"EFFECT OF TAMOXIFEN ON EXPERIMENTALLY INDUCED CARDIAC HYPERTROPHY"

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IN

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BY

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UNDER THE GUIDANCE OF

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This is to certify that ***Mr. VISHAL J. DESAI*** *has prepared his thesis entitled "Effect of Tamoxifen on Experimentally induced Cardiac hypertrophy" in partial fulfillment for the award of M. Pharm. degree of the Nirma University, under my guidance. He has carried out the work at the Department of Pharmacology, Institute of Pharmacy, Nirma University.*

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Vishal J. Desai

Dedicated to
my Beloved
Parents,
Teachers and
Friends

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1. ABSTRACT

Cardiovascular diseases are most prevalent disease worldwide. Cardiac hypertrophy is an independent risk factor for heart failure, arrhythmia, myocardial infraction and other cardiovascular disease. So, it necessary to treat cardiac hypertrophy. Protein kinase C is important key regulator of cardiac hypertrophy and involved in cardiac remodeling and hypertrophic gene regulation. Tamoxifen is reported to inhibit protein kinase C. The aim of present study was to evaluate the effect of Tamoxifen on isoproterenol induced cardiac hypertrophy and partial abdominal aorta constriction (PAAC) induced cardiac hypertrophy.

In Isoproterenol induced cardiac hypertrophy, isoproterenol (5 mg/kg/day, i.p.) was administered for 10 days in health adult wistar rats of either sex. Control group received normal saline and treated group received Tamoxifen (2 mg/kg/day, p.o.) for 10 days. After 10 days animals were sacrificed and various biochemical and cardiac parameters. For PAAC induced cardiac hypertrophy, in healthy adult wistar rats, abdominal aorta was ligated by 4-0 silk thread along with 7.0mm diameter blunt needle. Then needle was removed to leave aorta partially constricted and received tamoxifen (2 mg/kg/day, p.o.) for 30 days. PAAC control group and sham control group received normal saline. After 30^{th} day animals were sacrificed and various biochemical and cardiac parameters were evaluated. Parameter evaluated were serum lipid profile, cardiac markers like Creatinine kinase-MB, Lactate dehydrogenase, C reactive protein, hypertrophic parameters like cardiac hypertrophy index, Left ventricular hypertrophic (LV) index, hemodynamic parameters, LV collagen, prooxidant and antioxidant levels.

Isoproterenol (ISO) control and PAAC control rats significantly produced significant increase in serum triglyceride, total cholesterol and LDL while decrease in HDL levels. Treatment with tamoxifen (2 mg/kg/day, p.o.) significantly reduced serum total cholesterol and LDL levels in hypertrophic treated rats; but there was no effect on triglyceride and HDL level. There was a significant increase in serum cardiac markers like Lactate dehydrogenase (LDH) and Creatine kinase- MB (CK-MB) in ISO and PAAC control rats. Treatment with tamoxifen significantly reduced serum level of LDH and CK-MB in hypertrophic treated rats. Further, hypertrophic control rats

exhibited increase levels of C reactive protein (CRP). Treatment with tamoxifen significantly reduced CRP level in hypertrophic treated rats.

Moreover, ISO control and PAAC control rats exhibited significantly increased in cardiac hypertrophy index (CHI), Left ventricular (LV) hypertrophic index. Treatment with tamoxifen significantly reduced CHI, LV hypertrophic index in hypertrophic treated rats. There was significant increase in LV collagen level and decreased in hypertrophic control rats. Treatment with tamoxifen significantly reduced LV collagen levels in hypertrophic treated rats.

