CARDIOPROTECTIVE EFFECT OF *Delonix elata* (L.) LEAF EXTRACT AGAINST DOXORUBICIN INDUCED MYOCARDIAL DAMAGE IN WISTAR RATS

A Dissertation submitted to

THE TAMIL NADU Dr. M.G.R MEDICAL UNIVERSITY

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In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY
IN
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Submitted by

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MAY 2019

CERTIFICATE

This is to certify that this dissertation work entitled **"CARDIOPROTECTIVE EFFECT OF *DELONIX ELATA* (L.) LEAF EXTRACT AGAINST DOXORUBICIN INDUCED MYOCARDIAL DAMAGE IN WISTAR RATS"** submitted by **Reg. No.261726051** in partial fulfillment of the requirements for the award of degree in Master of Pharmacy in Pharmacology by The Tamil Nadu Dr. M.G.R. Medical university, Chennai-600 0032, is a bonafide work done by his during the academic year 2018-2019 under the guidance of Dr.Indumathy,M.Pharm.,Ph.D, Assistant Professor, Institute of Pharmacology, Madras Medical College,Chennai-600003.

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ABBREVIATIONS

CVD	- Cardiovascular Disease
DALY	- Disability-adjusted Life Year
LMIC	- Low and Middle Income Country
HIC	- High Income Country
ROS	- Reactive Oxygen Species
IHD	- Ischemic Heart Disease
CHF	- Congestive Heart Failure
DNA	- Deoxyribonucleic Acid
RNA	- Ribonucleic Acid
VCAM-1	- Vascular Cell Adhesion Molecule - 1
LDL	- Low Density Lipoprotein
HDL	- High Density Lipoprotein
CRP	- C-reactive Protein
DOX	- Doxorubicin
CK-MB	- Creatinine Kinase - Muscle/Brain
HK	- Hexokinase
LDH	- Lactate Dehydrogenase
CTN	- Cardio Troponin
MI	- Myocardial Infarction
ECG	- Echocardiography
MYO	- Myoglobin
CT	- Computed Tomography
ACE	- Angiotensin Converting Enzyme
ARB	- Angiotensin Receptor Blocker
PG	- Prostaglandin

NO	- Nitric Oxide
ALL	- Acute Lymphocytic Leukemia
AML	- Acute Myeloid Leukemia
CLL	- Chronic Lymphocytic Leukemia
SOD	- Superoxide Dismutase
DPPH	- 1, 1-diphenyl-2-picrylhydrazyl
MTT	- 3-(4, 5 dimethyl thiazole 2yl) - 2,5- diphenyl tetrazolium bromide
RSA	- Radical Scavenging Activity
IAEC	- Institutional Animal Ethics Committee
CPCSEA	- Committee for the Purpose of Control and Supervision of Experiments on Animals
OECD	- Organisation for Economic Co-operation and Development
RBC	- Red Blood Cell
WBC	- White Blood Cell
ADP	- Adenosine Diphosphate
ATP	- Adenosine Triphosphate
EDTA	- Ethylene diamine tetra acetic acid
CAT	- Catalase
GPx	- Glutathione Peroxidase
TBARs	- Thiobarbituric acid reactive Substance
MDA	- Malondialdehyde
ANOVA	- Analysis Of Variance

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1. INTRODUCTION

Cardiovascular Diseases (CVDs) represents the major cause of morbidity and mortality globally, as well as in India. Over the past decade, cardiovascular disease has emerged as the single and most important cause of death world wide. CVDs takes the lives of 17.9 million peoples every year, 31% of all global deaths[1]. According to world health report 2002, CVDs will be the large cause of death and disability by 2020 in India[2]. In 2010, CVD caused an estimated 16 million deaths and led to 293 million disability-adjusted life years (DALYs) lost- accounting for approximately 30% of all deaths and 11% of all DALYs lost. Like many high-income countries (HICs) during the past century and now, low and middle income countries (LMICs) are seeing an alarming and accelerating increase in CVD rates[3]. CVDs caused more than 2.1 million deaths in India, in 2015 at all ages or more than a quarter of all deaths. Nearly half of this deaths are likely to occur in the age of 30-69 years of total population[4]. A perusal of recent epidemiological survey indicates that urbanization/ changes in life style may also increase the risk of CVD[5]. Most CVDs can be prevented by addressing behavioural risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol using population-wide strategies. People with CVD or who are at high cardiovascular risk (due to the presence of one or more risk factors such as hypertension, diabetes, hyperlipidaemia or already established disease) need early detection and management using counselling and appropriate medicines[6].

CVD is a group of disorders or diseases of the heart and blood vessels, which also include atherothrombotic diseases such as myocardial and cerebral infarction commonly called as heart attack and stroke respectively. The sequence of events leading to CVD includes endothelial dysfunction, atherosclerotic plaque formation, and rupture of atherosclerotic lesion[7]. Types of CVD include coronary heart disease, stroke, rheumatic heart disease, deep vein thrombosis and pulmonary embolism and congenital heart disease.

Atherothrombotic diseases such as MI and cerebral infarction are serious consequences of a thrombus formed in blood vessels. MI is the end result of the accumulation of atheromatous plaques within the walls of the coronary arteries that supply the myocardium with oxygen and nutrients[8].

Damage to the myocardial cells arises due to generation of toxic reactive oxygen species (ROS) such as super oxide radical, hydrogen peroxide and hydroxyl radical[9]. ROS (Reactive Oxygen Species) is a key factor causing many diseases like cancer, CVDs, acceleration of ageing and immunological disorders. ROS causes oxidative damage to variety of cellular components which displays a significant role in the etiology of several cardiovascular diseases[10]. Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage[11].

Adriamycin (also named as doxorubicin) is an anthracycline antibiotic that has been used for more than 30 years for the treatment of a wide variety of cancers. It is obtained from Streptomyces peucetius, although a total chemical synthesis is now possible. The tumours that respond better to adriamycin are breast and esophageal .carcinomas, osteosarcoma, kaposi's sarcoma, soft-tissue sarcomas, and Hodgkin's and non- Hodgkin's lymphomas. The clinical use of DOX is often limited because of its undesirable serious cardiotoxic side effects, which frequently lead to congestive heart failure, Oxidative stress causes increased toxicity in the cardiomyocytes, irreversible damage of DNA and alteration of cardiac energetic levels[12].

Modern drugs although effective in preventing the disorders, are of limited use owing to their adverse reactions. A wide array of plants and its phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties with minimal side

effects. Moreover, the plant kingdom represents a largely unexplored reservoir of biologically active compounds[13]. The herbal medicines are effective in the treatment of various ailments. With only a few exceptions, most herbal treatments have not been tested for safety and efficacy utilizing scientific studies or clinical trials. The herbal drugs are unscientifically exploited and are improperly used. Therefore these plant drugs deserve detailed studies in the light of modern science. The detailed investigation and documentation of plants used in local health traditions and pharmacological evaluation of these plants can lead to the development of invaluable plant drugs for many dreaded diseases.

The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds, many of which have been the basis for the development of new lead chemicals for pharmaceuticals.

Many of the herbal plants are claimed for the cardioprotective property and few of the species were scientifically proved. Based on the active phytochemical constituents such as flavonoids, cardiac glycosides, Terpenoids and saponins were present in *Delonix elata* and are known to responsible for antioxidant activity. Hence, this plant may be considered to be potential cardioprotective effect. The objective of the present study was designed to investigate the protective effect of the *Delonix elata* leaf extract on experimentally induced myocardial toxicity.

2. DISEASE PROFILE

INTRODUCTION[14]

The heart is a muscular pump that ejects blood into the vascular tree with sufficient pressure to maintain optimal circulation. Heart is divided into four chambers : a right and left atrium both lying superiorly and a right and left ventricle both lying inferiorly and are larger.

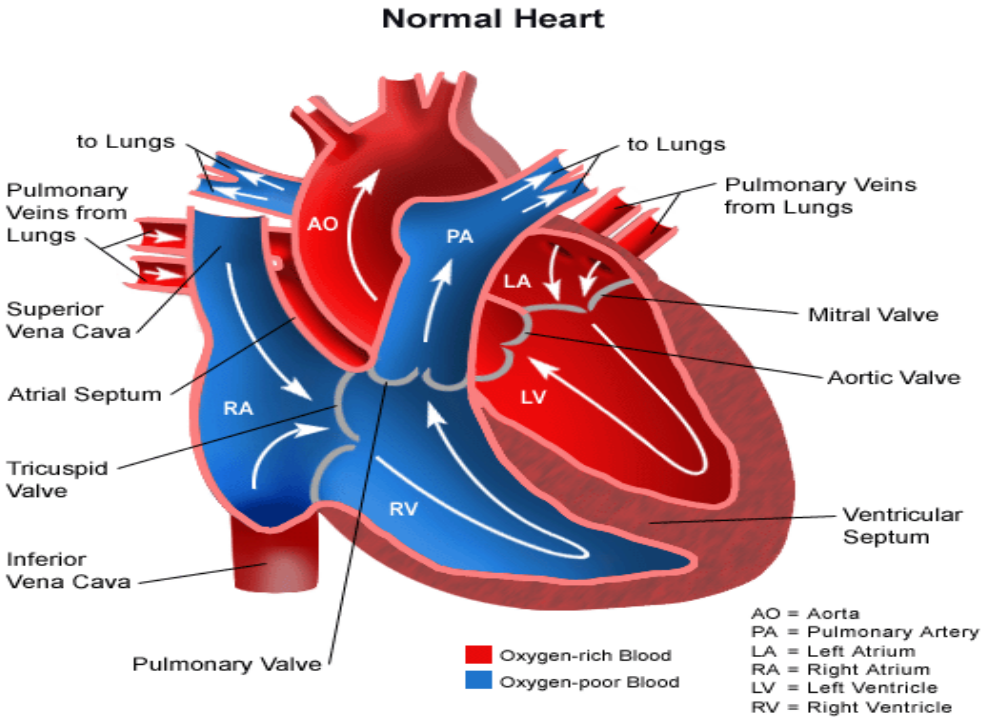
The blood in the heart chambers moves in a carefully prescribed pathway:

Venous blood from systemic circulation → Right atrium → Right ventricle → Pulmonary arteries → Lungs → Pulmonary veins → Left atrium → Left ventricle → Aorta → Systemic arterial supply

The transport of blood is regulated by cardiac valves: two loose flap like atrioventricular valves, tricuspid on the right and mitral (bicuspid) on the left and two semilunar valves with three leaflets each, the pulmonary and aortic valves guarding the outflow tracts.

Wall of the heart consists maily of the following three layers. They are, Myocardium - covered externally by thin membrane; Epicardium and Endocardium - lined internally by another thin layer.

Figure 1: Normal heart structure



Myocardium is the muscle tissue of the heart composed of syncytium of branching and anastomosing transversely striated muscle fibres arranged in parallel fashion. The space between myocardial fibres contains rich in capillary network and loose connective tissue and they are connected to each other by irregular joints (intercalated discs). The cardiac myocyte is very rich in mitochondria which are the source of large amount of ATP required for cardiac contraction. The cardiac muscle fibre has abundant sarcoplasmic reticulum corresponding to endoplasmic reticulum and other cells.

CARDIOVASCULAR DISEASES[15]

Ischemic heart disease: IHD represents a group of pathophysiologically related syndromes resulting from myocardial ischemia - an imbalance between myocardial supply and cardiac demand for oxygenated blood.

IHD can present as one or more of the following clinical syndromes: Atherosclerosis, Angina pectoris and Myocardial infarction.

Atherosclerosis: A process of progressive thickening and hardening of the walls of medium-sized and large arteries as a result of fat deposits on their inner lining.

Angina pectoris: It is a pain syndrome due to induction of an oxygen supply and demand situation in a portion of the myocardium.

Myocardial infarction: It is commonly referred to as "heart attack", the death of cardiac muscle due to prolonged severe ischemia.

Arrhythmia: Abnormalities in myocardial conduction can be sustained or sporadic (paroxysmal).

Cardiomyopathy: Cardiomyopathies are a heterogenous group of disorders of the myocardium associated with mechanical and/or electrical dysfunction that usually exhibit an inappropriate ventricular hypertrophy.

Myocarditis: It is an inflammatory process cause myocardial injury.

Heart failure: It often called Congestive heart failure (CHF), is a common usually progressive condition with a prognosis. The heart is unable to pump blood at a rate sufficient to meet the metabolic demands of the tissues.

Hypertensive heart disease: It is the disease of the heart resulting from systemic hypertension of prolonged duration and manifesting by left ventricular hypertrophy.

OXIDATIVE STRESS IN CVD

The stress purported by the various reactive oxygen species (ROS) which threaten to oxidize biological moieties involved in various organ systems and thus inducing damage is called "oxidative stress". Oxidative stress represents an imbalance between the production of ROS and a biological system's ability to readily detoxify or repair the resulting damage.

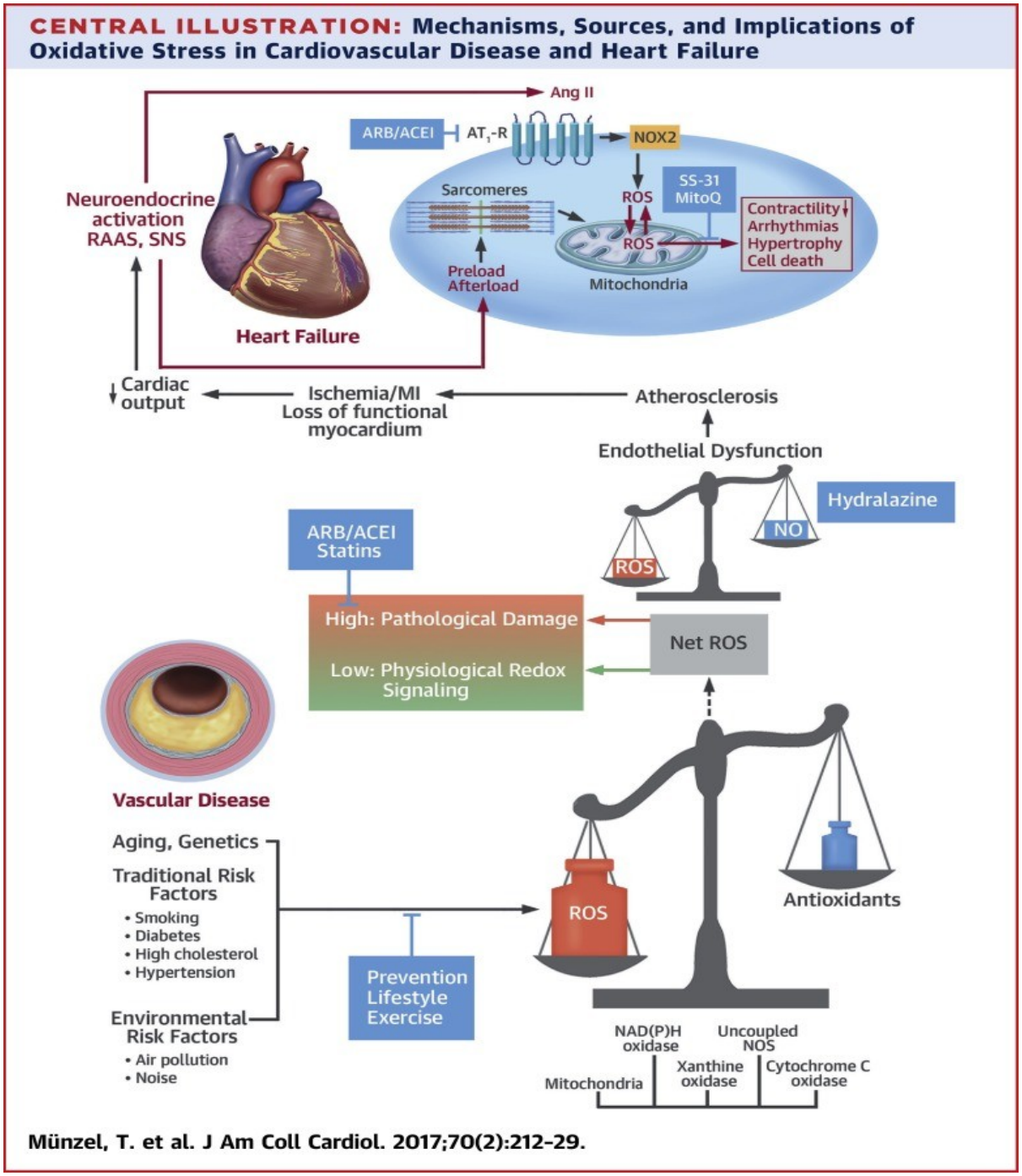
The ROS are highly active chemical moieties, which contain oxygen and are unstable radical like hydrogen peroxide (H_2O_2) and superoxide (O_2) are produced in a number of cellular reactions by various enzymes such as lipoxygenases, peroxidises, NADPH oxidase and xanthine oxidase as a result of energy production from mytochondria.

Oxidative stress has the potential to be harmful to the human body and its cells by damaging lipids, proteins, RNA and DNA, etc,. Oxidative stress associated with number of diseases including Cancer, CVDs, diabetes, immunological disorders and also acceleration of ageing process.

The vascular endothelium is extremely sensitive to oxidative damage mediated by reactive oxygen metabolites, which play a role in the pathogenesis of atherosclerosis by an important contribution to plaque development[16,17]. Oxygen free radicals were implicated in Ischemia-reperfusion injury to endothelium of heart, lung and other vital organs and contributing to development of atherosclerosis and pulmonary toxicity. Endothelial injury due to oxidative stress was believed to be the result of calcium influx and resultant activation of calcium dependent proteases[18]. Oxidative stress, when associated with electrocardiographic aspects of coronary artery disease indicates an accelerated course of atherosclerosis in the onset phase and an additional thrombogenic risk in the ischemic disease

phase and when associated with chronic cardiac failure, it indicates a progressive form with replacement fibrosis.

Figure 2: Oxidative stress in CVD



The role of a molecular linkage between an antioxidant sensitive transcriptional regulatory mechanism and VCAM-1 gene expression expanding on the notion of oxidative stress and it being implicated in genesis of atherosclerosis[19,20].

Oxidative stress indicators were found to be abnormal in unstable angina. Free radicals and toxic oxygen species were proven experimentally to bear adverse effects of myocardial contractile dysfunction, myocardial electrical stability, endothelial mediated vaso dilation and coagulation[21].

Increased blood pressure cause continuing adaptive responses in the blood vessels, be it larger arteries or the microvasculature. The normal endothelium exhibits an inhibitory influence on vascular smooth muscle cell growth, which when becomes dysfunctional, contributes to vascular smooth muscle growth and narrowing of the lumen.

Hyperlipidaemia induces oxidative stress in the arterial wall to trigger the development of hypertension, presumably by inactivating endothelium derived nitric oxide and thus mitigating vasodilator mechanism. An elevation of blood pressure was the destruction of nitric oxide by excessive production of oxygen free radicals.

RISK FACTORS

Recently, two major studies reporting observations from nearly 500,000 patients have emphasized the importance of multiple risk factor management by establishing that over 80% of patients with coronary heart disease have at least one of the major risk factors. Clearly, early diagnosis and active management of multiple risk factors are the goals of cardiovascular care; too many people remain undiagnosed and untreated for these conditions while the evidence shows that CVD is preventable. The majority of CVD is caused by risk factors that can be controlled, treated or modified, such as high blood pressure, cholesterol,

overweight/obesity, tobacco use, lack of physical activity and diabetes. Life style changes can reduce the risk for heart diseases[22,23].

Most risk factors for heart disease are related to lifestyle and environmental factors. There are several risk factors for heart diseases: some are modifiable, others are non-modifiable risk factors include gender, age, family history of heart disease, post menopausal, race. Controllable risk factors include smoking, low density lipoprotein (LDL), and high density lipoprotein (HDL), uncontrolled hypertension (high blood pressure), physical activity, obesity, uncontrolled diabetes, stress, anger, alcohol, high-C reactive protein (CRP) values (it is only present during episodes of acute inflammation).

Non-modifiable risk factors[24,25]

Gender

Coronary heart diseases are much more common in middle aged man. Women are likely to have angina than men. Younger women with heart disease often do not have the same symptoms as their male counterparts and may be less likely to be diagnosed.

Age

Age is among the strongest risk factor and its relationship with cardiovascular mortality is exponential. It is an important factor to consider in total cardiovascular risk estimation, but its non-modifiable nature limits its use in the management of risk. Other non-modifiable risk factors are gender and a family history of premature CVD. Total risk levels in women tend to resemble those of men 10years younger. Thus, risk is merely differed by 10years and ultimately more women than men die from CVD.

Sedentary lifestyle

Systemic reviews and meta-analysis of observational studies have evidenced reduced cardiovascular mortality risk in physically active subjects. All available evidence indicates that the association between physical activity pattern and CVD is casual. Physical activity has

both a direct protective effect on the development of risk for CVDs. Physical inactivity increases the risk of heart disease and stroke by 50%. Obesity is a major risk for cardiovascular disease and predisposes you to diabetes and diabetes is a risk factor for CVD

Modifiable risk factors[26-28]

The major modifiable risk factors are smoking, cholesterol and other lipids, uncontrolled hypertension, physical inactivity, obesity, uncontrolled diabetes, high CRP, uncontrolled stress and anger, ROS.

Hypertension is the single biggest risk factor for stroke. It also plays a significant role in heart attacks. It can be prevented and successfully treated but only if diagnose and stick to recommended management plans.

Abnormal blood lipid levels that include high total cholesterol, high levels of triglycerides, and low-density lipoprotein or low levels of high-density lipoprotein (HDL) cholesterol which increases the risk of heart disease and stroke. Changing to a healthy diet, exercise and medication can modify blood lipid profile.

Tobacco use increases risks of CVD. The risk is especially high in young smokers and heavily smoking woman. Passive smoking is also a risk factor for CVD. Stopping tobacco use can reduce the risk of CVD significantly; no matter how long one smoked.

CARDIAC MARKERS IN THE DIAGNOSIS OF MYOCARDIAL INFARCTION[29]

The analysis of cardiac biomarkers has become the frontline diagnostic tools for AMI, and has greatly enabled the clinicians in the rapid diagnosis and prompt treatment planning, thereby reducing the mortality rate to a great extent.

- Creatine kinase
- Lactate dehydrogenase (LDH)
- Troponins
- Myoglobin

Creatine kinase

It is an enzyme chiefly found in the brain, skeletal muscles, and heart. An elevated level of creatine kinase is seen in heart attacks, when the heart muscle is damaged, or in conditions that produce damage to the skeletal muscles or brain. There are three different forms of creatine kinase that can be measured.

- CK-MM (located in the skeletal muscles and heart)
- CK-MB (mainly located in the heart)
- CK-BB (located in the brain)

An elevated level of creatine kinase, specifically CK-MB, occurs within hours of a heart attack as the heart muscle cells die. The enzyme level continues to rise for the first 18 to 24 hours after a heart attack and slowly returns to normal after a few days. Trauma and other conditions that damage the skeletal muscle are also associated with an elevated creatine kinase level. In some cases, the test may be used to detect muscle conditions such as polymyositis (condition characterized by the inflammation of muscles) or to estimate the degree of muscle damage. Stroke and other forms of brain damage can also result in an elevated creatine kinase level. Creatine kinase (CK) has several functions in cellular energy metabolism. It catalyzes the reversible transfer of high-energy phosphate from ATP to creatine, facilitating storage of energy in the form of phosphocreatine. In muscle cells, this extra energy buffer plays a pivotal role in maintaining ATP homeostasis.

Lactate dehydrogenase

Lactate dehydrogenase (LDH) is an enzyme present in many different cells. There are five isoenzymes, each with different specificities for different types of tissue. In the case of cardiac injury, LDH isoenzyme1 is higher than isoenzyme-II. But under normal circumstances, isoenzyme II is present in greater amounts than isoenzyme I. The LDH starts going up in 12-24 hours following an MI, and it dissipates within a week or two. This test has been supplanted by the other markers.

Troponins

Troponins are the best overall markers; they have the best combination of sensitivity, specificity, and ease of test performance of all the markers. They are 9 complex of 3 protein subunits located on the thin filaments of the skeletal and cardiac muscle fibers. They are;

• Troponin C -Calcium-binding component in the skeletal and cardiac muscle, but it is not extremely specific for myocardial injury.

• Troponin T - Tropomyosin-binding component

• Troponin I - Inhibitory component

The isoforms of troponin-T and troponin-I differ in the skeletal and the cardiac muscle, and thus are extremely specific for cardiac tissue necrosis. Troponin-T is present chiefly in the bound form to the contractile elements of the myocardial cells and it is also present free in the cytoplasm.

Troponin-T exhibits a dual release initially of the cytoplasmic component and later of the bound component. Troponin-I is extremely specific for the cardiac muscle and has not been isolated from the skeletal muscle. This absolute specificity makes it an ideal marker of myocardial injury. They are released into the circulation 6-8h after myocardial injury, peak at 12-24h and remain elevated for 7-10days.

Troponins are components of cardiac muscle that are released into the blood when myocardial cells are injured. They are very specific for myocardial muscle even more specific than CK-MB. Troponins go up within 3-12 hours after the onset of MI.

Myoglobin (MYO)

Myoglobin is a small cytoplasmic oxygen-binding protein found in the skeletal as well as the cardiac muscle. It is released extremely early into the serum, 1h after the onset of myocardial injury, peaks at 4-12h and returns to baseline values immediately. The major disadvantage of myoglobin is the lack of specificity to the cardiac tissue due to the presence of large amounts of MYO in the skeletal muscle. The levels of MYO can therefore not be used as a single diagnostic marker, but in conjunction with the troponins or CK-MB. Thus, serum levels of MYO can be used to rule out, rather than diagnose, myocardial infarction.

DIAGNOSIS[30,31]

The diagnosis of CVD is performed after assessing patient's complaints and performing a general physical examination. Changes in the ECG, coronary angiogram and the levels of cardiac biomarkers help to confirm the diagnosis of CVD. Through ECG, the site of myocardial damage can be identified.

Firstly, a chest radiograph and routine blood test will be done to diagnose CVD. An echocardiogram also confirms the presence of CVD. Imaging techniques such as myocardial perfusion imaging, stress echocardiography are used to confirm CVD.

Patients who are stable can be diagnosed using Technetium (99mTc) sestamibi ("MIBI scan") or thallium-201 chloride can be used in nuclear medicine to visualize areas of reduced blood flow in combination with physiologic or pharmacologic stress.

Echocardiography

Echocardiography is an outstanding real-time imaging technique with reasonable spatial and temporal resolution. Its strong point is the evaluation of myocardial thickness, thickening and motion at rest. This can be aided by tissue Doppler imaging.Echocardiographic contrast agents can advance endocardial visualization, but contrast studies are not yetfully authenticated for the detection of myocardial necrosis, although early work is heartening.

