6.1. Overview of the chapter

Cardio-protective role of Curcumin in drug induced cardiotoxicity was derived in vitro in previous chapters. Possible signaling molecules involved in Curcumin mediated effects were derived and analyzed by in silico analysis. These findings were further validated in the present chapter at transcriptomic and proteomic level in vitro and further extended in vivo for deriving possible signaling involved in Curcumin protective effects in NE and Doxorubicin induced cardiotoxicity. Molecular signaling involved in Curcumin mediated effects was derived by gene expression analysis by analyzing different biomarkers for cell death and survival using real-time PCR, western blotting and immunocytochemistry.

This chapter is structured as:

6.2. Introduction
6.3. Experimental design
   6.3.1. Expression studies in vitro
   6.3.2. Expression studies in vivo
6.4. Results
6.5. Discussion
6.6. Conclusion
6.2. Introduction

In our previous in vitro studies by various biochemical assays, we obtained convincing results for the possible cardio-protective role of Curcumin against Doxorubicin and NE induced stress in cardiomyoblasts. We have also analyzed the binding energies of Curcumin with potential signaling molecules reported in cardiac stress that might be involved in mediating its protective effects. To validate these findings, it is very important to study these Curcumin effects at molecular level as well as in vivo models where the effect of different parameters of biological entities will be analyzed on whole organism. The findings will give a clear understanding of drug mediated cardiac side-effects as well a protective effects of Curcumin in different drug induced states.

Gene expression analysis at proteomic and transcriptomic level aids in understanding the exact modulations at molecular level and comprehend mechanisms involved in normal biological and disease processes [274]. In the present chapter, gene expression analysis was done for cardiomyocytes induced with drug stress and treated with Curcumin alone, and in combinations. Different biomarkers for cardiac stress, oxidative stress, cardiac hypertrophy, inflammation, and apoptosis were studied by western blotting and real-time PCR. The findings were then further validated in animal models.

For designing efficient and relatable in vivo studies, it is very important to choose appropriate animal model system as well as the right dosage of compounds to be tested. As NE, Doxorubicin and Curcumin are well studied molecules in different horizons of research, literature for their in vivo doses was amply available. Thorough literature search was done to derive the doses to best fit our study design [275-280]. Also, SD rats have been studied well for spontaneous cardiomyopathy and refered as an ideal model system for cardiac stress studies. It has been shown to have comparable cardiac-specific serum troponin levels as reported in pathological cardiomyopathy [37].

The outcomes of the present chapter facilitated us to understand multiple level of gene regulation and specific signaling mechanisms involved in Curcumin mediated protective effects in drug induced cardiac stress. The findings also helped in establishing Curcumin as a potential candidate
compound to be used in therapeutics for preventing or reducing cardiotoxicity of different classes of drugs without compromising their actual drug effects.

6.3. Experimental design

The gene expression studies done in the present chapter were conducted in two phases:

6.3.1. Expression studies in vitro

Following experimental sets were used:

I) UT control cells  
II) 2.5 µM NE (Hypertrophy)  
III) 50 µM NE (Transition)  
IV) 15 µM Doxorubicin  
V) 50 µM NE + 15 µM Curcumin  
VI) 2.5 µM NE + 10 µM Curcumin  
VII) Curcumin + Doxorubicin (Parallel)  
VIII) Curcumin + Doxorubicin (Pre)  
IX) 15 µM Curcumin

Set I, II, III, V, VI and VII were incubated for 48 hours. Set IV and VII were incubated for 24 hours. Set VIII was incubated for 24 hours with Curcumin followed by 24 hours with doxorubicin without media change.

6.3.2. Expression studies in vivo

All the in vivo experiments were conducted at NIB under the supervision of Dr. Shikha Yadav and approved by IAEC ethical approval committee (Appendix A). Following experimental sets were used:

I) 30 mg/kg body weight Doxorubicin administered for 15 days alternatively  
II) 200 mg/kg body weight Curcumin for 15 days daily
II) 200 mg/kg body weight Curcumin for 15 days followed by 30 mg/kg body weight Doxorubicin for 15 days alternatively and Curcumin daily for 15 days

IV) 1.5 mg/kg body weight NE and 200 mg/kg body weight Curcumin daily for 15 days

V) 1.5 mg/kg body weight NE daily for 15 days

VI) 15 µM Curcumin control UT: Normal saline

VII) Control vehicle for NE: Ascorbic acid

VIII) Control vehicle for Curcumin: Carboxy methyl cellulose (CMC)

Male SD rats were used and divided into above mentioned sets with six animals in each set, weighing approximately 200-220 gms each. Doses for NE and Doxorubicin was given intra-peritoneally, Curcumin was administered by oral gavage.

