“Knowledge can only be got in one way, the way of experience there is no other way to know”

- Swami Vivekananda
1. INTRODUCTION

1.1 THE HEART

Heart is the central organ of the cardiovascular system located amidst of lungs in our body. Two-third portion of the heart resides on the left part of breast bone and one third to the right. The adult heart is about the size of two clenched fists with its shape resembling cone. Heart weighs about 1 pound which may vary in different individuals. An average adult heart is about 14 cm long and 9 cm wide. Heart is the hollow muscular pump in a circulatory system connected with many small and large elastic vessels to conduct blood for the removal of metabolic wastes and to transport oxygen and nutrients throughout the body. Every beat of heart nourishes 300 trillion cells in human body; an average human adult heart pumps about 2,100 gallons of blood per day. Heart diseases are the growing causes of death more than multitude of cancers, it is expected to be up to 23.6 million deaths in 2030; for every 90 seconds some are die in heart disease from United States. Inspite of the aetiology or the types of cardiovascular diseases, the identification of populations with broad spectrum of therapeutic values to treat or prevent this deadful disease’s incidence may be a fascinating area of current pharmacology to promote the healthy survival of the victims.

1.2 ANATOMY OF THE HEART

The heart has four chambers; the upper two chambers called atria, and lower two chambers called ventricles. Atria are divided by inter-atrial septum and ventricles are divided by inter-ventricular septum. Right side receives blood from the body, which is pumped into the lungs, where carbon-dioxide is removed and
oxygen is added. Left side of the heart collects oxygenated blood from the lungs and circulates it to whole body.

Figure 1.2 Anatomy of Heart structure (Anatomy and Physiology., 2014)

1.2.1 Valves of the Heart

The heart has four valves namely,

- Tricuspid valve, situated between right atrium and the right ventricle.
- The pulmonary (pulmonic) valve, located in between right ventricles and the pulmonary artery.
- The mitral valve lies between the left atrium and left ventricle.
- The aortic valve located between the left ventricle and the aorta.

Table 1: Location and actions of heart valves (Marieb and Hoehn Human et al., 2014)
1.2.2 Walls and Coverings of the Heart

Heart is intrinsically lined with a protective layer of cells that forms a smooth membrane called endocardium constituted by many elastic and collagenous fibers.

- Cardiac muscles covering the major proportion of heart are called myocardium and are richly supplied with blood capillaries, lymph capillaries and nerve fibers.

- The layer by which heart is protected from friction is called pericardium, which consists of connective and adipose tissues.

<table>
<thead>
<tr>
<th>Heart valve</th>
<th>Location</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tricuspid valve</td>
<td>Between right atrium and left ventricles</td>
<td>During ventricular contraction, it prevents blood from moving from right ventricle into right atrium.</td>
</tr>
<tr>
<td>Pulmonary valve</td>
<td>At entrance to pulmonary trunk</td>
<td>During ventricular relaxation, it prevents blood from moving from pulmonary trunk into right ventricle.</td>
</tr>
<tr>
<td>Mitral bicuspid valve</td>
<td>Between left atrium and left ventricles</td>
<td>During ventricular contraction, it prevents blood from moving from left ventricle into left atrium.</td>
</tr>
<tr>
<td>Aortic valve</td>
<td>At entrance to aorta</td>
<td>During ventricular relaxation, it prevents blood from moving from aorta into left ventricle.</td>
</tr>
</tbody>
</table>
1.3 FUNCTIONS OF HEART

A function of heart is mainly co-ordinates the effective actions of chambers. The atrial systole contracts as the ventricles relax, likewise ventricles contract as atria relax (atrial diastole). The brief period of relaxation of both atria and ventricles occurs; this complete series of events makes up a heartbeat, called cardiac cycle.

Cardiac cycle initiates pressure in the chambers of heart to rise and fall and allow valves to open and close completely. During diastole pressure in ventricles was low causing the AV valves to open and ventricles to fill with blood. Nearly 70% of returning blood enters the ventricles before contraction, once atria contracts, remaining 30% is pushed into ventricles. As ventricles contract, pressure in the ventricles rises. When ventricular pressure exceed the AV valves closes and papillary muscles contract, preventing the cups of the AV valves from bulging into the atria excessively. In ventricular contraction, AV valves are closed and atrial
pressure is low. Blood flow continues in atrial walls of ventricles contract, so that the atrial are prepared for another cardiac cycle.

1.4 CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is one of the uniform term which was describes all the diseases related to heart. Circulation conditions for cardiovascular disease includes everything from conditions that are mainly diagnosed as inherited or birth and developed conditions such as coronary heart disease, atrial fibrillation, heart failure, stroke, atherosclerosis, hypertension, congestive heart failure, hypertrophy, arrhythmias, ventricular fibrillation, myocardial infarction, cardiomyopathy, ventricular tachycardia (Krstevska et al., 2001; Towbin et al., 2001; Missov et al., 2001; Lopez et al., 2001). In many European countries and other countries all over the world, cardiovascular disease is one of the major causes of death. For example, 17 million people died from cardiovascular disease in 2005, and it is estimated to be 30% of all global deaths, among these ranges 7.2 million were due to heart attacks and 5.7 million were due to stroke. About 80% of this death occurred in many countries. If this continues, by 2030 it is estimated that 23.6 million of people would die because of cardiovascular disease. Over 80% of cardiovascular disease deaths take place in low-middle countries and it occurs equally in both men and women.

1.4.1 Cardiomyopathy

Cardiomyopathy is important heterogenous group of diseases. The awareness and information about this disease in public and medical communities
and has been impaired by persistent confusion of disease. Awfully cardiomyopathy is diseases of heart muscles, characterized by abnormalities in heart chambers size and wall thickness. Sometimes, the abnormalities are due to dysfunction in functional contraction importantly systolic and diastolic dysfunction in the absence of coronary artery disease, hypertension congenital heart disease and valvular disease (Elliott et al., 2008). Cardiomyopathy is generally classified as primary and secondary confined heart muscles damages, genetic/non-genetic and was partially classified as primary by myocardial damages to systemic and multiorgan disease may comes under secondary cardiomyopathies (Moron et al., 2002). Depending on morphological and functional criteria it was follow up by various categories like dilated cardiomyopathy (DCM), Hypertrophic cardiomyopathy (HCM), Restrictive cardiomyopathy (RCM) and Arrhythmogenic right ventricular cardiomyopathy/ dysplasia (ARVC/D). Cardiomyopathies can be primary myocardial disorders or many develop secondary modulating variety including myocardial ischemia, inflammation infection, increased myocardial pressure or volume loads and by some toxic agents.