Radionuclide imaging

Several radionuclide tracers permit viable myocytes to be imaged unswervingly, including thallium-201, technetium-99m MIBI, tetrofosmin, and [18F] 2 fluorodeoxyglucose (FDG). The vigor of the techniques are that they are the only regularly available direct methods of evaluating viability, although the moderately low resolution of the images disadvantages them for perceiving small areas of infarction. The regular single photonemitting radio-pharmaceuticals are also tracers of myocardial perfusion and so the techniques readily sense areas of infarction and inducible perfusion abnormalities. ECG-gated imaging offers are liable assessment of myocardial motion, thickening and global function.

Magnetic resonance imaging[32]

The specific change in the resonating frequency of a nucleus in different chemical compounds is the basis of NMR signal. In Professor R Damadien and P Lautebur described the concept of magnetic resonance imaging and in 1973, 2D MR image of a heterogenous object was obtained. MRI depends on the tissue behaviour when placed in an external magnetic field. The wide range of tissue contrast provides the potential for myocardial tissue characterization. This produces excellent delineation of soft tissue pathology in the myocardium. The recent advancement of intravenous contrast agent (gadolinium) and faster

imaging technology has enabled MR angiography, which has opened up newer applications in Coronary artery disease (CAD).

X-Ray computed tomography[33]

Infarcted myocardium is originally visible to CT as a focal area of diminished LV enhancement, but later imaging shows hyper improvement as with late gadolinium imaging by MRI. This verdict is clinically pertinent because contrast-enhanced CT may be executed for alleged embolism and aortic dissection, circumstances with clinical features that overlap with those of acute myocardial infarction.

TREATMENT[34,35]

Vasodilatating agents

Organic nitrates like Nitroglycerine, Isosorbidedinitrate are used to decrease the ultimate size infarct and preserves the viable tissues by reducing the oxygen demand of the myocardium.

α & β - blockers

It produces vasodilatation and has additional antioxidant/ free radical scavenging activity.

ACE inhibitors

ACE inhibitors increases the cardiac work. These agents reduce the long time mortality. It can be safely used in patients with IHD.

Calcium channel blockers

Calcium channel blockers are used to reduce the intracellular calcium level. Ca2+ agents will alters the contraction of cardiac and smooth muscle contraction by blocking the entry of the ca2+ into the myocytes.

Angiotensin receptor blockers (ARBs)

ARBs causes vasodilation, myocardial cell apoptosis, fibrosis intercellular matrix changes and remodelling and preventing ventricular hypertrophy. It decreasing Ang II production and increase the level of kinins which stimulate generation of cardioprotective NO and PGs.

Cardiac glycosides

Cardiac glycosides has increased the efficiency of failing heart. It increase the myocardial contractility and output ina hypodynamic heart without a proportionate increase in O_2 consumption.

Analgesics

In this condition opioid analgesic like morphine injection is used to decrease the pain and apprehension.

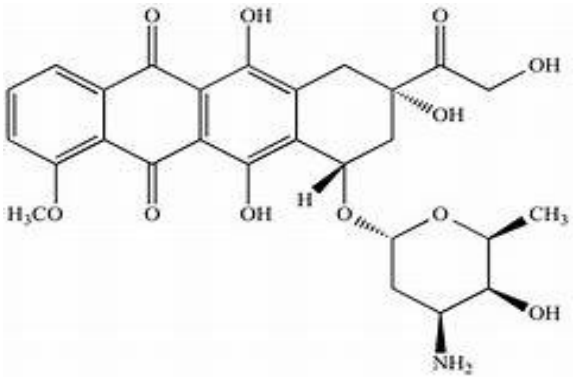
DOXORUBICIN

Doxorubicin (Adriamycin) was the first anthracycline antibiotic isolated from Streptomyces peucetius in the year of 1963 by both Italian and French group of scientists. Italian group isolated natural product doxorubicin from Streptomyces peucetius var. caesius. The French group produced semi synthetic derivatives. Doxorubicin exerts broad-spectrum activity against a variety of hematologic malignancies and solid human cancers. These drugs have the ability to produce free radicals and cause an irreversible, unusual cardiomyopathy which is related to exposure of the total dose of the drug[36].

CHEMICAL STRUCTURE[37]

The anthracyclines have a tetra cyclic ring structure attached to daunosamine which is an unusual sugar. These drugs having quinone and hydroxyquinone moieties on neighbor rings which permit the loss and gain of electrons. Their chemical structures differ from daunorubicin by only a single hydroxyl group on Carbon -14.

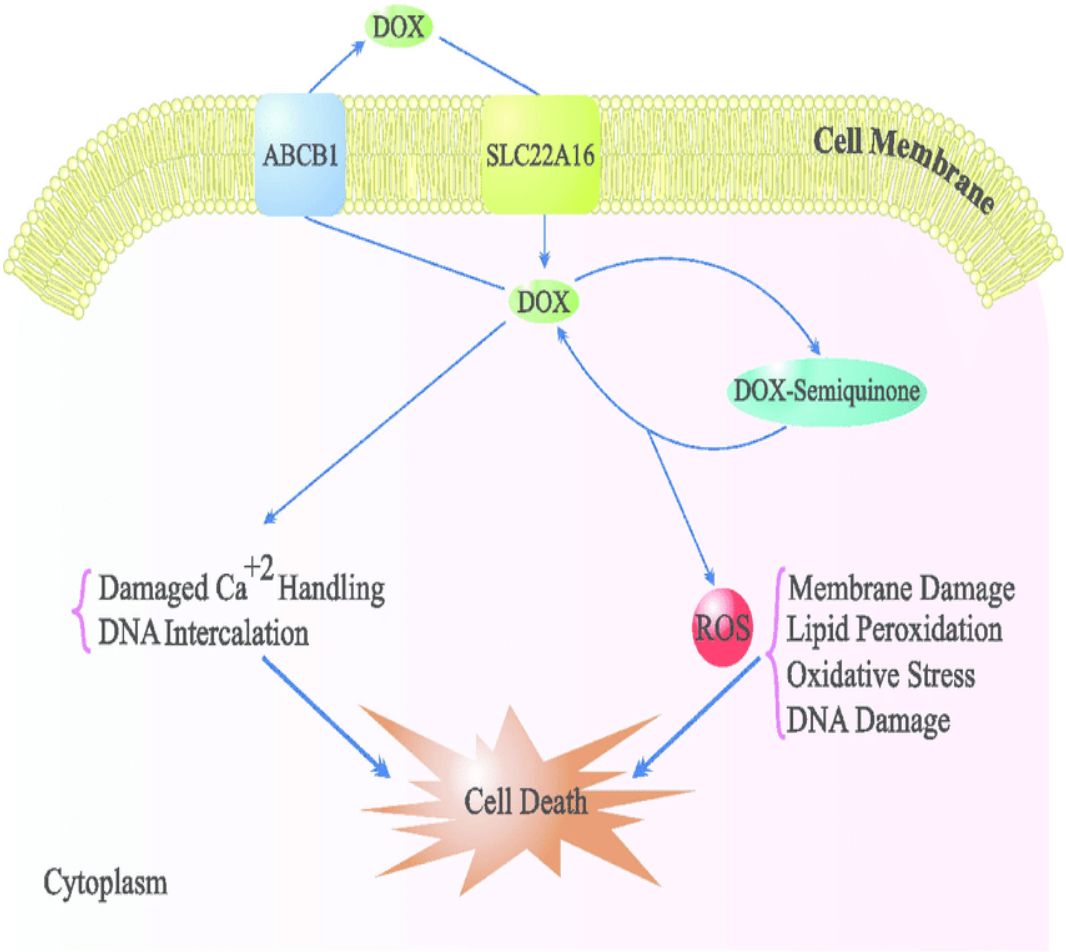
Figure 3: Chemical structure of Doxorubicin



MECHANISM OF ACTION[38]

Anthracyclines are directly affecting the transcription and replication of the neoplastic cells by intercalating with DNA. Their important action is mediated by their ability to form a tripartite complex with DNA and topoisomerase II. Topoisomerase II is an ATP-dependent enzyme that binds to DNA and causes double-strand nicks at the 3'-phosphate backbone and allowing strand passage and uncoiling of super-coiled DNA. Then topoisomerase II religates the DNA strands. This enzymatic function is important for DNA replication and repair. The tripartite complex formation with anthracyclines or with etoposide inhibits the re-ligation of the broken DNA strands, which leads to apoptosis. Any defect in DNA double-strand break repair sensitizes neoplastic cells to damage by these drugs. But over expression of transcription-linked DNA repair may lead to drug resistance.

Figure 4: Mechanism of action - Doxorubicin



Anthracyclines generates free radicals in solution and in both normal and malignant tissues because of their quinone moieties. Anthracyclines can form semiquinone radical intermediates that can react with O_2 to produce superoxide anion radicals which is responsible of their antitumor activity and also its cardiotoxicity.

USES

Hodgkin' s lymphoma, ALL , AML, CLL, Non Hodgkin' s lymphoma, Multiple myeloma, Mycosis fungoides, Mantle cell lymphoma, Kaposi sarcoma, Breast cancer (adjuvant and advanced disease), Advanced Prostate cancer, Gastric cancer, Ewing's sarcoma, Thyroid cancer.

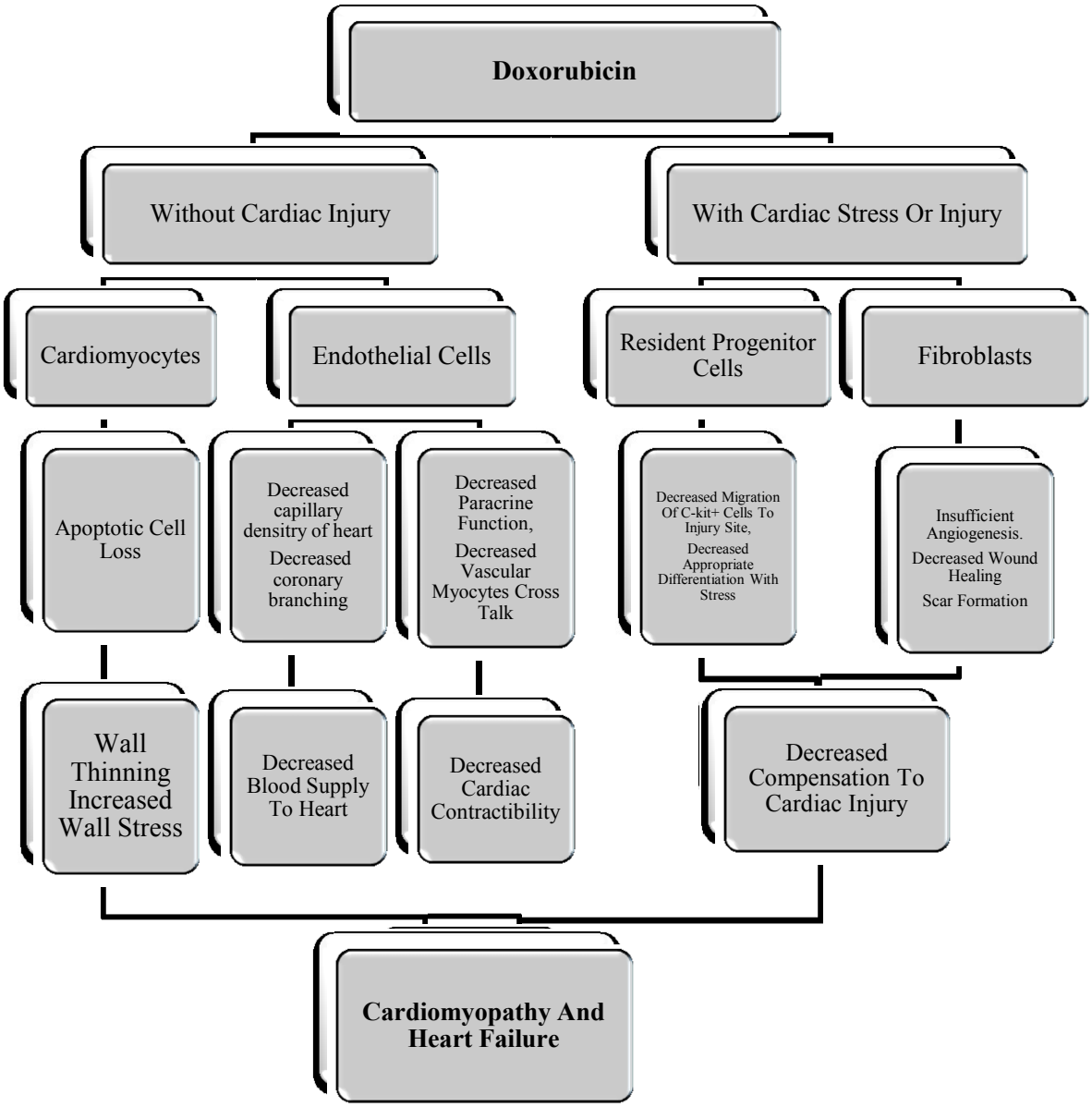
In combination with cyclophosphamide, it is an important component of different regimens used as adjuvant chemotherapy and in metastatic carcinoma of the breast.

DOXORUBICIN INDUCED CARDIOTOXICITY[39,]

The anthracyclines which are currently approved in the united states (US), doxorubicin, daunorubicin, epirubicin, idarubicin and a related compounds. All anthraxcyclines are associated with both early and late toxicity. Early toxicity may be manifested as a myopericarditis with non specific ST- segment and T-wave abnormalities on the ECG; arrhythmia may be part of the clinical presentation. Late anthracycline toxicity is cumulative dose related, and at sufficiently high dosages, it may result in LV dysfunction leading to life threatening heart failure. The mechanism is thought to be direct myocardial injury because of free radical formation. The incidence of cardiomyopathy increases significantly for patients who receive cumulative doses of Doxorubicin that exceed 450mg/m^2.

Anthracyclines cause a unique pattern of histologic cardiac changes, including the vacuolization of myocardial cells, myofibrillar disarray and loss, necrosis.

Figure 5: Effect of Doxorubicin in Cardiovascular System



MECHANISM OF ANTHRACYCLINE INDUCED CARDIOTOXICITY[40]

The mechanism of the cardiac damage caused by anthracycline exposure has not been fully elucidated, however free radical formation is believed to be an important factor for cardiotoxicity.

Free radicals can injure lipid structures in the myocardial cell, and the resultant peroxidation of thes structures impairs the function of the sarcoplasmic reticulum and mitochondria. Cardiac myocytes are more prone to these degenerative changes, since they lack catalase, superoxide dismutase and they are less able to metabolize free radicals than other cells. Cell necrosis is end result of this cardiac damage.

There is an increasing evidence that the generation of the oxygen free radicals is mediated through and Fe-doxorubicin complex. Doxorubicinol is one of the principal metabolite of Doxorubicin, and has a greater effect on the calcium pump of the sarcoplasmic reticulum than does the parent compound, which suggests that the antitumor effect is distinct from the cardiotoxic effect.

DIAGNOSIS[41,42]

Endomyocardial biopsy is the most sensitive method

Recently the use of biomarkers of injury - BNP , hs-TnI

Serologic tests include a complete lipid count and blood glucose level

THERAPEUTIC AGENTS[35]

ACE inhibitors, ARBs, β-blockers, loop diuretics, aldosterone antagonist, vasopressin antagonist, nitrates & hydralazine, digoxin.

Cardiac devices such as permanent pacemaker, implantable cardiac defibrillator, biventricular pacemaker and ultrafiltration.

3. REVIEW OF LITERATURE

Phytochemical investigation of *Delonix elata* (L.) Gamble

- NT Selvi *et al* (2015)., have been investigated the phytochemical constituent evaluation of the *Delonix elata* leaf extracts like aqueous, ethanol, acetone, petroleum ether and chloroform extracts. Phytochemical screening detected the presence of active ingredients such as tannins, saponins, quinones, terpenoids, steroids, flavonoids, phenols, alkaloids,cardiac glycosides, coumarins and betacyanin in the leaf extracts[43].

- Aravindhan TR *et al* (2015)., have been analyzed the phytochemical constituents present in the leaves of *Delonix elata* extracted with five different solvents like aqueous, ethanol, chloroform, petroleum ether and acetone. The resultant extract was shows the presence of phenols, cyanins, quinones, terpenoides, tannins, flavonoids, steroids and glycosides and aqueous extract was found to be rich in phytochemical constituents and it can be used for obtaining therapeutic benefits[44].

- Murugananthan and Shamanna (2014)., have been studied the preliminary phytochemical constituents of *Delonix elata* bark extracted using 70% alcohol and determined total phenolic and flavonoidal content using UV/Visible spectrophotometer. An equivalent 75 mg/gm of the total polyphenol and 57.4 mg/gm of the flavonoid content was found in the alcoholic extract. Apigenin and caffeic acid were isolated and characterized for the first time and these constituents are found in *Delonix elata* bark where comparable to other plant-based sources[45].

- Yashwanth kumar D R and Joy Hoskeri H (2013)., have been reported the phytochemical constituents and pharmacological activities of *Delonix elata* that belong to the family Fabaceae. They concluded that plant possess array of biological and pharmacolgical properties which includes antibacterial activity, anti-inflammatory activity, psychosomatic maedicinal for scorpion bite, antimalarial activity, anti-ovicidal activity, antioxidant and chelating activity[46].

Pharmacological investigation of *Delonix elata*(L.) Gamble

Antioxidant activity

- Telkit G A *et al* (2016)., have been evaluated the phytochemical constituents and have high levels of total phenolic and flavonoid compounds of flower extract of *Delonix elata.* The flower extract contain saponin, alkaloid, terpenoids, flavonoids, steroids, phenols, cardio glycosides, quinine coumarins and Tannins. They possessed strong antioxidant potential and was thus capable of inhibiting, quenching free radicals to ter-minate the radical chain reaction[47].

- Amala B and Poonguzhali T V (2015)., have studied the phytochemical constituent, total phenol, flavonoid and antioxidant activity of *Delonix elata* flower extract. Antioxidant activity was determined by the method of DPPH free radical scavenging assay. The flower extract possessed strong antioxidant potential and was thus capable of inhibiting, quenching free radicals to terminate the radical chain reaction and the plant material would be helpful in prevention of various oxidative stresses[48].

- Babu K *et al* (2015)., have been evaluated the phyto-chemical constituents, estimation of tannin and anti-oxidant activity from the leaf extract of *Delonix elata* Preliminary screening involved the qualitative methods to detect the presence of terpenoids, flavonoids, phenols, tannins, steroids, quinones, saponins, cardiac glycosides and alkaloids. *In vitro* antioxidant activity of petroleum ether, chloroform, acetone, aqueous and ethanol extracts was evaluated by studying (DPPH) 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity using the standard procedure[49].

- Suriyavathana M and Sivanarayan V (2013)., have analyzed the various medicinal plants (C*assia auriculata, Catharanthus roseus,* ***Delonix elata****, Hibiscus sabdariffa, Mukia maderasptna, Ruellia tuberosa, Vitex negundo*) of their phytochemicals and antioxidant activity. Methanolic extract of these plants showed high antioxidant activity and *Delonix elata* possessed the highest percent inhibition of DPPH, reducing power, nitric oxide scavenging activity and hydroxyl radical scavenging activity amongst all studied plants. The presence of substantial amount of bioactive constituents comprising phenol content (31.7mg in 0.5 ml of 1:10 g ml-1) and flavonoids (29.4mg in 0.5 ml of 1:10 g ml-1) where *Delonix elata* was observed comparatively richer source of these phytochemicals[50].

- Chitra M *et al* (2011)., have been evaluated the antioxidant activity of ethanolic extract of *Delonix elata* L. leaf was studied for its free radical scavenging on different *in-vitro* models like 1,1-diphenyl 1-2-picryl hydrazine (DPPH), hydrogen peroxide, total antioxidant capacity and peroxy radical model. The extract showed good dose-dependent free radical scavenging property and antioxidant property when compared to standards. The study indicated that the leaves are a source of natural antioxidant[51].

Antimicrobial activity

- Pradeepa K *et al* (2012)., have screened the *in-vitro* antibacterial activity of *Delonix elata* leaf extract against both gram positive and gram negative bacteria. The methanol extract of leaf showed significant antibacterial activity against *Klebsiella pneumonia* (18.40 ± 0.80) and *Bacillus subtilis* (17.60 ± 0.55) with minimal inhibitory concentration of 1mg ml^{-1} and exhibited less bactericidal activity against *P. vulgaris* (9.83±0.31). *In silico* docking of Luteolin, a bioactive compound from leaf extract with a key enzyme of bacterial glucosamine-6-phosphate synthase, showed significant inhibition with minimum docking energy -10.12 kJmol-1, binding energy -10.07 kJ mol-1 and inhibition constant 4.14e-008. The molecular docking study was comparatively evaluated with the standard drug ciprofloxacin with an inhibition constant 4.2e-007[52].

- M. Vijayasanthi and V. Kannan (2014)., have been evaluated the methanol and aqueous extracts of *Delonix elata* and *Spathodea campanulata* for their antimicrobial activities against nine bacterial species: *Bacillus cereus, Staphylococcus aureus, Streptococcus pneumonia, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi, Proteus vulgaris* and *Shigella flexneri* and two fungal species: *Aspergillus niger* and *Candida albicans*. The susceptibility of the microorganism to the extracts of these plants was compared with each other and with selected antibiotics. The methanol extract of these two plants showed zone of inhibition against isolated human pathogens with varied diameter[53].

Anti-hyperlipidemic activity

- Sajja Ravindra Babu and Priyanka Goud K (2018)., have been investigated the antihyperlipidemic activity of aerial part of ethanolic extract of *Delonix elata* (EEDE) in high-fat diet induced hyperlipidemic rats. The ethanolic extract has the ability to reduced triglycerides levels, by stimulating the lipolytic activity of plasma lipoprotein lipase and reduction in oxidative stress may be responsible for the antihyperlipidemic activity[54].

Anti inflammatory activity

- Amala B and Poonguzhali T V (2015)., have studied the phytochemical screening and *in-vitro* anti-inflammatory activity of ethanolic extract of *Delonix elata* leaves by human red blood cell (HRBC) membrane stabilization method. Inflammation is the complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells. Many substances, interfering with the inflammatory response have been isolated from medicinal plants. Natural products with anti inflammatory activity mainly contain active principles viz, flavonoids, terpenoids, steroids, Phenolic compounds, saponins and alkaloids[55].

- Suriyavathana M and Sivanarayan V (2014)., have been evaluated the anti inflammatory activity of *Delonix elata* on collagen induced paw edema in mice as for controlling inflammatory disorders.The pro-inflammatory cytokines induced by collagen, such as TNF- α, IL-6 and IL-1 β, play a key role in the process of inflammatory diseases. Excessive production of these pro-inflammatory cytokines will result in a systemic inflammatory response syndrome, such as septic shock. Western blot analysis revealed that the methanolic crude extract inhibited TNF- α, IL-6 and IL-1 β production in collagen induced mice (expression of TNF - α, IL-6 and IL-1 β lesser

than that of inflammation induced groups). The anti-inflammatory cytokines counteracted the cytotoxic effects of pro inflammatory cytokines during inflammation. Thus, IL-10 and IL-4 levels are increased in the collagen induced mice, whereas the levels are reduced in the methanolic crude extract[56].

- Wijayasiriwardena C *et al* (2009)., have been investigated the anti-inflammatory activity methanolic extract of *Delonix elata* leaf, by carrageenan induced paw edema and cotton pellet granuloma models, along with the antioxidant potential underpinning its role as traditional medicine for joint disorders. Methanol extract of leaf was found to be active in the last phase of paw edema at 300 mg/kg, compared to other doses and fractions. At the same dose it was found to have potent antioxidant capacity, which may play its role in reducing inflammation[57].

Analgesic activity

- Ravichandran S and Panneerselvam P (2012)., have been evaluated the antinociceptive activities of combined petroleum ether extract of *Cardiospermum halicacabum* and *Delonix elata*. The analgesic investigations were carried out against two types of noxious stimuli, chemical (formalin-induced pain) and thermal (hotplate tests). It decreased the lickings of formalin-induced pain. These results showed that these plants had both central and peripheral acting effects and this was confirmed by its effect on both phases of formalin-induced pain[58].

- Seetharam Y N *et al* (2002)., have been evaluated the anti microbial and analgesic activity of ethanolic extract of *Delonix elata* and *Delonix regia* plant due to the presence of secondary metabolites like flavonoids and alkaloids. Ethanolic extract of

plants indicated the analgesic activity at a concentration 200mg/kg bodyweight have shown good analgesic activity after 60 minute of drug administration when compared to the standard analgin[59].

Hepatoprotective activity

- Pradeepa K *et al* (2014)., have studied the *in vitro* antioxidant and hepatoprotective effect of the stem bark extracts of *Delonix elata* by CCl_4 induced liver damage in rats. The ethanolic extract showed significant *in vitro* antioxidant effect in radical scavenging , metal chelating and lipid peroxidation inhibition assays. Ethanolic extract and isolated quercetin showed significant prophylactic effects by restoring the liver function markers (AST, ALT, ALP, Serum albumin and total Protein)[60].

- Ravichandran S and Panneerselvam P (2014)., have been evaluated the *invivo* antioxidant properties of combined petroleum ether extracts of *Cardiospermum halicacabum* and *Delonix elata* leaves on ccl4 induced erythrocyte damage in rats. The study suggested that oxidative stress and lipid peroxidation rise might occur after CCl_4 administration. The study results showed that CPCD significantly decreased lipid peroxidation and increased superoxide dismutase, catalase, Glutathione Reductase and Glutathione peroxidase. The presence of flavonoids and polyphenols in petroleum ether extract might be responsible for their observed antioxidant activity. The alteration of bio-membrane lipid profile disturbs its permeability, fluidity, activity of associated enzymes and transport system. The cumulative effect of CCl_4 intoxication resulted that microviscosity of a membrane increases markedly with increases in cholesterol to phospholipid ratio thus leading to cellular rigidity. Intoxication of experimental animals with carbon tetrachloride altered membrane structure and function as shown by the increases in cholesterol and subsequent decreases in

phospholipid concentrations, hence increased cholesterol to phospholipid ratio. Thus CPCD plays a key role in peroxidation by inhibiting the free radical attack on bio-membranes[61].

Wound healing activity

- Pradeepa K *et al* (2016)., have evaluated the wound healing activity of *Delonix elata* stem bark ethanolic extract (DSE) and its isolated constituent quercetin-3-rhamnopyranosyl-(1-6) glucopyranoside (QRPG) on collagen synthesis and their modulatory role in Col 1 α (I) gene expression which is a significant factor contributing to the normal wound healing process. The study showed promising and stronger wound healing promoting activity than the standard povidone-iodine ointment[62].

