CHAPTER 1

1.1 INTRODUCTION

Cardiovascular anomalies are the leading cause of deaths around the world as reported by World Health Organisation recently, where, cardiototoxicity has become very critical for scientific community in past two decades [1]. Compromised cardiac cells and tissues functioning upon any chemical treatment results in cardiotoxic responses. Cardiac toxicity is a multi-factorial process that ultimately leads to cardiac failure by activating terminal downstream events [2]. In response to the drug on non-target organs, primary defense mechanism elevates free radicals generation within cells considerably, thereby altering the balance of free radicals and inbuilt anti-oxidant machinery. This imbalance further activates other stress responses that ultimately leads to cardiomyocyte death [3]. Drug-induced cardiotoxicity has become an important concern for developing cardiovascular complications because present day non-cardiovascular drugs used for treating broad range of diseases are reported to have cardio-toxic side effects. It is a matter of serious concern as different classes of drugs including Rosiglitazone, Prenylamine, Rofecoxib, Levomethadyl acetate have etc. have been withdrawn from the market as a result of associated severe cardiototoxicity [4-7]. As a result of drug induced cardiotoxicity, patients recover from one disease but develop cardiovascular complications in the longer run [8]. Earlier, many drugs were not screened for associated cardiotoxicity but it is now an essential concern for drug discovery. Clinicians still prescribe many cardio-toxic drugs either because they outweigh the risk of cardiac deformities or due to the non-availability of other safer alternatives.

Natural products supplementation for reducing the cardio-toxic side effects has drawn ample attention as they are associated with least or no toxic effects [9-12]. Supplementing drugs with natural products may increase the therapeutic index of drugs in addition to various protective effects [13-14]. Curcumin is one such natural compound present in nutritionally rich Curcuma longa, also known as turmeric, which is known for its various protective effects including anti-oxidant, anti-inflammatory, anti-cancerous and anti-microbial properties. [15-20]. The cardio-protective effects of Curcumin are also recently reported [21-25].
In the present study, we studied the effects of Curcumin against cardiotoxicity induced by different classes of drugs. Oxidative abnormalities are believed to be a major factor for drug induced cardiac toxicity and Curcumin, being an established anti-oxidative compound, holds a great potential for preventive therapeutics. We selected two different classes of well-known cardio-toxic drugs- Levophed or Noerepinephrine (NE) - an anti-hypotensive drug and Doxorubicin - an anti-cancerous drug. NE is a neurotransmitter and also acknowledged as sympathomimetic amine having α- and β-adrenergic actions for peripheral vasoconstriction and increasing blood flow respectively [26]. Various studies suggest the role of NE in heart related complications and relationship of various pathways to be involved but the exact mechanism involved is poorly defined [27-29]. As the therapeutic use of NE cannot be avoided clinically, interventions for avoiding its toxic cardiac side effects should be established. Anti-cancer drug induced cardiotoxicity is also a major concern at present where most of the cancer therapeutics are associated with current cardiac side-effects [30-31]. Doxorubicin is one such drug that is commonly used as a chemo-therapeutic agent for wide range of cancer including breast, lungs, ovarian, lymphoma, leukemia cancers etc., but its effectiveness reduces significantly because of the associated severe cardio-toxic effects [32-33]. Clinical reports suggest that approximately 11% incidences are reported for anti-cancer drug induced cardiotoxicity every year, out of which Doxorubicin alone accounts for more than 20% cases that further results in dilated cardiomyopathy and heart failure [34]. Hence, there is an urgent need for advancing the anti-cancer treatments and to develop cardio-oncological therapeutic interventions.

The present study was designed to understand the mechanism of NE and Doxorubicin induced cardiotoxicity and to further investigate the effect of natural polyphenol- Curcumin against these drugs induced cardiotoxicity. H9C2 cardiomyocytes were used to establish in vitro drug induced stress. It is an established model system for studying cardiomyopathy and stress related studies because it display similar stress responses as observed in primary cardiomyocytes [35-36]. In silico molecular docking studies were also done to derive the exact mechanism of Curcumin mediated effects in NE and Doxorubicin induced cardiac stress individually. The findings were further validated in vivo using Sprague Dawley (SD) rats as they represent a suitable animal model system for studying spontaneous cardiomyopathy and acute cardiac necrosis associated with drug-induced cardiac muscle injuries [37].
1.1.1 Origin of the proposed work

Drug induced cardiotoxicity has emerged as one very important cause for developing cardiovascular complications. Present day therapies used for treating various life style and chronic diseases are reported to have cardio-toxic side effects. Supplementing cardio-protective natural compounds with existing cardio-toxic therapeutics may have potential to suppress the side effects of chemical based drugs as well as improve the cardiovascular health of the individuals. In this regard, Curcumin, a well-known anti-oxidant, anti-inflammatory and anti-cancerous molecule can be a good choice as it has recently been studied for its potent cardio-protective potential. Hence, it may hold a great potential in preventing drug induced cardiotoxicity. With increasing cardiac patients day by day, there is an urgent need to develop safe and effective therapies with long-term relief and no associated side effects. The synergistic potential of Curcumin may also be beneficial for developing future cardio-oncological therapeutics. The proposed study is therefore designed to investigate the effect of Curcumin against drug induced cardiotoxicity against different classes of well-known cardiotoxic drugs.

1.1.2 The relevance and expected outcomes of the proposed study

Proposed work aims to study the cardio-protective effects of Curcumin, a polyphenol derived from Curcuma longa, against toxicity of two different classes of cardiotoxic drugs: Levophed and Doxorubicin. Studying the mode dependent effects of Curcumin against drug mediated cardiotoxicity would help in developing natural products based cardiovascular therapeutics. The study also aims to analyze the synergistic therapeutic potential of Curcumin for dual efficacy of anti-cancer and cardio-protection effects. This study would contribute to develop natural products based cardio-oncological therapeutic interventions without compromising the actual anti-cancerous drug effects.
1.1.3 Key questions

The key questions of the proposed studies were-

1. Is Curcumin cardio-protective against drug induced cardiotoxicity?

2. Are the effects of Curcumin in drug induced cardiotoxicity dose dependent or mode dependent?

3. Are the Curcumin mediated cardio-protective effects drug specific?

4. What is the mechanism of dual role of Curcumin and “Death vs Survival” responses?

5. What is the possible signalling mechanism involved for Curcumin responses in drug induced cardiotoxicity?
1.2 REVIEW OF LITERATURE