1.4.2 Global Ratio of Cardiovascular Diseases

Cardiovascular disease is one of the world wide diseases, and is mainly responsible for over 17.3 million deaths per year, and is leading cause of death worldwide. Increases in cardiovascular disease mortality rate is due to behavioural risk factors like tobacco usage, physical inactivity, unhealthy food habits like food rich in (salt and calories) and harmful usage of alcoholic beverages etc.,
Epidemological studies that have been noted in WHO (World Health Organization, 2011) of mortality rate of disease was described below.

Figure 1.4.2  World map showing the global distribution of burden of CVD, in male (age standardized /100,000).  [http://www.who.int/healthinfo/global_burden_disease/cod_2008_sources](http://www.who.int/healthinfo/global_burden_disease/cod_2008_sources)

Figure 1.4.2  World map showing the global distribution of burden of CVD, in female (age standardized /100,000)  [http://www.who.int/healthinfo/global_burden_disease/cod_2008_sources](http://www.who.int/healthinfo/global_burden_disease/cod_2008_sources)
1.4.3 **Risk Factors**

Other risk factors for cardiovascular disease are

- Poverty and low educational level
- Gender
- Inherited features
- Psychological factors (stress and depression)
- Body over weight and obesity.

1.5 **CHEMOTHERAPY RELATED CARDIOMYOPATHY**

Cancer is one of the major social-economic health problems worldwide. Whereas, current statistical data insist that 1 in 3 women and 1 in 2 men in United States develop cancer once in his or her life time (Siegel, R et al., 2012). Over the last few decades treatment for cancer has marked much improvement in cure rates. Though some of the latest chemotherapeutic agents are very effective for the treatment of most of the cancers, such new chemotherapeutic drugs are highly associated with one of the prominent side effects associated to that of heart, as cardiotoxicity. Mostly cardiotoxicity ranges from mild level of blood pressure to potentially dangerous heart failure (Kufe et al., 2003; Hershman et al., 2008). The risk of cardiac failure may be highly increased when compared to the risk of patient’s death due to cancer. So it may also cause severe morbidity changes mostly in cancer of the paediatrics. Further, conventional chemotherapy has been known for many decades to form improper effects on cardiac and peripheral vasculature. Wide use of new chemotherapeutic agents may develop harmful targets against heart function (Yeh et al., 2009; Bickford et al., 2009). The adverse side effects of
chemotherapy are due to close limiting factor of the cancer patients compromising with tumour response (Tan et al., 1967; Tasaka et al., 1967). Some of the antibiotic classes of chemotherapeutic drugs remain as main cause for severe cardiotoxicity. Anthracyclines, alkylating agents, anti-microtubule agents and anti-metabolites are highly used around in many chemotherapy procedures (Siegel, R et al., 2012). Since 1967, an anthracycline class of antibiotic is the most common chemotherapeutic agent causing cardiotoxicity. Some of the major cardiotoxic complications are Arrhythmia, Pericarditis, Myocardial ischemia and Cardiomyopathy. Therefore, overall increase in survival of cancer patients have partially increased in adverse cardiac complications from cancer chemotherapy (Jone et al., 2007; Pinder, MC et al., 2007; Siegel, R et al., 2012). Therefore, the close relationship in between cardiologist and oncologists are important for the cancer patients who are receiving cardiotoxic agents as treatment regimen in future (Shapira et al., 1990).

To proceeds a good quality of life and to decrease mortality rate due to chemotherapy induced cardiotoxicity and good knowledge about mechanism of cardiomyopathy is needed to improve strategies for cardioprevention by early diagnosis and treatment such side effects.

Figure 1.5  Chemotherapy related Cardiovascular diseases (Shapira et al., 1990).
1.5.1 Drugs Induced Cardiomyopathy

Cardiac failure is one of the major cause of death in many parts of the world. Mortality results from severe complications such as cardiac contractile dysfunctions due to ventricular arrhythmias (Shin et al., 1984). There are several reasons for patients who under goes chemotherapy for cardiac failure. Some of the reasons are (i) drugs and cardiotoxic substances leading to heart failure in terms of abrupt contractile performances (ii) drugs affecting channels or ion pumps in some cases leading to prolongation in cardiac repolarisation. Many chemotherapeutic substances may cause acute cardiac depression and lower heart rate, contractility and conduction leading to severe cardiac arrest. Some of the specific chemotherapeutic drugs are anthracycline, taxanes, fluoropyrimidine, cyclophosphamide, bevacizumab, trastuzumab, lapatinib, sunitinib, sorafenit (Siegel, R et al., 2009) cause severe cardiac complication.

1.5.2 Chemotherapeutic Drugs Associated with Cardiac Injury or Cardiomyopathy Anthracyline

Among many anti-cancer agents, anthracyclines were the first line for creating heart failure. It has been reported that anthracycline related cardiotoxicity was irreversible non-ischemic toxic cardiomyopathy. Its severity form may be due to Left Ventricular (LV) systolic dysfunction and CHF (Hwang, SL., 2008). These antineoplastic drugs were mainly approved as anthracyclines, Adriamycin, daunorubicin and epirubicin (Hwang, SL., 2008). Adriamycin were used as treatment drug for hematologic and many solid malignancies (Siegel, R et al., 2009).
**CYCLOPHOSPHAMIDE**

Unlike anthracycline, cyclophosphamide does not cause cardiomyopathy in cumulative doses during administration. Cyclophosphamide cardiotoxicity was due to the single dose associated with toxicity. The administration of high doses of the drug leads to lethal acute pericarditis and hemorrhagic myocarditis (Tebbi, CK et al., 2007). Mechanism of cyclophosphamide cardiac damage was not completely understood but reason for cardiac damage was due to oxidative stress (Spallarossa, P et al., 2006; Cardinale, D et al., 2004) and also increases the level of free radicals. It may also result in oxazaphosphorine induced cardiotoxicity (Lip, P et al., 1999).