List of Review on The Model Used for Cardioprotective Evaluation

Various models used to investigate the Cardioprotective activity are,

- Doxorubicin induced cardiotoxicity[65-69]
- Cyclophosphamide induced cardiotoxicity[63]
- Isoprotenerol induced cardiotoxicity[64]

Doxorubicin induced cardiotoxicity is one of the commonly used models for the evaluation of cardioprotective activity. The cardioprotective activity was carried out by utilising Doxorubicin model as an inducing method. Some of the plant have been evaluated for cardioprotective activity using Doxorubicin model are *Allium sativum* (aged garlic extract)[65], *Aloe barbadensis* Miller[66], *Curcuma longa*[67], *Ixora coccinea*[68], Pomegranate fruit extract[69].

4. PLANT PROFILE

Botanical Name : *Delonix elata* (L.) Gamble

Synonym : Poinciana elata

Family : Fabaceae (Caesalpinaceae)

Common Name : White Gulmohur, White poincian

Vernacular Name[70]

Tamil - Vadhanarayanan, Perungondrai

English - Yellow Gulmohur, Tiger bean, Creamy peacock flower

Telugu - Chinna seibiseri, Chitti keshwaramu

Kannada - Nirangi, Vatanarayana

Hindi - Waykaran, Sandeshra

Marathi - Sanchaila, Sankasura

Sanskrit - Siddhesvara

Taxonomic Classification

Kingdom - Plantae

Phylum - Tracheophyta

Class - Magnoliopsida

Order - Fables

Family - Fabaceae

Genus - Delonix

Species - *Delonix elata* (L.) Gamble

Habitat - Terrestrial

Distribution[71]

The species is globally distributed in tropical areas like Africa, Egypt, Arabia to India (Kathiawar and South India).

Description

Delonix elata is a perennial, medium-sized, deciduous tree about 2.5-15 mtr tall with a spreading, rather rounded crown and very attractive white fragment flowers. Crooked poor stem form and drooping branches. Bark smooth, shining; sometimes flaking.

Leaves 3-6 or more, bipinnate; pinnae usually 4-6 pairs; leaflets 10-14 pairs oblong or oblanceolate-oblong, 0.6-1.2 cm long. Leaflets 1.25-4 mm wide.

Flowers in terminal corymbs; stalks pubescent, lowest flowers stalks longest. Flowers open one at a time. Sepals 1.8 cm long, with a broadly ovate or rotundate-cuneate lamina narrowing into a distinct claw. Petals rounded in outline and crisped on margins 1.6-3.8 cm long, 1.8-4.2 cm wide; upper one smaller than rest, pale yellow; the remainder white; later all turning apricot. Staminal filaments pale brown or reddish, hairy at the base, 5-10 cm long; pedicels up to 3.75 cm. Ovary pubescent or tomentose all over

Phytochemical constituents

Flavanoids, Tannin, Saponin, Quinone, Terpenoids, Steroids, Alkaloid, Phenol, Cardiac glycosides, Coumarins.

Medicinal uses

The leaf extracts are anti-inflammatory, a root decoction is drunk for abdominal pains. A psychosomatic medicinal use relating to scorpion bite treatment. Anti-oxidant, Anti rheumatism and Antibacterial activity. The plant used for siddha and folk medicine.

Figure 6: *Delonix elata* Linn.

Leaves

Flower

5. AIM AND OBJECTIVE

AIM

Plants are rich source in phytochemical constituents. Active phytochemical constituents such as flavonoids, cardiac glycosides, terpenoids and saponins are known to responsible for antioxidant activity and hence maybe considered to be potential cardioprotective effect. The preliminary review of literature indicates that plant possess natural antioxidant property and belonging to the family Fabaceae.

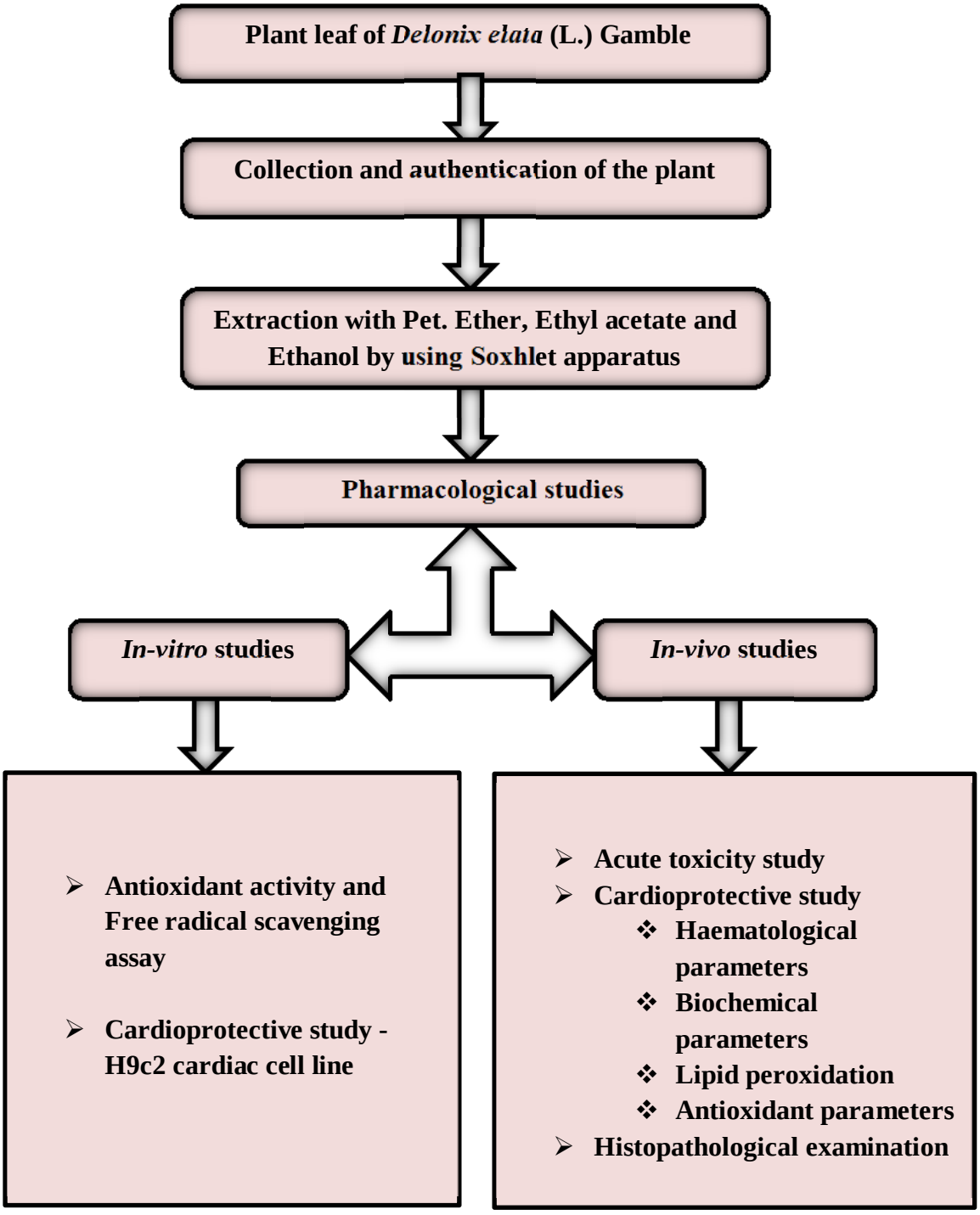
The aim of the present study is to evaluate the cardioprotective effect of *Delonix elata* (L.) Gamble leaves by *in vitro* and *in vivo* method.

OBJECTIVE

- Successive extraction of leaves of *Delonix elata* using various solvents like petroleum ether, ethyl acetate and ethanol by hot continuous extraction using Soxhlet apparatus.
- Evaluation of *in-vitro* antioxidant activity of *Delonix elata* (L.) Gamble leaves extracts by antioxidant free radical scavenging assay
    - SOD assay
    - DPPH radical scavenging assay
- Evaluation of *in-vitro* cardioprotective activity of *Delonix elata* (L.) Gamble leaves extracts by using H9c2 rat cardiomyocyte cell line.
    - Cell viability assay (MTT Assay)

- Evaluation of *in-vivo* cardioprotective effect of *Delonix elata* (L.) Gamble leaf extract in doxorubicin induced myocardial damage in rats.
    - Acute toxicity study
    - Cardioprotective study
        - ❖ General observation, Body weight and Heart weight
        - ❖ Haematological parameters - Haemoglobin, RBC, WBC
        - ❖ Biochemical parameters - CK-MB, LDH, CTn I
        - ❖ Lipid peroxidation assay
        - ❖ Antioxidant parameters - SOD, CAT, GPx
    - Histopathological examination

6. PLAN OF WORK



7. MATERIALS AND METHODS

Plant Collection and Identification

The fresh leaf of *Delonix elata* (L.) Gamble was collected from Tharamangalam, Salem District, Tamilnadu (India) in the month of August 2018. The plant was authenticated by Dr. K. N. Sunil kumar, Research officer and HOD of Pharmacognosy, Siddha Central Research Institute, Anna Govt. Hospital campus, Arumbakkam, Chennai.

Preparation of Plant extract

The freshly collected leaf of this plant was shade dried for one week. The dried material was powdered and passed through 10-mesh sieve. The powder was then extracted with petroleum ether (500ml), ethyl acetate (500ml) and ethanol (500ml) separately for 24 hours in a Soxhlet apparatus.

Procedure

80g powdered material was placed inside the extraction chamber, which was loaded into the main chamber of the Soxhlet extractor. After packing, the content was extracted using petroleum ether, ethyl acetate and ethanol successively and poured until the siphon tube was filled. The Soxhlet is then equipped with a condenser and the solvent is heated to reflux. The solvent vapour travels upto a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapours cools and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle has desired compound concentrated in the distillation flask. At the completion of extraction procedure the

solvent was removed by means of evaporation at room temperature, yielding the extracted compound.

$$\text{Yield Value} = \frac{\text{Weight of the residue}}{\text{Weight of the dried plant}} \times 100$$

IN-VITRO STUDIES

ANTIOXIDANT AND FREE RADICAL SCAVENGING ASSAY

ESTIMATION OF SUPEROXIDE DISMUTASE (SOD)[72]

Principle

The method involves generation of superoxide radical of riboflavin and its detection by nitrite formation from hydroxylamine hydrochloride. The nitrite reacts with sulphanilic acid to produce a diazonium compound which subsequently reacts with naphthylamine to produce a red azo compound whose absorbance is measured at 543 nm.

Reagents

- 50 mM Phosphate buffer, pH 7.4
- 20 mM L-Methionine
- 1 % (v/v) Triton X-100
- 10 mM Hydroxylamine hydrochloride \
- 50mM EDTA
- 50 µM Riboflavin
- Greiss reagent: 1 % sulphanilamide, 5 % phosphoric aeid and 0.1 % naphthylethylene diamine dihydrochloride.

Procedure

Pipetted out 1.4 ml aliquot of the reaction mixture in a test tube. 100 U of the sample was added followed by a pre incubation at 37^0C for 5 min. 80 µl of riboflavin was added and the tubes were exposed for 10min to 200W Philips fluorescent lamps. The control tube contained equal amount of buffer instead of sample. The sample and its respective control were run together. At the end of the exposure time, 1.0ml of Greiss reagent was added to each tube and the absorbance was measured at 543nm.

One unit of enzyme activity was defined as the amount of SOD capable of inhibiting 50% of nitrite formation under assay condition.

DPPH radical scavenging assay[73]

Principle

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a red coloured stable free radical. Antioxidants react with DPPH and turn it to yellow. The degree of discolouration indicates the scavenging potentials of the antioxidant compounds.

(DPPH) + (H-A) ⟶ DPPH-H + (A)

Procedure

The free radical-scavenging activity of *D.elata* plant extract was measured by DPPH radical scavenging assay. For this, 1ml of DPPH solution (0.1mM) in methanol was added to different concentrations of extract (200-1000µg/ml) of extract. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance was measured at 517nm using a UV-Visible spectrophotometer. A control was prepared without adding extract. Quercetin was used as a standard. The % radical scavenging activity of the extract was calculated using the formula

$$\% \text{ RSA} = \frac{\text{Abs control - Abs sample}}{\text{Abs Control}} \times 100$$

IN-VITRO CARDIOPROTECTIVE ACTIVITY USING H9c2 (Rat cardiomyocyte) CELL LINE

Cell culture

Normal H9c2 (rat cardiomyocyte) cell line were obtained from National Centre for Cell Sciences (NCCS), Pune. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were grown in 100 mm dishes at 37^0C in 5% CO_2 and subcultured when they reached 70% confluence. Experiments are performed with cells from passages.

CELL VIABILITY (MTT) ASSAY[74]

Principle

The principle involved in the cleavage of tetrazolium salt MTT (3-(4,5 dimethyl thiazole 2yl) - 2,5- diphenyl tetrazolium bromide) into a blue coloured (formazon) by mitochondrial enzyme succinate dehydrogenase. The number of cells are found to be proportional to the extent of formazon production by the cell used.

Procedure

- The monolayer cell culture was trypsinized and the cell count was adjusted to $1x10^5$cells /ml using medium containing 10% newborn calf serum.
- To each of the 96 well microtitre plate 0.1ml of the diluted cell suspension (10,000 cells) were added.

- After 24hrs, when a partial monolayer was formed the supernatant was flicked off, washed once with PBS and 100ml of different extract concentration was added to the cells in microtitre plates.
- The plates were incubated at 37^0C for 3 days in 5 % CO_2 atmosphere and microscopic examination was carried out and observation recorded every 24 hours.
- After 72hrs the extract solution in the well was discarded and 50m1 of MTT was added to each well.
- The plates were gently shaken and incubated for 3hrs at 37^0C in 5% CO_2 atmosphere.
- The supernatant was removed and 50ul of propanol was added and gently solubilize the formed formazon.

The absorbance was measured using a microtitre plate reads at a wavelength of 540nm. The percentage growth inhibition was calculated. Using the formula below:

$$\% \text{ growth inhibition} = \frac{100 - \text{Mean OD of test group}}{\text{Mean of OD control group}} \times 100$$

IN VIVO STUDIES

Experimental Animals

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee (IAEC) and this protocol met the requirements of national guidelines of **CPCSEA/IAEC approval no.1917/ReBi/s/16/CPCSEA/25.10.2016 and 27/AEL/IAEC/MMC, Date: 16.11.2018**. The Wistar albino rats (150 - 250 g) used for this study were procured from Animal house, Madras Medical College, Chennai, Tamil Nadu, India.

Quarantine and Acclimatization

Quarantine is the process of separation of newly received animals from those already in facility until the health and possibly microbial status of the newly received animals have been determined. The newly procured wistar rats was quarantined for period of one week to minimize the chance of introduction of pathogens into established animals and allowed to develop the psychologic, physiologic and nutritional stabilization before their use.

Housing

The animals were housed in well ventilated animal house which was maintained at a constant temperature and relative humidity of 55 to 70%. The animals were housed in spacious polypropylene cages and paddy husk was utilized as bedding material. The bedding material was changed twice a week.

Diet and Water

The animals were maintained on standard pellet diet and purified water. The animals were provided with food and water ad libitum.

Animal Identification

All animal cages were used in the study had a proper identification i.e., labels. Each animal in the cage was marked either on head or body or tail with picric acid for their appropriate identification.

TOXICITY STUDIES

ACUTE TOXICITY STUDY[75]

Acute toxicity study was designed as per the OECD guidelines (423).

Principle and Purpose

The main purpose of acute toxicity study is to evaluate the degree of toxicity in a quantitative and qualitative manner with the purpose of comparing it with other drug substance (eg. Other drug candidates for the same indication).

Experimental Animals

Three healthy adult wistar albino rats were weighing between 150 - 250g was selected for the study. For all the three animals food, but not water was withheld overnight prior to dosing.

Selection of dose levels and administration of doses

Being a traditional herbal medicine, the mortality is unlikely at the highest starting dose level (2000 mg/kg body weight). Hence a limit test at one dose level of 2000mg/kg body weight was conducted in all the three animals.

Observations

The animals were observed individually after dosing once during the first 30 minutes, periodically for the first 24 hours, with special attention given during the first 4 hours and daily thereafter for a total of 14 days. The following clinical observations were made and recorded.

- Toxic signs: All rats are observed for any toxic signs.
- Pre - terminal deaths: All rats are observed for any pre - terminal deaths.
- Body weight: Individual body weight is recorded for all the animals.
- Cage side observation : The faeces colour and consistency, Changes in skin and fur, eyes of the animals are observed.

- Physical examination : Physical observation include changes in respiratory system (rate), cardiovascular system (heart rate), autonomic nervous system (salivation, lacrimation, piloerection, urinary incontinence and defecation), central nervous system (ptosis, drowsiness, convulsions, motor activity, writhing, straub's test, motor incoordination, righting reflex, pinna reflex, corneal reflex, tremors) were recorded.

IN VIVO CARDIOPROTECTIVE STUDY

The cardioprotective activity of *Delonix elata* (L.) Gamble leaf extract was evaluated in wistar albino rats.

Induction of cardiac toxicity: Cardiac toxicity was induced by intraperitonial injection of Doxorubicin was dissolved in normal saline attain cumulative dose 25 mg/kg body weight.

Table 1: Experimental Design

Group	Group name (n=6)	Treatment schedule
I	Normal Control	Normal saline or water
II	Disease Control	Doxorubicin (2.5mg/kg i.p) alternate days for 3weeks
III	EEDE leaves (200mg/kg)	Doxorubicin (i.p) alternate days for 3weeks + Ethanolic extract of *D.elata* (L.) leaves (200mg/kg) orally (day 1 to day 28)
IV	EEDE leaves (400mg/kg)	Doxorubicin (i.p) alternate days for 3weeks + Ethanolic extract of *D.elata* (L.) leaves (400mg/kg) orally (day 1 to day 28)

After the experimental regimen, the animal was anesthetized by using isoflurane anesthesia and blood was collected through cardiac puncture method for hematological and

biochemical analysis. Then animals were sacrificed and heart was excising out for histopathological analysis.

EVALUATION PARAMETERS

HEMATOLOGICAL PARAMETERS[76, 77]

ESTIMATION OF HEMOGLOBIN

Principle

In alkaline medium, hemoglobin and its derivatives are oxidized in the presence of potassium ferricyanide and get converted to methemoglobin which then reacts with potassium cyanide to form purple red coloured cyanomethemoglobin complex, the intensity of which is measured at 546nm.

Reagents

- Drabkin's solution
- Cyanomethemoglobin standard: 65 mg/dl

Procedure

Pipetted out 0.02ml of serum and 5.0ml of Drabkin's solution into a test tube. Simultaneously, a blank was set up with Drabkin's solution and distilled water. Mixed well the above tubes and allowed to stand at room temperature for 5minutes. Measured the absorbance of test at 546 nm. Take the absorbance of cyanomethemoglobin standard directly without adding working reagent against blank at 546nm. The results were expressed as g/dl in serum.

ESTIMATION OF RED BLOOD CELLS

Principle

The blood specimen is diluted (usually 200 times) with red cell diluting fluid which does not remove the white cells but allows the red cells to be counted under 400X magnification in a known volume of the fluid. Finally the number of cells in undiluted blood is calculated and reported as the number of red cells /mm3.

Reagents

- RBC diluting fluid: (Trisodium citrate-3g, distilled water~99ml, and formalin-1ml).

Procedure

The whole blood was taken into the RBC pipette exactly up to the 0.5 mark (Thoina pipette mark 101) and the diluting fluid (formal citrate solution) is immediately drawn up to the mark 101. The pipette was rotated between the thumb and the forefinger. This gave a dilution of 1:200. The cover glass was placed in position over the ruled area using gentle pressure. The suspension was mixed thoroughly by rotating the pipette for about a minute, holding it in horizontal position, and finally shook it sidewise. The fluid was expelled from the stem of the pipette and filled the chamber immediately by holding the pipette at an angle of 45° and slightly touching the tip against the edge of the cover glass. There should not be any bubbles under the cover glass. Then the red corpuscles were allowed to settle for 2 to 3 minutes. The number of RBCs was counted in 180 small squares (4 squares of 16 at each four corners and one of 16 at centre). The cells touching the lower and right hand lines were not counted, but the cells touching the upper and left hand lines was counted. The cells counted were expressed as million cells /mm^3 blood.

Calculation

Number of RBCs/mm^3 "Number of cells counted in 5 squares x 10000.

ESTIMATION OF WHITE BLOOD CELLS

Principle

Blood is diluted with acid solution which removes the red cells by hemolysis and also accentuates the nuclei of the white cells, counting is done with a microscope under the low power (100X magnification) and knowing the volume of the fluid examined and dilution of the blood, the number of white cells in undiluted whole blood is calculated and reported as the number of WBCs/ mm^3.

Reagents

- WBC diluting fluid (Turk solution): (Acetic acid-3ml, distilled water-97ml).

Procedure

The whole blood was taken up to the mark 0.5 in WBC pipette and diluted up to the mark 11 with WBC fluid as described in RBC counting and filled the counting chamber in the same manner. Then the cells area was allowed to settle for 3 minutes. The neubaur counting chamber was used to count the cells in the four comers and each of these 4sq mm. Area was subdivided into 16 squares by using the low power objective and a medium ocular. While counting, the cells included were those touching the lines on the left and bottom. The difference between the two squares millimeter area as thousand cells /mm of blood.

Calculation

Number of WBCs/mm^3 = Number of cells counted x 50.

BIOCHEMICAL ANALYSIS OF CARDIAC MARKER ENZYMES

ESTIMATION OF CREATINE KINASE- MB ACTIVITY[78]

Principle

The sample is incubated in the CK-MB reagent which includes the anti-CK-M antibody. The activity of the non-inhibit CK-B is then determined using the following series of reactions:

$$\text{CK ADP + Creatine Phosphate} \longrightarrow \text{Creatinine + ATP}$$

$$\text{ATP + Glucose} \xrightarrow{\text{HK}} \text{- ADP + Glucose-6-phosphate}$$

$$\text{G6PDH G-6-P + NAD+} \longrightarrow \text{6-Phosphogluconate + NADH + } H^{+}$$

CK-B catalyzes the reversible phosphorylation of ADP, in the presence of Creatinine phosphate, to form ATP and creatine. The auxiliary enzyme hexokinase (HK) catalyzes the phosphorylation of glucose by the ATP format, to produce ADP and glucoses-phosphate (G-6-P) is oxidized to 6-phosphogluconate with the concomitant production of NADH. The rate of NADH formation, measured at 340nm, is directly proportional to serum CK-MB activity.

Reagent

- **CK-MB Reagent:**

  Creatinine Phosphate 30mM

  Adenosine-5-Phosphate 2mM NAD

  2mM Hexokinase (Yeast) > 3000U/L G-6-PDH (Bacterial) > 2000U/L Anti Human CK-M antibody (Goat) - sufficient amount to inhibit up to 1500 U/L of CK-MM.

Procedure

To the test tubes added 1000µl of the reagent and 50ml of the sample. The mixture was mixed and incubated at 37^0C. The absorbance was measured after 300seconds. Two additional absorbance was taken at 1minute interval. The mean absorbance change/minutes

(AA/min) was calculated. The change in absorbance/ minute was multiplied by factor 3376 that is equal to CK-MB.

ESTIMATION OF LACTATE DEHYDROGENASE (LDH) [79]

Principle

The lactate is acted upon by lactate dehydrogenase to form pyruvate in the presence of NAD. The pyruvate forms pyruvate phenyl hydrazine with 2, 4 dinitrophenyl hydrazine. The color developed is read in a spectrophotometer at 440nm.

Reagents

- Glycine buffer 0.1M, (pH 10): 7.505 g of glycine and 5.85 g of sodium chloride was dissolved in 1 litre of water.
- Buffered substrate: 125ml of glycine buffer and 75ml of 0.1N NaOH were added to 4g of lithium lactate and mixed well.
- Nicotinamide Adenine Dinucleotide: l0mg of NAD was dissolved in 2ml of water.
- 2, 4 - Dinitrophenyl hydrazine: 200mg of DNPH was dissolved in 100ml of IN HC1.
- 0.4N NaOH.
- Standard pyruvate, l umole/ml: 11mg of sodium pyruvate was dissolved in 100ml of buffered substrate (1 umole of pyruvate /ml).
- NADH solution, l umole/ml: 8.5 mg/l0ml buffered substrate.

Procedure

Placed 1.0ml buffered substrate and 0.lml sample into each of two tubes. Added 0.2ml water to the blank. Then to the test added 0.2ml of NAD. Mixed and incubated at 37°C for 15 mins. Exactly after 15 mins, 1.0ml of dinitrophenyl hydrazine was added to each (test and control). Left for further 15mins. Then added 10ml of 0.4N Sodium hydroxide and the

color developed was read immediately at 440nm. A standard curve with sodium pyruvate solution with the concentration range is 0.1 -l.0µmole.

LDH activity in serum was expressed as umoles of pyruvate liberated / L and in liver homogenate as nmoles of pyruvate liberated / minute / mg protein.

TROPONIN ASSAY

Materials

- Deionized water for diluting Read Buffer.
- Phosphate buffered saline + 0.05% Tween-2Q (PBIJ-T) fox plate washing.
- Adhesive plate seals.
- Microtitre plate shaker.
- Liquid handling equipment, or other efficient multi-channel pipetting equipment that must accurately dispense 25 and 150uL into a 96-well micro plate.
- Automatic plate washer or multi-channel pipette for washing 96-well plates

Procedure

Begin with a MULTI-SPOT 96-well 4 Spot Human CTn I plate. Add 25µL/well of Detection Antibody Solution. Add 25µL/well Calibrator or sample, cover with an adhesive plate seal, and incubate at room temperature with shaking for 1 hour. Prepare SECTOR Imager so that the plate can be read immediately after Read Buffer addition. Wash plates 3 times with PBS-T. Add 150uL/well IX Read Buffer to avoid bubbles. The use of an electronic multi-pipettor at moderate speed setting was recommended. Analyze immediately with SECTOR Imager.

ASSAY OF ANTIOXIDANT ENZYMES IN HEART HOMOGENATE[80]

GENERAL PROCEDURE FOR HOMOGENIZATION

The isolated rat heart was homogenized (10% w/v) in 50mM ice-cold sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA. Then the homogenate was centrifuged for 60minutes. The supernatant was used for the estimation of lipid peroxidation and antioxidant enzyme levels.

ESTIMATION OF LIPID PEROXIDATION IN HEART HOMOGENATE[81]

Principle

Determination of lipid peroxidation levels in terms of Thiobarbituric acid reactive substances (TBARS) provides valuable information about the cellular/tissue oxidative stress. MDA is the end product of lipid peroxidation that reacts with TBA. The Absorbance of which is measured at 532 nm.

Procedure

In this study, the levels of TBARs were analyzed spectrophotometrically in the heart of controls and experimental rats based on the method described by Ohkawa *et al*. The heart (10% W/V) was homogenized in homogenization buffer containing 1.15% KCl solution. To the homogenate (2.5 ml), 0.5 ml of saline (0.9% sodium chloride) and 1.0 ml of (20% W/V) Trichloroacetic acid were added and centrifuged for 20 min at 4000 rpm. To the resultant supernatant (1.0 ml), 0.25 ml of TBA reagent was added and the mixture was incubated at 95°C for 1h. Finally, an equal volume of n-butanol was added to the mixture and the contents were centrifuged for 15 min at 4000 rpm. The organic layer was carefully transferred into a clear tube, and its absorbance was measured spectrophotometrically at 532 nm. The absorbance was recorded and compared against the blank. The rate of lipid peroxidation was expressed as μmoles of Malondialdehyde formed/gram wet weight of tissue.