1.2.1. Cardiovascular diseases

Cardiovascular diseases (CVDs) majorly include coronary and rheumatic heart disease, atherosclerosis, cardiomyopathy, ischemic strokes, and other cardiac complications, and have emerged as the leading cause of deaths worldwide. CVDs develops when there is a difficulty in upholding the sufficient cardiac output, and majorly include hypertension and coronary or valvular heart diseases. In recent years, lifestyle diseases have developed dynamically as a result of increasing life expectancy and modern life style and play a central role in increasing CVD occurrence [38]. The World Health Organization has declared CVDs as a global epidemic and also highlighted the role of lifestyle activities in their occurrence as well as prevention, where 75% of cardiovascular mediated deaths can be avoided by adapting proper lifestyle modifications including healthy diet and regular exercise routine [39]. Figure 1.1 illustrates various modifiable and unmodifiable lifestyle factors leading to the cardiovascular risk factors like hypertension, diabetes, obesity etc. If not cured, these factors result in the onset of cardiac myopathy and heart failure.

Fig. 1.1. 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. (Adopted from Kokubo, 2014, Hypertension, 63:655-660 [40])
The modifiable factors, also called cardio-metabolic factors, can be prevented or reverted back by adapting better lifestyle practices and most effective when started at early stages. Apart from these factors, epigenetics also play major role in cardiovascular pathophysiology [41]. Epigenetics in CVD is a new and emerging field that focuses on chromatin-based gene expression regulation without any alterations in the DNA sequences. Epigenetic factors majorly include DNA base modifications, post-translational modifications of histone proteins, and nuclear RNA-based mechanisms [42].

Statistics suggest that developing countries accounts for up to 80% of total CVDs influenced deaths [43-44]. In India, CVD frequency has increased exponentially over last two decades due to aging, increase in population, and a steady age-adjusted CVD mortality rate [45]. India witnessed approximately 62.5 million deaths in 2016 due to age-adjusted CVDs as compared to estimated 12.7 million deaths in the United States. To achieve significant decline in these mortality ratios, steps should be taken at wide-population levels for primary preventive measures as well as, simultaneous expansions in secondary and acute care among CVD patients [46].

Being a leading cause of mortality, cardiovascular research extract huge amount of healthcare expenditure for developing better therapeutic strategies. Various pharmacologic approaches are present to address different CVD risk factors, including antagonists for endo-cannabinoid receptors, peroxisome proliferator-activated receptor (PPAR)-α and γ inhibitors, drugs modulating glucagon-like peptide-1 activity etc. These agents generally target individual CVD risk factors, and clinicians prescribe combination treatments for overall improved cardiovascular health. Hence, there is an urgent requirement for developing single medication having combined effect on all the CVD risk factors that may also help clinicians to reduce the long-term CVD risk of chemical based drugs [47].

1.2.1.a. Cardiac hypertrophy

Cardiomyocytes are terminally differentiated cells that do not proliferate under normal conditions but can respond towards different environmental demands by altering cellular size or dying ultimately in response to intolerable stresses. Cardiac hypertrophy is defined as remodeling or compensatory increase in cardiomyocyte size upon different form of stress responses where the global shape, size and working of heart modifies to accommodate the increased work load due to
stress [48]. It is classified as physiological or pathological when related with normal cardiac function or cardiac dysfunction respectively. Physiological hypertrophy is temporary in nature and occurs in presence of temporary stress or overload including normal body growth in younger age, pregnancy, vigorous exercise or in athletes. On the contrary, pathological hypertrophy is characterized by prolonged or irregular hemodynamic stress as a consequence of hypertension, myocardial infarction or myopathy. This hypertrophy is further accompanied with fibrosis, capillary rarefaction, increased levels of pro-inflammatory cytokines, altered cellular signaling, autophagy suppression, and irregular cardiac and non-cardiac cell interactions leading to detrimental epigenetic variations, cardiac remodeling and heart failure [49]. Figure 1.2 illustrates the different forms of hypertrophy and the associated characteristics in a pictorial form.

**Fig. 1.2.** Pathological and physiological hypertrophy with possible causing factors and significant associated characteristics (Adopted from Heineke and Molkentin, 2006, Nat Rev Mol Cell Biol. 7(8):589-600 [50])
Hypertrophy is predominantly specified by increase in protein synthesis, cellular size, and thickened cardiac chamber walls. If these characteristics sustain in cardiac system for longer duration, they may act as triggering factor for arrhythmia and cardiac failure [51]. Cardiac hypertrophy is shown to be associated with altered histone acetylation where histone acetyltransferases positively regulate hypertrophy and results in overexpression of CREB binding protein and p300 in cardiomyocytes [52]. Cardiac gene expression also gets altered in hypertrophic conditions where activated p38, JNKs and ERKs activate multiple intracellular targets by phosphorylation thereby initiating interlinked signaling involving various transcription factors [53]. Hypertrophy also results in extra cellular matrix (ECM) remodeling thereby causing fibrosis and angiogenesis. Fibrosis results in accumulation of various ECM proteins including collagens, matrix metalloproteinases (MMPs) and their inhibitor proteins (TIMPs), fibronectins etc. in cardiac system [54]. Myocardial angiogenesis results in cardiac remodeling as a consequence of altered signaling between cardiac cells and the vasculature [55]. Cardiac fibroblasts provide mechanical scaffold and produce ECM components, hence responsible for efficient cardiac contractions in normal conditions. However, during hypertrophy, these fibroblasts results in cardiac remodeling by providing growth factors and other ECM components [56]. Free radical mediated modifications also play central role in many hypertrophy aspects where myocardial oxygen uptake increases as well as generation of reactive oxygen species (ROS) takes place [57]. Autophagy is a common process of self-digestion of cells under non favorable circumstances, and required for maintaining cellular homeostasis. However, excessive autophagy responses may also lead to cardiac hypertrophy and targeting cardiac autophagy is an emerging area for research for curing cardiac hypertrophy [58]. Though cardiac hypertrophy is a compensatory response, prolonged hypertrophic responses may serve as a critical factor for developing cardiovascular complications including myocardial infarction and heart failure. Hence, hypertrophy serves as an important therapeutic marker to be targeted to reduce the heart disease progression as well as the occurrence [59].