**TRASTUZUMAB**

It is a monoclonal antibody used as a treatment drug for HER+ breast cancer (Pegram, M et al., 2000 & Baselga, J et al., 2001). Administration of trastuzumab causes cardiomyopathy to nearly 1/3rd of the patients during chemotherapy. Cardiac dysfunction ranges from 2% to 7%, when trastuzumab is used as monotherapy. In combination process, trastuzumab was used up to 2% to 13% with paclitaxil. Finally it was used up to 27% in combination with anthracyclines and cyclophosphamide (Pegram, M et al., 2000; Baselga, J et al., 2001).

**SUNITINIB**

Sunitinib was one of the chemotherapeutic drugs that inhibit both tumour cell proliferation and angiogenesis in tumour cells (Naumann, D et al., 2013). Patients treated with sunitinib have increased development of hypertension, left
ventricular dilations and other cardiac complications. Studies elaborate that around 11% of patients are prone to develop cardiovascular events, including myocardial infarction and Congestive Heart Failure (CHF). However the mean time for the development of CHF in cancer patients ranges from 22 days to 27 weeks. At the same time patients with already shown complication of coronary artery disease have an increase in rate of HF, CVD mediated cardiotoxicity. Specific clinical mechanisms for sunitinib injury are not clearly known. However sunitinib inhibit some of the growth factor receptors like VEGFRs, PDGFRs, C-Kit, FLT3, CSFIR and RET.

**SORAFENIB**

Cardiotoxic effect of sorafenib is lower than the cardiac dysfunction created by sunitinib. And its treatment appears to be reversible when compared with sunitinib. Its cardiotoxic effect was observed in 2.9% of patients. However hypertension is also one of the major adverse affects that occur in about 17-43% of patients in general. Possible explanation was observed to be inhibition of DAF1 was the reason behind the drug induced toxicity (De Azambuja, E et al., 2014).

**1.6 LIVE AND DEATH FACTOR OF CHEMOTHERAPEUTIC DRUG ANTHRACYCLINE**

Anti-cancer drug are predominantly recognized as strong cardiotoxic causing agent. However, anthracyclines have been discussed more, which hardly affects the cardiac muscle. They were discovered in the late 1960s and remain one of the main stay in modern therapeutics of cancer. Anthracyclines are categorized into
two groups. First group Daunorubicin (also known as Daunomycin and Rubidomycin) and the second group Doxorubicin (also known as Adriamycin). Anthracyclines were isolated from the species of actino-bacterium known as *Streptomyces peucetius* (Tan et al., 1967; Arcamone et al., 1969). Anthracyclines are highly efficacious against various haemopoietic and solid tumours such as breast cancer, sarcoma, ovarian and bronchogenic carcinoma as well as lymphoma, and certain forms of leukemia. However, the treatment of cancer patients with adriamycin may be complicated by acute and chronic side effects. The acute side effects such as nausea, vomiting and arrhythmias are either reversible or clinically manageable (Lefrak, EA et al., 1973; Singal, PK et al., 1987). The chronic side effects are represented by the development of cardiomyopathy and ultimately congestive heart failure (Lefrak, EA et al., 1973; Buja, LM et al., 1973; Singal, PK et al., 1983). Consequently, once the cumulative dose exceeds 500 mg/M$^2$ body surface area, Adriamycin is generally excluded from the patient’s chemotherapeutic regimen.

Table 2: Features of Anthracyclines (Simbre, VC et al., 2001)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Acute cardiotoxicity</th>
<th>Early on-set, chronic progressive cardiotoxicity</th>
<th>Late on-set, chronic progressive cardiotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset</td>
<td>Within the first week of anthracycline treatment</td>
<td>&lt;1 year after the completion of anthracycline treatment</td>
<td>≥1 year after the completion of anthracycline treatment</td>
</tr>
<tr>
<td>Risk factor dependence</td>
<td>Unknown</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Clinical features in adults</td>
<td>Transient depression of myocardial contractility</td>
<td>Dilated cardiomyopathy</td>
<td>Dilated cardiomyopathy</td>
</tr>
<tr>
<td>Clinical features in children</td>
<td>Transient depression of myocardial contractility</td>
<td>Restrictive cardiomyopathy, dilated cardiomyopathy, or both</td>
<td>Restrictive cardiomyopathy, dilated cardiomyopathy, or both</td>
</tr>
<tr>
<td>Course</td>
<td>Usually reversible when anthracycline is discontinued</td>
<td>Can be progressive</td>
<td>Can be progressive</td>
</tr>
</tbody>
</table>
Adriamycin (doxorubicin) is one of the cytotoxic drugs that are widely used as an anti-neoplastic agent for many cancers. Administration of Adriamycin was one of the intravenous modes of treatment in cancer therapy. However, anti-tumour activity of Adriamycin was greater than daunorubicin against various cancers (Di Marco, A et al., 1969). Even now, Adriamycin is still considered as one of the drugs that possesses high spectrum anti-neoplastic property (Weiss, RB et al., 1992). Although microbial biosynthesis of adriamycin remains the primary source, it can also be chemically synthesized from daunorubicin (Arcamone, F et al., 1972) and total chemical synthesis is now feasible (Arcamone, F; Penco, S et al., 1988). The molecules of ADR contain daunosamine, amino sugar linked through glycosidic bond to ADR one, with a red pigmented nucleues napthacenequinine. Due to the arrangement of its unique structure, it is highly lipophilic with long half-life in the body.

Figure 1.7 Structure of Adriamycin (ADR)
Adriamycin consists of a tetracyclic glycone structure linked to an amino sugar (called daunosamine) by a C-7 glycosidic bond in ring A. This amino sugar produces a hydrophilic centre for doxorubicin. A short chain with a carbonyl group at C-13 and a hydroxyl group at C-14 attaches to ring A at C-9. A quinine group presents in ring C and a hydroquinone group presents in ring B. A methoxyl substituent at C-4 links to ring D (Arcamone., 1969; Minotti, et al., 2004). The only structural difference between doxorubicin and daunorubicin is that it is a hydroxyl group at C-14 in doxorubicin, whereas it is a methyl group at C-14 in daunorubicin. The complex structure of doxorubicin is closely related to its main mechanisms of action and its metabolism.