ENZYMATIC ANTIOXIDANT ACTIVITY[82-84]

SUPEROXIDE DISMUTASE

Principle

The method involves generation of superoxide radical of riboflavin and its detection by nitrite formation from hydroxylamine hydrochloride. The nitrite reacts with sulphanilic acid to produce a diazonium compound which subsequently reacts with naphthylamine to produce a red azo compound whose absorbance was measured at 480nm.

Procedure

The activity of SOD was determined by the method of Mishra and Fridovich. SOD was assayed in the microsomal fraction according to its ability to inhibit the auto-oxidation of epinephrine at alkaline medium. The reaction mixture contained 0.05 M carbonate buffer (pH 10.2), 30mM epinephrine (freshly prepared) and the tissue homogenate. Changes in absorbance were recorded at 480 nm and measured at 10secs intervals for 1min in a spectrophotometer. The enzyme activity was expressed as units/mg protein/min.

CATALASE

Principle

Catalase causes rapid decomposition of hydrogen peroxide to water.

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

The method is based on the fact that dichromate in acetic acid reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchloric acid as an unstable intermediate. The chromic acetate thus produced was measured colorimetrically at 610 nm. Since dichromate has no absorbance in this region, the presence of the compound in

the assay mixture did not interfere with the colorimetric determination of chromic acetate. The catalase preparation is allowed to split H_2O_2 for different periods of time. The reaction is stopped at specific time intervals by the addition of dichromate / acetic acid mixture and the remaining H_2O_2 was determined by measuring chromic acetate colorimetrically after heating the reaction.

Procedure

The activity of CAT was determined by based on its ability to decompose hydrogen peroxide (H_2O_2), following the method of Chance and Machly. The reaction mixture in a volume of 2.5 ml contained 0.05M phosphate buffer (pH 7.0), 19 mM H_2O_2 and 1ml amount of tissue homogenate. Then, the absorbance was read at 240 nm and measured at 10secs intervals for 1min in a spectrophotometer. CAT activity was expressed as μM of H_2O_2 metabolized/mg protein/min.

GLUTATHIONE PEROXIDASE

Principle

A known amount of enzyme preparation is allowed to react with hydrogen peroxide in the presence of GSH for a specified time period. Then the remaining GSH were measured by the method of Ellman *et al.*

Procedure

The activity of GPx was estimated by the method of Mohandas *et al*. The reaction mixture contained 1.59 ml of 100 mM phosphate buffer (pH 7.6), 0.1 ml of 10 mM EDTA, 0.1 mL of 10 mM sodium azide, 0.1 ml glutathione reduced, 0.01 ml of 0.2 mM hydrogen peroxide, and 0.1 ml of tissue homogenate. Immediately, the contents were read at 340 nm against blank, at 10 s intervals for 3 min on a spectrophotometer. The activity of GPx was expressed as nanomoles of NADPH oxidized/mg protein/min.

HISTOPATHOLOGIACAL STUDIES

The heart samples were excised from the experimental animals of each group and washed with ice-cold normal saline. A small portion of the heart sample from each group was quickly fixed in 10% buffered neutral formalin for 48 hours and then with bovine solution for 6 hours. Then the heart tissues were processed for paraffin embedding by standard histopathological technique i.e. dehydration through graded DOX propyl alcohol, cleaning through xylene and impregnated in paraffin wax for two hours, paraffin sections were taken at 5mm thickness- using microtome and processed in alcohol xylene series. The heart sections was stained by alum-haematoxylin and resin and the sections were examined microscopically and photographed for the evaluation of histopathological changes.

STATISTICAL ANALYSIS

The results were expressed as Mean ± SEM. One –way Analysis of variance (ANOVA) followed by Dunnet multiple comparison test was used to correlate the difference between the variables. $P<0.05$, $P<0.01$ and $P<0.001$ is considered as statistically significant.

8. RESULTS AND DISCUSSION

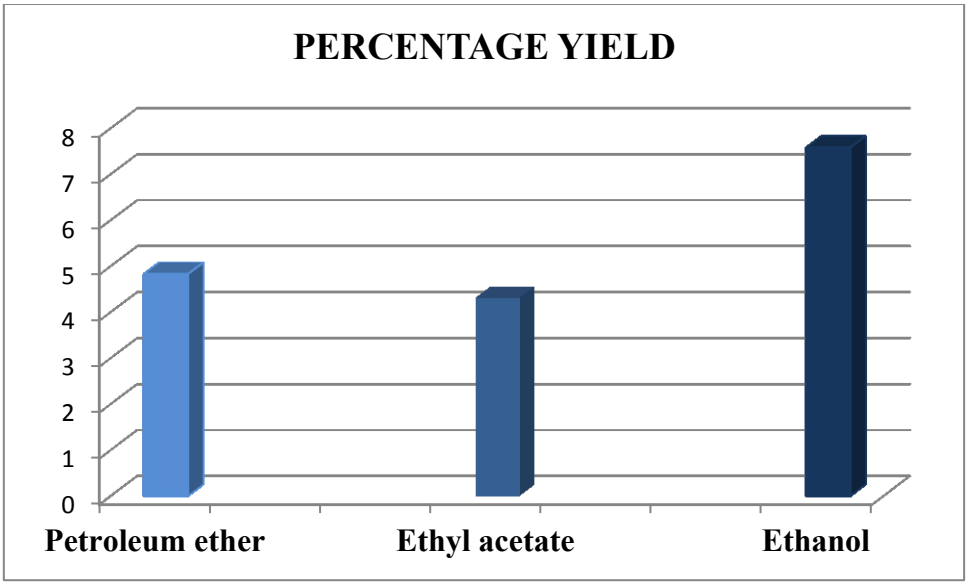
PERCENTAGE YIELD

The fresh leaves of *Delonix elata* (L.) Gamble was collected in the month of August, 2018. The leaves dried in shade and coarse powdered. Then the powder was extracted using various solvents like petroleum ether, ethyl acetate and ethanol by using Soxhlet apparatus. The percentage yield of various extracts are calculated and shown in **Table 2** and **Figure 7.**

Table 2: Percentage yield of Plant extract in various solvents

S.No	Type of Extract	Yield value (%w/w)
1	Petroleum ether	4.83
2	Ethyl acetate	4.31
3	Ethanol	7.58

Figure 7: Percentage yield of Plant extract in various solvents



It results shows that the percentage yield of ethanol extract (7.58%) was higher than the petroleum ether extract (4.83%) and ethyl acetate extract (4.31%).

IN-VITRO STUDIES

ESTIMATION OF ANTIOXIDANT ACTIVITY

Antioxidants are the substance which is used to delay or prevent the oxidation of the substrate. It may help the body to protect itself against various types of oxidative damage caused by reactive oxygen species, which are linked to a variety of diseases including cancer, diabetes, arthritis and acceleration of ageing process[85]. Free radicals and other reactive oxygen species are considered as an important causative factor for various diseases like neuro degenerative diseases, cancer and cardiovascular diseases[86]. Antioxidants may act by decreasing oxygen concentration, intercepting singlet oxygen, preventing first chain initiation by scavenging initial radicals, binding metal ion catalysts, decomposing primary products to non-radical compounds and chain breaking to prevent continued hydrogen abstraction from substances.

The oxidative damages caused by ROS on lipids, proteins and nucleic acids may trigger various chronic diseases, such as coronary heart diseases, atherosclerosis, cancer and aging[87]. The health-promoting effect of antioxidants from plant materials is thought to arise from their protective effects by counteracting ROS[88]. Assessing the antioxidant capacity of foods, botanicals and other nutritional antioxidant supplements. As plants expressed their significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity.

Many medicinal plants possessed large amounts of antioxidants such as polyphenols (phenolic acids, flavonoids and anthocyanin) which can play an important role in adsorbing

and neutralizing free radicals[89]. There is increasing interest in natural antioxidant products for use as medicines and food additives. Many studies have shown that antioxidants present in plants at higher level and these biomolecules exert a potential protective effect against oxidative damage.

SUPEROXIDE DISMUTASE ASSAY

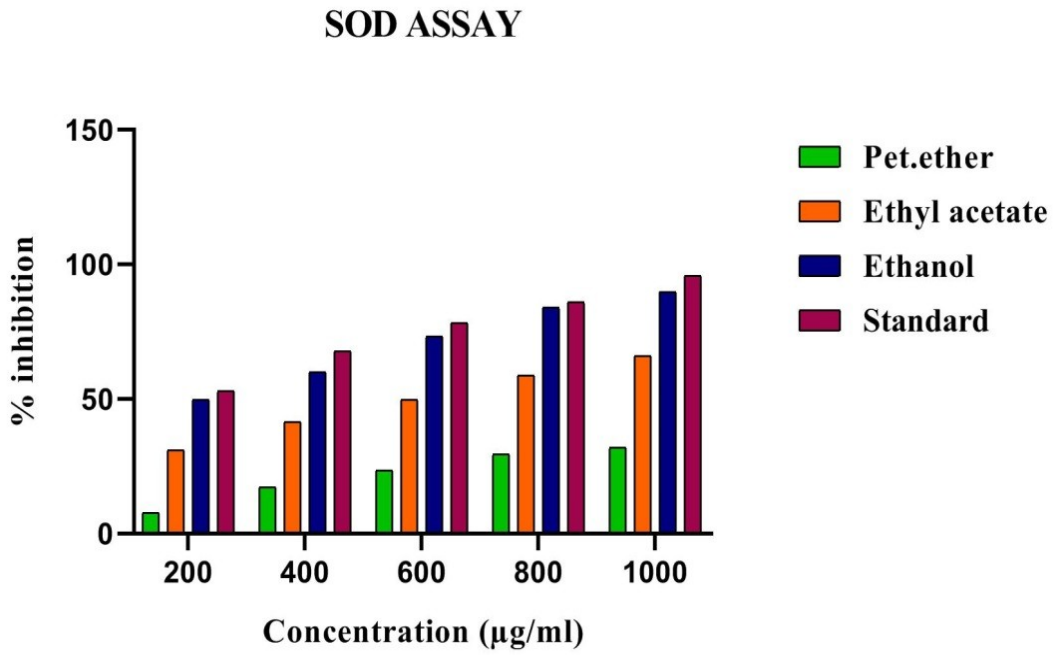
Superoxide dismutase (SOD) is a primary antioxidant enzyme and it was directly involved in the elimination of ROS. SOD is an enzyme that alternately catalyzes the dismutation of superoxide radical into their ordinary molecular oxygen or hydrogen peroxide and provides cellular defence against reactive oxygen species. The antioxidant effect of petroleum ether, ethyl acetate and ethanol extracts of *Delonix elata* (L.) leaves was evaluated and the results are tabulated in **Table 3 and Figure 8.**

Table 3: Estimation SOD activity of *Delonix elata* (L.) leaf extract

S.No	Concentration (µg/ml)	SOD (U/mg Protein)			
		Standard (Ascorbic Acid)	Pet. Ether Extract	Ethyl acetate Extract	Ethanol Extract
1	200	52.89	7.84	30.95	49.71
2	400	67.83	17.23	41.46	60.08
3	600	78.31	23.39	49.86	73.24
4	800	85.9	29.41	58.82	83.89
5	1000	95.78	31.93	66.11	89.92
6	**IC_{50} value**	**166.59**	**1528.08**	**613.33**	**209.03**

Values are expressed as triplet determination

Figure 8: Estimation SOD activity of plant extract of *Delonix elata* (L.)



IC_{50} Values of Standard (Ascorbic acid) and Petroleum ether, Ethyl acetate and Ethanolic Extract of *Delonix elata* (L.) leaves on SOD assay

Figure 9: Standard (Ascorbic acid)

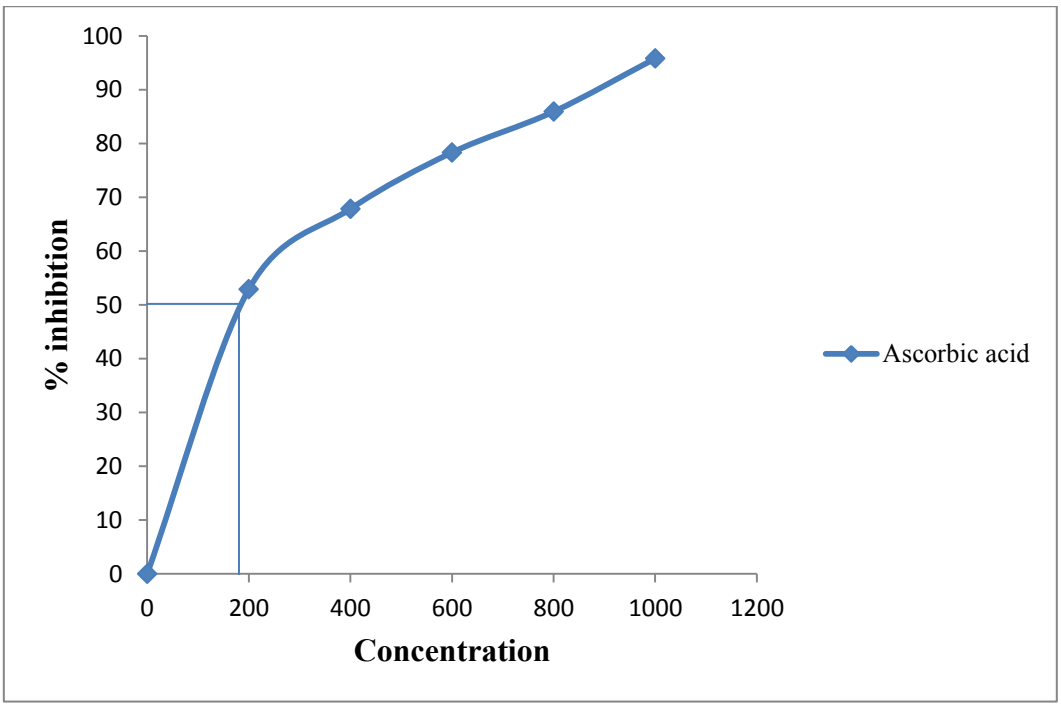


Figure 10: Petroleum ether extract

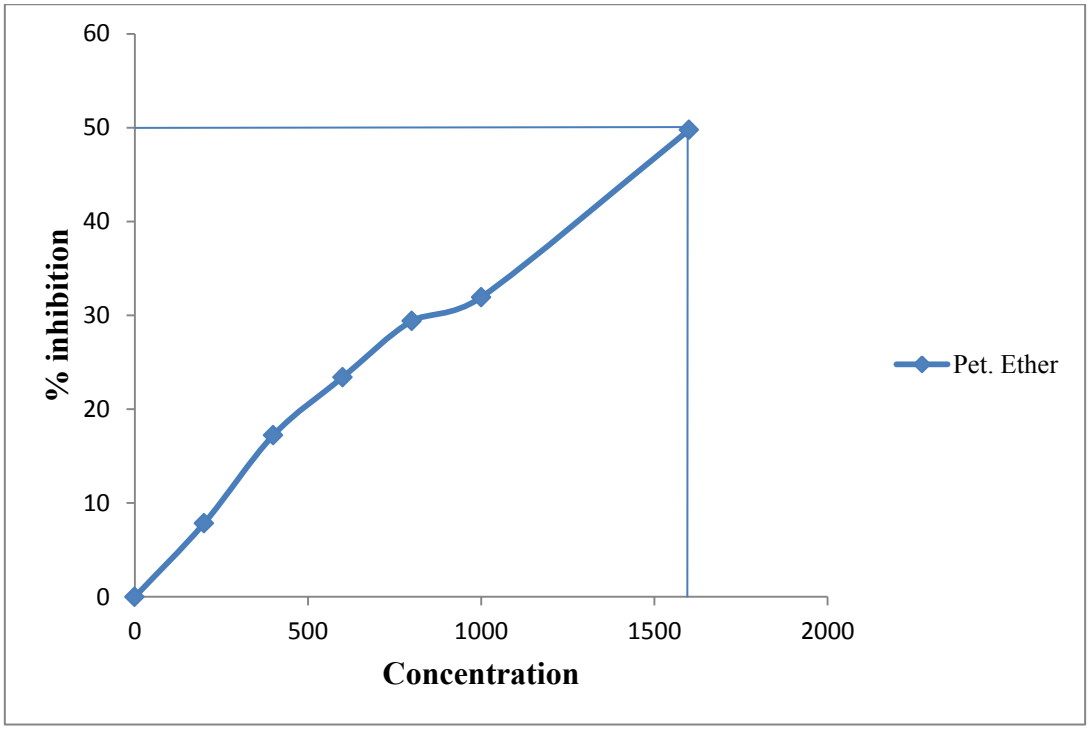


Figure 11: Ethyl acetate extract

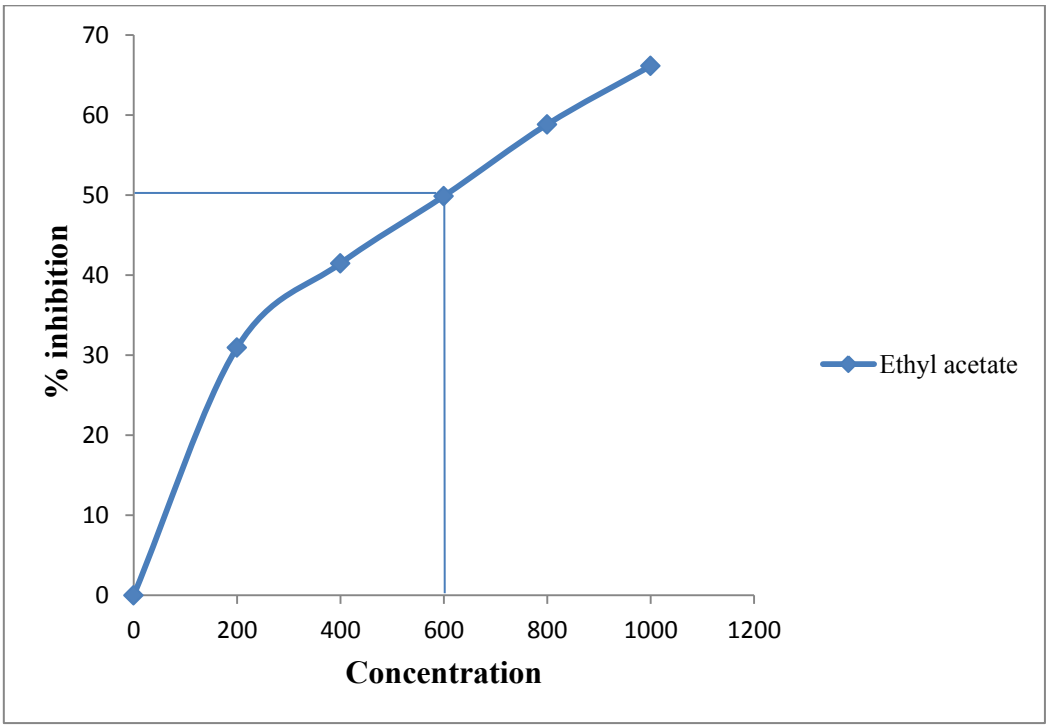
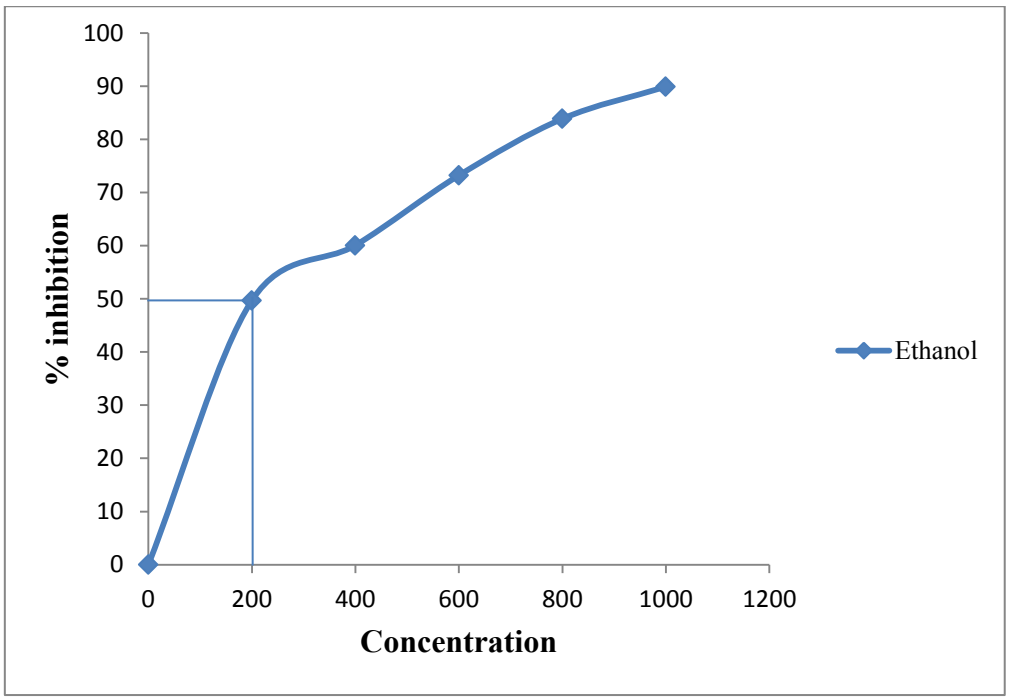


Figure 12: Ethanol extract



From the results the IC_{50} values of various plant extracts and standard are depicted. The IC_{50} values of plant extract was compared with the IC_{50} value of standard (Ascorbic acid). The ethanolic extract of *Delonix elata* (L.) leaf showed significant IC_{50} value (209.03 U/mg Protein) than petroleum ether (1528.08 U/mg Protein) and ethyl acetate (613.33 U/mg Protein) extract. The IC_{50} value of ethanolic extract of *Delonix elata* (L.) leaves has produced significant IC_{50} value near to standard (AA) 166.59 U/mg Protein.

DPPH ASSAY

DPPH radical scavenging activity is widely used method for assessing antioxidant effect of the plant extracts. DPPH is a well-known stable radical and scavenger for other radicals. The rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. A strong absorption band centered at 520nm. DPPH radical has a deep violet colour in solution and it becomes colourless or pale yellow

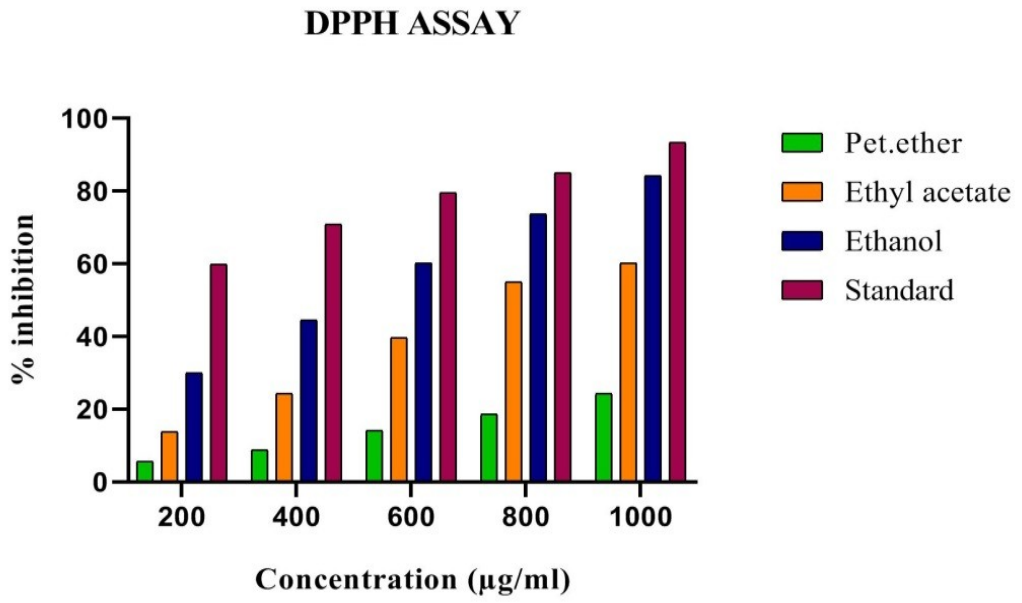
when it neutralised. The DPPH radical scavenging activity of petroleum ether, ethyl acetate and ethanol extract of *Delonix elata* (L.) leaves was given in following **Table 4 and Figure 13.**

Table 4: DPPH free radical scavenging assay of *Delonix elata* (L.) leaf extract

S.No	Concentration (µg/ml)	DPPH (U/mg Protein)			
		Standard (Quercetin)	Pet Ether Extract	Ethyl acetate Extract	Ethanol Extract
1	200	61.53	5.66	13.76	29.96
2	400	70.85	8.91	24.29	44.53
3	600	78.54	14.17	39.67	60.32
4	800	85.02	18.62	55.06	73.68
5	1000	91.49	24.29	60.32	84.21
6	**IC_{50} value**	**142.14**	**2117.48**	**784.28**	**476.09**

Values are expressed as triplet determination

Figure 13: DPPH free radical scavenging assay of *Delonix elata* (L.) leaf extract



IC_{50} Values of Standard (Quercetin) and Petroleum ether, Ethyl acetate and Ethanolic Extract of *Delonix elata* (L.) leaves on DPPH assay

Figure 14: Standard (Quercetin)