1.2.1.b. Heart failure

Heart failure is a systemic and multifactorial disease that results from intricate genetic predispositions and other environmental factors. Several mechanisms including structural, cellular, molecular and neuro-humoral get activated upon any form of cardiac injury and act in
synchronization to activate complex coordinated processes for maintaining the proper cardiac functioning. This results in elevated sympathetic activity, circulation re-distribution and volume overload that further leads to develop signs and symptoms that can be detected by various diagnostic tools to get a clear picture of the underlying cause for failing heart [60]. Based on circulatory system, cardiac function, and other pathophysiological factors, heart failure is categorized as: i) Systolic versus diastolic heart failure; ii) Pressure-overload versus volume-overload heart failure; iii) Low-output versus high-output heart failure; and iv) Right-sided versus left-sided (unilateral) heart failure [61-65]. Different clinical manifestations of heart failure include pulmonary (congestion, dyspnea, orthopnea, bendorpnea, paroxysmal nocturnal dyspnea and cardiac asthma), vascular, anemia, gastro-intestinal, cardiac cachexia, renal and cerebral complication individually or in combinations as illustrated in Figure 1.3 [66].

Fig. 1.3. Different non cardiac mechanisms contributing in heart failure: pulmonary arterial hypertension, renal mechanisms, ventricular–vascular mechanisms etc. (Adopted from Sharma et al, Circulation Research 2014,115:79-96 [66])
Different epigenomic studies in heart failure patients have reported that three different genes regulating angiogenesis gets differentially methylated in end stage heart failure and imitate common pathway in cardiac remodeling and vasculature [67]. Apart from epigenetic modifications, ischemic heart disease that occurs as a result of impaired myocardial perfusion and ischemia, vulvular heart diseases, idiopathic and toxin induced cardiomyopathies are the most common cause for heart failure. Literature also suggest that one in every three cases of heart failure represents dilated cardiomyopathy [68]. Heart failure occurrence is significantly more in older population and as life expectancy is increasing day by day, the probability of increase in number of patients is also there. Although, there are improved therapeutics being developed, there is a need of advanced management is also required as the advanced therapeutics are also associated with toxic side effects [69].

1.2.2. Drug induced toxicity

Drug induced toxic effects arise due to biological modifications during its metabolism in the body as a result of unwanted reactions with various intermediate nucleophiles generated in the course of normal cellular mechanisms. Another important mechanism behind drug induced toxicity is hyper pharmacological response of the drug molecule at non-target sites as a result of either overdose or altered metabolism of the drug molecule itself. Drug induced toxicity can be classified majorly into five classes as i) on target toxicity, ii) off target toxicity, iii) toxic reactions arise as a result of hypersensitivity and other immunological reactions; iv) as a result of biological activation of drug molecule and v) idiosyncratic toxicity. These classes have some mechanistic overlaps despite having distinct defining characteristics [70].

Hepatic toxicity and nephrotoxicity were recognized as major toxicity issues associated with drugs of different classes in pre-clinical developmental stage of drugs as well as in later stages, but recently, cardiac side-effects have also emerged as an important toxicity to be considered as it is also a reasons for drug withdrawal. Organ specific toxicity of drugs may take place as a result of drug accumulation in any particular tissue or cell types within the organ [71].

Cumulative detrimental effects of oxidative stress and ROS have been observed in various drug-induced toxicities. ROS generation is a primary defense of cell upon any unwanted exposure to a
toxic molecule, and if this oxidative stress is not neutralized by the inbuilt cellular anti-oxidants, this may result in the activation of numerous pathways including DNA damage, lipid peroxidation, protein oxidation, mitochondrial imbalance etc. ultimately leading to cell death [72]. Fig 1.4 depicts series of events getting activated upon any chemical or drug molecule is introduced in the biological system and tend to exert toxic effects.

**Fig. 1.4.** Events associated with the toxicity of drugs. (Adopted from Liebler and Guengerich, *Nat Rev-Drug Disc*, 2005, 4:410-420 [70])
Drug induced toxicity is also associated with mitochondrial dysfunction and altered lipid metabolism. Drugs mediated side effects may result in lipid accumulation in the liver thereby causing necro-inflammation and fibrosis. These events are also attributed to the oxidative stress generated as a result of damaged mitochondria. Drug induced metabolic toxicity may occur because of altered drug molecule structure, genetic predispositions, alcohol uptake etc. [73]. Non-desirable drug mediated effects in biological system may directly alter the electron transport chain thereby obstructing the process of oxidative phosphorylation that further result in mitochondria mediated ROS generation and reduced DNA replication, transcription or translation. As evident from mitochondrial mediated toxicity upon drug reactions, it is very critical to detect these mitochondrial events in early drug development process [74].

1.2.3. Drug induced cardiotoxicity

As mentioned above, drug induced cardiotoxicity is an emerging field of concern for both clinicians and researchers. Also, screening of drugs for possible cardiotoxicity is now must during pre-clinical and clinical stages of a new drug before getting launched in market [75]. It may take place as a result of adverse reaction of either cardiac or non-cardiac drugs on the cardiovascular system [76]. Drug induced cardiotoxicity is classified broadly into three categories: sub-acute, acute, and chronic type. Acute and sub-acute cardiotoxicity occur for shorter duration till the drug is being used and involve symptoms like chest distress, shortness of breath with myopericarditis, ventricular repolarization, electrocardiographic QT-interval changes and pericarditis or myocarditis-like syndromes [77]. Chronic or late cardiotoxicity may take place within one year after the therapy or in the longer duration with symptoms including asymptomatic systolic and diastolic left ventricular dysfunction leading to severe congestive cardiomyopathy, which can prove to be fatal. Chronic cardiotoxicity usually occurs in adult patients with cardiomyopathy who have undergone chemotherapy for cancer during childhood or adolescence [78]. Different classes of drugs are reported to be associated with both acute and chronic cardiotoxicity issues and lead to different cardiac abnormalities including hypotension, tachycardia, arrhythmia, and transient depression of left ventricular function [79].
1.2.3.a. Different classes of cardiotoxic drugs

Different classes of drugs including anthracyclines (Doxorubicin, Daunomycin, Epirubicin And Idarubicin); monoclonal antibodies (Pertuzumab, Trastuzumab); Taxanes (Paclitaxel); signal transduction inhibitors (Imatinib, Lapatinib); central nervous system stimulators (Amphetamines and Metamphetamines) etc. are reported to have severe cardiotoxicity issues (Table 1).