1.8 ANTI-CANCER ACTION OF ADRIAMYCIN

Since the introduction of doxorubicin into clinical practice in the early 1970s, it has become one of the most effective chemotherapeutic agents for a broad range of malignant tumours such as lymphomas and various solid tumours such as breast cancer, small cell lung cancer and ovarian cancer (Ogura et al., 2001). Various mechanisms of action of doxorubicin–induced anticancer cytotoxicity have been discovered. However, some issues remain as a matter of debate and are yet unclear. Thus, the mechanism of action has been the centre of intensive investigation. Inhibition of topoisomerase II (topo II) triggered by DNA intercalation, oxidative damage mediated by generation of free radicals, apoptosis induced by tumour protein p53-dependent and/or tumour protein p53-independent pathway and interaction with proteasome to mediate-transport doxorubicin into the nucleus (Minotti et al., 2004).
Anthracycline induced cardiac damage may occur in acute, sub-acute and chronic stages. Pericarditis, myocarditis or arrhythmias is generally normal and reversible that occur due to acute cardiotoxicity during some course of treatment immediately, where as chronic cardiotoxicity undergoes even decades after the treatment completed for many years was significantly clinical and substantially affecting complete morbidity and mortality for long term therapy. Generally Adriamycin as one of the important drugs for treatment of many types of cancer that generate high amount of ROS (Reactive Oxygen Species) emphasizes adriamycin induced cardiac complication depends on cumulative doses of chemotherapeutic drug.

Figure 1.8  Mechanism action of Adriamycin (ADR) in cancer cell (Ogura et al., 2001)
1.9 CELLULAR AND MOLECULAR MECHANISMS OF ADRIAMYCIN RELATED CARDIOMYOPATHY

Multiple cellular and molecular mechanisms are involved in Adriamycin induced cardiac failure. Strongest point of cardiac damage induced by Adriamycin is due to cardiac oxidative stress. Reactive oxygen species is followed by an increased amount of oxidative stress in heart. Also, formation of reactive oxygen species results in the lipid peroxidation (Takemura, G et al., 2007, Singal, PK et al., 1987, Kalyanaraman, B et al., 1980 & Doroshow, JH et al., 1983), decreased level of antioxidant and sulfhydryl groups (Singal, PK et al., 1987; Segstro, RJ et al., 1985; Doroshow, JH et al., 1979; Olson, RD et al., 1980; Odom, AL et al., 1992).

Myofibrillar deterioration and intracellular calcium dysregulation, inhibition of nucleic acid and protein synthesis (Arena E et al., 1979; Monti E et al., 1995), release of vasoactive amine (Bristow, MR et al., 1980), altered adrenergic function (Tong, J et al., 1991) and decreased expression of cardiac-specific genes are other proposed mechanisms mediates cardiomyopathy (Takemura, G et al., 2007). Not only cardiomyocytes a target for doxorubicin induced apoptosis apart from that endothelial cell are also affected by activation of caspase and inter nucleosomal DNA degradation. Furthermore, the cardiac toxicity associated with doxorubicin administration is mediated at least in part, by changes in the high-energy phosphate pool, endothelin-1 levels, and disturbances of myocardial adrenergic signaling.
1.9.1 Oxidative Stress

Oxidative stress is the main reason for Adriamycin induced cardiotoxicity. Mechanisms of oxidative stress are due to formation of reactive oxygen species. Chemically reactive molecules containing oxygen are termed as Reactive Oxygen Species (ROS). These substances are generally unpaired electrons apart from that there are also some of the reactive non radical species like hydrogen peroxide (H$_2$O$_2$), peroxidase and free oxygen ions. These substances are generated during normal metabolism of oxygen or by diverse enzymatic pathway. Mitochondria are the best source for the production of reactive oxygen species. During normal oxidative phosphorylation, superoxide radicals (O$_2^-$) are formed as the by product of all reactions. O$_2^-$ is highly reactive with Nitric Oxide (NO), generating Reactive Nitrogen Species (RNS) such as peroxynitrite and further downstream nitrogen species, including NO, peroxynitrite, and nitrogen dioxide via, the activity of enzymes such as inducible Nitric Oxide Synthase 2 (NOS2) and NADPH oxidase (NOX).

ROS have many roles in normal cell and maintain homeostasis (Cai, H et al., 2000; Harrison, D. G et al., 2000). O$_2^-$ may act to limit the duration of the response to NO, a key mediator in vascular functions, including regulation of smooth muscle tone and blood pressure, platelet activation, and vascular cell signaling (Cai, H et al., 2000; Griendling, K et al 2000). However, production of ROS also depends on the response of stressors as toxicant exposure, radiation damage and also resulting in local oxidative stress.
Normal cells have many defense systems to fight against the level of ROS under physiological conditions. Some of the anti-oxidants and anti-oxidant enzymes like ascorbic acid, vitamin-E, glutathione, superoxide dismutase, catalase and glutathione peroxidase prevent cell from intercellular damage and ameliorate the harmful effect by ROS. Mitochondrial manganese Superoxide Dismutase (MnSOD) is a key enzyme which converts superoxide radicals into hydrogen peroxide, which further breakdown into water and peroxidases (Griendling, K et al., 2003). But when the level of this defense system is low, ROS involve in cellular damage results in modification of intracellular targets like DNA damage, protein oxidations and modulate several signaling cascade. When mitochondrial DNA is the target of oxidation, it can lead to mutations, rearrangements, and transcriptional errors that impair important mitochondrial components, leading to more oxidative stress and eventual cell death. Molecular modifications in surviving cells can cause alterations in gene expression; depending on the severity and duration of ROS exposure, pro-survival or pro-apoptotic response pathways may be activated.

Oxidative stress leads to induced damage in DNA and many macromolecules which may be associated with many diseases like cardiovascular diseases and neurological degenerative diseases. Adriamycin is an anti-neoplastic drug associated with numerous oxidative stress mechanisms leading to toxicity in tissues and may play a key role in initiating cell death.
1.9.2 ROS mediating Mitochondria Damage by Adriamycin Induced Cardiomyopathy

Oxidative stress by adriamycin is due to the accumulation of reactive oxygen species in mitochondria of cardiac myocytes. Mitochondria are one of the most extensively injured subcellular organelles. Main reason behind adriamycin inducing cardiotoxicity may be due to cationic charge of drug were retained into the inner membrane of mitochondria and forming irreversible complex with cardiolipin (Goormaghtigh, E et al., 1980). Cardiolipins are the proteins that play an important role in Electron Transport Chain. Doxorubicin disrupts the cardiolipin-protein interface forming more superoxide’s (Schlame, M et al., 2009) Apart from cardiolipin some of the other proteins are also responsible for the carnitine transfer are also affected by Adriamycin and result in low mitochondrial functions. (Sweatman; TW et al., 1990). Due to mitochondrial disturbance production of ATP
is totally disturbed to 90% and it is not utilized by cardiomyocytes leading to pathological complications such as mitochondrial swelling and cristae disfiguration in the mitochondria (Cole, MP et al., 2000) Changes in shifting is a common feature of heart failure while doxorubicin initiates oxidative stress inducing signaling mechanisms to cause the shift in metabolism of long-chain fatty acid (Suliman, HB et al., 2007). And it is well established that mitochondria plays a significant role in pathogenesis of doxorubicin induced cardiomyopathy.