ISO control and PAAC control rats exhibited significantly increase blood pressure and decrease heart rate. Treatment with tamoxifen did not produce any significant change in blood pressure and heart rat. Moreover, there was significant reduction in rate of pressure development and decay in hypertrophic control rats. Treatment with tamoxifen significantly increased rate of pressure development and decay. Further, ISO control and PAAC control rats showed increase pro oxidant level i.e. malondialdehyde (MDA) and reduced antioxidant levels such as superoxide dismutase (SOD) and reduced glutathione (GSH) levels. Treatment with tamoxifen significantly reduced MDA levels and increased SOD and GSH levels in hypertrophic treated rats. Reduction of cardiac hypertrophy with treatment of tamoxifen in hypertrophic treated rats was further supported by histopathological studies of left ventricle, which shows marked reduction in fibrosis and increase extracellular space as compare to ISO control and PAAC control rats.

In conclusion, our data suggests that tamoxifen produces beneficial effects on cardiac hypertrophy as evident specially from reduction in hypertrophic parameters including collagen levels and thereby preserves LV systolic and diastolic dysfunction which may thereby prevent heart failure.

2. Introduction

Cardiovascular diseases (CVDs) are major cause of morbidity and mortality over worldwide. CVDs are the number one cause of death globally, more people die annually from CVDs than from any other cause. According to World Health Organization (WHO) report, almost 23.6 million people will be affected from CVDs by 2030.

Cardiac hypertrophy (CH) is an important predictor of cardiovascular morbidity and mortality, independent of other cardiovascular risk factors associated with diastolic dysfunction (Glennom et al., 1995). Although left ventricular hypertrophy is an adaptive response to pressure and volume overload, this process becomes maladaptive if left untreated and pathologic cardiac hypertrophy then becomes an important and independent risk factor for the development of heart failure. The heart adapts in response to an array of mechanical, hemodynamic, hormonal, and pathological stimuli due to the increased demand for cardiac activity by increasing myocardial mass through the induction of a hypertrophic response (Hunter and Chien, 1999). Also, cardiac hypertrophy is a risk factor for QT-prolongation and sudden cardiac death. Recent studies in human patients and animal models have demonstrated that cardiac hypertrophy significantly affects myocardial electrotonic cell-to-cell coupling, leading to disturbance in action potential duration and potential malignant arrhythmia and sudden cardiac death (Kang YJ, 2006). Myocardial remodeling and the transition from compensated hypertrophy to failure of the myocardium involve a series of complex events at the molecular and cellular levels such as myocyte growth or hypertrophy, changes in myocyte phenotype resulting from re-expression of fetal gene programs and decreased expression of adult gene programs, alterations in the expression or function, or both, of proteins involved in excitation – contraction (E-C) coupling and contraction, myocyte death caused by necrosis or apoptosis and changes in the extracellular matrix (Oka and Komuro, 2008).

Protein kinase C (PKC) is a group of closely related serine– threonine protein kinases, further classified as the (a) Classical PKCs (α, βI, βII, and γ), the diacylglycerol (DAG) and calcium-dependent enzymes,(b) The novel PKCs (θ, ε, δ, and η), which require DAG, but not calcium, for activity, and (c) The atypical PKCs (ζ, λ), which are not stimulated by DAG or calcium, but are stimulated by other lipid-derived

second messengers. Downstream signaling from activated PKC involves two main pathways that are indirect regulators of nuclear events (i) PKC can phosphorylate Raf directly or indirectly via Ras, thereby activating Raf and initiating the ERK pathway which results in activation and nuclear translocation of ERK. (ii) PKC can phosphorylate proteins, such as IκB, that function as a cytoplasmic anchor for proteins that have nuclear functions such as the transcription factor NFκB. Upon phosphorylation of IκB, NFκB is released and subsequently translocated to the cell nucleus where it exerts its function. Additionally, PKC may modify [Ca^{+2}] via Raf and MEK (components of the ERK pathway).

In addition to PKC and other mechanisms, estrogen also plays an important role on cardiovascular system. Estrogen effects are mediated by 2 different nuclear hormone receptors, ERα and ERβ, both of which are expressed in cardiac myocytes, fibroblasts, and vascular cells in human and rodent heart (Grohe et al., 1997; Babiker et al., 2002). ERα and ERβ agonist 17β-estradiol (E2) attenuates cardiac hypertrophy (Pelzer et al., 2005). Cardiovascular mortality in postmenopausal women receiving estrogen replacement therapy (ERT), with estrogen alone or in combination with progesterone appears to be lower than in untreated women. Estrogen also increase atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), which activates guanylate cyclase A receptor and inhibits PKC and ERK activation (Pedram et al., 2005).