Table 1.1. Different classes of drugs with reported cardiotoxicity and their cardiovascular implications in brief.

<table>
<thead>
<tr>
<th>Classes of drugs</th>
<th>Drug names</th>
<th>Cardiovascular indicators</th>
</tr>
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<tbody>
<tr>
<td>Anthracyclines</td>
<td>Doxorubicin, daunomycin, epirubicin and idarubicin</td>
<td>Left ventricular dysfunction, heart failure</td>
</tr>
<tr>
<td>Monoclonal antibodies</td>
<td>Pertuzumab, trastuzumab</td>
<td>Left ventricular dysfunction, heart failure, ischemia</td>
</tr>
<tr>
<td>Taxanes</td>
<td>Paclitaxel</td>
<td>Sinus bradycardia, heart failure</td>
</tr>
<tr>
<td>Alkylating agents</td>
<td>Cisplatin</td>
<td>Heart failure, ventricular tachycardia</td>
</tr>
<tr>
<td>Antimetabolites</td>
<td>5-fluorouracil</td>
<td>Ventricular tachycardia, ischemia</td>
</tr>
<tr>
<td>Small molecule tyrosine kinase inhibitors</td>
<td>Imatinib</td>
<td>Thrombo-metabolism</td>
</tr>
<tr>
<td>anaesthetic agent</td>
<td>Bupivacaine</td>
<td>Dysrhythmias, hypotension, and depression of cardiac output</td>
</tr>
<tr>
<td>antiretroviral drug</td>
<td>Azidothymidine(AZT)</td>
<td>Neuropathy, cardiac dysfunction</td>
</tr>
<tr>
<td>traditional dietary supplement</td>
<td>Ephedra</td>
<td>Stroke and adverse cardiac events such as myocardial infarction, cardiomyopathy</td>
</tr>
<tr>
<td>metamphetamines</td>
<td>3,4-methylenedioxy-methamphetamine</td>
<td>Myocardial infarction, arrhythmias, and cardiomyopathy</td>
</tr>
<tr>
<td>Steroids</td>
<td>Quabain</td>
<td>Cardiomyopathy</td>
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Anthracyclines are effective anti-neoplastic agents that prevent cell division by disrupting the structure and function of DNA in cancer cells [80]. This class of drugs follow the free radical hypothesis and also generate reactive nitrogen species (RNS) in addition to ROS, which exert stress on heart [81]. Genetic factors also play an important role in developing anthracycline-induced cardiotoxicity. Identification of polymorphic genes encoding three proteins: NADP(H) oxidase, MRP1 and MRP2 (doxorubicin efflux transporters) has been found linked to cardiotoxicity induced by anthracyclines [82]. Trastuzumab, a monoclonal antibody, is an inhibitor of the tyrosine kinase-associated Her2 receptor for breast cancer treatment, is found to have adverse effect on cardiac cells [83]. In contrast to anthracycline-induced cardiomyopathy, trastuzumab-induced cardiomyopathy is reversible. Trastuzumab administration blocks the activation of cell survival pathways leading to decreased cardiomyocyte growth [84]. Paclitaxel, a type of taxane, stimulates the polymerization of tubulin, leading to the development of dysfunctional microtubules which blocks normal cell division eventually leading to cell death. Approximately 29% of the patients treated with paclitaxel have adverse cardiovascular damage that may lead to cardiac arrest or heart failure [85]. Imatinib is a signal transduction inhibitors which selectively blocks tyrosine kinase and down-regulates the activity of Bcr-Abl also have cardiotoxic effects [86]. It also inhibits c-Kit (the receptor for stem cell factor) and platelet-derived growth factor receptors-α and -β. Cardiomyocytes in adults do not express c-Kit, but do express PDGFRs and Abl (the normal tyrosine kinase expressed in all cells of the body) leading to cardiotoxicity. Sunitinib, a tyrosine kinase inhibitor, was developed to inhibit cell survival/proliferation in tumour angiogenesis and is effective in treating renal cell carcinoma. The drug inhibits vascular endothelial growth factor receptors [87]. Lapatinib is a dual kinase inhibitor of ErbB2 and EGFR/ErbB1, and has been recently introduced as an alternative to an additional therapy, with trastuzumab. It reversibly binds to intracellular ATP-binding site of kinases, thereby blocking phosphorylation and activation of receptor. Since it does not interact with extracellular domain of ErbB2 it can inhibit trastuzumab resistant tumour cells that express p95 ErbB2. As cardiomyocytes express ErbB1, lapatinib treatment selectively induces cardio-toxicity [88].

Capecitabine and its metabolite 5-fluorouracil are chemotherapeutics used to treat solid cancers and cardiotoxicity is a major limitation associated with this treatment that results in myocardial ischemia, cardiac arrhythmias, hyper- and hypotension, left ventricular dysfunction, cardiac arrest
and sudden death [89]. Limited studies have reported mechanisms of capecitabine-induced cardiotoxicity suggesting vascular endothelial damage followed by coagulation, ischemia secondary to coronary artery spasm and direct toxicity on the myocardium and thrombo-genicity due to altered rheological factors [90]. Central nervous system including amphetamines and metamphetamines induce euphoria, exaggerate aggression and alertness, intensify emotions, and alter self-esteem. They also affect the receptors present on the heart cells due to which there is an accelerated growth of cardiomyocytes. Cardiovascular symptoms related to their treatment include chest pain, palpitations and dyspnea. It has been reported that subjects abusing methamphetamine have 18% more incidences of cardiomyopathy [91-92].

Extensive research is going on to derive the mechanisms involved in different drug induced cardiotoxicity and measure to minimize the same.