1.9.3 Role of NOS in ROS Production by Adriamycin Mediating Cardiomyopathy

Studies illustrate that Reactive Oxygen Species (ROS) was formed by binding of adriamycin to eNOS reductase domain (Kremer, LC et al., 2002). Electron reduction by eNOS would result in the formation of Adriamycin semiquinone radicle, which subsequently reduces oxygen molecules to form \( \text{O}_2^\cdot \). Consequences between the drug and enzymes was that, when there was increase in adriamycin concentration eNOS will be carried out from nitric acid to produce superoxide. Uncoupling of eNOS plays a major role in cardiac injury (Moens, AL et al., 2009), and there is also some clinical proof for the eNOS dependent ROS formation developing myocardial injury. Some of the studies elaborated that disruption in eNOS transcription protect heart form cardiac dysfunction, injury and mortality (Neilan, TG et al., 2007).
1.9.4 Role of Iron in ROS Production by Adriamycin Induced Cardiomyopathy

Similar to eNOS iron is also prone for the production of reactive oxygen species. Formation of ROS promotes oxidative myocardial damage and this oxidative damage by anthracycline is accounted by the drug that easily undergoes redox cycling reaction and free radical production. Adriamycin contains naphthalene quinone nucleus linked through glycosidic bond to an amino sugar, daunosamine. Addition of electron to the quinone moiety in “C” ring of Adriamycin results in the formation of semiquinone and it quickly regenerates its parent quinone by reducing oxygen into ROS molecules like super-oxide anion and hydrogen peroxidase. This reaction is generally said to be futile cycle and it is supported by an enzyme NADPH oxidoreductase (Cyto p450 or b5 reductase, mitochondrial NADH dehydrogenase, xanthine dehydrogenase, endothelial nitric oxide synthase (reductase domain). Super oxide anion is catalyzed by super oxide dismutase enzymes and hydrogen peroxide is eliminated by defense system like catalase and Gpx. In the presence of iron, H₂O₂ and O₂⁻ can readily form highly toxic radical (OH⁻) which is catalyzed by iron in 2 step formation

\[ \text{O}_2^- + \text{H}_2\text{O}_2 = \text{O}_2 + \text{OH}^- + \text{OH}^- \ldots \ (1) \]

\[ \text{O}_2^- + \text{Fe}^{3+} = \text{O}_2 + \text{Fe}^{2+} \ldots \ldots \ (2) \]

\[ \text{H}_2\text{O}_2 + \text{Fe}^{2+} = \text{OH}^- + \text{OH}^- + \text{Fe}^{3+} \ldots \ (3) \]

Figure 1.9.4 Free Radical Formation
Formed OH group reacts with polyunsaturated fatty acids forming lipid peroxides, conjugated dienes and malonyldialdehyde and initiates cellular damage followed by cellular dysfunction. Production of ROS affects proteins, nucleic acid and most predominately ion channels and ion transpoters (Halliwell; Gutteridge et al., 2007). Formation of Adriamycin semiquinone followed by superoxide anion is accompanied by iron released from ferritin. Ferritin contains 24 subunits of protein, store up to 4500 atoms of iron. Anthracycline semiquinone is large enough to enter into ferritin, where as superoxide radical enter into ferritin and has reduction potential and stored in ferritin core. Polynuclear and steric factor promote superoxide anion to release iron from ferritin (Harrison et al., 1986; Theil., 1987). Intercellular iron homeostasis is regulated and controlled by iron regulatory protein and iron - responsive element in target gene sequences. Adriamycin totally disrupts the Fe-S cluster, and inhibits IRP-1 whose metabolic role is to maintain iron levels. Second mechanism by which Fe promotes oxidative damage is by (DOX-Fe) complex. This reaction is carried out by the enzymes NADPH cytochrome p450 reductase which converts DOX-Fe (III) complex into DOX-Fe (II) and releases superoxide radicals and converts DOX quinone into DOX semiquinone free radicals. Hence Adriamycin treatment in cancer patients have prominent role in producing elevated amount of iron from redox cycle and increase in the production of Reactive Oxygen Species (ROS), which generally create oxidative damages in heart when compared to other organ injury.
1.9.5 Impair Calcium homeostasis in Adriamycin Induced Cardiomyopathy

Anthracyclines are powerful chemotherapy agents, widely limited due to the onset of fatal cardiotoxicity which includes arrhythmogenesis and heart failure. Several proteins which are important in intracellular Ca^{2+} signaling have been identified as drug binding targets, including the cardiac ryanodine receptor Ca^{2+} release channel (RyR2), the Ca^{2+} binding protein calsequestrin (CSQ2) and the Sarco/Endoplasmic Reticulum Ca-ATPase (SERCA2a). The effects of the drug metabolites have been poorly characterized but are believed to be important in the devastating cardiac effects.

The functional effect of doxorubicin and its metabolite, doxorubicinol on RyR2 was assessed by adding clinically relevant drug concentrations to single RyR2 channels in lipid bilayers. Both of these drugs cause biphasic modulation of RyR2 activity where there was an early increase in channel activity followed by a later,
inhibitory phase. RyR2 channel activation, but not inhibition, could be reversed by drug washout, typical of a ligand binding effect (Muller, D et al., 1984). Conversely, the irreversible nature of the inhibitory effect suggested a non ligand binding effect. Treatment with doxorubicin/doxorubicinol reduces the number of thiols on RyR2, indicative of drug-induced thiol oxidation. Additionally, doxorubicinol abolished the response of RyR2 to changes in luminal Ca$^{2+}$. Further experiments revealed that the loss of luminal Ca$^{2+}$ sensing was due to an interaction between dox-ol and CSQ2. Finally, we found that doxorubicinol inhibits SERCA2a Ca$^{2+}$ uptake into SR vesicles and was prevented by pretreatment with DTT. These results provide novel insight into the cellular mechanisms of anthracyclines (Solem, L.E et al., 1994) Adriamycin targeting multiple Ca$^{2+}$ handling proteins in cardiac muscle, anthracyclines severely disturb cardiomyocyte Ca$^{2+}$ homeostasis and that these effects have an important role in the onset of anthracycline-mediated arrhythmia and heart failure (E. Chacon, D et al., 1991).