6.4. Results

Caspase expression was checked by western blotting in drug induced cardiomyoblasts. Expression of caspase 3 was increased by 2-folds in parallel treated sets and as compared to pre-treated sets. And, expression of active caspase 9 was found to be decreased significantly in Curcumin pre-treated sets as compared to Doxorubicin and parallel treatment sets. Also, the expression of pro-inflammatory marker, TNF-α, was increased upon Doxorubicin treatment and decreased significantly Curcumin pre-treated sets (Fig. 6.1). Expression analysis of caspase 2 and hypertrophic marker- β-MHC was then studied in cardiomyoblasts treated with NE, Doxorubicin and Curcumin alone and in combinations. β-MHC expression was decreased by 3.5 folds upon Curcumin treatment at hypertrophic dose of NE and interestingly, its expression increased significantly at NE transition dose. Expression of caspase 2 was also reduced significantly in Curcumin treated sets (Fig. 6.2).

Expression analysis upon Curcumin treatment in NE and Doxorubicin induced cardiomyoblasts was studied by Real-time PCR and SYBER green probe for caspase 3, TNF-α, SOD and catalase genes. qPCR results obtained were normalized against β-actin and relative expression was calculated by ΔΔCt method. Transcriptomic expression pattern for caspase 3 and TNF-α further validated the above proteomic levels (Fig. 6.3-6.4). Also, the expression pattern of SOD and
catalases confirmed our previous findings of these enzyme overexpression in presence of Curcumin treatment (Fig. 6.5-6.6). Immunocytochemistry was done to analyze the expression of NFκB and Doxorubicin induced up-regulation of NFκB was found to be significantly decreased upon pre-treatment with Curcumin (Fig. 6.7). The results further validated Curcumin mediated cardio-protective effects in NE and Doxorubicin induced cardiomyoblasts.

Next, the cardio-protective effects of Curcumin were studied in drug induced in vivo models to further validate our findings. These effects were studied in Doxorubicin and NE induced SD rats. Cardiotoxicity was induced with the selected doses from literature and body mass index studies of animals treated with these drugs were done carefully. Body weight was recorded on the daily basis.

We observed significant reduction in overall body weight of Doxorubicin treated rats, and inverse effect was observed in NE treated rats as compared to their respective control groups. These effects were found to be restricted in Curcumin treated rats (Table 6.1). Heart weight and size was recorded for the animals at the time of sacrifice and significant reduction in the heart weight of Doxorubicin treated rats was observed and NE treated heart size was increased significantly representing NE mediated characteristic hypertrophy (Fig. 6.8).

Effect of Curcumin treatment on the concentration of serum biomarkers for cell death, cardiac stress, and oxidative stress were analyzed from the blood collected from heart by terminal puncture. The results further confirmed that Curcumin treatment restricts the biochemical and oxidative stress by modulating the levels of LDH, MDA and catalases (Table 6.2).