1.2.3.b. **Risk factors prevalent among cardiotoxic patients**

As cardiac side effects are majorly dose mediated, dosage of administered drugs is a major risk factor for developing cardiovascular complications. In addition to cumulative drug dosage, age, gender, history of radiation therapy and hypertension are other major risk factors [93]. Patients with pre-existing left ventricular dysfunction are at increased risk of developing cardiotoxicity. Further, survivors with trisomy of chromosome 21 are at increased risk for LV dysfunction [94]. Genetic factors and history of different diseases among patients are also considered as important risk factors. Pre-existing risk factors associated with cardiotoxicity include the presence of cardiovascular disease and non-cardiac medical conditions such as pulmonary disease, musculoskeletal disease, renal disease, hepatic disease, history of alcohol use, energy drink, prematurity and other genetic disorders [95]. These factors should be pre-evaluated and considered before initiating any chemotherapies as this may help in identifying high cardiovascular-risk populations for cancer treatment and further strategizing for reduction or modification of these risk factors, use of cardio-protective strategies, and minimizing the use of cardio-toxic cancer therapies. Females are found to be more susceptible than males for developing cardiotoxicity and display higher rates of left ventricular dysfunction [96].
1.2.3.c. Prognostic techniques to monitor drug induced cardiotoxicity

Prevention of cardiotoxicity is very important for patients undergoing treatment with known cardiotoxic medications. Different screening methods are recommended before, during and after the completion of the treatment and series of diagnostic and prognostic methods have been suggested for detecting cardiotoxicity including clinical, imagistic, serological, or molecular methods. Imagistic methods include radionuclide ventriculography, positron emission tomography, cardiac magnetic resonance, and echocardiography techniques that monitor any alterations in cardiac function during anti-cancer chemotherapy. Radionuclide ventriculography uses radioactive material, usually Technetium-99m, for labelling red blood cells followed by monitoring cardiac blood pumping patterns [97-98]. It is also used to evaluate coronary artery disease, valvular heart disease, congenital heart diseases, cardiomyopathy, and other cardiac disorders. Cardiotoxicity can also be monitored by analyzing medical history of the patient, ECG report and echocardiogram. Modern techniques have also been introduced for examining left ventricle function including cardiac magnetic resonance tagging, contrast echocardiography, 3D echocardiography and tissue Doppler strain [99]. Clinical studies are done in vitro or in vivo for monitoring the cardiotoxicity at the drug level but this has many limitations like difficulty in managing long term cardiotoxicity, different route of administration and relevance of the data in humans [100]. Human pluripotent stem cell-derived cardiomyocytes offers a human cell-based model for both drug development and cardiotoxicity screening [101]. It has been shown that hPSC-derived cardiomyocytes show early indication of drug-induced ion-channel dysfunction in a human cardiomyocyte [102]. There is a requirement for multi-scale prediction methods, either computational or experimental, that can be utilized for initial screening of any drug molecule for possible toxicity [103]. Different biomarkers have been discovered for diagnosing cardio-toxic side effects, for example, cardiac troponins. Cardiac damage is allied with outflow of cardiac enzymes into the blood. Indeed, the elevated concentrations of troponin, elongation of QT-interval and increased levels of natriuretic peptides are associated with anthracyclin-induced cardiotoxicity. While several biomarkers have been studied, troponin-I is a well-known biomarker for detecting changes in left ventricular function. Other biomarkers include N-terminal pro-peptide of B-type natriuretic peptide. Elevated levels of NT-proBNP and BNP are most notably associated with cardiac failure [104-106].
1.2.3.d. Amelioration of drug induced cardiotoxicity

Various interventions for avoiding or reducing the cardiotoxicity of drugs have been developed including supplementing natural cardio-protective molecules or using modified drugs with nanomaterial based delivery or simply using an alternative non-toxic medicines. Vitamin E is a major lipid soluble antioxidant and used in combination of anti-cancer drugs for the treatment of large spectrum of malignancies [107]. Coenzyme Q, another anti-oxidant, is an important part of the mitochondrial respiratory chain and its supplementation has shown to prevent anthracycline-induced cardiotoxicity in both preclinical and clinical studies. Recently a study has shown that coenzyme Q treatment reduced the incidence of cardiotoxicity in children treated with Doxorubicin [108]. L-Carnitine is naturally occurring amino acid that protects heart from damage against drug induced lipid peroxidation of cardiac membranes by its anti-oxidant action. Further, it has an ability to inhibit long-chain fatty acid production. Therefore, L-carnitine supplementation may protect against the acute and chronic effects of anthracycline-induced cardiotoxicity [109].

Various studies have done with other naturally derived bioactive compounds and their cardio-protective effects in drug induced cardiotoxicity is established. For example, Curcumin and epicheatchin have shown positive effects on drug induced cardiac cells and helped in reducing cardiotoxicity. Its cardio-protective potential is shown by reverting the drug induced cardiac hypertrophic conditions in rat cells [110-111]. (-)epigallocatechin gallate (EGCG), a principle polyphenols in green tea is found to possess good anti-cancerous, anti-inflammatory and anti-oxidative properties. In rats, they have been found to have protective role against drug induced toxicity by acting on mitochondrial lipids, lipid peroxides, Na(+)/K(+) ATPase, calcium and adenosine triphosphate thereby reverting the alterations and maintaining normal mitochondrial functions [112].

Modifications in the structural forms of existing drugs has also gained importance for reducing cardiotoxicity [113]. This include drug encapsulation by liposomes or microscopic vesicles consisting of a phospholipid bilayer, when placed around an active drug alter the pharmacologic and pharmacokinetic profiles of conventional drugs, which may decrease the drug toxicity. Polyethyleneglycol (PEG) coating around the liposome bilayer provides protection from enzymatic degradation of drug molecule. These liposomal drugs do not penetrate cardiac cell tight junctions consequently lowering their concentration in the heart and reduce cardio toxic effects [114].
Tumor-specific formulations are new techniques developed for delivering anthracyclines in ways that reduce their absorption in cardiac tissue. The method for targeted delivery of Doxorubicin to tumor includes liposomes, which offers a good degree of cardio-protection by increasing the drug’s molecular size through encapsulation and extending its elimination time. This enables the drug to remain in the organism for longer with fewer adverse effects by keeping it away from organs that have normal capillary junctions, while easily penetrating areas with immature vascular systems, such as tumors [115].