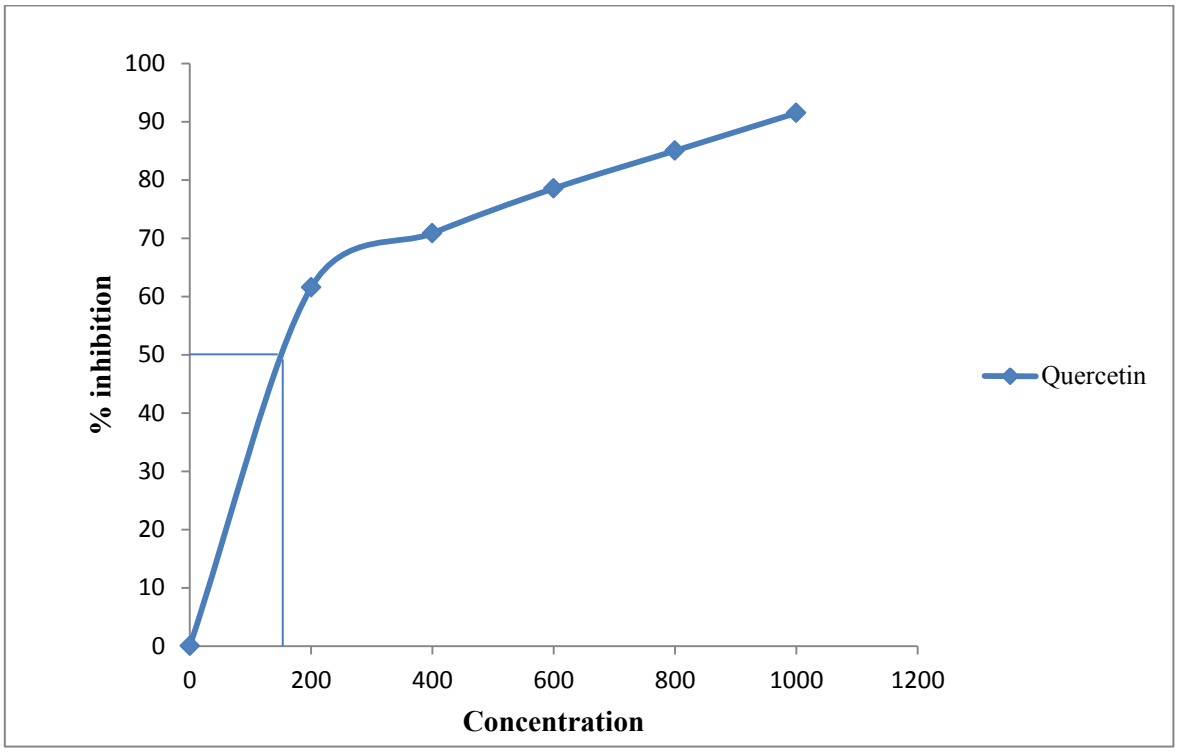


Figure 15: Petroleum ether extract

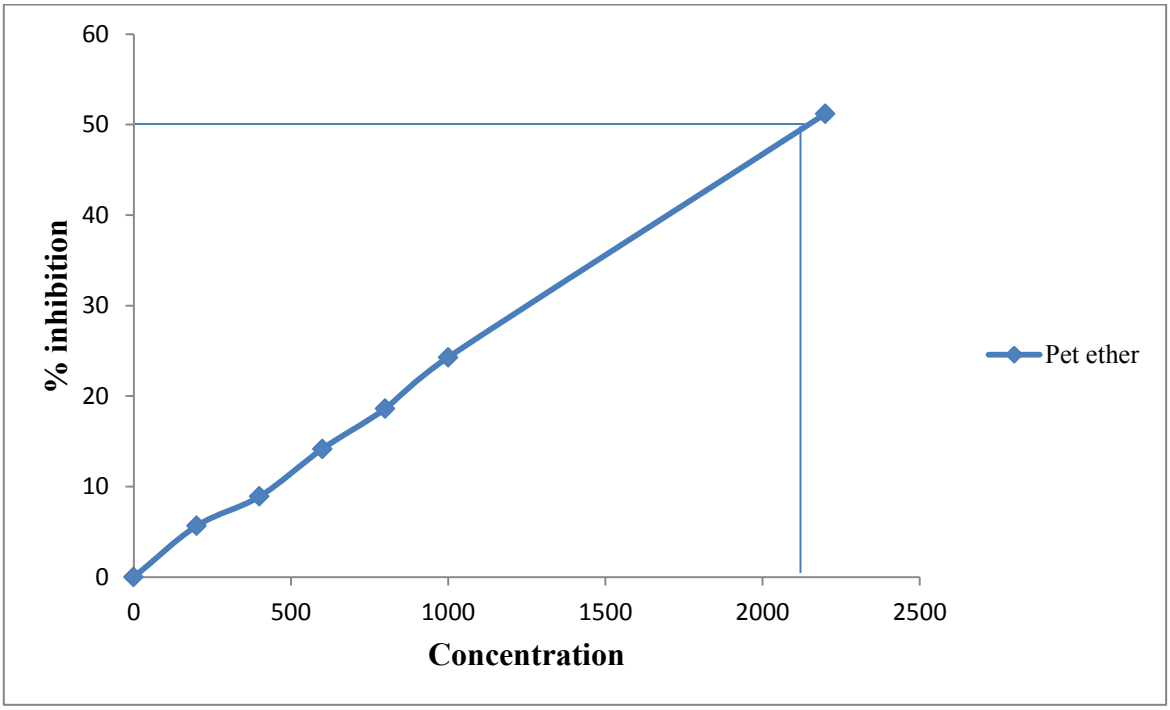


Figure 16: Ethyl acetate extract

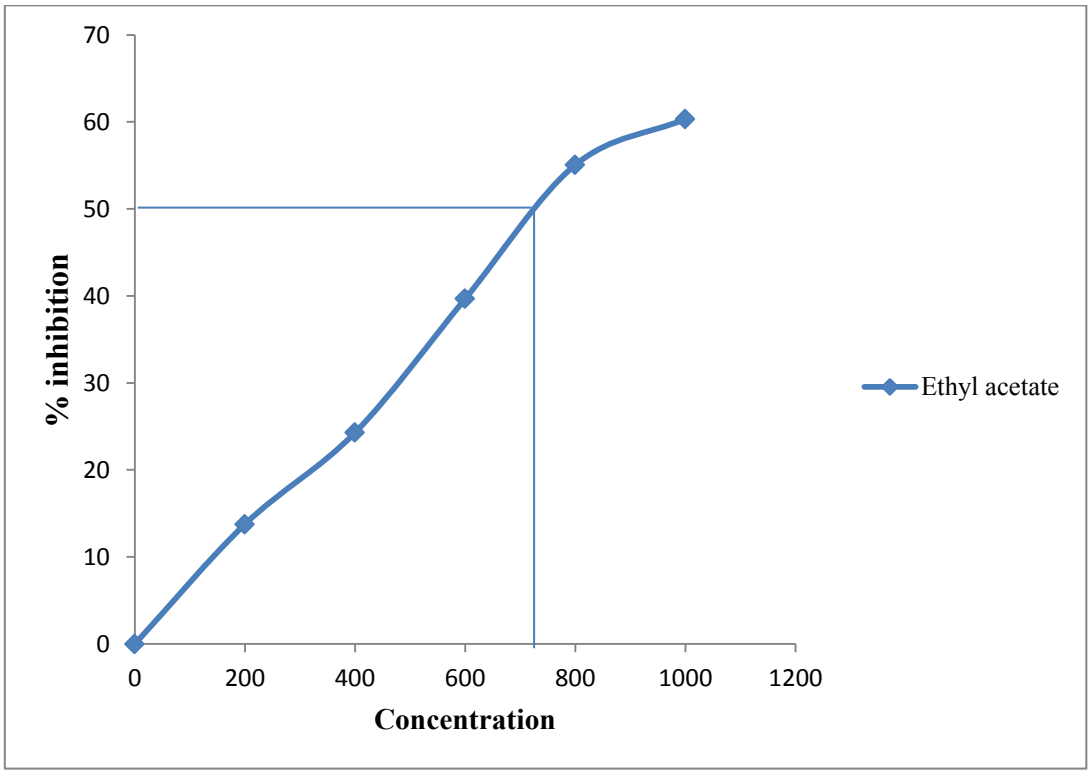
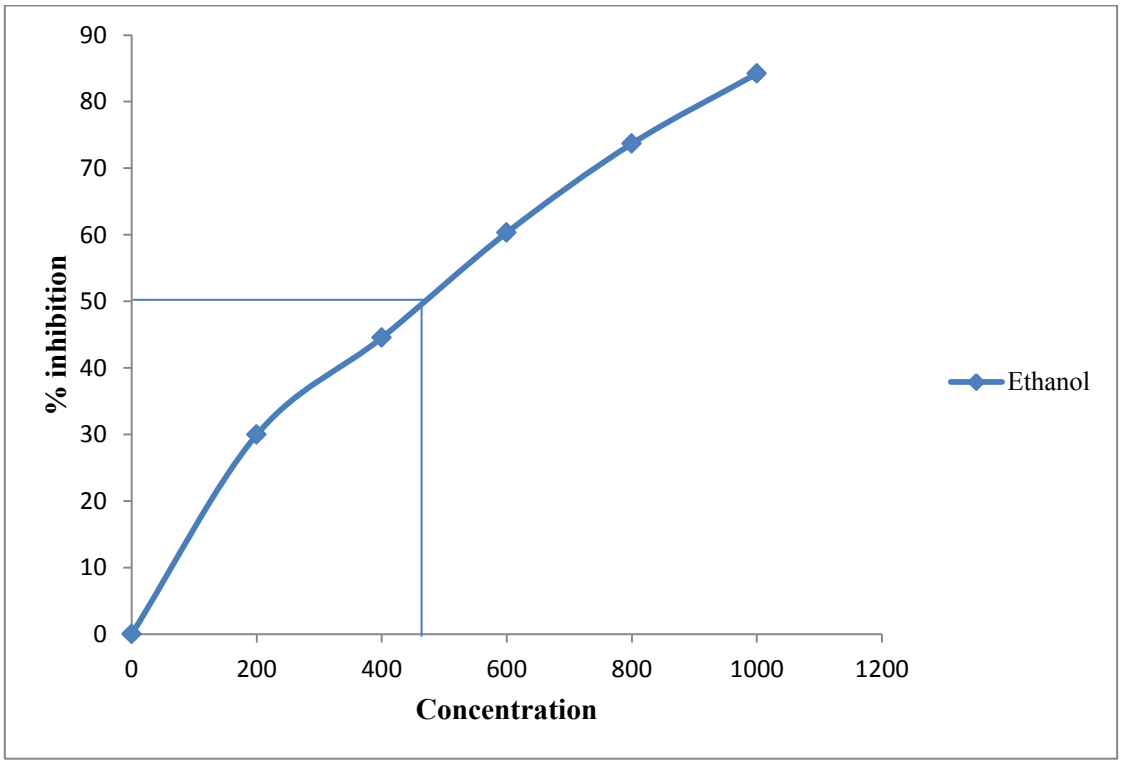


Figure 17: Ethanol extract



The free radical scavenging activities of extracts depends on the ability of antioxidant compounds to loss hydrogen and structural conformation of these components. The DPPH radical's binding ability with hydrogen (H) is considered as a radical scavenging property. A solution of DPPH radicals prepared in methanol is converted into DPPH-H molecules in the presence of antioxidants, as it shown in following equation.

$$DPPH^{\bullet} + \overleftarrow{A}\text{-}H \leftrightarrow DPPH\text{-}H + A^{\bullet}$$

From the results the IC_{50} values of various extracts of *Delonix elata* (L.) leaf and standard are depicted. The IC_{50} values of plant extract was compared with the IC_{50} value of standard (Ascorbic acid). The ethanolic extract of *Delonix elata* (L.) leaf showed significant IC_{50} value (476.09 U/mg Protein) than petroleum ether (2117.48 U/mg Protein) and ethyl acetate (784.28 U/mg Protein) extract. The IC_{50} value of ethanolic extract of *Delonix elata* (L.) leaves has produced significant IC_{50} value near to standard (Quercetin) 96.59 U/mg Protein.

From the results of SOD and DPPH assay showed that the ethanolic extract of *Delonix elata* (L.) leaves possess the potent antioxidant effect than petroleum ether and ethyl acetate extract of *Delonix elata* (L.) leaves.

IN-VITRO CARDIOPROTECTIVE STUDY USING H9c2 (RAT CARDIOMYOCYTE) CELL LINE

H9c2 (rat cardiomyocyte) cell line derived from rat heart ventricle of embryo and it was widely used as an *in-vitro* cellular cardiac model because they have most of the molecular and functional features of adult cardiomyocytes. Cardiac hypertrophy is a major risk factor for heart failure. H9c2 cell line has the advantage of being animal-free alternative

and it can accurately mimic the hypertropic responses[90, 91]. So this finding are helps to choose H9c2 cell line as a model for the study of *In-vitro* cardioprotective effect of *Delonix elata* (L.) leaf extracts.

CELL VIABILITY ASSAY (MTT ASSAY)

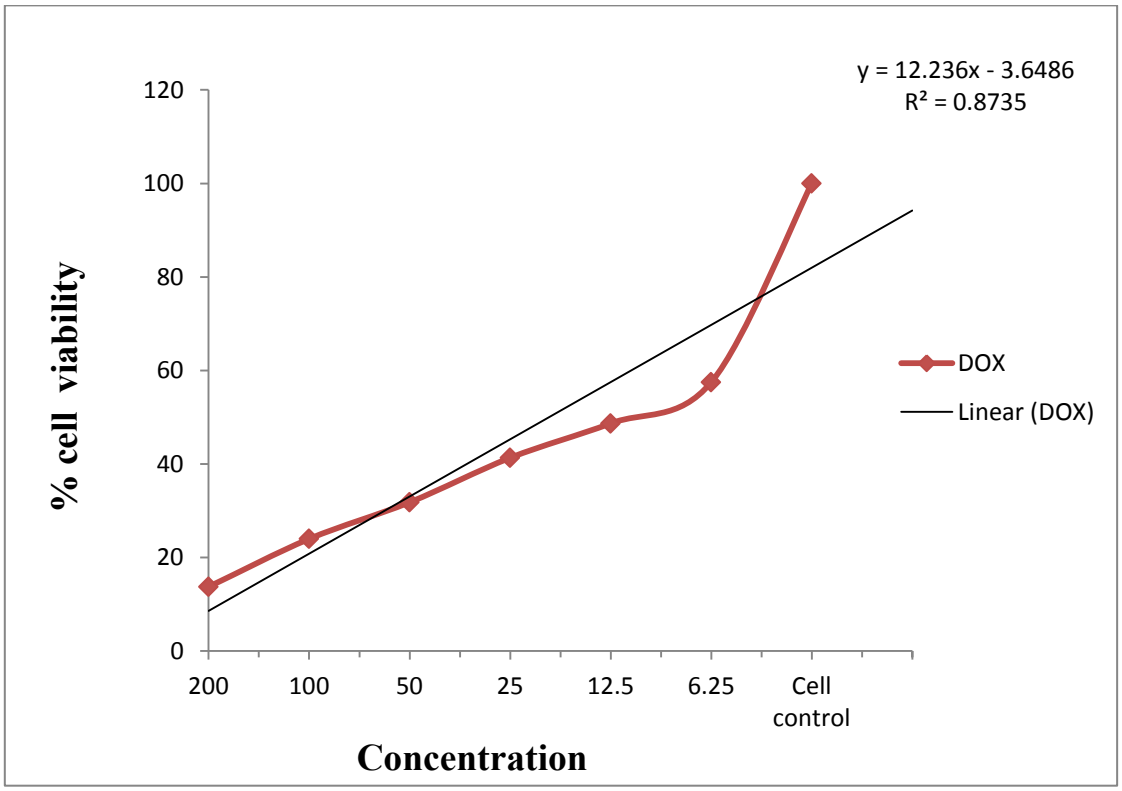
The MTT assay is most commonly used method for determining the viability of the cells. It is colorimetric assay for assessing the cell metabolic activity. Viable cell contains NAD (P) H - dependent oxidoreductase enzymes which reduce the MTT reagent to formazan, an insoluble crystalline product with a deep purple colour. Formazan crystals are dissolved using a solubilising solution and absorbance was measured at 500-600nm. When the cells are die they lose their ability to convert MTT into formazan. If more cells are in viable they shows thick colour formazan formation. The colour formation serves as a convenient marker of the viable cells. The cell line is treated with Doxorubicin alone and Plant extract with doxorubicin and percentage of cell viability is depicted in following **Table 5 and Figure 18.**

Table 5: Cytotoxic effect of different concentrations of Doxorubicin on H9c2 cell line

S.No	Concentration (µg/ml)	Dilutions	Cell viability (%)
1	200	1:1	13.75
2	100	1:2	23.99
3	50	1:4	31.82
4	25	1:8	41.33
5	12.5	1:16	48.69
6	6.25	1:32	57.48
7	Cell control	-	100

Values are expressed as triplet determination

Figure 18: Cytotoxic effect of different concentrations of Doxorubicin on H9c2 cell line



From **Table 6, 7, 8 and Figure 19** shows the result of cytotoxic effect of doxorubicin induced cardiotoxicity on H9c2 cell line after 24hrs. In doxorubicin treated cell line significantly decreased the cell viability in a dose dependent manner and it shows increased cell death because of its excessive ROS production acitivity. Doxorubicin has the several mechanism for the cardiotoxic effects, but an excessive generation of free radicals (ROS) is the major mechanism behind drug induced toxicity[92]. Doxorubicin has Quinone moiety in their molecule which is converted enzymatically or non-enzymatically by cytochrome P_{450} into its semiquinone moiety by the acquisition of electrons. This semiquinone form is then oxidised by molecular oxygen to yield free radicals which is leading to cardiotoxicity. Doxorubicin was reduced the half percentage of cell viability on H9c2 cell line at the concentration of 4.38µg/ml after 24hrs. So the Doxorubicin has cardiotoxic effect in a dose dependent manner.

Table 6: Cardio protective effect of Pet. Ether extract of *Delonix elata* (L.) leaves on H9C2 cell line induced cardiotoxicity using Doxorubicin

S.No	Concentration (µg/ml)	Dilutions	Cell viability (%)
1	200	1:1	70.48
2	100	1:2	62.17
3	50	1:4	50.55
4	25	1:8	39.69
5	12.5	1:16	29.01
6	6.25	1:32	19.76
7	Cell control	-	100

Values are expressed as triplet determination

Table 7: Cardio protective effect of Ethyl acetate extract of *Delonix elata* (L.) leaves on H9C2 cell line induced cardiotoxicity using Doxorubicin

S.No	Concentration (µg/ml)	Dilutions	Cell viability (%)
1	200	1:1	89.31
2	100	1:2	77.60
3	50	1:4	64.37
4	25	1:8	52.16
5	12.5	1:16	38.93
6	6.25	1:32	26.72
7	Cell control	-	100

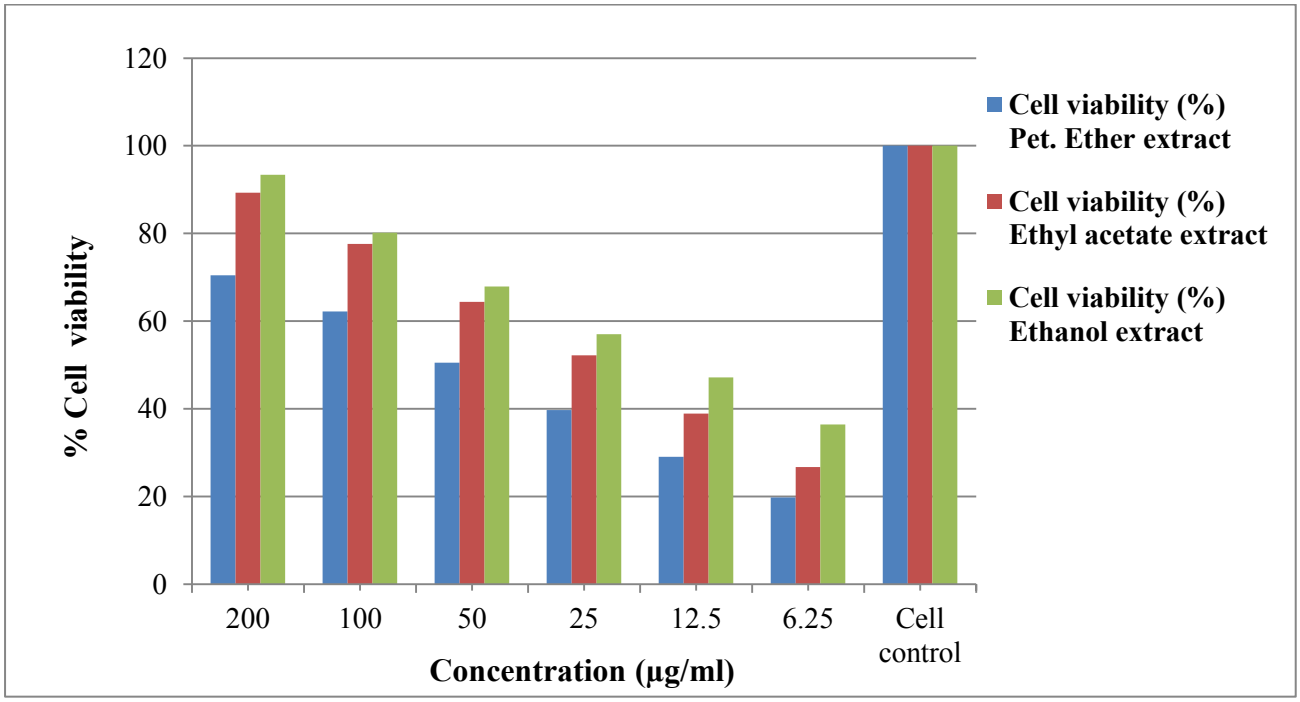
Values are expressed as triplet determination

Table 8: Cardio protective effect of Ethanol extract of *Delonix elata* (L.) leaves on H9C2 cell line induced cardiotoxicity using Doxorubicin

S.No	Concentration (µg/ml)	Dilutions	Cell viability (%)
1	200	1:1	93.38
2	100	1:2	80.15
3	50	1:4	67.93
4	25	1:8	56.99
5	12.5	1:16	47.16
6	6.25	1:32	36.39
7	Cell control	-	100

Values are expressed as triplet determination

Figure 19: Percentage cell viability of H9c2 cell line at various concentrations of Plant extracts against Doxorubicin induced cardiotoxicity



From **Tables 6, 7, 8 and Figures 19** shows the result of the cardioprotective effect of petroleum ether, ethyl actetate and ethanol extract of *Delonix elata* (L.) leaf against doxorubicin induced toxicity in H9c2 cell line. The plant extracts treated cell lines are significantly increased the cell viability by inhibiting the excessive ROS production activity of the doxorubicin.

The cell viability of pre-treatment with ethanolic extract of *Delonix elata* (L.) leaf significantly increased when compared with petroleum ether and ethyl acetate extract. The percentage cell viability of petroleum ether extract at **200µg/ml** is **70.48%**, ethyl acetate extract is **89.31%** and ethanol extract is **93.38%**. Hence, the ethanolic extract of *Delonix elata* (L.) leaf possess more cardioprotective effect than other two extracts.

From the result of antioxidant study and cardioprotective cell line study (cell viability assay) the ethanolic extract of *Delonix elata* (L.) leaf was possess significant antioxidant and cardioprotective activity than petroleum ether and ethyl acetate extract. So, furtherly the ethanolic extract of *Delonix elata* (L.) leaf was chosen for *in-vivo* cardioprotective study.

Figure 20: Normal H9c2 cell line

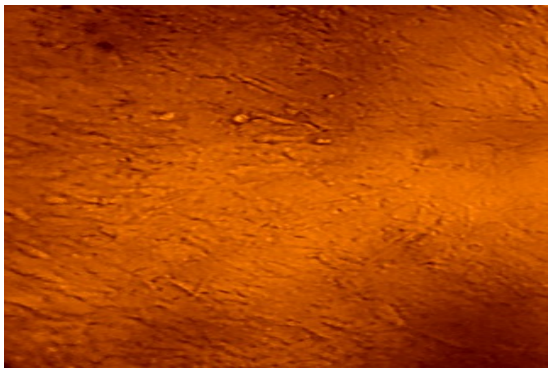
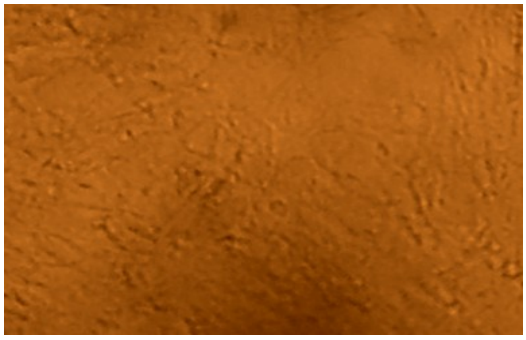
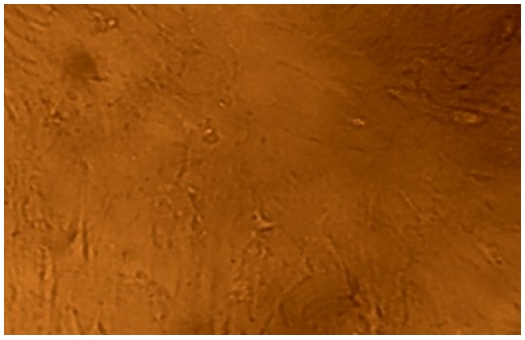


Figure 21: H9c2 cell line treated with different concentrations of Doxorubicin

6.25µg/ml



25µg/ml



100µg/ml

Figure 22: H9c2 cell line treated with petroleum ether extract of *D.elata* leaves and Doxorubicin

50µg/ml **100µg/ml**

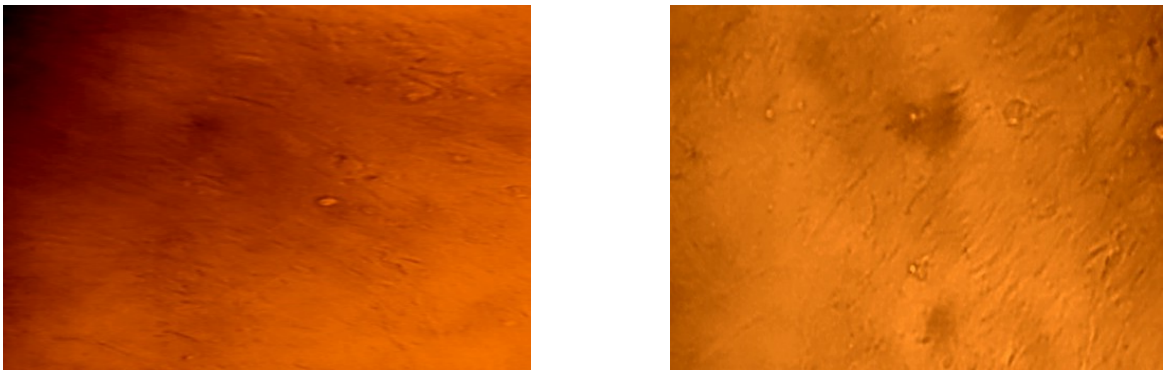


Figure 23: H9c2 cell line treated with ethyl acetate extract of *D.elata* leaves and Doxorubicin

50µg/ml **100µg/ml**

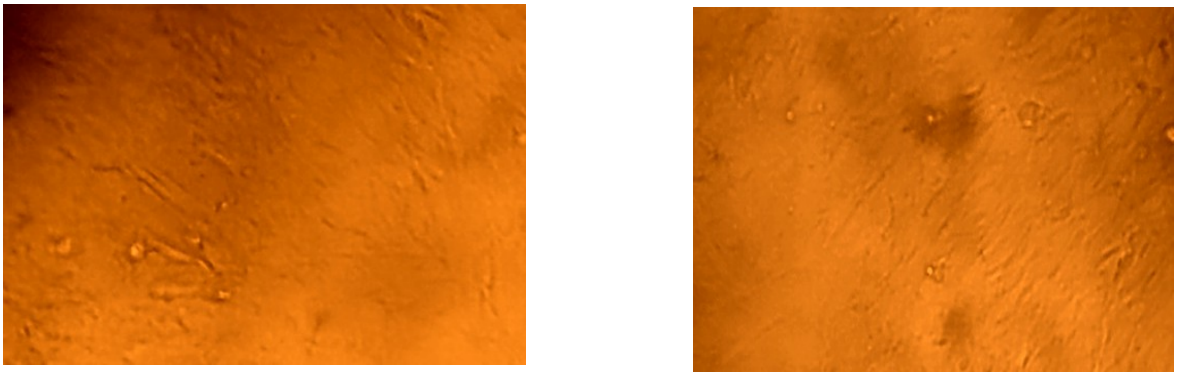
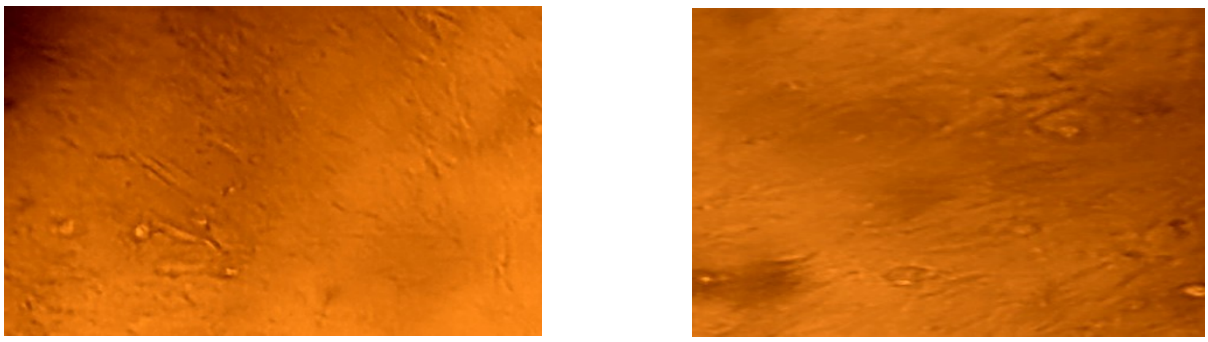


Figure 24: H9c2 cell line treated with ethanolic extract of *D.elata* leaves and Doxorubicin

50µg/ml **100µg/ml**



IN-VIVO STUDIES

ACUTE ORAL TOXICITY STUDY - OECD 423

Acute toxicity studies demonstrates the adverse effects of finite duration occurring within a short period of time (upto 14 days) after administration of single dose of a test substance or after multiple doses within 24hrs of starting point. To evaluate the safety, toxicity and dose determination of ethanolic extract of *Delonix elata* (L.) leaf, the acute oral toxicity was carried out using female wistar rats as per OECD guideline-423. A set of 3 animals were taken for oral administration dose of 2000mg/kg body weight. The behavioural changes and physical observation parameters was observed and the results were given in following **Table 9.**

Table 9: Acute oral toxicity study

Days	**1**				**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Observation parameters	**30 ms**	**1 hr**	**2 hr**	**4 hr**													
Alertness	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aggressiveness	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Touch response	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gripping	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motor co-ordination	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catatonia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Righting reflux	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Corneal reflux	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pinna reflux	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin and fur	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Lacrimation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Writhing effect	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Death	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ Present; - Absent; N- Normal

Animals were observed for appearance any toxic signs after administration of ethanolic extract of *Delonix elata* (L.) leaf (EEDE). The table showed there is no toxic symptoms and behavioural changes were observed during 14 days. The results of acute toxicity study revealed that the administration of EEDE leaf upto 2000mg/kg dose not produce any toxic effect.