Further, protein levels of TNF-α, catalase and Bcl2, biomarkers for inflammation, oxidative stress and anti-apoptosis were studied by western blotting. Curcumin treatment significantly reduced NE and Doxorubicin induced levels of TNF-α by 1.5 and 2 folds respectively. Increased levels of catalase and anti-apoptotic biomarker Bcl2 was witnessed upon Curcumin treatment. These observations confirmed the cardio-protective effects of Curcumin in vivo (Fig. 6.9). This was further validated at transcriptomic level by real-time PCR for the gene expression of caspase 3, catalase and TNF-α. Significant up-regulation of caspase 3 and catalase activity in Curcumin treated rats and down-regulation of TNF-α further validated Curcumin mediated significant reduction in drug mediated toxic effects by altering cell death and survival pathways (Fig. 6.10-6.12).
Fig. 6.1. Effect of mode dependent Curcumin effects on apoptotic and inflammatory marker expression in Doxorubicin induced cardiomyoblasts. Expression of Caspase 3, caspase 9 and TNF-α was analyzed by western blotting using specific antibodies. The band intensity was quantified using NIH ImageJ software and plotted. One-way ANOVA and student’s t-test were done to evaluate the significance of differences in the data obtained (*P<0.05).
Fig. 6.2. Effect of Curcumin on protein expression analysis of apoptotic and hypertrophic marker in drug induced cardiotoxicity and Curcumin treatment. Western blotting for Caspase 2 and β-MHC was done using specific antibodies and band intensities were quantified using NIH ImageJ software and plotted. Experimental sets are as follows: A: Untreated Control Cells, B: 2.5 µM NE (Hypertrophy); C: 50 µM NE (Transition); D: 15 µM Doxorubicin, E: 50 µM NE + 15 µM Curcumin, F: 2.5 µM NE+ 10 µM Curcumin, G: Curcumin Doxorubicin (Parallel), H: Curcumin+Doxorubicin (Pre), I: 15 µM Curcumin. One-way ANOVA and student’s t-test were done to evaluate the significance of differences in the data obtained (*P<0.05).
Fig. 6.3. Expression analysis of Caspase 3 upon Curcumin treatment in NE and Doxorubicin induced cardiomyoblasts by Real-time PCR and SYBER green probe. qPCR results obtained were normalized against β-actin and relative expression was calculated by ΔΔCt method and plotted as a histogram. Two-way ANOVA and student’s t-test were done to evaluate the significance of differences in the data obtained (*P<0.05).
Fig. 6.4. Expression analysis of TNF-α upon Curcumin treatment in NE and Doxorubicin induced cardiomyoblasts by Real-time PCR and SYBER green probe. qPCR results obtained were normalized against β-actin and relative expression was calculated by ΔΔCt method and plotted as a histogram. Two-way ANOVA and student’s t-test were done to evaluate the significance of differences in the data obtained (*P<0.05).
Fig. 6.5. Expression analysis of SOD upon Curcumin treatment in NE and Doxorubicin induced cardiomyoblasts by Real-time PCR and SYBER green probe. qPCR results obtained were normalized against β-actin and relative expression was calculated by ΔΔCt method and plotted as a histogram. Two-way ANOVA and student’s t-test were done to evaluate the significance of differences in the data obtained (*P<0.05).
Fig. 6.6. Expression analysis of Catalase upon Curcumin treatment in NE and Doxorubicin induced cardiomyoblasts by Real-time PCR and SYBER green probe. qPCR results obtained were normalized against β-actin and relative expression was calculated by ΔΔCt method and plotted as a histogram. Two-way ANOVA and student’s t-test were done to evaluate the significance of differences in the data obtained (*P<0.05).
Fig. 6.7. Expression of NFκB transcription factor by Immunocytochemistry in Doxorubicin induced cardiotoxicity: Anti-NFκB p65 primary antibody and FITC conjugated secondary antibody were used and images were captured at 100X magnification. Scale bar corresponds to 10 µm.