1.2.4. Molecular mechanisms involved in drug induced cardiotoxicity

Studying the different molecular and cellular mechanisms involved in drug mediated side-effects in cardiac system have become possible with the advancement of molecular tools and techniques. Different cardiac stress responses are reported to get activated upon drug induced cardiotoxicity and behave in interlinked or independent manner to exhibit deleterious effects [116-117]. Sub-acute drug induced cardiotoxicity may result in reversible pathological responses like cardiac arrhythmia whereas acute or prolonged drug stress may stimulate transcription factors such as AP-1 by elevating intra-cellular calcium and calcineurin. Myocardial cells undergo vigorous biochemical alterations at molecular and cellular level and may result in cardiac hypertrophy to cardiomyocyte death depending on the extent of drug stress and cellular strength of resisting the induced stress. Compensatory hypertrophy causes tissue hypo-perfusion and generation of stress hormones including angiotensin II and NE that further results in upregulation of natriuretic peptides, pro-inflammatory cytokines etc. If these events get prolonged and not prevented, may result in activation of cell death pathways either by necrosis or apoptosis [118-119].

1.2.4.a. Oxidative stress and apoptosis

ROS mediated oxidative stress is a major player in acquiring drug induced cardiotoxicity as it target key cellular components including DNA, lipids, and proteins (Fig. 1.4).
Fig. 1.5. Series of events taking place inside a cell upon drug induced oxidative stress generation and ultimately leading to programmed cell death. (Adopted from Deavall et al, J Toxicol, 2012, 1-12 [72])

ROS activates kinase cascade of signaling that modulate cell survival pathways in drug induced oxidative stress in cardiomyoblasts. This ROS induced p38 mitogen activated kinase activation in drug induced myocardial injury or death is shown to induce apoptosis in ischemia–reperfusion-treated hearts [120-121]. Drug induced ROS generation is also reported to results in DNA damage and if it exceed the inbuilt cellular repair potential, it may results in inducing mutations or cell death. Upon ROS mediated DNA damage, tumor suppressor and transcription factor p53 gets activated and initiate cell cycle arrest, repair protocols and apoptosis [122]. Lipid peroxidation is another well reported ROS mediated side effect in cardiomyoblasts where free radicals convert the plasma membrane lipids into polar lipid hydro-peroxides. This disturbs membrane fluidity, hinder membrane-bound receptors and enzymes, and further downstream processes leading to
atherosclerosis and myocardial infarction [123]. Direct and indirect protein and amino acid damage is another ROS mediated event upon drug stress. ROS oxidize important metabolic enzymes having role in basic cellular signaling and may contribute further to oxidative stress [124]. Myocardial apoptosis upon drug induced stress has been studied in detail recently and it has been shown that the loss of cardiac myocytes is an important factor for the myocardial injury that further initiate and extends cardiomyopathy [125-126].

1.2.4.b. Mitochondrial dysfunction

Mitochondria are known as power house for eukaryotic cells as they generate ATP through oxidative phosphorylation of pyruvate (end product of glycolysis). They are also furnished by several enzymes responsible carrying out detoxification process of ammonia [127]. However, the most recent and critical role of mitochondria identified is carrying out energy dependent cell death by the release of cytochrome c from inter-membrane space upon drug induced toxicity [126]. Toxins mediated mitochondrial disruption results in altered trans-membrane potential, permeabilisation, and swelling [128]. Drug induced mitochondrial toxicity could have either positive effects (in case of anticancer drugs) or negative effects (in case of insulin resistance). As the role of majority of drugs is to keep cells in healthy and flourishing state, drug induced toxicity resulting in mitochondrial dysfunction is a critical side effect that can lead to cell death and consequent organ failure [129]. In context to cardiac functioning, mitochondria play a critical role in maintaining homeostasis and providing essential energy requirement for continues cardiac functioning. A number of anti-cancer drugs have been proven to promote mitochondrial dysfunction [130]. Thus, along with inhibiting cancer progression they cause severe cardiotoxicity via mitochondrial dysfunction.

Mitochondrial toxicity develops due to various mechanisms involving interference with the mitochondrial respiratory chain (like uncoupling) or inhibition of the important mitochondrial enzymes (oxidative phosphorylation, mitochondrial DNA replication, ADP/ATP trans-locator etc.). The final phase of mitochondrial dysfunction induces loss of mitochondrial membrane potential and an increase in mitochondrial oxidative/nitrative stress, eventually culminating into cell death.
Fig. 1.6. Different mechanisms for mitochondria mediated drug-induced cardiotoxicity. (Adopted from Chan et al, Expert Opin Drug Metab Toxicol, 2005, 1(4):655-669 [74])

1.2.5. Norepinephrine (NE)

NE is one of the first-line prescribed critical-care medicines for septic shock apart from Dopamine as recommended by various international organizations [131]. It is commercially available as Levophed and prescribed to the patients with consistent low blood pressure as a result of orthostatic or systemic hypotension [132-134]. Shafer first reported the medical application of NE or Levophed as a vasopressor agent in 1951 followed by case study report in 1956 indicating its therapeutic use in critical care patients having myocardial infarction where Levophed was used as a part of treatment for maintaining blood pressure and successful recovery was witnessed [135-136].
1.2.5.a. Mechanism NE in hypotension

It is an intrinsic neurotransmitter as well as a vasopressor agent having vasoconstrictor effects as a result of α- and β-adrenergic agonist effects and improves cardiac output [137-138]. Increase in systemic blood pressure and blood flow in coronary artery take place as a result of its α-adrenergic action mediated peripheral vasoconstriction and β-adrenergic action mediated inotropic stimulation following dilating coronary arteries in heart [24]. Structure of NE or Levophed is as shown in Fig. 1.7.

