYBER green probe

6.6 Expression analysis of Catalase upon Curcumin treatment in NE and Doxorubicin induced cardiomyoblasts by Real-time PCR and SYBER green probe

6.7 Expression of NFκB transcription factor by Immunocytochemistry in Doxorubicin induced cardiotoxicity

6.8 Effect of Curcumin on heart size in NE and Doxorubicin induced SD rats

6.9 Gene expression analysis by western blotting for biomarker of inflammation, oxidative stress and anti-apoptosis

6.10 Expression analysis of Caspase 3 upon Curcumin treatment in NE and Doxorubicin induced SD rats by Real-time PCR and SYBER green probe

6.11 Expression analysis of Catalase upon Curcumin treatment in NE and Doxorubicin induced SD rats by Real-time PCR and SYBER green probe

6.12 Expression analysis of TNF-α upon Curcumin treatment in NE and Doxorubicin induced SD rats by Real-time PCR and SYBER green probe
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Figure Number</th>
<th>Caption</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Different classes of drugs with reported cardiotoxicity and their cardiovascular implications in brief.</td>
<td>13</td>
</tr>
<tr>
<td>2.1</td>
<td>List of Primers used for semi-quantitative and Real-time PCR</td>
<td>39</td>
</tr>
<tr>
<td>5.1</td>
<td>List of signaling molecules reported in cardiac stress</td>
<td>129</td>
</tr>
<tr>
<td>5.2</td>
<td>Binding energy (kcal/mol) derived by AutoDock Vina for Doxorubicin, Curcumin and NE with the cardiac stress signaling molecules</td>
<td>131</td>
</tr>
<tr>
<td>6.1</td>
<td>Effect of Curcumin on body weight, heart weight and mortality of SD rats treated with Doxorubicin and NE</td>
<td>149</td>
</tr>
<tr>
<td>6.2</td>
<td>Effect of Curcumin on serum biomarkers for biochemical and oxidative stress</td>
<td>151</td>
</tr>
</tbody>
</table>
THESIS STRUCTURE

The thesis is structured as follows:

**Chapter 1** - Introduction, Review of literature, Aim & Objectives

**Chapter 2** - Material and Methods

**Chapter 3** – Effects of Curcumin against Levophed/ Norepinephrine induced cardiotoxicity.

**Chapter 4** – Effects of Curcumin against Doxorubicin induced cardiotoxicity.

**Chapter 5** – To analyze possible mechanisms involved in Curcumin mediated effects by *in silico* Molecular Docking.

**Chapter 6** – Transcriptomic and proteomic analysis of Curcumin mediated effects.

**Conclusion and Future perspectives**