Tamoxifen is well known drug from selective estrogen receptor modulator (SERMs) class; basically, used in treatment of breast cancer. In addition to its effect on estrogen receptor, it is also reported to inhibit Protein kinase C (Gundimeda et al., 1996; O'Brain et al., 1985). Tamoxifen shows anti- estrogenic and estrogenic effect based on receptor locations in different tissues. It shows anti-estrogenic effect on breast tissue and estrogenic effect on bone, serum lipids, vaginal and endometrial proliferation (Agnusdei and Iori, 2000). Also, Tamoxifen exerts a direct relaxant effect on vascular smooth muscle (Figtree et al., 2000). Rutqvist and Mattsson (1993) suggested that tamoxifen decreases cardiac morbidity with long term treatment with tamoxifen.

Despite above mentioned facts, direct reports of effect of tamoxifen on cardiac hypertrophy are not available. Hence, objective of the present study was to evaluate the effect of tamoxifen on experimentally induced cardiac hypertrophy.

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3. REVIEW OF LITERATURE

Cardiac hypertrophy refers to the cardiac remodeling process in response to a variety of intrinsic and extrinsic stimuli that stress the heart. Hypertrophy is defined by anatomic findings of increased cardiac muscle mass, which results from increased wall thickness, due to enlarged cardiac myocytes and/or increased myocardial interstitial fibrosis usually occurs with occurs with preserved systolic but impaired diastolic function. Cardiac hypertrophy is an early milestone during the clinical course of heart failure and an important risk factor for subsequent cardiac morbidity and mortality (Hunter & Chien, 1999).

Cardiac hypertrophy is induced by pathological stimuli (e.g., pressure or volume overload) or physiological stimuli (e.g., developmental growth, exercise training). When disease causes pressure or volume overload (e.g., hypertension, valvular disorders) of the heart, the resulting cardiac hypertrophy is initially a compensatory response to the increased load. Cardiac hypertrophy is classified as physiological hypertrophy and pathological hypertrophy. In physiological hypertrophy, which occurs mainly in athletes and during pregnancy, there is increased cardiomyocyte size and total heart weight in response to increased workload. Cardiac function is improved and contractile abnormalities are absent. Pathological CH occurs as a consequence of biomechanical stress, such as prolonged arterial pressure overload or valvular heart disease. This form of CH is maladaptive because heart enlargement, although it abrogates the initial stimuli, is characterized by contractile dysfunction and a decrease in heart performance. Prolonged pathological hypertrophy is associated with a significant increase in the risk for progression to heart failure, ischemic heart disease, and sudden death (Errami et al., 2008; Kannel et al., 1969). Pathological hypertrophy occur after conditions such as myocardial infarction (pressure overload), inflammatory myocardial disease, with idiopathic dilated cardiomyopathy, or with volume overload. There is a transition, which may result from a maladaptation of the coronary circulation relative to the increased cardiac myocyte size. The reduced supply of oxygen and substrates can lead to a fall in contractile function. During this process, there is increased expression of embryonic genes, such as natriuretic peptides and fetal contractile proteins, and development of cardiac fibrosis. The increased synthesis of collagen fibers, which both support the myocytes and provide a

framework against which the myocytes contract is initially adaptive and probably reversible at this stage of the process. Therefore, the onset of fibrosis, in itself, does not indicate a pathophysiological condition. However, with progressive and maladaptive hypertrophy, which is due, in part, to unrestrained positive feedback of the neurohormonal system, excessive fibrosis can eventually contribute to a fall in contractile function. From this aspect, the blockade of some critical signaling pathways, such as myocardial protein synthesis, may lead to decreased oxygen demand by cardiomyocyte at the cellular level. (Nakagami et al., 2003)