IN VIVO CARDIOPROTECTIVE STUDY

After completion of the acute toxicity study, the animals were grouped and the cardioprotective effect of ethanolic extract of *Delonix elata* (L.) leaves against DOX induced cardiotoxicity was established by assessing haematological, biochemical and histopathological studies.

GENERAL OBSERVATIONS

The general observations like behavioural changes and appearance of the animals was noted throughout the experimental period. In DOX treated group have shown the characteristic changes as the animal fur became scruffy, had red exudates around the eyes and nose, soft watery faeces, abdomen enlargement and animals looked weaker and lethargic when compared with normal control. These observations are markedly less in the animals treated with ethanolic extract of *Delonix elata* (L.) leaves (EEDE) when compared with DOX treated group.

BODY WEIGHT

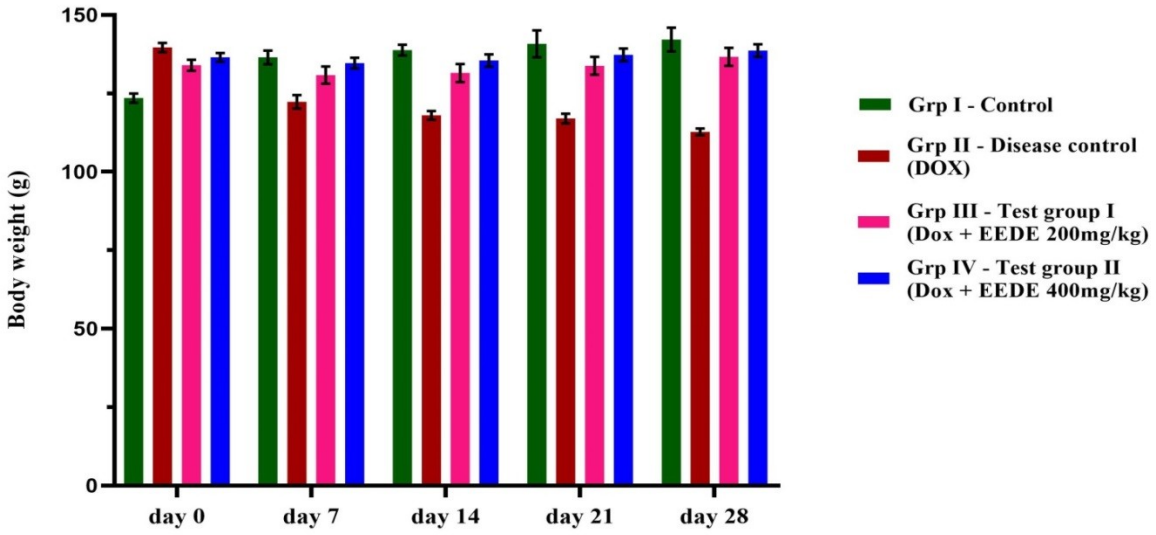
The body weight changes of the animals was measured thoughout the study period and given it into the **Table 10 and Figure 25.**

Table 10: Body weight changes

Days	Body weight (g)			
	Normal control	Disease control	EEDE (200mg/kg)	EEDE (400mg/kg)
0	123.5±1.43	139.66±1.47	134±1.73	136.5±1.38
7	136.5±2.15	122.33±2.12$^{\#\#}$	130.83±2.75^{NS,*}	131.5±1.72 ^{NS,**}
14	138.83±1.70	118±1.41$^{\#\#}$	131.5±2.92 ^{NS,*}	135.5±1.96 ^{NS,**}
21	140.83±4.26	117±1.52$^{\#\#}$	133.83±2.85 ^{NS,*}	137.33±1.97 ^{NS,**}
28	142.16±3.77	112.75±1.07$^{\#\#}$	136.66±2.84 ^{NS,*}	138.66±1.98 ^{NS,**}

- **Values are expressed as Mean ± SEM (n=6)**
- **$^{\#\#}$P≤0.01, ^{NS}P≥0.05 compared with Normal control**
- ***P≤0.05, **P≤0.01 compared with Disease control**

Figure 25: Body weight changes



The results tabulated shows the DOX treated group significantly decreased in body weight when compared with the normal control group, and it may occurred due to reduced food intake and weakness caused by Doxorubicin administration. The ethanolic extract of *Delonix elata* (L.) leaves treated group significantly increased the body weight when compared with disease control (DOX treated) group. The high dose of EEDE group ($P<0.01$) most significantly increased the body weight than low dose of EEDE group ($P<0.05$).

HEART WEIGHT

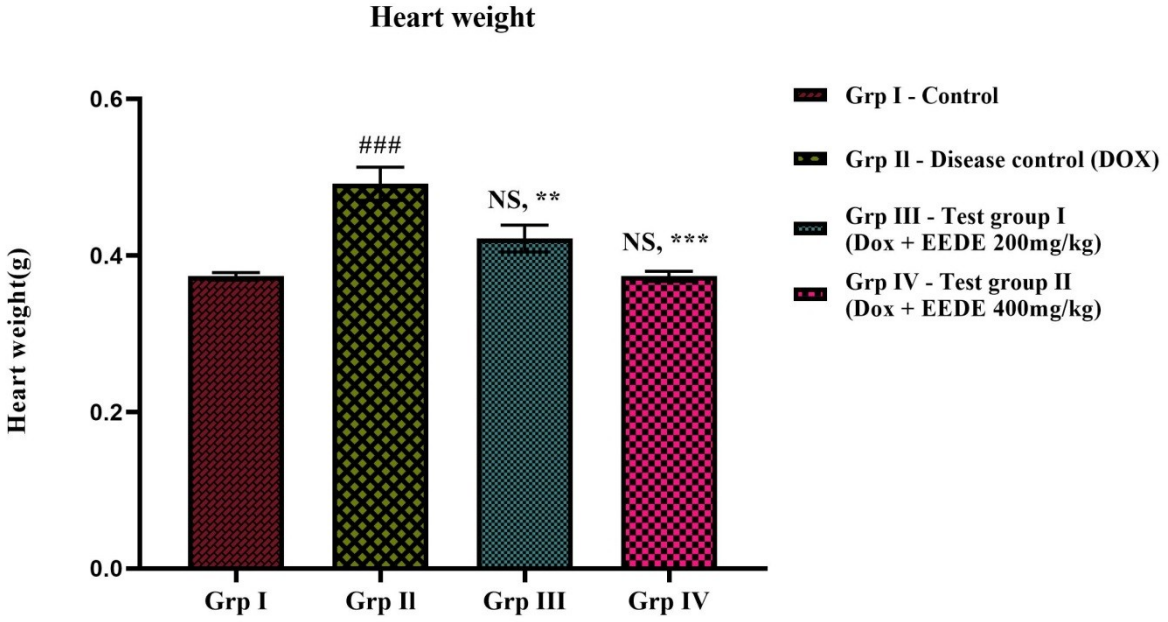
After end of the treatment period the heart weight of the animals was measured and tabulated in **Table 11 and Figure 26.**

Table 11: Heart weight changes

S.NO	GROUP	HEART WEIGHT (g)
1	Normal control	0.37±0.0049
2	Disease control	$0.49\pm0.0210^{\#\#\#}$
3	EEDE (200mg/kg)	$0.42\pm0.0170^{NS,**}$
4	EEDE (400mg/kg)	$0.37\pm0.0066^{NS,***}$

- **Values are expressed as Mean ± SEM (n=6)**
- **$^{\#\#\#}P\leq0.001$, $^{NS}P\geq0.05$ compared with Normal control**
- **$^{**}P\leq0.01$, $^{***}P\leq0.001$ compared with Disease control**

Figure 26: Heart weight changes



The results showed that the DOX treated group significantly increased in heart weight when compared with the normal control group, and it may occurred due to enlarged, dilated and the edema as well as infiltration of inflammatory cells. The ethanolic extract of *Delonix elata* (L.) leaves treated group significantly decreased the heart weight when compared with disease control (DOX treated) group. The high dose of EEDE group ($P<0.001$) most significantly decreased the heart weight than low dose of EEDE group ($P<0.01$).

HEMATOLOGICAL PARAMETER ANALYSIS

Blood is an important index for physiological and pathological index for humans and animals and the parameters usually measured are haemoglobin (Hb), RBC and WBC. Alterations in blood parameters may be due to the changes in cellular integrity and membrane permeability of cells or even exposure to toxic chemicals[93]. DOX intoxication might be lead

to anaemia as a result of either altered activity of hematopoietic tissue, impaired erythropoiesis and defective iron metabolism.

HAEMOGLOBIN

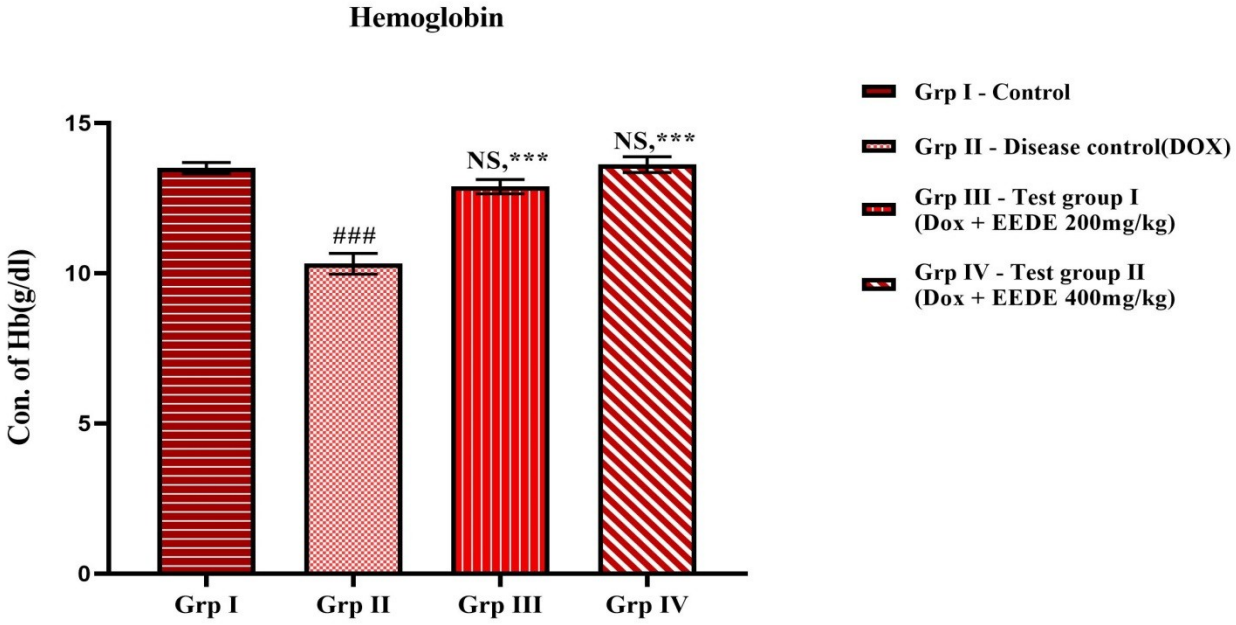
Haemoglobin (Hb) is the oxygen carrying protein which is present in RBC. Hb concentration may affect the cardiovascular system through oxygen supply and viscosity[94]. An increased or decreased levels of Hb are independently associated with increased risk factor of CVDs. The Hb concentration of the animals were given in following **Table 12 and Figure 27.**

Table 12: Concentration of Hemoglobin

S.NO	GROUP	HEMOGLOBIN (g/dl)
1	Normal control	13.5±0.18
2	Disease control	$10.3\pm0.34^{###}$
3	EEDE (200mg/kg)	$12.8\pm0.24^{NS,***}$
4	EEDE (400mg/kg)	$13.6\pm0.26^{NS,***}$

- **Values are expressed as Mean ± SEM (n=6)**
- **$^{###}P\leq0.001$, $^{NS}P\geq0.05$ compared with Normal control**
- **$^{***}P\leq0.001$ compared with Disease control**

Figure 27: Concentration of Hemoglobin



From the **Table 12 and Figure 27,** the result showed that the DOX treated group (P<0.001) significantly decreased the Hb content when compared with the normal control group. In EEDE leaves treated group both low and high dose group (P<0.001) significantly increasing the Hb level.

TOTAL RBC

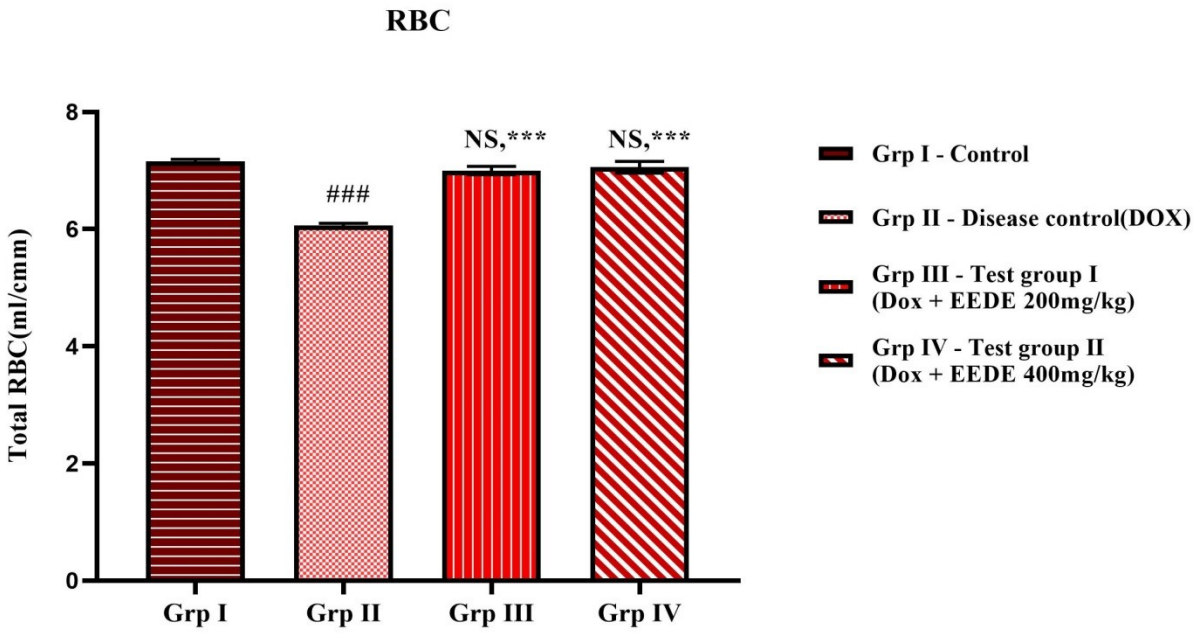
Red Blood Cells the are constituents of intravascular clots and carry oxygen. RBCs may play a prothrombotic role in blood coagulation by increasing blood viscosity and forcing platelets towards the vessel wall. Incorporation of RBCs into fibrin clot affects clot structure and mechanical properties. Even small structural differences in RBCs may have large influence on pathophysiology. RBCs may actively participate in thrombin generation[94]. The reduction in RBC level may increasing the heart rate and cause chest pain. The Total RBC level of animals were given in following **Table 13 and Figure 28.**

Table 13: Concentration of RBC

S.NO	GROUP	TOTAL RBC (ml/cmm)
1	Normal control	7.16±0.033
2	Disease control	$6.06\pm0.039^{###}$
3	EEDE (200mg/kg)	$7.001\pm0.071^{NS,***}$
4	EEDE (400mg/kg)	$7.05\pm0.102^{NS,***}$

- **Values are expressed as Mean ± SEM (n=6)**
- **$^{###}P\leq0.001$, $^{NS}P\geq0.05$ compared with Normal control**
- **$^{***}P\leq0.001$ compared with Disease control**

Figure 28: Concentration of RBC



From the **Table 13 and Figure 28,** the result showed that the DOX treated group ($P<0.001$) significantly decreased the total RBC level when compared with the normal control group. In EEDE leaves treated group both low and high dose group ($P<0.001$) significantly increasing the total RBC level.

TOTAL WBC

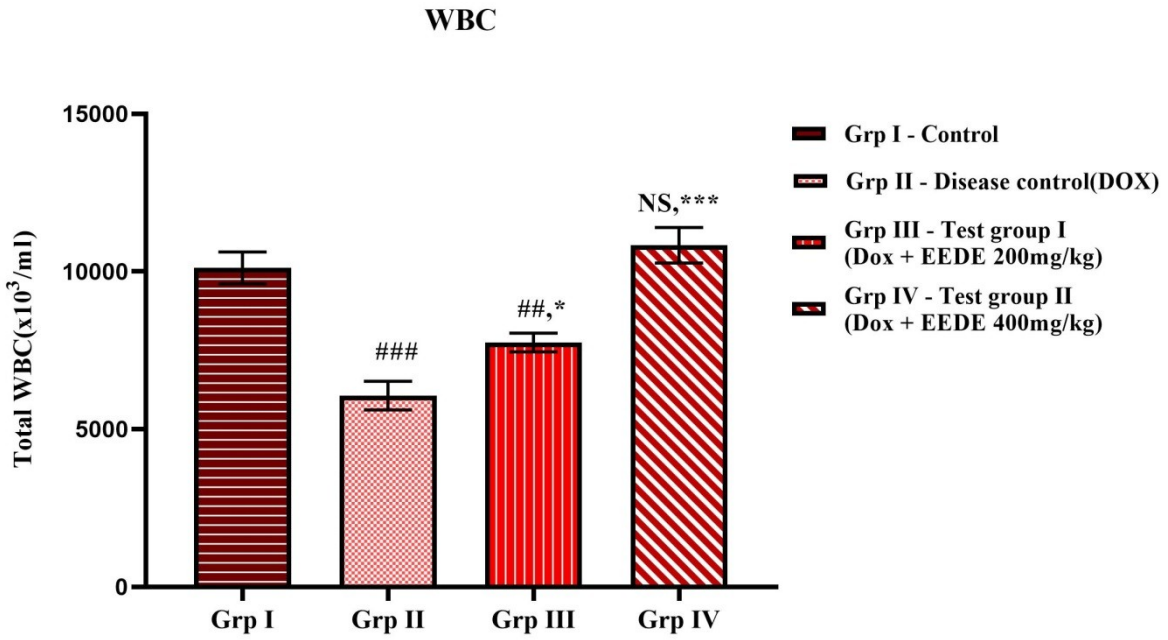
The WBC and WBC subtypes are important for body immune system. The increased and decreased level of WBC may cause inflammatory responses, auto immune disorder and other diseases. In cancer, the chemotherapy drugs are destroying the bone marrow and reduce WBC count and cause several infections and disorders. The total WBC concentrations of animals are given in following **Table 14 and Figure 29.**

Table 14: Concentration of WBC

S.NO	GROUP	TOTAL WBC($x10^3$/m)
1	Normal control	10116.67±504.25
2	Disease control	$6066.67±456.55^{###}$
3	EEDE (200mg/kg)	$7783.33±314.55^{##,*}$
4	EEDE (400mg/kg)	$10833.33±560.16^{NS,***}$

- **Values are expressed as Mean ± SEM (n=6)**
- **$^{###}P\leq0.001$, $^{##}P\leq0.01$, $^{NS}P\geq0.05$ compared with Normal control**
- **$^{*}P\leq0.05$, $^{***}P\leq0.001$ compared with Disease control**

Figure 29: Concentration of WBC



From results of haematological parameters showed that the DOX treated group ($P<0.001$) significantly decreased the total WBC content when compared with the normal control group. In EEDE leaves treated group significantly increasing the total WBC level. The high dose of EEDE leaves treated group showed more significantly ($P<0.001$) increased the WBC level than the low dose of EEDE leaves ($P<0.05$).

The results of haematological parameters showed that the Doxorubicin treated animals significantly decreased the Hb, RBC and WBC counts by changing the cellular integrity and permeability. Dox toxicity leads to the condition of anaemia, which increase the oxygen demand in the blood supply associated with leukopenia. Groups pre-treated with EEDE has been reversed the Dox toxicity and significantly increased the Hb, RBC and WBC contents. Therefore, the results suggested that the extract has a significant protective effect on the hematopoietic system.

SERUM PARAMETER

ASSESSMENT OF CARDIAC MARKER ENZYMES

Cardiac markers enzymes (CK-MB, LDH, CTN I) are important diagnostic factor for CVDs. The elevated levels of marker enzymes in serum may indicate the risk of CVDs. Deficiency of oxygen supply and glucose may cause damage in the myocardial cell membrane leading to permeable and may rupture which resulting in leakage of enzymes[95] (specific biomarkers).

CREATININE KINASE - MB (CK-MB)

Creatinine kinase (CK) is a muscle specific enzyme mainly for heart, and brain and it is the diagnostic marker for myocarditis, cardiac insufficiency, arrhythmias and MI. It has 3 sub types of isoenzymes, they are CK-MM, CK-MB and CK-BB. CK-MB is the main

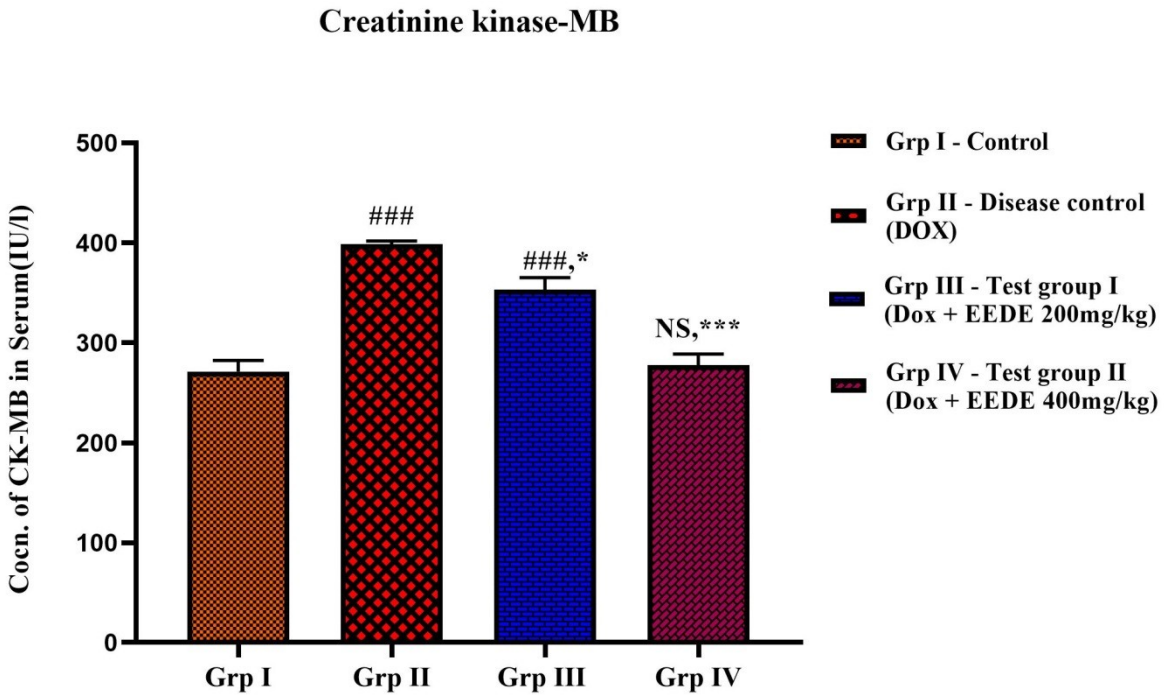
diagnostic marker enzyme for heart. This enzyme released from heart muscle to blood during myocardial damage. The level of CK-MB enzyme was estimated in blood serum and results were given in the following **Table 15 and Figure 30.**

Table 15: Concentration of CK-MB

S.NO	GROUP	CONC. OF CK-MB (IU/l)
1	Normal control	271.33±11.12
2	Disease control	398.83±2.99$^{###}$
3	EEDE (200mg/kg)	353±12.16$^{###,*}$
4	EEDE (400mg/kg)	277.5±11.28^{NS,***}

- **Values are expressed as Mean ± SEM (n=6)**
- **$^{###}$P≤0.001, ^{NS}P≥0.05 compared with Normal control**
- ***P≤0.05, ***P≤0.001 compared with Disease control**

Figure 30: Concentration of CK-MB



From the result, the DOX treated group showed significantly increased (P<0.001) CK-MB level in serum when compared with normal control group. The pre-treatment with EEDE leaves against DOX induced cardiotoxic group significantly decrease the marker enzyme level. The high dose of EEDE leaves most significantly (P<0.001) decreased the CK-MB levels in serum when compared with low dose of EEDE leaves (P<0.05).