Figure 1.9.5 Oxidative stress by calcium homeostasis
1.9.6  Role of ROS in Extracellular Matrix Changes by Adriamycin Induced Cardiomyopathy

Apart from the reactive oxygen species and oxidative stress, ADR disturbs both cellular and extracellular damage in cardiomyocytes. During myocardial remodeling cellular and extracellular factors plays a lead role, while ADR weakens the collagenous matrix proteins and contributes some pathological remodelling by MMP-2 and MMP-9 expressed in ADR cardiomyopathy due to ROS generation (Goetzenich, A et al., 2009; Spallarossa, P et al., 2006). The level of MMP-2 and MMP-9 also depends on production of NADPH oxidase partially generated by ROS. Tissue inhibitor of metalloproteinase-3 (TIMP-3) also decreased during adriamycin administration (Tokarska-Schlattner, M et al., 2010). Due to accumulation of MMP-2 and MMP-9, stability of membrane structure in cardiac tissue was expressed lesser and showed membrane impairment and loss of structural potential was also suspected (Singh, BK et al., 2010).

1.10  INFLAMMATORY Response of ADR Induced Cardiomyopathy

Apart from many clinical use of ADR, limitation was due to cumulative dosage of cardiotoxicity which promote various heart diseases (Hrdina, R et al., 2000; Scully, RE et al., 2007; Zucchi, R et al., 2003). Several causes for ADR mediating cardiomyopathy are widely higher. However cardiac inflammation and generation of oxidative stress liberally govern in participating in clinical changes.

ADR a neonatal drug induces significant increases in level of inflammatory responses by elaborating the level of inflammatory markers like interleukin (IL-6),
interleukin (IL-1β), tumor necrosis factors-α (TNF-α) and Cyclo-oxygenase-2 (COX-2). In cellular levels, inflammatory cytokines like IL-1β and IL-16 are regulated by many molecular pathways especially Mitogen Activated Protein Kinase (MAPK) pathway (Baldassare, JJ et al., 1999; Kent, LM et al., 2009; Obata, T et al., 2000). Same like interleukins, cyclooxygenase activity was also induced by ADR (Adderley, S et al., 1999). Generally COX catalyses the steps involved in the conversion of arachidonic acid to prostaglandin (Hemler, M et al., 1976). COX-1 is isoform expressed in most normal cardiomyocytes. COX-2 is highly absent from cells but it is initiated by various factors (Hla, T et al., 1992) like free radicals and by oxidative stress. Apart from two gene COX-2 promoter containing inflammatory response elements (Fletcher, B.S et al., 1995; Xie, W et al., 1994). ADR induces COX-2 gene expression and promote inflammatory response in many cardiomyocytes (Hemler, M; W.E.M.; Smith, W.L et al., 1976).

ADR activates MAPKs, including the Jun N-terminal kinases (JNK) and p38 MAPK, which are involved in various biochemical processes and regulate apoptosis and the production of many inflammatory mediators.

1.11 ADRIAMYCIN INDUCED CARDIOMYOPATHY BY APOPTOSIS

1.11.1 Apoptosis

“Cell loss” is unifying features found mostly in all form of cardiomyopathy (Nishida, K et al., 2008; Choi, AM et al., 2013; Liang, C et al., 2006) and decrease in number of myocytes is highly predicted to enhance cell slippage, wall thinning, decrease in contractility and finally chamber dilatation. Best understanding form of cell loss and cell death in cardiomyocytes is due to apoptosis.
The term Apoptosis is regulation of cell death in various multicellular organisms. It is very difficult for sculpting tissue during development, and activated by certain tissue damages due to formation of reactive oxygen species, finally damaged cells have to be eliminated subsequently (Wongcharoen, W et al., 2009).

Apoptosis is initiated by various biochemical and morphological changes. Apoptosis is generally characterized by cellular and nuclear shrinking, chromatin condensation, cell membrane bledding, apoptotic body formation and DNA fragmentation (Venkatesan, N et al., 1998).

Figure 1.11 Mechanism of Apoptosis

Although there are many reasons for adrimaycin induced cardiomyopathy that were noted, there are several investigations for the ADR
induced cardiotoxicity, particularly onset cardiomyopathy is been investigating for many decades. But, exact mechanism of toxicity and heart failure are still difficult to explain. Among the following categories, reactive oxygen species plays a vital role for the production of oxidative damage and finally ends up with apoptosis. Apoptosis can be initiated by several triggering process and it was followed by two steps namely the intrinsic and extrinsic pathways. The main concept of ADR induced cardiomyopathy is carried out in the intrinsic pathway (Vedam, K et al., 2010).

The mitochondria are major cell organelles believed to be primary source for ADR inducing cardiac damages. Furthermore, mitochondria play a key role in regulating apoptosis by liberating cytochrome-c (Singal, PK et al., 1998; Kremer, LC et al., 2002). The level of cytochrome-c is partly controlled by many anti-apoptotic and pro-apoptotic Bcl-2 family proteins which is located in the outer membrane of mitochondria (Gilleron, M et al., 2009; Wojnowski, L et al., 2005; Blanco, JG et al., 2008). Oxidative stress induced by ADR involves in liberation of cytochrome-c from mitochondria and activate caspase-3 and caspase-9 leads to apoptosis. Pro and anti-apoptotic proteins like (Bcl-2: Bax) also triggers apoptosis by ADR.