![Fig. 1.7. Structure of Norepinephrine](image)

1.2.5.b. Norepinephrine induced cardiotoxicity

Literature supports that NE induced stress is an important cause of compromised heart that can additionally lead to cardiomyocyte apoptosis *in vitro* [139]. Compromised cardiac output upon NE administration was reported initially by Pugh et al. in 1952 and it has been studied by researchers since then [140-141]. It causes evident myocardial hypertrophy, necrosis as well as progressive cardiac muscle damage by activating pathways leading to apoptosis in cultured cardiomyocytes [142-143]. Increased NE levels have shown to increase intracellular ROS generation and phosphorylation of ERKs, cJNKs, and p38 signaling molecules, ultimately leading to cardiomyocyte apoptosis [144]. NE treatment has been linked with β-adrenergic pathways and protein kinase A, resulting in cardiac apoptosis *in vitro* and *in vivo* [145-146]. NE induced apoptosis in neonatal rat cardiomyocytes have been shown to be associated with decreased Bcl2 expression
and activation of mitochondrial dependent caspase-2 expression [147]. These alterations may results in developing severe cardiac complications leading to heart failure.

1.2.6. Doxorubicin

Doxorubicin is a well-known anti-cancer drug used against broad range of cancer types. It is a hydroxy derivative of another well-known anti-cancer drug- Daunorubicin, and the structure is as shown in Fig. 1.8. It is an antibiotic that falls under anthracycline category [148]. It is isolated from caesius variety of bacterium Streptomyces. Its efficiency is majorly limited because of severe associated cardio-toxic side effects, as discussed in next sections.

Fig. 1.8. Structure of Doxorubicin

1.2.6.a. Mechanism in Cancer

Being an anthracyclin, Doxorubicin inhibits topoisomerase enzyme thereby altering DNA replication process in cancer cells [149]. It also intercalates in DNA helix thereby disrupts base pair bonds and inhibit DNA replication and translation [150]. Zhou et al have also shown that Doxorubicin activates PI3K/Akt and MAPK signaling pathways and results in apoptotic cellular death [151]. Other than these mechanisms, Doxorubicin mediated anti-proliferative and cytotoxic effects in tumor cells may be free radical generation mediated that may result in lipid peroxidation and alkylation of DNA strands [152].
1.2.6.b. Doxorubicin induced cardiotoxicity

The cardio-toxic effects of Doxorubicin are generally dose dependent but largely depend from patient to patient. After the successful treatment of cancer, cardio-toxic effects sometimes take several years to appear. Doxorubicin interfere with different intracellular processes, which makes it difficult to determine the exact molecular mechanism involved with its associated cardiotoxicity. Different studies have reported that different cardiac abnormalities associated with the Doxorubicin treatment [153]. Free radical generation and the consequent oxidative stress are considered as major factors contributing to these toxic effects. Different cellular pathways have been studied in relation its cardiotoxicity as illustrated in Fig. 1.9 in detail.

Fig. 1.9. Doxorubicin induced activation of different pathways leading to cellular death by necrosis, apoptosis or autophagy and resulting in cardiomyopathy. (Adopted from Shi et al, Herz 2011 · 36:296–305) [154]
Briefly, Doxorubicin induced oxidative stress results in mitochondrial disruption, DNA damage, hindered DNA replication and translation, myofiber degeneration, and cardiac myocyte apoptosis that ultimately results in cardiomyopathy.

Doxorubicin has shown to initiate AMP-activated protein kinase (AMPK) activation in cultured rat embryonic myocardial cells that results in altered cell survival or death regulation in response to various pathological stresses [155]. This further modulate various downstream signaling targets including JNK, p53 etc and initiate programmed cell death in cardiomyoblasts [156]. Also, ROS-dependent LKB1 activation serves as the upstream signal for AMPK activation in response to Doxorubicin stress [157]. Its treatment is also associated with increased p53 tumor suppressor protein expression that in turn inhibit mTOR pathway and considered as a predominant contributor to acute Doxorubicin cardiotoxicity [158]. Doxorubicin induced stress in cardiomyoblasts targets mitochondria and results in an iron accumulation and increase in oxidative stress thereby activating other ROS mediated downstream pathways. Doxorubicin can also form a complex with iron by directly interactions [159].

Several other pathways have also established but the exact mechanism involved is still not completely understood. This fact hinders in developing new therapies for preventing and reverting the associated toxicity.

**1.2.7. Natural products in cardio-protection**

Functional food products are broadly defined as the food components that deliver additional health benefits away from sustaining hunger and providing the basic nutritional values. Functional food displays beneficial effects on various body functions by improving the state of health and reducing the disease risk apart from its nutritional importance [160]. Recent trends indicate substantial awareness in elderly population for consuming products with functional food rich diet worldwide [161]. More than 80% of world’s population relies on natural and herbal food products for medicinal purposes due to their low side-effects, easy availability, traditional acceptance etc. [162]. There occur a number of antioxidants such as flavonoids, vitamins, and polyphenol responsible for providing health benefits. Natural products have always been an invaluable source for the development of new drugs. With the advancements in technology for developing new medicines,
bioactive compounds have rigorously been utilized for deriving natural product derivatives with wide range of protective effects [163-164]. Numerous studies have established therapeutic potential of plant-derived phenolic compounds during cardiovascular diseases [165].

1.2.8. Curcumin

Curcumin is a natural product with wide history of its application in various health conditions. It is a major constituent of the perennial herb spice named as turmeric (*Curcuma longa*). Roughley and Whitting in 1973 reported that Curcumin was first isolated in year 1815 and structurally defined it as bis-α, β-unsaturated, β- diketone, which exists in equilibrium with its enol tautomer in turmeric [166]. Curcumin is known to be used as a remedy for different wounds, burns, infections, and skin diseases and is known to be associated with various biological activities including anti-oxidant, anti-inflammatory, anti-tumor, anti-angiogenesis, anti-microbial, anti-viral, anti-diabetic etc. These properties make Curcumin a very potential and promising functional food and nutraceutical ingredient. More than 30,000 publications and extensive research in relation with Curcumin exhibits its significance in the fields of medicinal chemistry, healthcare, food chemistry, pharmacology, and analytical chemistry. Curcumin is a very promising natural compound and has been studied for broad range of biological and chemical properties.