Table 6.1. Effect of Curcumin on body weight, heart weight and mortality of SD rats treated with Doxorubicin and NE. One-way ANOVA and student’s t-test were done to evaluate the significance of differences in the data obtained (*P<0.05)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Experimental Group</th>
<th>Body weight (gms)</th>
<th>Heart weight (gms)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control- UT</td>
<td>247.2±1.5</td>
<td>296.2±2.5</td>
<td>0.65±0.01</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle- NE</td>
<td>234±3.2</td>
<td>276.6±2.9</td>
<td>0.63±0.04</td>
</tr>
<tr>
<td>3</td>
<td>Vehicle- Curcumin</td>
<td>240.3±2.5</td>
<td>302.5±1.6</td>
<td>0.67±0.07</td>
</tr>
<tr>
<td>4</td>
<td>Curcumin alone</td>
<td>238.8±2.7</td>
<td>287.4±2.3</td>
<td>0.68±0.12</td>
</tr>
<tr>
<td>5</td>
<td>Pre Curc + Dox</td>
<td>235.3±1.9</td>
<td>263.2±2.2</td>
<td>0.63±0.13</td>
</tr>
<tr>
<td>6</td>
<td>Dox alone</td>
<td>246.4±2.4</td>
<td>235.6±3.1</td>
<td>0.75±0.21</td>
</tr>
<tr>
<td>7</td>
<td>NE alone</td>
<td>242.4±3.1</td>
<td>265.3±1.9</td>
<td>0.84±0.12</td>
</tr>
<tr>
<td>8</td>
<td>NE + Curc</td>
<td>234.7±2.6</td>
<td>260.3±1.3</td>
<td>0.72±0.09</td>
</tr>
</tbody>
</table>
Fig. 6.8. Effect of Curcumin on heart size in NE and Doxorubicin induced SD rats. Images were captured at the day 15 after collecting blood by cardiac puncture under deep anesthesia.
Table 6.2. Effect of Curcumin on serum biomarkers for biochemical and oxidative stress.
One-way ANOVA and student’s t-test were done to evaluate the significance of differences in the data obtained (*P<0.05)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Experimental Group</th>
<th>LDH (nmol/ml)</th>
<th>ROS biomarkers</th>
<th>Catalse (nmol/l/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>MDA</strong></td>
<td><strong>Catalse</strong></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Control- UT</td>
<td>238±19.5</td>
<td>48±1.1</td>
<td>28±2.3</td>
</tr>
<tr>
<td>2.</td>
<td>Vehicle- NE</td>
<td>240±21.2</td>
<td>40±1.4</td>
<td>27±4.5</td>
</tr>
<tr>
<td>3.</td>
<td>Vehicle- Curcumin</td>
<td>222±18.4</td>
<td>38±1.2</td>
<td>22±3.2</td>
</tr>
<tr>
<td>4.</td>
<td>Curcumin alone</td>
<td>225±17.4</td>
<td>45±2</td>
<td>25±6.7</td>
</tr>
<tr>
<td>5.</td>
<td>Pre Curc + Dox</td>
<td>280±22.5</td>
<td>49±1.3</td>
<td>33±7.5</td>
</tr>
<tr>
<td>6.</td>
<td>Dox alone</td>
<td>415±17.3</td>
<td>75±0.8</td>
<td>45±4.3</td>
</tr>
<tr>
<td>7.</td>
<td>NE alone</td>
<td>389±20.2</td>
<td>69±0.9</td>
<td>39±3.4</td>
</tr>
<tr>
<td>8.</td>
<td>NE + Curc</td>
<td>310±12.4</td>
<td>50±1.1</td>
<td>31±5.6</td>
</tr>
</tbody>
</table>
Fig. 6.9. Expression analysis for biomarker of inflammation, oxidative stress and apoptosis. Western blots for TNF-α, catalase and Bcl2 were quantified using NIH ImageJ software and plotted. One-way ANOVA and student’s t-test were done to evaluate the significance of differences in the data obtained (*P<0.05)
Fig. 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. qPCR results obtained were normalized against β-actin and relative expression was calculated by ΔΔCt method and plotted as a histogram. Two-way ANOVA and student’s t-test were done to evaluate the significance of differences in the data obtained (*P<0.05)
Fig. 6.11. **Expression analysis of Catalase upon Curcumin treatment in NE and Doxorubicin induced SD rats by Real-time PCR and SYBER green probe.** qPCR results obtained were normalized against β-actin and relative expression was calculated by ΔΔCt method and plotted as a histogram. Two-way ANOVA and student’s t-test were done to evaluate the significance of differences in the data obtained (*P<0.05).
Fig. 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. qPCR results obtained were normalized against β-actin and relative expression was calculated by ΔΔCt method and plotted as a histogram. Two-way ANOVA and student’s t-test were done to evaluate the significance of differences in the data obtained (*P<0.05).
6.5. Discussion