LACTATE DEHYDROGENASE (LDH)

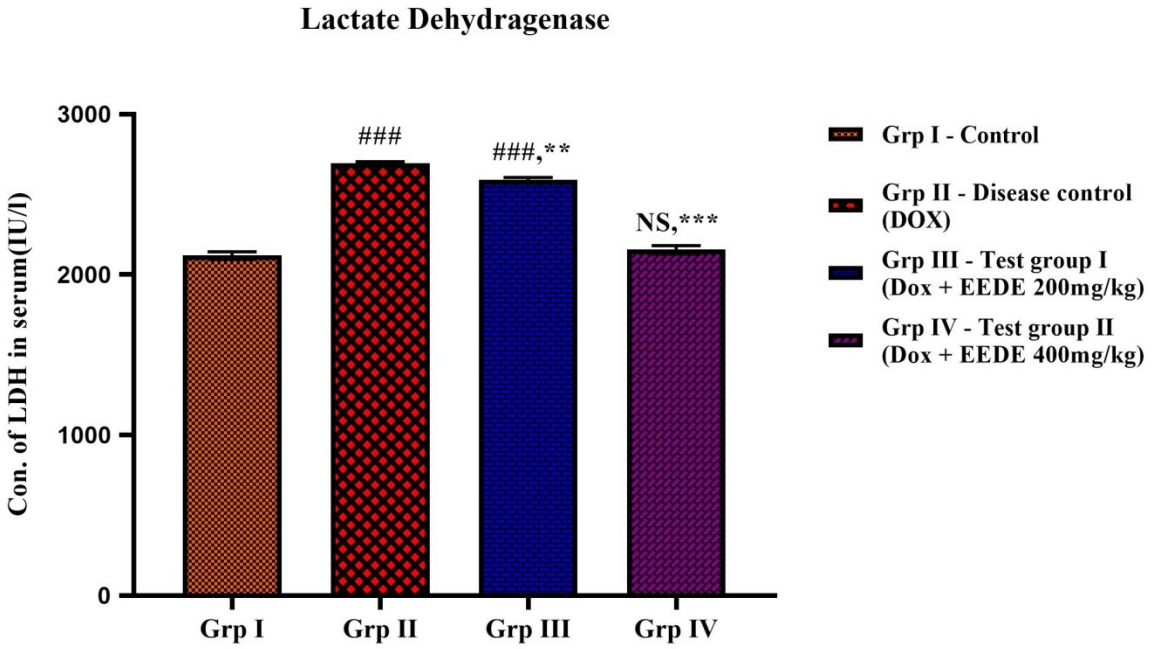
LDH is a protein and expressed extensively in body tissues such as, blood cells and heart muscle. It is released in blood stream during tissue damage and it is a common marker enzyme for cardiac failure. The LDH level in blood serum was estimated and the results were given in the following **Table 16 and Figure 31.**

Table 16: Concentration of LDH

S.NO	GROUP	CONC.OF LDH (IU/l)
1	Normal control	2118.5±24.54
2	Disease control	$2694.17\pm10.82^{\#\#\#}$
3	EEDE (200mg/kg)	$2590.17\pm15.94^{\#\#\#,**}$
4	EEDE (400mg/kg)	$2155.17\pm26.05^{NS,***}$

- **Values are expressed as Mean ± SEM (n=6)**
- **$^{\#\#\#}$P≤0.001, ^{NS}P≥0.05 compared with Normal control**
- ****P≤0.01, ***P≤0.001 compared with Disease control**

Figure 31: Concentration of LDH



From the result, the LDH level in blood serum was significantly increased ($P<0.001$) in DOX treated group and it is compared with normal control group. The pre-treatment with EEDE leaves against DOX induced model showed significantly decreased in LDH level in serum. The high dose of EEDE leaves showed most significant decreased ($P<0.001$) in LDH level in blood serum than the low dose group ($P<0.01$).

CARDIOTROPONIN I (CTN I)

Cardiac troponin T and I are cardiac regulatory proteins that control the calcium mediated interaction between actin and myosin. These regulatory proteins are coded by specific genes and theoretically have the potential of being unique to the myocardium. The measurement of serum CTn I and CTn T is superior sensitivity and specificity to cardiac muscle enzyme measurements in the identification of cardiac muscle damage. The elevated level of cardiac troponin concentrations as the standard biochemical marker of CVDs. The

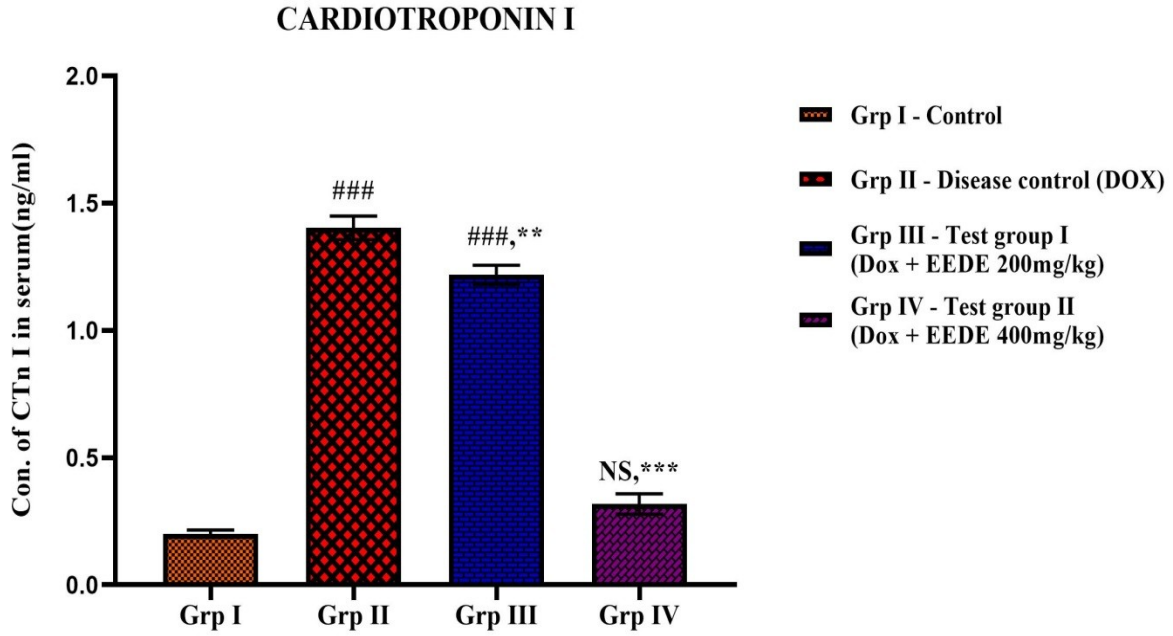
concentration of CTn I in experimental animal serum was estimated and the results were given in the following **Table 17 and Figure 32.**

Table 17: Concentration of CTn I

S.NO	GROUP	CONC. OF CTn I
1	Normal control	0.2016±0.014
2	Disease control	$1.4033\pm0.046^{###}$
3	EEDE (200mg/kg)	$1.2183\pm0.037^{###,**}$
4	EEDE (400mg/kg)	$0.3183\pm0.039^{NS,***}$

- **Values are expressed as Mean ± SEM (n=6)**
- **$^{###}$P≤0.001, ^{NS}P≥0.05 compared with Normal control**
- ****P≤0.01, ***P≤0.001 compared with Disease control**

Figure 32: Concentration of CTn I



From the result, the CTn I level in blood serum was significantly increased (P<0.001) in DOX treated group and it was compared with normal control group. The pre-treatment with EEDE leaves against DOX induced model showed significantly decreased in cTn I level

in serum. The high dose of EEDE leaves showed most significant decreased (P<0.001) in cTn I level in blood serum than the low dose group (P<0.01).

The results showed that the cardiac marker enzymes in blood serum are significantly increased in Doxorubicin treated group. Doxorubicin caused damage in myocardial cells and leaks the marker enzymes which increased in the blood serum and decreased in the cardiac muscle. The group of animals which was pre-treated with EEDE prevents the myocardial cell damage and leakage of cardiac marker enzymes into blood serum. Therefore, the results showed that the plant extract possess significant cardioprotective activity.

ESTIMATION OF LIPID PEROXIDATION

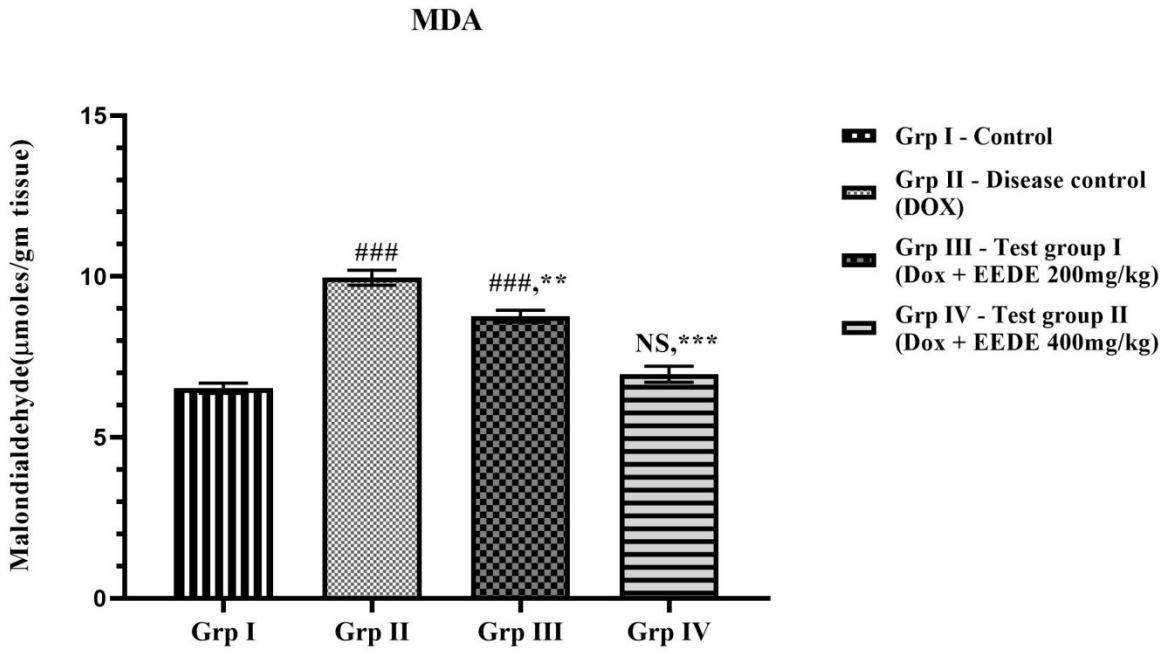
The enzymatic antioxidant defence systems are the natural protector against lipid peroxidation. Lipid peroxidation is one of the fundamental mechanisms of cellular damage caused by free radicals including hydrogen peroxide and hydroxyl radicals. Extents of Lipid peroxidation is evaluated by estimating MDA levels, which is a stable end product of Lipid peroxidation. The level of MDA in experimental animals heart homogenate was estimated and given in the following **Table 18 and Figure 33.**

Table 18: Effect of EEDE leaf extract on Lipid peroxidation

S.NO	GROUP	MDA (µmoles/gm tissue)
1	Normal control	6.53±0.16
2	Disease control	$9.96\pm0.23^{\#\#\#}$
3	EEDE (200mg/kg)	$8.77\pm0.18^{\#\#\#,**}$
4	EEDE (400mg/kg)	$6.96\pm0.25^{NS,***}$

- **Values are expressed as Mean ± SEM (n=6)**
- **$^{\#\#\#}$P≤0.001, ^{NS}P≥0.05 compared with Normal control**
- ***P≤0.01, ***P≤0.001 compared with Disease control**

Figure 33: Effect of EEDE leaf extract on Lipid peroxidation



From the result it was seen that the DOX treated group significantly increased ($P<0.001$) the MDA level. The pre-treatment with EEDE leaves on DOX induced cardiotoxic model significantly decreased the enzyme MDA level. The dose of 400mg/kg EEDE leaves treated group ($P<0.001$) has most significant antioxidant activity than the dose of 200mg/kg group ($P<0.01$).

The most studied free radical reaction is the process of lipid peroxidation. It is oxidative conversion of PUFAs to product known as MDA, usually measured as TBARs or lipid peroxides[96]. It is an important pathogenic event in myocardial necrosis and accumulation of lipid hydroperoxides reflects damage of the cardiac constituents. The lipid peroxides are significantly increased in Doxorubicin treated group. Pre-treated with EEDE group showed decreased MDA level in heart homogenate and the result showed that the plant extract which reduced oxidative stress. Therefore, the results confirmed that the plant extract possess significant antioxidant activity.

ASSESSMENT OF ANTIOXIDANT PARAMETER

Oxidative stress considered as an important factor for the pathogenesis of many diseases CVDs, cancer, etc,. Doxorubicin and other chemotherapy drugs cause oxidative stress in myocardium and induce myocardial damage by increasing level of free radicals (ROS). Antioxidant enzymes are a first line defence system against excessive ROS production. Free radical scavenging enzymes namely catalase, superoxide dismutase, glutathione peroxidase are the first line cellular defence against oxidative injury by decomposing O_2 and H_2O_2 before their interaction to form the more reactive hydroxyl radical (OH*). The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles. Excessive production of free radicals may result in alterations in the biological activity of cellular macromolecules. Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of free radicals (ROS).

SUPEROXIDE DISMUTASE (SOD)

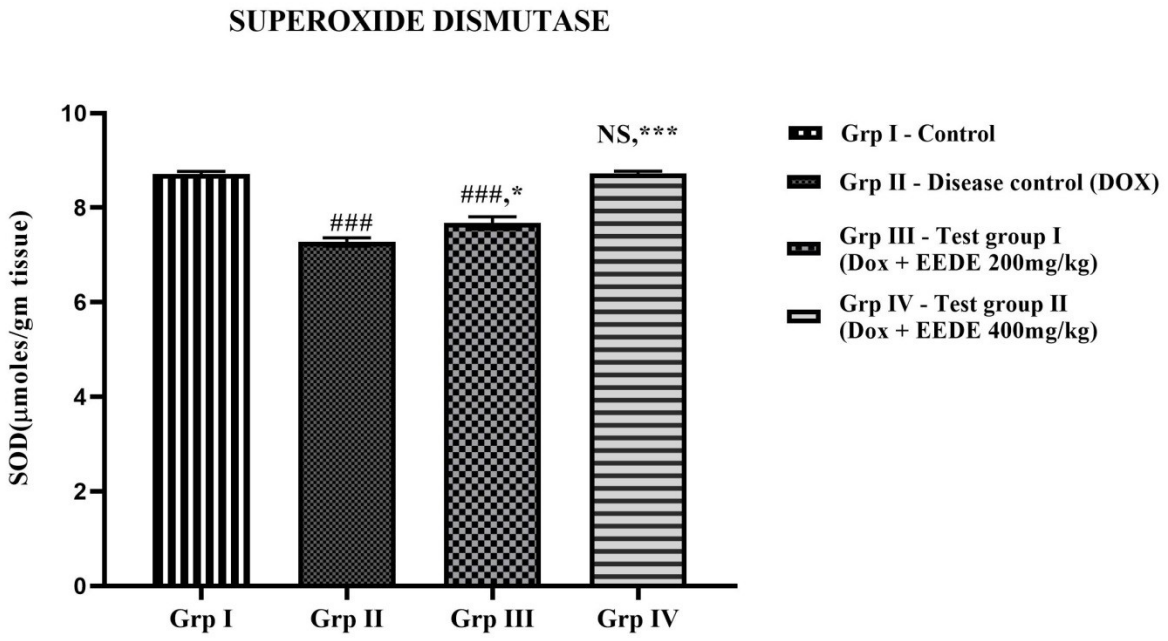
SOD is an important scavenger activity against free radicals (ROS) in the cells by catalysing dismutation of superoxide ion to H_2O_2 and molecular oxygen. The excessive ROS production may reduce the SOD level which is leading to further accumulation of free radicals and enhanced the tissue damage. The level of SOD enzyme in experimental animals heart homogenate was estimated and the results were given in the following **Table 19 and Figure 34.**

Table 19: Effect of EEDE leaf extract on SOD

S.NO	GROUP	SOD (µmoles/gm)
1	Normal control	8.72±0.048
2	Disease control	$7.27\pm0.089^{###}$
3	EEDE (200mg/kg)	$7.68\pm0.131^{###,*}$
4	EEDE (400mg/kg)	$8.73\pm0.042^{NS,***}$

- **Values are expressed as Mean ± SEM (n=6)**
- **$^{###}P\leq0.001$, $^{NS}P\geq0.05$ compared with Normal control**
- **$^{*}P\leq0.05$, $^{***}P\leq0.001$ compared with Disease control**

Figure 34: Effect of EEDE leaf extract on SOD



From the results showed the DOX treated group significantly decreased (P<0.001) the SOD level because of their oxidative stress induced mechanism. The pre-treatment with EEDE leaves on DOX induced cardiotoxic model significantly increased the enzyme SOD level. The dose of 400mg/kg EEDE leaves (P<0.001) treated group of animals has most significant antioxidant activity than the dose of 200mg/kg group (P<0.05).

CATALASE

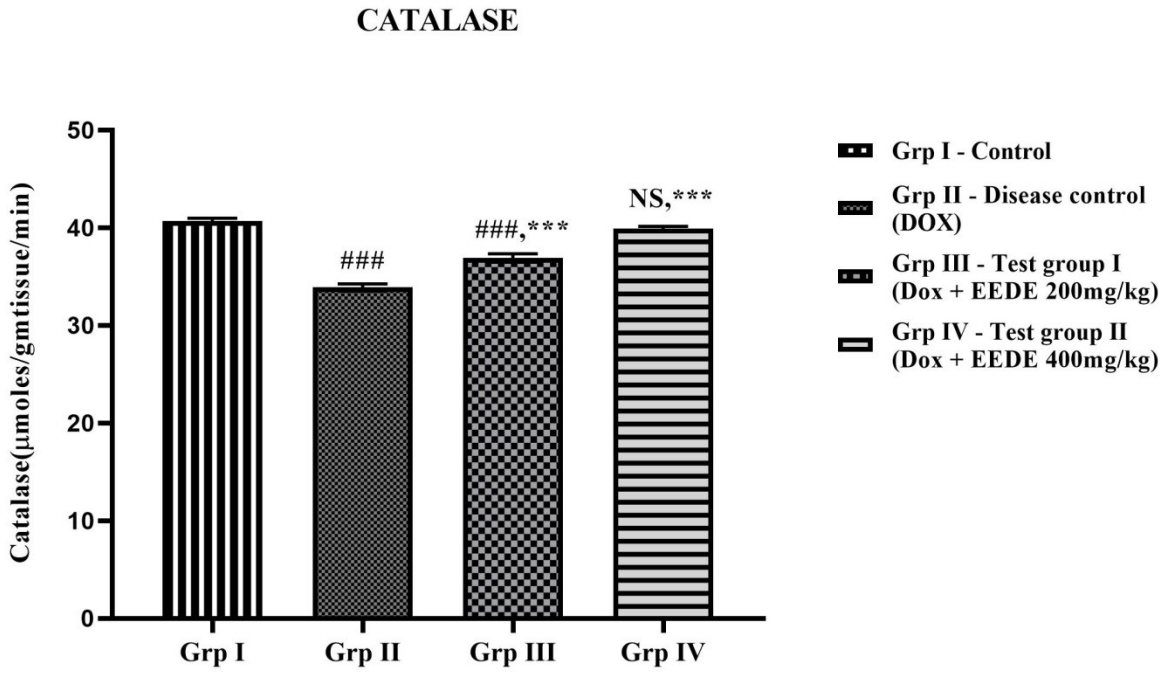
CAT is a key component of the antioxidant defence system. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical induced cellular damage. The level of Catalase enzyme in experimental animals heart homogenate was estimated and the results were given in the following **Table 20 and Figure 35.**

Table 20: Effect of EEDE leaf extract on Catalase

S.NO	GROUP	CAT (µmoles/gm tissue/min)
1	Normal control	40.72±0.25
2	Disease control	$33.90\pm0.36^{###}$
3	EEDE (200mg/kg)	$36.92\pm0.42^{###,***}$
4	EEDE (400mg/kg)	$39.90\pm0.24^{NS,***}$

- **Values are expressed as Mean ± SEM (n=6)**
- **$^{###}P\leq0.001$, $^{NS}P\geq0.05$ compared with Normal control**
- **$^{***}P\leq0.001$ compared with Disease control**

Figure 35: Effect of EEDE leaf extract on Catalase



From the result showed the DOX treated group significantly decreased (P<0.001) the CAT level because of their oxidative stress induced mechanism. The pre-treatment with EEDE leaves on DOX induced cardiotoxic model significantly (P<0.001) increased the enzyme CAT level.

GLUTATHIONE PEROXIDASE (GPx)

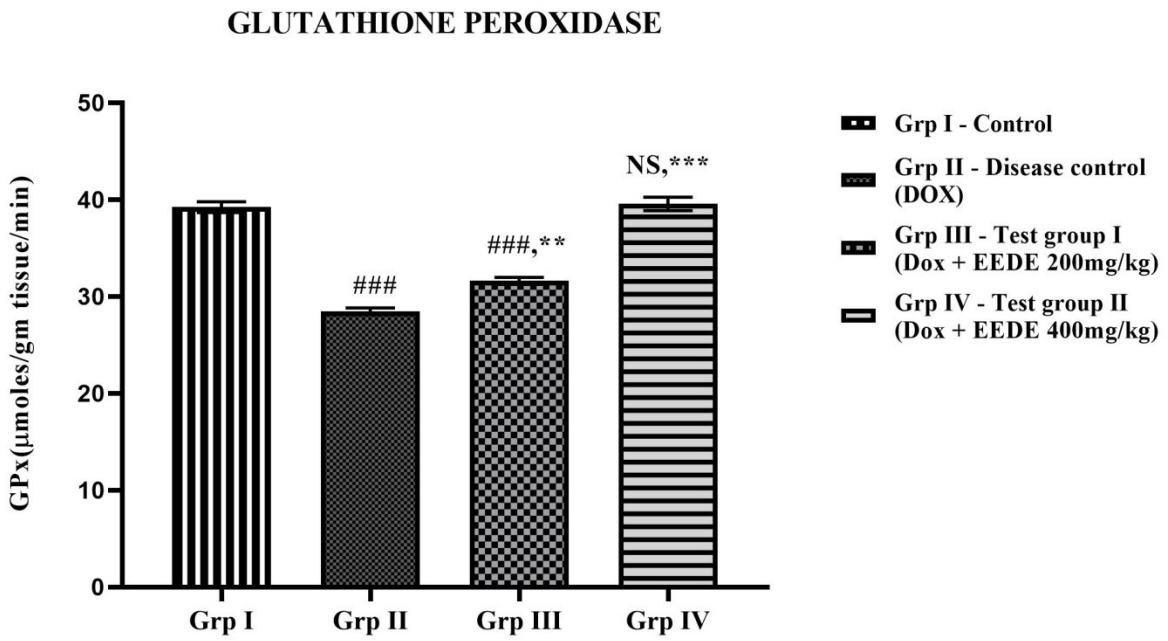
GPx is particularly important for maintaining the structural and functional integrity of the cytosolic and mitochondrial compartments. GPx is a seleno- enzyme, two third of which (in liver) is present in the cytosol and one third in the mitochondria. It catalyzes the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. The level of GPx enzyme in experimental animals heart homogenate was estimated and the results were given in the following **Table 21 and Figure 36.**

Table 21: Effect of EEDE leaf extract on Lipid peroxidation

S.NO	GROUP	GPx (µmoles/gm tissue/min)
1	Normal control	39.23±0.55
2	Disease control	$28.46\pm0.35^{\#\#\#}$
3	EEDE (200mg/kg)	$31.63\pm0.36^{\#\#\#,**}$
4	EEDE (400mg/kg)	$39.57\pm0.70^{NS,***}$

- **Values are expressed as Mean ± SEM (n=6)**
- **$^{\#\#\#}$P≤0.001, ^{NS}P≥0.05 compared with Normal control**
- ****P≤0.01, ***P≤0.001 compared with Disease control**

Figure 36: Effect of EEDE leaf extract on GPx



From the result showed the DOX treated group significantly decreased ($P<0.001$) the GPx level because of their oxidative stress induced mechanism. The pre-treatment with EEDE leaves on DOX induced cardiotoxic model significantly increased the enzyme GPx level. The dose of 400mg/kg EEDE leaves ($P<0.001$) treated group animals has most significant antioxidant activity than the low dose group ($P<0.01$).

The results of *in vivo* enzymatic antioxidant study showed that the antioxidant enzymes like SOD, CAT and GPx are significantly decreased in Doxorubicin treated group by excessive generation of free radicals (ROS). Pre-treatment with EEDE group reduce the free radical formation and increased the level of antioxidant enzymes in heart homogenate.