Also, cardiac hypertrophy is also classified as concentric hypertrophy and eccentric hypertrophy. In concentric hypertrophy, ventricular wall becomes thick and increase the wall mass. End diastolic volume remains normal or mildly increase. This leads to an increase in mass to volume ratio and increase width of cardiomyocytes. In eccentric hypertrophy, both ventricular mass and volume increase but wall thickness may remain unchanged or moderately increases. This result in ventricular dilation with decrease in mass to volume ratio and relatively greater increase in the length than in the width of myocytes has been found.(Hunter & Chien, 1999)

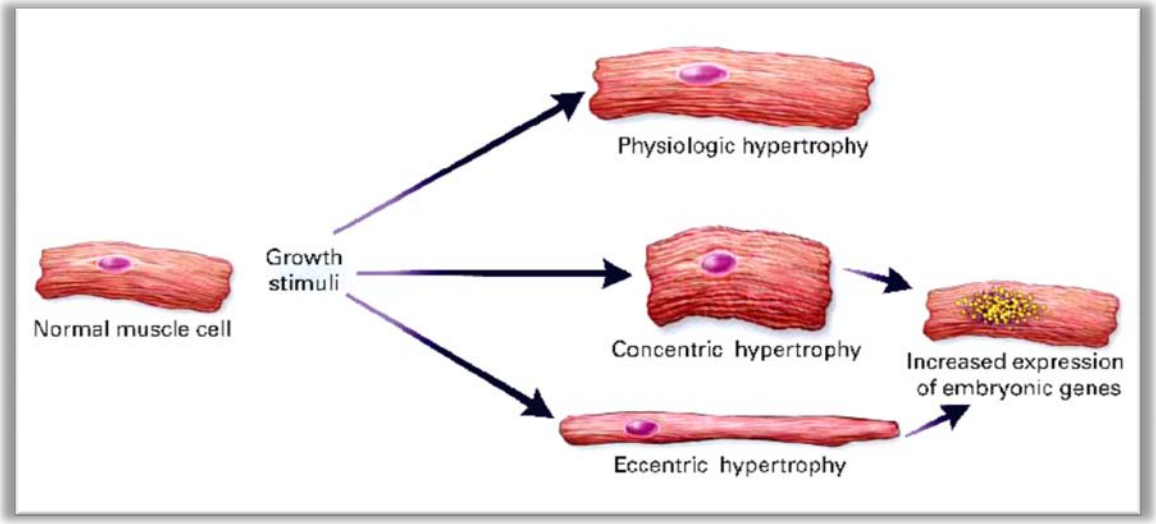


Figure 1: Morphology of Ventricular Muscle Cells in Cardiac Hypertrophy

3.2 TYPES OF CARDIAC HYPERTROPHY

Diastolic dysfunction plays a role in the presentation of some cardiomyopathies, variety of diseases that affect the myocardium in either a diffuse or multifocal manner or that frequently result in heart failure. The terminology and classification used for the cardiomyopathies are confusing because of overlap among the disease and/or classification schemes. Cardiomyopathies are sometimes defined according to their cause. Primary cardiomyopathies are disorders that affect either their structure or function of myocardium in absence of other known cases of heart disease or systemic disease known to affect the heart. Secondary forms of cardiomyopathy are condition in which a recognized factor is causing the myocardial abnormality. Infectious agents, inflammation, metabolic disorders, infiltrative disease and toxins are a few of the causative factors of secondary cardiomyopathy. Therefore, another commonly used categorization of the cardiomyopathies is based on the structural and/or fictional abnormalities present (Wynne and Braunwald, 1997; Masn J.W., 1994).