In the present chapter, gene expression studies were done for validating the cardio-protective effects of Curcumin against selected cardio-toxic drugs. Curcumin mediated modulation of signaling molecules involved in cell survival and cell death were studied by various expression studies, where, gene expression studies were conducted at proteomic and transcriptomic levels to derive the mechanism of Curcumin responses.

Curcumin mediated cardio-protective effects as observed in previous chapters were validated by analyzing the expression of caspase 3 and 9 in Doxorubicin induced cells treated with Curcumin at different time points. Caspases are chief modulators of programmed cell death and their expression decide cell transition to apoptosis. The expression of caspases was reduced significantly upon Curcumin pre-treatment compared to Doxorubicin and parallel treatment sets. The expression of pro-inflammatory marker, TNF-α, was also observed to be down-regulated significantly in Curcumin pre-treated sets (Fig. 6.1). These findings confirmed the Curcumin mediated inhibition of apoptosis in cardiomyoblasts upon Doxorubicin induced cardiac stress. Expression of hypertrophic marker - β-MHC was also studied in cardiomyoblasts treated with Doxorubicin and NE and Curcumin in combination or alone, and it was significantly down-regulated upon Curcumin treatment at hypertrophic dose of NE as well as Doxorubicin induced compensatory stress. Expression of caspase 3 was found to be increased in NE induced transition towards cell death and Curcumin shown to decrease this significantly (Fig. 6.2). These expression studies were further studied at mRNA level for gene expression of different stress biomarkers. Expression Caspase 3 genes were found to be significantly up-regulated in drug induced cardiac cells and Curcumin treatment shown to restrict this increased expression (Fig. 6.3). TNF-α is a well-known cytokine and a marker for inflammatory responses [281]. Curcumin showed significant reduction in drug induced expression of TNF-α as well (Fig. 6.4). SOD and catalase are two-important anti-oxidant enzymes present in cells and genes responsible for their expression were found to be up-regulated significantly upon Curcumin treatment thereby justifying its anti-oxidative effects for preventing cardiac stress induced by NE and Doxorubicin (Fig. 6.5-6.6). NFκB is another important signaling molecule that regulates DNA transcription, cytokine production and cell survival pathways [282]. Doxorubicin has shown to induce NFκB expression in cardiomyoblasts [283]. Doxorubicin induced up-regulation of NFκB was found to be significantly decreased upon pre-treatment with Curcumin.
These observations validate the role of Curcumin in preventing drug-induced cardiotoxicity \textit{in vitro} and further analyzed \textit{in vivo} in the following experiments for validation.

As we observed mode-dependent preventive effects of Curcumin against Doxorubicin-induced cardiac stress only in pre-treatment of Curcumin, experimental set for parallel treatment of Curcumin was not considered for animal studies. However, for NE, simultaneous treatment with Curcumin was done. \textit{In vivo} doses for Doxorubicin, NE and Curcumin were derived from literature as 6 mg/kg body weight (on alternative days for 15 days for making the cumulative dose of 50 mg/kg body weight of Doxorubicin) and 1.5 mg/kg body weight of NE (for 15 days daily) and 200 mg/kg body weight of Curcumin of 15 days respectively. Doxorubicin and Curcumin induced cardiomyopathy were validated on SD rats by inducing the drugs followed by serum enzyme biomarkers for cardiac stress and body mass index analysis. The drugs displayed contrasting effects, where significant decrease in body was observed in Doxorubicin treated rats. In NE induced experimental sets, 20% mortality was witnessed (Table 6.1) Significant increase in heart weight of NE induced rats was observed whereas, it was reduced significantly in Doxorubicin induced animals as compared to all the controls (Fig. 6.8). Increase in heart size of NE treated rats represents cardiac hypertrophy, whereas Doxorubicin induced decrease in heart size and weight represents cardiomyopathy. These effects were significantly restricted in Curcumin treated experimental sets.

To study the effect of Curcumin on Doxorubicin induced cardiomyopathy, rats were treated with Curcumin for two weeks before starting Doxorubicin induction. Also, Curcumin was administered as well in these pre-treated rats along with Doxorubicin. This was done to maintain the Curcumin presence in the biological system, as it gets rapidly eliminated from the body [284]. After treatment, blood was collected by cardiac puncture under deep anesthesia and serum was prepared for studying the enzyme biomarkers for oxidative and cardiac stress. LDH levels are generally studied for early phase detection of suspected ischemic myocardial injuries and its levels were found to be significantly higher in rats treated with Doxorubicin and NE. Also, Curcumin treatment reduced this elevated expression significantly thereby confirming drug-induced cardiotoxicity \textit{in vivo}. Catalase and MDA levels were also studied in blood serum of all the experimental sets as they are critical oxidative stress biomarkers where their expression upon Curcumin treatment was found to be reduced significantly.
Following this, expression studies were done by western blotting and Real-time PCR for apoptotic, pro-apoptotic, oxidative stress and inflammatory markers and the Curcumin mediated effects were validated. Expression of TNF-α, catalase and Bcl2, biomarkers for inflammation, oxidative stress and anti-apoptosis respectively, were studied by western blotting. Significant reduction in TNF-α expression as well as increase in catalase and Bcl2 expression was witnessed in Curcumin treated animals (Fig. 6.9). This was validated by gene expression analysis using real-time PCR for expression of caspase 3, catalase and TNF-α. Curcumin mediated significant reduction in drug mediated toxic effects were observed by altering cell death and survival pathways (Fig. 6.10).