HISTOPATHOLOGICAL STUDIES

The histopathological study carried out from the isolated heart under light microscopy. The morphological changes are showed in following **Figures 37.**

Figure 37: Histopathological studies

Fig.a - Normal control

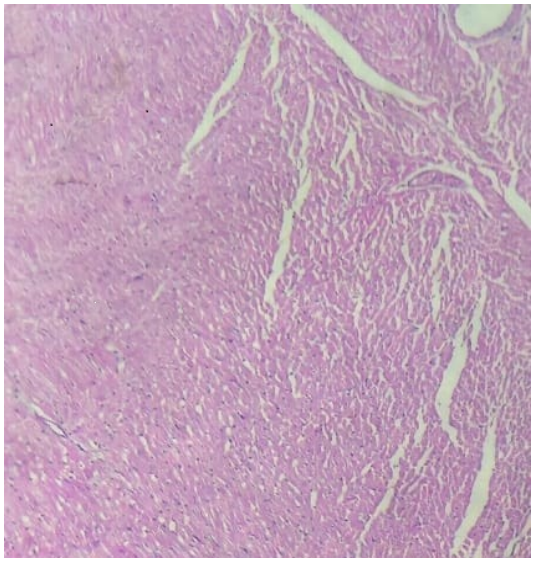


Fig.b - Disease control

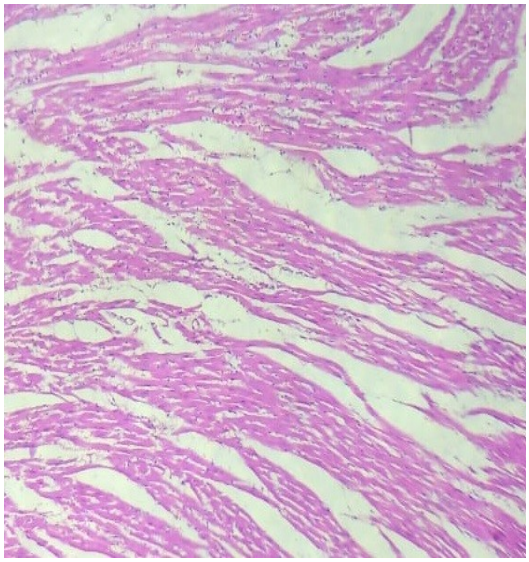


Fig.c - EEDE (200mg/kg)

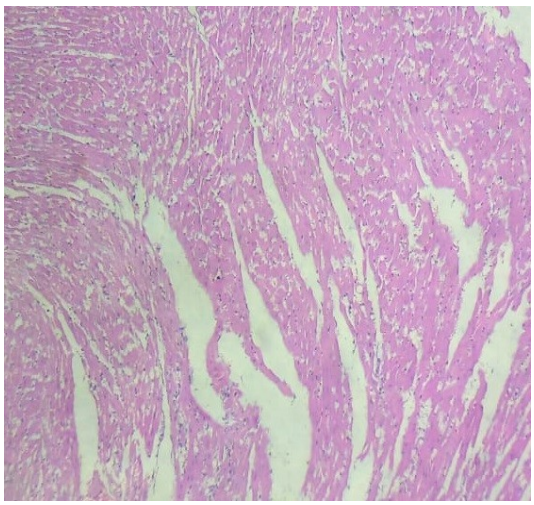
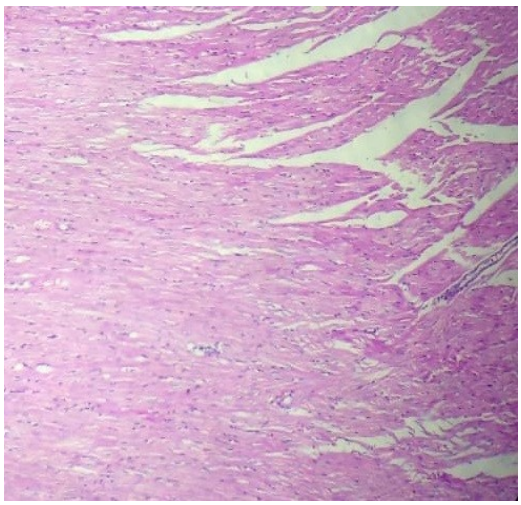


Fig.d - EEDE (400mg/kg)



Group I - Normal control group

The figure did not show any morphological changes and heart muscle showed normal architecture. Myocardial fibres were found to be uniform size, shape and configurations with no inflammatory cell infiltrates.

Group II - DOX treated group

DOX treated group shows enormous changes in myocardial cell with degeneration of myocardial tissue, myofibril loss and infiltration of inflammatory cells, vacuolisation of cardiomyocytes.

Group III and IV - Pre-treatment of EEDE leaf (low and high dose) to DOX

The pre-treatment of low dose 200mg/kg and high dose 400mg/kg of EEDE leaves was protects the heart muscle from DOX induced myocardial damage and restore the normal architecture of the cardiac muscle.

9. SUMMARY AND CONCLUSION

The present study was chosen for the leaf of *Delonix elata* (L.) for the scientific evaluation of cardioprotective potential based on the presence of bioactive compounds which is used to prevent cardiotoxicity.

Doxorubicin is an anthracycline antibiotic which is effectively used in the treatment of different malignancies. Nowadays its use is limited because of the drug induce cardiotoxicity in dose dependent manner. Several mechanisms are involved in DOX induced cardiotoxicity, but the major mechanism is oxidative stress. Antioxidant properties of several plants are experimentally proved and widely used as most effective agents against oxidative stress.

ROS is the major causative factor for many diseases. Antioxidants are prevent the deleterious effect caused by these free radicals. *In vitro* antioxidant study and cardioprotective study was carried out in three extracts (petroleum ether, ethyl acetate and ethanol) of *Delonix elata* (L.) to choose effective extract for further *in vivo* studies. The antioxidant effect was evaluated by SOD assay and DPPH radical scavenging assay. The cardioprotective study was evaluated by using H9c2 (Rat Cardiomyocyte) cell line. Cell viability assay (MTT assay) was carried out. From this, the ethanolic extract of *Delonix elata* (L.) leaf possess potent antioxidant effect which exihibit their action at lower concentration. In cell viability assay the DOX treated cells showed decreased cell viability and increased cell death. The pretreatment with the plant extracts increased the cell viability and reduce the cell death. From this also the ethanolic extract of *Delonix elata* (L.) possess potent cardioprotective activity. So the ethanolic extract was chosen for further *in vivo* studies.

Acute oral toxicity study was carried out for evaluating the safety and toxic effect of ethanolic extract of *Delonix elata* (L.) leaf. From this study the result showed the ethanolic

extract of *D.elata* leaves extract does not have any toxic effect upto the dose of 2000mg/kg body weight.

The *In vivo study* was conducted with 4 groups of wistar rats, 6 animals in each group. For assessing the cardioprotective activity, Doxorubicin induced cardiotoxicity model was used. The doses of plant extract was chosen for the test to be 200 and 400mg/kg body weight of the animal.

In doxorubicin induced cardiotoxic rats showed significant decrease in body weight and looked very weaker when compared with the normal control group. The pre-treatment of EEDE leaf to Dox administered animals showed significant increase in body weight. The heart weight of Dox treated rats showed significant increase when compared with normal control group. The pretreatment of EEDE leaf to Dox administered animals showed significant decrease in heart weight.

In Dox induced cardiotoxic group significantly decreased the haematological parameter values and the pretreatment of EEDE leaf to Dox administered animals shows that the haematological parameter values were increased. The elevation of cardiac marker enzyme level in serum was showed in Dox induced cardiotoxic group and it confirms the onset of myocardial injury. The pretreatment of EEDE leaf to Dox administered animals significantly decreased the cardiac marker enzyme level in serum.

The antioxidant enzyme (SOD, CAT, GPx) level was decreased in Dox induced cardiotoxic rats heart homogenate which is compared with normal control group. The pretreatment of EEDE leaf to Dox administered animals showed significantly increased antioxidants level in heart homogenate. Dox induced cardiotoxic group increased lipid peroxidation parameter like MDA level and it was significantly decreased in EEDE treated group.

Histopathological studies in isolated heart revealed that the pretreatment of EEDE leaf prevent the myocardial damage and restored the normal architecture of the heart.

From this study, it is concluded that the ethanolic extract of *Delonix elata* (L.) was found to possess the cardioprotective activity. Further findings are needed to explore the possible mechanism of action of isolated compound from this plant against cardiotoxicity.

REFERENCES

1. Cardiovascular disease. Available from: https://www.who.int/cardiovascular_diseases/en/
2. Non-communicable disease resource on national, CVD database. Available from: http://www.searo.who.int/india/topics/cardiovascular_diseases/NCD_Resources_National_CVD_database-Final_Report.pdf?ua=1
3. Mann, Zipes, Libby, Bonow. Braunwald's Heart Disease, A textbook of Cardiovascular Medicine. An imprint of Elsevier Inc. 2015; 1:1-28.
4. CVD related death rates. Available from: https://www.livemint.com/Politics/fKmvnJ320JOkR7hX0lbdKN/Rural-India surpasses-urban-in-heart-diseaserelated-deaths.html
5. Jeemon P, Reddy KS. Social Determinants of Cardiovascular Disease Outcomes in Indians. J Med Res. 2010; 132:617.
6. Rost K, Pyne JM, Dickinson LM, LoSasso AT. Cost-effectiveness of Enhancing Primary care Depression Management on An Ongoing Basis. Ann Fam Med. 2005; 3:7-14.
7. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons From Sudden Coronary Death A Comprehensive Morphological Classification Scheme for Atherosclerotic Lesions. Atheroscler Thromb Vasc Biol. 2000; 20:1262-1275.
8. Kalogeris T, Baines CP, Krenz, Korthuis RJ. Cell biology of Ischemia/Reperfusion Injury. Int Rev Cell Mol Biol. 2012; 228:229.
9. Bandyopadhyay U, Das D, Banarjee RK. Reactive Oxygen Species: Oxidative Damage and Pathogenesis. Curr Sci. 1999; 77:658-666.
10. Brown JM, Terada LS, Grosso MA, Whitman GJ, Velaso SE, Patt A, *et al.* Hydrogen peroxide mediates reperfusion injury in the isolated rat heart. Mol cell Biochem 1988; 84:173-5.
11. Vijay T, Dhanarajan MS, Cardioprotective Activity of Endangered Indian Medicinal Plants, Shodhganga: A Reservoir of Indian thesis @ INFLIBNET. 2015.
12. Tripathi KD. Essentials of Medical Pharmacology, 7th edition. New Delhi: Jaypee publications. 2013: 867.
13. Kritikar KR, Basu BD. Indian medicinal plants, 2nd edition. Allahabad: International Book Distributors, Book Sellers and Publisher. 1999; 1139-1140.

14. Harsh Mohan, Textbook of Pathology, 6th edition. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd. 2010: 417.
15. Kumar, Abbas, Aster. Robbins & Cotran, Pathologic Basis of Disease, South Asia edition. Reed Elsevier India Pvt Ltd. 2015; 1:523-578.
16. Whorton AR, Montgomery ME, Kent RS. Effects of hydrogen peroxide on prostaglandin production and cellular integrity cultured porcine aortic endothelial cells. J Clin Invest. 1985; 76:295-302.
17. Hennig B, Boissonneault GA, Wang Y. Protective effects of vitamin E in age-related endothelial injury. Int J Vitam Nutr Res. 1985; 59:273-9.
18. Geeaerts MD, Ronveaux-Dupal MF, Lemasters JJ, Herman B. Cytosolic free calcium and proteolysis in lethal oxidative injury in endothelial cells. Am J Physiol. 1991; 261:889-96.
19. Marui N, Offermann MK, Swerlick R, Kunsh C, Rosen CA, Ahmad M, Alexander RW, Medford RM. Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. J Clin Invest. 1993; 92:1866-1874.
20. Offermann MK, Medford RM. Antioxidants and atherosclerosis: a molecular perspective. Heart Dis Stroke. 1994; 3:52-57.
21. McMurray J, Chopra M, Abdullah I, Smith WE, Dargie HJ. Evidence for oxidative stress in unstable angina. Br Heart J. 1992; 68:454-457.
22. Grundy SM, Brewer HB, Cleeman JI, Smith SC, Lenfant C. Definition of metabolic syndrome report of the National Heart, Lung, and Blood Institute/American Heart Association Conference on scientific issues related to definition. Circulation. 2004; 109:3: 433-438.
23. Svenungsson E, Jensen-Urstad K, Heimbürger M, Silveira A, Hamsten A, De Faire U, Frostegard J. Risk factors for cardiovascular disease in systemic lupus erythematosus. Circulation. 2001; 104:1887-1893.
24. Mendis S, Puska P, Norrving B. Global atlas on cardiovascular disease prevention and control. World Health Organization. 2011.
25. Li J, Siegrist J. Physical activity and risk of cardiovascular disease - a meta-analysis of prospective cohort studies. Int J Environ Res Public Health. 2012; 9:2: 391-407.

26. Ferini-Strambi L, Walters AS, Sica D. The relationship among restless legs syndrome (Willis–Ekbom Disease), hypertension, cardiovascular disease, and cerebrovascular disease. J neurol. 2014; 261:6:1051-1068.
27. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Turner MB. Executive summary: heart disease and stroke statistics - 2012 update a report from the American Heart Association. Circulation. 2012; 125:188-197.
28. Gang Liu, Marta Guasch-Ferre, Yang Hu, Yanping Li, Frank B Hu, Eric B Rimm, JoAnn E Manson, Kathryn M Rexrode, Qi Sun. Nut consumption in relation to Cardiovascular disease incidence and mortality among patients with diabetes mellitus. Circ Res. 2019; 920-929.
29. Mythili S, Malathi N. Diagnostic markers of acute myocardial infarction. Biomed Rep. 2015; 3:743-48.
30. Korosoglou G, Labadze N, Hansen A, Selter C, Giannitsis E, Katus H, Kuecherer H. Usefulness of real-time myocardial perfusion imaging in the evaluation of patients with first time chest pain. Am J Cardiol. 2004; 94: 1225–1231.
31. Mahmarian JJ, Moye L, Verani MS, Eaton T, Francis M, Pratt CM. Criteria for the accurate interpretation of changes in left ventricular ejection fraction and cardiac volumes as assessed by rest and exercise gated radionuclide angiography. J Am Coll Cardiol. 1991; 18: 112–119.
32. Damadian R. Tumor detection by Nuclear magnetic resonance. Science. 1971; 117:1151-1153.
33. Gosalia A, Haramati LB, Sheth MP, Spindola-Franco H. CT detection of acute myocardial infarction. Am J Roentgenol. 2004; 182: 1563–1566.
34. Rang HP, Dale MM, Ritter JM, Moore PK. Rang & Dale's Pharmacology, 5th edition. America: Churchill livingstone. 2003; 271.
35. Tripathi KD. Essentials of Medical Pharmacology. 7th edition. New delhi: Jaypee brothers medical publishers (P) Ltd. 2013; 492-574.
36. Sean C Sweetman. Martindale: The complete drug reference. 36th edition. Pharmaceutical Press. 2009; 712-715.
37. Ilango K, Valentina P. Textbook of Medicinal Chemistry. 2nd edition. Chennai: Keerthi Publishers. 2015; 2:253-255.
38. Waun Ki Hong, Robert C Bast Jr, William N Hait, Donald W Kufe, Raphael E Pollock, Ralph R Weichselbaum, James F Holland, Emil Frei III. Holland -Frei, Cancer Medicine. People's Medical Publishing House - USA. 2010; 1840-1846.

39. Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. Cancer. 2003; 97:2869-2879.
40. Billingham ME, Mason JW, Bristow MR, Daniels JR. Anthracycline cardiomyopathy monitored by morphologic changes. Cancer Treat Rep. 1978; 62:865-72.
41. Mann, Zipes, Libby, Bonow. Braunwald's Heart disease, A textbook of cardiovascular medicine. An imprint of Elsevier Inc. 2015; 2:1613-1623.
42. Fuster, Walsh, Harrington, hunt, King III, Nash, Prystowsky, Roberts, Rose. Hurt's The Heart. 13th edition. McGraw-Hill Companies. Inc. 2011; 2:2012-2017.
43. Thiruvalar Selvi N, Fernandus Durai M. Preliminary Phytochemical Screening of *Delonix elata* Linn. Leaf Extracts. J Acad Ind Res. 2015; 5.
44. Aravindhan T Ranganathan, Chitra R Chandran, Pradeep Kumar Y, Parthasarathi T, Priyanka K Cholan. Preliminary Phytochemical Analysis of Leaf extract of *Delonix elata*. Int J Pharm Res Bio sci. 2015; 4:78-82.
45. Gopal Murugananthan, Mohan Shamanna. Phytochemical Studies On *Delonix Elata* Bark. Int J Pharm Sci Res. 2014; 5: 982-988.
46. Yaswanth Kumar D R, Joy Hoskeri H, *Delonix elata* – A Potent Medicinal Plant : A Review, Int J Pharm Pharm Sci. 2013; 5.
47. Telkit gebregiogis Amabye, Afework Mulugeta Bemnzabh, Firehiwot Mekon-nen Tadesse. Phytochemical Constituents and Antioxidant Activity of *Delonix elata L*. in Flower Extract. J Anal Pharm Res. 2016; 2.
48. Amala B, Poonguzhali TV. Studies on Phytochemical Screening and *in- vitro* anti-inflammatory activity of *Delonix elata* L. leaves. Int J Adv Res. 2015; 3:1425 – 1429.
49. Babu K, Samundeeswari A, Chitti Babu CV. Studies on phytochemical screening, estimation of tannin and antioxidant activity of *Delonix elata* Linn. Int J Curr Sci. 2015; 15: 37-42.
50. Suriyavathana M, Sivanarayan V. Comparative Analysis of Phytochemical Screening and Antioxidant Activity of Some Medicinal Plants. Int J Pharm Sci. 2013; 18:72-76.
51. Chitra M, Sathyapriya R, Gayathri U, Hema H. *In vitro* Antioxidant activity of *Delonix elata* L. Asian J Pharm Health Sci. 2011; 1.
52. Pradeepa K, Krishnan V, Harish BG, Venkatesh, Santosh Kumar SR, Girish Kumar K. Antibacterial activity pf leaf extract of *Delonix elata* and molecular docking studies of luteolin. J Biochem Technol. 2012; 3: 193-197.

53. Vijayasanthi M and Kannan V. Antimicrobial activities of *Delonix elata* (Bojer ex Hook.) Raf. and *Spathodea campanulata* P. Beauv.,. Afr J Microbiol Res. 2014; 8: 697-701.
54. Sajja Ravindra Babu, Priyanka Goud K. Evaluation of Anti-Hyperlipidemic and Antioxidant Activity of Ethanolic Extract of Delonix elata on High-Fat Diet Induced Rats. Int J Pharm Pharm Res. 2018; 12.
55. Amala B, Poonguzhali TV. Studies on phytochemical screening and *in-vitro* anti-inflammatory activity of *Delonix elata* L. leaves. Int J Adv Res. 2015; 3:1425-1429.
56. Suriyavathana M, Sivanarayan V. Anti-Inflammatory Activity Of *Delonix Elata* On Collagen Induced Paw Edema In Swiss Albino Mice. Indo Am J Pharm Res. 2014; 4.
57. Wijayasiriwardena C, Chauhan MG, Sharma PP, Lahiri SK, Shah MB. Anti-inflammatory activity of *Delonix elata* (L.) gamble. J Nat Rem. 2009; 9(2): 209-215.
58. Ravichandran S and Panneerselvam P. Anti-Nociceptive Activities Of Combined Extract Of *Cardiospermum Halicacabum* L. And *Delonix Elata* L. Leaves. Int J Biol Pharm Res. 2012; 3(6): 762-766.
59. Seetharam YN, Vijay G, Sharanabasappa N, Srinivas Murthy, Sangamma VR, Antimicrobial and Analgesic Activities of *Delonix Elata* Gamble And *Delonix Regia* Raf.,. Aryavaidyan. 2002; 16:51-53.
60. Pradeepa Krishnappa, Krishna Venkatarangaiah, Venkatesh, Santosh Kumar Shivamogga Rajanna, Rajesh Kashi Prakash Gupta. Antioxidant and Prophylactic Effects of *Delonix elata* L., Stem Bark Extracts, and Flavonoid Isolated Quercetin against Carbon Tetrachloride-Induced Hepatotoxicity in Rats. BioMed Res Int, 2014: 14.
61. Ravichandran S, Panneerselvam P. *Invivo* Antioxidant Properties Of Combined Extracts Of *Cardiospermum Halicacabum L.* And *Delonix Elata L.* Leaves On Ccl4 Induced Erythrocyte Damage In Rats. Int J Preclinic Pharma Res. 2014; 5(1): 19-24.
62. Pradeepa Krishnappa, Krishna Venkatarangaiah, Venkatesh, Santosh Kumar Shimoga Rajanna, Rebijith Kayattukandy Baland. Wound healing activity of Delonix elata stem bark extract and its isolated constituent quercetin-3-rhamnopyranosyl-(1-6) glucopyranoside in rats. J Pharm Anal. 2016; 6: 389–395.
63. Ayshath Afroos Shahana AR, Sanjiv karale, Jagadish V Kamath. Cardio protective effect of *Mentha longifolia* against cyclophosphamide induced cardiotoxicity in rats:

A biochemical electrocardiographic and histopathological study. Int J Pharm Pharm Sci. 2016; 8(9): 214-217.

64. Nabeel Kinattingal, Mruthunjaya K, Mahalakshmi AM, Manjul SN. Cardioprotective Effect of *Tamarindus indica Linn* against Isoproterenol Induced Myocardial Infarction in Rats. Int J Pharm Pharm Sci. 2016; 8(5).
65. Alkreathy H, Damanhouri ZA, Ahmed N, Slevin M, Ali SS, Osman Am. Aged Garlic extract Protects against Doxorubicin-induced Cardiotoxicity in rats. Food Chem Toxicol. 2010; 48: 951-956.
66. Kaithwas G, Dubey K, Pillai KK. Effect of Aloe vera (*Aloe barbadebsis* Miller) gel on Doxorubicin-induced Myocardial oxidative stress and Calcium Overload in albino rats. Indian J Exp Biol. 2011; 49: 260-268.
67. Agadihiremath Viswanatha Swamy, Sumeet Gulliaya, Agadihiremath Thippeswamy, Basavaraj C Koti, Donnahalli V Manjula. Cardioprotective effect of curcumin against doxorubicin-induced myocardial toxicity in albino rats. Indian J Pharmacol. 2018; 44(1).
68. Firoz N Momin, Bharatesh R Kalai, Tabassum S Shikalgar, Nilofar S Naikwade. Cardioprotective effect of Methanolic extract of *Ixora coccinea* Linn. Leaves on Doxorubicin-induced cardiac toxicity in rats. Indian J Pharmacol. 2012; 44(2).
69. Fard MH, Ghule AE, Bodhankar SL, Dikshit M. Cardioprotective effect of whole fruit extract of Pomegranate on Doxorubicin-induced cardiotoxicity in rat. Pharm Biol. 2011; 40: 377-382.
70. Plant profile. Available from: http://. www.flowers of india.net.
71. Phytoconstituents and uses of *Delonix elata.* Available from: http://envis.frlht.org/plant details.php?disp-id=722.
72. Das K, Samantha L, Chainy GBN. A modified Spectrometric assay of Superoxide radicals. Indian J Biochem Biophys. 2000; 37: 201-204.
73. Mensor LL, Menezes FS, Leitao GG, Reis AS, Dos Santos TC, Coube CS, Leitao SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytoether Res. 2001; 15:127-30.
74. Rabkin SW, Kong JY, Nitroprusside Induces Cardiomyocyte death: interaction with hydrogen peroxide. Am J Physiol Heart Circ Physiol. 2000; 279: H3089-H3100.
75. OECD guideline for testing of chemicals 423 [online]; 2001 December; [cited on 2015 Feb 2011] Available from URL.

76. Drabkin D JL, Austin JH. Spectrometric studies Spectrometric constants for common Haemoglobin derivatives in humans, dog and rabbit blood. Journal Biological Chemistry. 1932; 98:719-733.
77. Chesbrough, Arthur MC. Determination of RBC & WBC count, Laboratory manual of rural tropical Hospitals. The English language book society and Churchill Livingstone. 1972: 145.
78. Neumeir D, Tissue specific distribution of Creatinine kinase isoenzyme. H.Lang, editor, Springerverlag, Newyork. 1981: 85-109.
79. King J. The Dehydrogenase or Oxidoreductase - Lactate dehydrogenase, In: Practical clinical enzymology van.D.Eds.Norstand company Ltd. 1965: 83-93.
80. Kanchana Ganga U, Hemalatha C, Kishori B. Protective role of Date fruit extract against Chlorpyrifos-induced reproductive toxicity in Albino male rats. Int J Green Pharm. 2018; 12(1): S113.
81. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95: 351-8.
82. Mishra H, Fridovich PI. The role of superoxide anions in the autoxidation of epinephrine and a simple assay of superoxide dismutase. J Biol Chem. 1972; 247: 3170-5.
83. Chance B, Machly AC. Assay of catalase and peroxidases. Methods Enzymol. 1955; 2: 764-75.
84. Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller DJ. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney. Possible implications in analgesic nephropathy. Biochem Pharmacol. 1984; 33: 1801-7.
85. Shahidi F. Natural antioxidant chemistry, Health effects and applications, US: Amer Oil Chemists Socity. 1997;1-3.
86. Eastwood MA. Interaction of dietary antioxidants *in vivo*: How fruit and vegetables prevent disease. Int J Med. 1999; 92:527-530.
87. Madhavi DL, Deshpande SS, Salunkhe DK. Food antioxidants: Technological, Toxicological and Health perspectives. New york: CRC press. 1996; 1-5.
88. Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). Clin Sci. 1996; 347:781-786.

89. Anderson KJ, Teuber SS, Gobeille A, Cremin P, Warehouse AL, Steinberg FM. Walnut polyphenolics inhibit *in vitro* human plasma and LDL oxidation. J Nutr. 2001; 131(11):2837-2842.
90. Zordoky BN, El-Kadi AO. H9c2 cell line is a valuble *in vitro* model to study the drug metabolizing enzymes in the heart. J Pharmacol Toxicol Methods. 2007; 56(3):317-322.
91. Watkins SJ, Borthwick GM, Arthur HM. The H9c2 cell line and primary neonatal cardiomyocyte cells show similar hypertrphic responses *in vitro*. *In vitro* Cell Dev Biol Anim. 2011; 47(2):125-131.
92. Mobaraki M, Faraji A, Zare M, Dolati P, Ataei M, Dehghan Manshadi HR. Molecular mechanisms of cardiotoxicity: A review on major side-effect of Doxorubicin. Indian J Pharm Sci. 2017; 79(3):335-344.
93. Maheswaran R, Devapaul A, Muralidharan S, Velmurugan B, Ignacimuthu S. Haematological studies of fresh water fish, *Clarias batrachus* (L.) exposed to mercuric chloride. Int J Integr Biol. 2008; 1(2):49-53.
94. Sadiqua Begum, Nausheen Rumana, Revathi Devi ML, Sadananda KS, Bharathi M. Hematological parameters in Cardiovascular diseases. Pak J Physiol. 2018; 14(1):23-26.
95. Senthil S, Sridevi M, Pugalendi KV. Protective effect of ursolic acid against myocardial ischemia induced by Isoproterenol in rats. Toxicol Mech Methods. 2007; 17(1):57-65.
96. Chole RH, Patil RN, Basak A, Palandurkar K, Bhowate R. Estimation of serum malondialdehyde in oral cancer and precancer and its association with healty individuals, gender, alcohol and tobacco abuse. J Cancer Res Ther. 2010; 6(4):487-491.