1.11.2 MAPK signaling

Most of the characteristic features of ADR induced cardiomyopathy was due to dose dependent refractoriness to isotropic support, membrane tubular structure dilations, drop out in myofibrils and lack of inflammatory up regulation (Tian, W et al., 2000; Ushio-Fukai, M et al., 1998; Muller, E et al., 1999). Other
major ADR induced apoptosis mainly involves the activation of major pathway like Mitogen Activated Protein Kinase family (MAPK) pathway. MAPK signaling are due to activation of primary inter mediator of apoptosis due to oxidative stress by generation of reactive oxygen species. Three major MAPK families include including extracellular signal-regulated kinases (ERKs), p38, and c-Jun NH2-terminal kinases (JNKs). In the cardiovascular system, ERK1/2 are potently and rapidly activated by growth factors and hypertrophic agents thereby mediating cell survival as well as offer cytoprotection (Thandavarayan, RA et al., 2008; Green, LC et al., 1982; Minotti, G et al., 2004). In contrast, JNKs and p38-MAPKs are activated by cellular stresses, including oxidative stress, and are thought to correlate with cardiomyocyte apoptosis and cardiac pathologies (Xuejiang, W et al., 1999; Wold, LE et al., 2005; Kitiyakara, C et al., 2003).

Several studies shown ADR induced cardiomyocytes apoptosis partially due to increased in expression of p53 tumour suppressor proteins. Further, reduction of p53 during via, genetic deletion (Silber, JH et al., 1993) of chemical inhibition (Biancaniello, T et al., 1980) is cardio protective during exposure of ADR treatment. P53 tumour suppressor gene can directly regulate the role of Bcl-2 family proteins (Bcl-2 anti-apoptotic proteins) and (Bax pro-apoptotic proteins). Activation of the protein can regulate the collapse of mitochondrial stability, permeability and involve in the activation of subsequent caspases which involve in Apoptosis.
1.12 MONOTERPENES

Monoterpenes are secondary metabolites of plants and it has various pharmacological properties like anti-bacterial, anti-fungal, anti-oxidant, anti-cancer, vasorelaxant, hypotension and anti-spasmodic effects (Garcia et al., 2008; Koto et al., 1990). 80% of the world population uses natural medicine as a primary health care (Balick et al., 1994). Monoterpenes belongs to the family of terpenoids and is also known as isoprenoids. Isoprenoids are formed by repetitive branched units of
five carbon similar to units of isoprene (Sharkey; Yeh, E et al., 2001). Terpenoids nomenclature mainly depends on the number of isoprene structure like monoterpene and polyterpenes. Generally terpenoids possess cyclic or acyclic structures, which results from change in isoprenoid chain reactions such as reduction, oxidation, cyclization, ring breaks or rearrangement (Chappell, J et al., 1995).

Monoterpenes are further categorized into three subgroups like acyclic, monocyclic and bicyclic. From this, they are again sub-grouped into unsaturated hydrocarbons, alcohols, aldehyde, ketones, lactones and tropolonas. Some of the examples for these groups are (d-Limonene) unsaturated hydrocarbons, menthol (alcohol), myrtenal, carvone (aldehyde and ketones) (Simoes et al., 2004). Monoterpenes are frequently used in many fields like agriculture, cosmetic, food industries and as general antiseptic. It was also used in many medical practices (Aeschbach, R et al., 1994; Lee, M et al., 1997; Manou, B.J et al., 1998). Based on these literature evidences, monoterpenes can also be used as a powerful agent for prevention and or treatment of cardiovascular diseases. Studies have shown that 33 monoterpenes have most of the cardiovascular activity, sixteen of them were carvacrol, (-) linalool, (+)-linaloo, citronellol, eucalyptol, myrtenal, menthol, myrtenol, Rotundifalolone, Sobrerol, thymol, limonene, α-terpine-4-ol, α-terpineol, p-cymene, perillyl alcohol, α-pinene and β-pinene.

1.12.1 Terpenoids

Terpenoids nomenclature mainly depends on numbering of isoprene structures. These isoprene structures were classified as monoterpene, sesquiterpene, diterpene, tetraterpene, polyterpene and triterpene.
1.12.1.1 \textit{d-LIMONENE}

\begin{quote}
\textit{d}-Limonene an oil nutrient, occurs naturally in lemon and orange peels. It is also found in certain bushes and trees. \textit{d}-Limonene is abundantly found in peels of citrus fruits, caraway, fennel and turpentine (Stromvall, H \textit{et al.}, 1992). Mean emission rate of limonene vary from several plant species ie, lemon, orange, pistachio and walnut (Areyetal, K \textit{et al.}, 1991). \textit{d}-Limonene found in natural fruits like grape fruit (95%), tangerine (94%), orange (91%), mandarine (72%), lemon (65%), elemi (50%). \textit{d}-Limonene is one of the best biodegradable solvent naturally, this drug showed enormous activity in health recovery like indigestion and acid reflux. In recent years it is used as anti-cancer agents in various animal models such as breast and colorectal cancer (Igimi, H \textit{et al.}, 1976; Sun, J \textit{et al.}, 2007; Safari, MR \textit{et al.}, 2001; Yang, X \textit{et al.}, 2010). Studies reported that monoterpenes have various pharmacological properties including anti-fungal, anti-bacterial, anti-oxidant, anti-
\end{quote}
cancer and anti-spasmodic (Garcia, R et al., 2008; Kato, T et al., 1990; Singh, P et al., 2010; Karkabounas, S et al., 2006; Magalhaes, PJC et al., 1998).

![Figure 1.11.1.1 Lemon Orange Tangerines](image)

1.13 STRUCTURE OF \(d\)-LIMONENE

\(d\)-Limonene (-methyl-4-(1-methylethenylcyclohexane) is a naturally occurring monocyclic monoterpenone, and it is a colourless liquid with lemon-like odour. Chemically \(d\)-limonene exists in two optical isomeric forms such as \(d\)-Limonene and \(l\)-Limonene and racemic mixture dipentene. Commercially available of \(d\)-Limonene occurs in 90-98% of purity.

![Figure 1.12. \(d\)-Limonene](image)
1.13.1 Absorption, Distribution and Metabolism

Oral administration of d-Limonene is generally absorbed in the gastrointestinal tract in both animals and humans. Hence, absorbed d-Limonene was distributed to several parts of the body such as liver, lungs and kidney (Igimi, M et al., 1974; Sun, J., 2007). Higher concentration was also detected in adipose tissue and mammary gland and is less in fatty tissues. Half-life of d-limonene was estimated to be 12-27 hr (Igimi, M et al., 1974). Amount of d-Limonene in urine is generally about 52-83% of the dose within 48 hrs. Oral dose of d-Limonene in human was found to be 25-30% as d-Limonene -8-9 diol and its glucuronide in urine and 7-11% was eliminated as perillic acid and its metabolites.