The three groups of cardiomyopathies are usually described as: dilated (congestive), hypertrophic and restrictive. In dilated cardiomyopathy (DCM), the cardinal feature is dilatation of ventricles. Systolic function is abnormal, leading to a decreased cardiac output. In those patients with hypertrophic cardiomyopathy (HCM), the ventricular muscle mass is increased. Ventricular cavity size is normal or decreased and systolic function is often preserved. Patients with restrictive cardiomyopathy have inadequate ventricular compliance, causing diastolic dysfunction as a result of endocardial and/or myocardial disease. The clinical presentation is similar to that of constrictive pericarditis. Other terms are frequently encountered in discussions of patients with cardiomyopathy. Familial cardiomyopathy denotes a condition found in more than one family member, genetic predisposition may occur in all three functional types. Patients with occlusive atherosclerotic coronary artery disease and left ventricular dysfunction are said to have ischemic cardiomyopathy.

Myocardial hypertrophy is one of the most important adaptive measures the failing heart uses to compensate for pressure ad volume overload conditions. However, these hypertrophied cells are not normal and this “cardiomyopathy of overload” may eventually lead to myocardial cell deterioration and death (Kaz A.M., 1994). The role of altered gene expression in the hypertrophied myocardium is an area of extensive

research. It appears that the phenotype of hypertrophied heart differs from that of normal heart (Schwartz et al., 1993). Following are different types of cardiac hypertrophy:

3.2.1 Hypertrophic cardiomyopathy

3.2.2 Dilated cardiomyopathy

3.2.3 Restrictive cardiac hypertrophy

3.2.1 Hypertrophic Cardiomyopathy

A primary myocardial disorder, HCM is characterized by a hypertrophied and nondilated left ventricle with no apparent cause. The distribution of hypertrophy is usually asymmetric, meaning segments of left ventricle are thickened to varying degrees. There may also be enlargement of atria, thickening of the mitral valve leaflets and fibrotic areas within the ventricular wall. In the past, HCM has been termed idiopathic hypertrophic subaortic stenosis and hypertrophic obstructive cardiomyopathy. These latter terms are used less frequently now, because they overemphasize the obstructive component of disease, which is present in only a minority of patients (Maron and Roberts, 1994). Treatment strategies are aimed at improving symptoms and preventing sudden cardiac death.

3.2.1A Left Ventricular Hypertrophy

The hypertrophy seen in HCM is usually diffuse, and it involves the septum and left ventricular anterolateral free wall to greater degree than the posterior segment. Asymmetric septal hypertrophy is a sensitive marker for HCM, bur is not specific for this disorders. In patients with outflow obstruction, the basal septum is usually markedly thickened at the level of mitral valve. In patients with nonobstuctive HCM, the outflow tract is larger ad septal hypertrophy that occurs has amore distal or apical distribution. Cellular disorganization is common histological finding of HCM. Morphologic abnormalities occur at the gross, microscopic and ultrastructural levels. The disarray of myocytes may contribute to diastolic and systolic dysfunction and may serve as nidus for ventricular arrthmias. The greater degree of left ventricular hypertrophy, the worse the clinical course.

3.2.1B Diastolic dysfunction

The most common abnormality found in patients with HCM is diastolic dysfunction. Approximately 80% of patients will exhibit symptoms associated with this finding. Examination of the left ventricle led to realization the diastolic dysfunction is the result of abnormalities in relaxation, distensibility and filling. These abnormalities can be both regional and global, and they lead to an incordination of contraction and relaxation. Abnormal relaxation is manifested by a prolong isovolumic relaxation period and a reduced rate of decline in left ventricular pressure. Filling of the left ventricle is prolonged in most patients. The presence of mitral regurgitation leads to normalize these abnormalities. β adrenergic stimulation can aggravate these abnormalities, where as β blockade may diminish them (Maron and Roberts, 1994).