6.6. Conclusion

The findings can be concluded as:

- Curcumin exerts cardio-protective effects in cardiac cells by up-regulating oxidative stress markers and reducing the expression of cell death markers.
- Expression analysis of ROS and cell death markers in both in vitro and in vivo models displayed the promising cardioprotective effect of Curcumin against drug induced cardiotoxicity.
FINAL CONCLUSIONS

Exponential increase in number of cases of CVDs has withdrawn ample attention of clinicians and researchers towards reducing the possible risk factors, where drug induced cardiotoxicity is a major concern. Natural products offers a huge potential for serving as an alternative for improved cardiac health as well as reduced cardiotoxicity. In this regard, present study was designed to analyze the role of Curcumin against drug induced cardiotoxicity induced by Levophed and Doxorubicin. Following are the overall thesis conclusions:

- Levophed or NE and Doxorubicin exert concentration mediated or dose dependent cardiotoxic side effects in cardiomyoblasts and in vivo models.
- Curcumin treatment upon NE induced stress can resist the altered responses of cardiac cells by reducing ROS production and suppressing expression of cellular death markers.
- Curcumin exerts mode-dependent responses in Doxorubicin induced cardiac stress where pre-treatment suppresses the cardiotoxicity and no protective events are witnessed in parallel treatment of Doxorubicin and Curcumin.
- Curcumin mediates its mode dependent activity in pre-treated cells by exaggerating the inbuilt anti-oxidant machinery of cardiomyoblasts to combat the Doxorubicin induced cardiac stress, thereby preventing the induced effects.
- Synergistic cardio-protective and anti-cancerous effects of Curcumin in the combination treatment was studied and the desired anti-cancerous in cancer cells was achieved at much lower concentration of Doxorubicin when administered with Curcumin without compromising the Doxorubicin activity, and hence, reduces the possibility of its dose mediated cardio-toxic effects.
• Doxorubicin displayed more binding energy than Curcumin towards the cardiac stress signaling molecules as compared to the binding energies of NE and Curcumin. This explained the observed cardio-protective effects of Curcumin only in pre-treatment of Doxorubicin and not in parallel treatment in cardiomyoblasts.

• Experiments were conducted on SD rats to validate the cardio-protective effects of Curcumin against drug induced cardiotoxicity. Expression analysis of ROS and cell death markers in both in vitro and in vivo models displayed the promising cardio-protective effect of Curcumin against drug induced cardiotoxicity.

In conclusion, Curcumin holds great potential for developing cardio-oncological therapeutic interventions. Also, the mode of treatment for Curcumin should be critically monitored for achieving the maximum prevention.
FUTURE PERSPECTIVES

Though the findings of our present study displays a significant role of Curcumin in reducing drug induced cardiotoxicity, these finding can still be validated by few more concluding studies.

- Molecular docking studies can further validated by Molecular Dynamic Simulation studies.
- The findings can further be extended to analyze ligand-ligand interactions for Doxorubicin-NE, Doxorubicin-Curcumin and Curcumin-NE. This would aid in understanding the observed mode dependent effects of Curcumin computationally.
- Further, molecular docking of signaling molecules with already bound ligands can be performed with other ligands, that is, docking of Doxorubicin and protein with Curcumin or docking of Curcumin and protein with Doxorubicin for understanding the mode dependent effects.
- Expression of various transcription factors such as GATA-4, AP-1, NFkB etc. can be analyzed by DNA binding assays and indirect immunofluorescence studies to further establishing the cardio-protective role of Curcumin in drug induced cardiotoxicity
- Curcumin has limited pharmacological efficacies due to low aqueous solubility and poor bioavailability. Therefore an efficient targeted delivery method for Curcumin is required to improve its bioavailability and to understand the mechanistic insight into its therapeutic potential as cardio-protective agent in drug induced toxicity.
- Bio-conjugates of two or more natural cardioprotective compounds can be prepared and analyzed for synergistic cardioprotective effects in drug induced cardiotoxicity.