Curcumin is known to be a pleiotropic compound that modulates molecular signaling pathways involved in cell survival, inflammation, apoptosis etc. Various studies have confirmed the beneficial pharmacological effects and therapeutic properties of Curcumin. These properties are associated with the well-established anti-oxidative and anti-inflammatory characteristics. Being a natural compound, Curcumin is safe and non-toxic compound and hence, holds exceptional pharmacological safety profile. Anti-bacterial, anti-viral and anti-cancer activities of Curcumin makes it a potential contrivance to be used against various malignant diseases including diabetes, allergies, arthritis, Alzheimer’s disease, and other chronic illnesses [167]. Curcumin is an established chemopreventive therapeutic agent with diverse pharmacological effects against chronic diseases. Numerous phase I and II clinical trials have been conducted to see the effects of Curcumin for the treatment of cancer and other chronic diseases [168-170]. Scientific studies have established that oral consumption of upto the dose of 12,000 mg Curcumin per day by humans is
non-toxic and safe [171]. As Curcumin influence multiple molecular targets including transcription factors, their receptors, cytokines, growth factors, enzymes and small non-coding RNA such as microRNA, it has exhibited excellent therapeutic benefits in medicinal applications.

1.2.8.a. **Structural characteristics of Curcumin**

Curcumin belongs to the group of compounds called “curcuminoids” along with demethoxycurcumin, bis-demethoxycurcumin and cyclic curcumin. Turmeric contains approximately 80% Curcumin, 18% demethoxycurcumin, and 2% bis-demethoxycurcumin [172]. Curcumin is also known as diferuloyl methane and its IUPAC name is (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione. It is soluble in organic solvents, alkaline solvent and solvents with extreme acidic pH. It is sparingly soluble in hydrocarbon solvents and insoluble in water [173]. Curcumin is a crystalline compound with a characteristic yellow color. It has a symmetric structure with two phenolic groups and one diketone moiety. These functional groups are present as two aromatic rings containing o-methoxy phenolic groups with a seven carbon linker having α,β-unsaturated β-diketone moiety. The β-diketo group present in Curcumin exhibits keto-enol tautomeration as per the acidity of the solution, where, it is present in the keto and enol forms in acidic/ neutral and alkaline solutions respectively as illustrated in Fig. 1.8.

![Curcumin tautomers](image)

**Fig. 1.10.** 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.
The heptadienone bonds of methoxyphenol rings in the keto form of Curcumin makes it majorly available in the cell membranes as a result of the presence of highly activated carbon atom [174]. The keto-enol tautomers of Curcumin also displays metal chelating ability and their interactions with biomolecules is contributed to its very sensitive spectroscopic properties. The higher stability of Curcumin at low pH is because of its conjugated diene structure that plays the role of a potent H-atom donor. Also, a higher pH, the enol forms act as an electron donor similar to that of phenolic antioxidants [175]. The chemical reactivity of Curcumin with ROS and hydrogen donation reactions leads to its oxidation and contributes to the well-established ROS-scavenging potential in biological system [176]. The presence of ortho-methoxy group and the structure–activity relationship of Curcumin plays a crucial role in displaying the characteristic antioxidant activity. The interactions between the hydrogen bonds, phenolic OH and ortho-methoxy groups of Curcumin leads to the abstraction of H-atom by free radicals and majorly contributes to its antioxidant characteristics. Methoxy substitutions on the aromatic rings of Curcumin differentiates the interactions of different Curcumin forms with the nucleophiles via Michael reaction inside the cellular micro environment because of different physicochemical and physiological activities. As a result of different methoxy substitutions, when Curcumin is administered orally, it undergoes glucuronidation and sulfation; and when administered intravenously or intraperitoneally, it undergoes reduction [177]. Also, different Curcumin analogs displays distinct biological activities that is based on different cell/ tissue/ and organism type. Curcumin is reported as a robust anti-oxidative and anti-inflammatory compound and possesses multidimensional therapeutic actions for the treatment of various chronic diseases.

1.2.8.b. Preventive role of Curcumin in CVDs

Literature and case reports strongly supports the pathogenesis of cardiovascular disorders, such as cardiomyopathy induced by diabetes inducible iNOS and endothelial eNOS play a central role [178]. Farhangkhoe et al suggested that curcumin treatment in the diabetic rats results in the higher levels of eNOS mRNA and iNOS mRNA in the myocardial tissueas compared to the control rats. Curcumin also halted eNOS mRNA and iNOS mRNA upregulation thereby decreasing the oxidative DNA impairment [179]. In a study, the effect of bioactive complex of Curcumin along
with other polyphenols like quercetin, selenium and catechins was studied on cardiovascular risk markers in a healthy population. These polyphenols were included in the diet of healthy individuals for two months and the levels of total cholesterol, HDL-cholesterol, C-reactive protein, homocysteine, vitamin B12, folic acid, cysteine, vitamin B6 and asymmetric dimethylarginine were closely monitored over the time period of study. The significant reduction in total cholesterol and LDL-cholesterol along with other biomarkers was recorded resulting in the better lipid profile thereby reducing the risk of cardiovascular abnormalities [180]. Role of curcumin for preventing vascular aging was supported by reducing arterial stiffening and endothelial dysfunction in a recent in vivo study [181]. Protective effects of curcumin has been reported in drug induced cardiotoxicity caused by different classes of drugs by enhancing cellular Glutathione S transferase and decreasing lipid peroxidation as a result of ROS scavenging properties of curcumin. In case of patients having co-morbidity due to diabetes and cardiomyopathy, curcumin has shown to down regulates NOS and NO production and reduced abnormal accumulation of various connective tissue constituents in the endothelia thereby stabilizing lysosomal membranes It also control hypertrophy in the aging heart by hindering the expression of Adenoviral transcription co-activator, p300 [182].

These reports have reported cardio-protective role of Curcumin in different stress responses with involvement of various signaling pathways. Its role in drug-induced cardiotoxicity is still poorly defined and need to be studied in detail.
1.3 AIM & OBJECTIVES

Proposed work was aimed to study the cardio-protective effects of Curcumin against drug induced cardiotoxicity. Different classes of cardio-toxic drugs were used to design drug-induced model *in vitro* and *in vivo*. Combination of different molecular and computational approaches were utilized to derive the signaling involved in Curcumin mediated effects in drug induced cardiotoxicity.

In order to answer the key questions proposed, the following main objectives were studied in detail-

**Objective 1**: To study the effects of selected cardiotoxic drugs on cardiomyoblasts.

**Objective 2**: To study the effects of Curcumin on cardiotoxicity induced by Levophed and Doxorubicin.

**Objective 3**: To study the mechanism of action and signaling of Curcumin against drug induced cardiotoxicity.