Myocardial relaxation is an energy dependent process that is sensitive to episodes of ischemia. Diastolic resequestration of calcium ions by the sarcoplasmic reticulum is also energy dependent process. In the event of ischemia, the sequestration of calcium is inhibited, allowing the calcium to continue its interaction with myofibrilar contractile proteins. Calcium channel blocking drugs have been used with some success in patients with diastolic dysfunction. Abnormalities in filling are also associated with changes in chamber stiffness that occur in HCM. This stiffness may be the result of myocardial fibrosis, cellular disorganization or the increase in myocardial mass. The decrease distensibility leads to abnormally steep slope of diastolic pressure volume curve such that an increase in left ventricular volume results in disproportionate increase in diastolic pressure (Von Dohlen and Frank, 1990).

3.2.1C Systolic function and outflow obstruction

Abnormalities of systolic also occur in patient with HCM. The hypertrophied left ventricle may cause a powerful, but sometimes uncoordinated contraction, presumably because of the abnormal architecture of myocardium. The increase seen in the left ventricular wall thickness result in decreased wall stress during systole. Therefore, the left ventricle contracts against a decreased afterload so that the left ventricle is described as being hyperdynamic rather that hypercontracile. Ejection fraction is often increased. The presence of a gradient (the systolic pressure difference between the body and outflow tract of the left ventricle) is indicative of

dynamic obstruction of left ventricular outflow tract. The obstruction that occurs usually shows spontaneous variability and interventions that decrease myocardial contractility may reduce it. Factors that increase contractility can augment the gradient (Maron and Roberts, 1994).

3.2.1D **Diagnosis**

It may be difficult to make the diagnosis of HCM, as the disorder may be confused with coronary artery disease, mitral regurgitation and aortic stenosis. Patients with HCM are often physically active. The physical signs of cardiac examination depend on the presence of systolic pressure gradient within the left ventricle. It a gradient is present, a late onset systolic murmur is often heard. The murmur is intensified by standing and valsalva maneuver and lessened with squatting or handgrip. Very rarely some patients develop end stage left ventricular dilation and decline left ventricular ejection fraction that is often confused with idiopathic DCM. Echocardiography is used to confirm the diagnosis. Finding consistent with HCM include a low normal or decrease end diastolic dimension, a septal wall thickness ration equal to or greater than 15 mm, a septal to posterior wall thickness ration equal to or greater than 1.3. The presence of hyperdynamic left ventricle and systolic antermotion of the anterior mitral leaflet increased the likelihood of diagnosis (Von Dohlen and Frank, 1990).

3.2.2 **Dilated cardiomyopathy**

Dilated cardiomyopathy (DCM) is applied to a form of cardiomyopathy characterized by progressive cardiac dilation and contractile (systolic) dysfunction, usually with concomitant hypertrophy. It is sometime called congestive cardiomyopathy. In DCM heart is usually enlarged, heavy (often weighing two to three times normal) and flabby, due to dilation of all chambers. Mural thrombi are common and may be a source of thromboemboli. There are no primary valvular alteration and mitral regurgitation. When present, results form left ventricular chamber dilation. Either the coronary arteries are free of significant narrowing or the obstructions present are insufficient to explain the degree of cardiac dysfunctions. DCM may occur at any age, including in childhood, but it most commonly affects individuals between the ages of 20 and 50. It presents with slowly progressive signs and symptoms of CHF such as shortness of breath, easy fatigability and poor exertion capacity. In the end stage, patients often have ejection fractions of less than 25% (Schoen & Mitchell, 2010).

3.2.3 Restrictive cardiomyopathy

Defined as heart muscle disease that result in impaired filling, with normal or decreased diastolic volume, restrictive cardiomyopathy is the leas common cardiomyopathy. Systolic function is normal earl in the course of the disease, but deteriorates later in the disease process. Restrictive cardiomyopathy is one type of diastolic dysfunction. It results from an increased stiffness of myocardium that causes ventricular pressure to rise dramatically with only small increase in volume. Either one or both of the ventricles may be affected. Therefore, restrictive cardiomyopathy may present as either left or right sided heart failure (Kushwaha et al., 1990).

3.3 EPIDEMIOLOGY

According to American heart association, in 2006 there were 81.1 million people have one or more form of cardiovascular disease and claims 8.31 million lives from CVD. Nearly 30% of all disability casesContributes to deterioration of the quality of life. According to the WHO, It is expected to rise to a loss of 17.9 million years in 2030 &CVD deaths account for one third of all deaths.