1.13.2 Therapeutic/Curative Effect of d-Limonene

Toxic effects of d-Limonene were found to be low, after many years of consumption (Sun, J., 2007). Many studies revealed that d-limonene provide antioxidant property, inhibit lipid peroxidation and fight against cell from free radical-induced cell damage. (Pandima Devi, K., 2004). Dietary strategies shown that increased consumption of fruits, vegetables and fishes are helpful in reducing the cardiovascular diseases mostly hypertension (Getz, G.S et al., 2007; Retelny, V.S et al., 2008) and physiological stress. Hence, d-Limonene works as a curative measure to prevent certain physiological stresses. d-Limonene was one of the best remedies for inflammatory diseases, and it is proved as anti-inflammatory, chemo preventive and chemotherapeutic drug against several types of cancers such as pancreas, stomach, colon, skin and liver cancer.
1.13.3 Remedial Effect of d-Limonene

Several studies has shown that d-limonene is one of the best chemotherapeutic drug (Elegbede, JA et al., 1984; Elsoce, M et al., 1988; Maltzan, TH et al., 1989; Wattenberg, H et al., 1983). They prevent carcinogenesis at both initiation/promotion and progression stages of cancer (Crowell; Gould et al., 1994). d-Limonene reduces cancer incidence in liver, lungs, fore stomach tumours, skin, and mammary tissue (Elson, C.E et al., 1998; Russin, V et al., 1994). Chemo protective activity of d-limonene enhances Cytochrome p450 phase I and II enzymes, which metabolizes into less toxic substances and inhibits the chemical carcinogen interaction with the DNA double helix (Vander logt, EM et al., 2004). Furthermore, oral administration of d-Limonene result in significant regression of chemical carcinogen like DNBA- and NMU- induced liver and breast carcinoma in dose dependent manner (Haag, J.D et al., 1992; Elegbede, J.A et al., 1986).

1.13.4 Salubrious Effect of d-Limonene on Inflammatory Cells

Peel of orange contain adequate amount of d-Limonene and specifically used as an anti-inflammatory agent (D’alesson, G et al., 2002). d-Limonene generate antibody production and antibody producing cells in some of the organs like spleen, bone marrow, cellularity and alpha-esterase positive cells suggesting it’s potential effect on immune system. Limonene effectively inhibits LPS-induced PGE2 and NO production that includes dose-dependent decrease in the expression of iNOS and COX-2 protein levels (Hirota, R et al., 2010). In humans, d-Limonene subjects with high inflammatory scores benefited from OPE supplementation through significant decrease of peripheral IL-6 levels, and on conclusion d-
Limonene acts on inflammatory cells by suppressing the pro-inflammatory activity of cytokines (Miller, JA et al., 2011). Furthermore anti-inflammatory properties of d-Limonene was proved in human eosinophilic leukemia HL-60 cells by measuring the level of MCP-1 (monocyclic chemoattract and protein-1) and significant role in reducing ROS, NF-kB and P38 mitogen activated proteins (MAPK).

1.13.5 Anti-Oxidant Potential of d-Limonene

Free radicals scavenging activity of many fruits, vegetables and medicinal plants have been studied extensively. Whereas d-Limonene possessed free-radicals scavenging property was mainly due to its natural monoterpenic unit (Carrari, F et al., 2003). Indeed, a number of dietary monoterpane have an anti-oxidant activity that can prevent progression of cancer. Hence, particularly alcohol and d-Limonene have a well protective activity on many cancers. (Growell et al., 1999). Some of the promising role of d-Limonene was confirmed in proliferative lymphomas and stimulate normal life of lymphocytes by activating anti-oxidant property. d-Limonene possess regular effects on immune system by enhancing WBC count and initiate the products of total anti-body and alveolar macrophages number and generate the potential without changing normal lymphocytes viability (Manuele, MG et al., 2008). d-Limonene as a potential anti-oxidant that effectively protects lymphocytes from mitochondrial dysfunction and oxidative stress.

1.13.6 Safety Measures of d-Limonene

d-Limonene is fairly low toxic and showed no risk of evidence in both the genders of rats. Subsequently d-limonene does not pose to be mutagenic,
cancerogenic or nephrotoxic risk in humans, and shows low toxicity after repeated
dose for up to many years.

The oral LD\textsubscript{50} for \textit{d}-Limonene in male and female rats was reported to be
4.4 and 5.1g/kg body weight respectively (IARC Monogr Eval Carcinog Risk Chem
Hum et al., 1999). Although \textit{d}-limonene was proved to be safe when subjected with
20g in single dose complained about increased bowel movement, and no
abnormalities were found in liver markers like (total protein, bilirubin, cholesterol,
AST, ALT and Alkaline phosphatase) also in kidney (BUN) and pancreatic
(amylose) (Igimi, H et al., 1992).

\textbf{1.13.7 Beneficial Effect of \textit{d}-Limonene in Gall Stone Dissolution}

In vitro, \textit{d}-Limonene dissolved human gallstones within two hours. In
animals, infusion of \textit{d}-limonene into the gallbladder dissolved and disintegrated
gallstones, which were excreted through the common bile duct. In patients post
gallstone surgery, infusion of 20 mL \textit{d}-Limonene every other day dissolved
gallstones overlooked during surgery. In some patients gallstone dissolution
occurred after only three infusions. A study with 200 patients reported a direct
infusion of 20-30 mL \textit{d}-Limonene (97\% solution) completely or partially dissolved
gallstones in 141 patients. Stones completely dissolved in 96 cases (48\%); partial
dissolution was observed in 29 cases (14.5\%); and in 16 cases (8\%) complete
dissolution was achieved with the inclusion of hexamethaphosphate (HMP), a
chelating agent that can dissolve bilirubin calcium stones. All the stones were
between 0.5 and 1.5 cm with an average diameter of 1.0 cm. The duration of the
treatment ranged from three weeks to four months (Igimi, H et al., 1991).
1.13.8 Other Clinical Uses

*d*-Limonene is considered to be a chemical with fairly low toxicity. Studies have determined *d*-Limonene does not pose a mutagenic, carcinogenic, or nephrotoxic risk to humans. *d*-Limonene has been clinically used to dissolve cholesterol-containing gallstones. It has also been used for relief of heartburn/GERD, because of its gastric acid neutralizing effect and improvement of peristalsis.