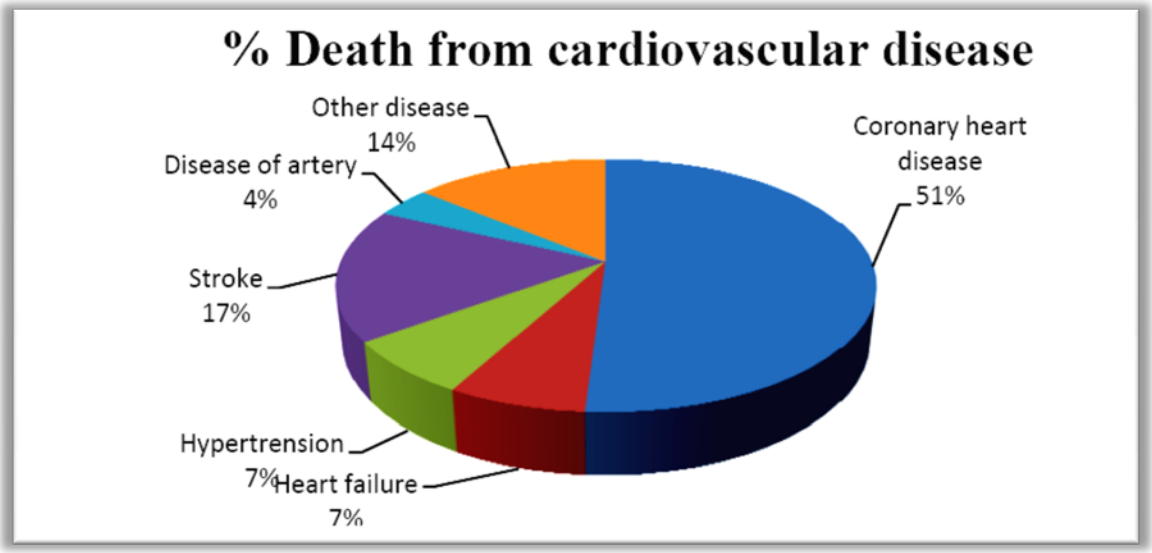


Figure 2: Percentage deaths from cardiovascular disease.

In the Framingham Heart Study, echocardiographic LVH was found to be present in 15% of the population and was independently associated with several cardiovascular endpoints, including coronary heart disease and stroke. Pressure-overload, cardiac

hypertrophy and resultant ischemia have been identified as primary pathophysiological mechanisms in people with diastolic heart failure. Cardiac hypertrophy is very prevalent in the DHF population; over 80% of unselected patients with acute DHF have increased left ventricular (LV) mass (Klapholz et al 2004; Hoenig et al., 2008).

3.4 MECHANISMS OF CARDIAC HYPERTROPHY

3.4.1 G proteins coupled receptors

Myocardial G-protein-coupled receptors (GPCRs) represent a group of seven transmembrane panning domain receptors, which play an important role in the regulation of acute hemodynamic and chronic myotrophic effects within the cardiovascular system (Rockman et al., 2002). Three functional classes of GPCRs principally correspond to three major classes of heterotrimeric GTP binding proteins: β-adrenergic receptors signal primarily to G_s, cholinergic receptors are typically connected to G_i, while members of the third class, including angiotensin II, endothelin, and α-adrenergic receptors, are linked to G_q and the functionally redundant G_{11}. All heterotrimeric G proteins consist of the subunits Gα and $G_{\beta\gamma}$, which dissociate upon receptor activation and independently activate intracellular signalling pathways (Koch et al. 2000).

The functional classes of cardiovascular receptors correspond to the three major classes of G proteins. Thus, α adrenergic receptors which couple primarily to $G_{\alpha s}$, mediate acute enhancement of heart rate and myocardial contractility in response to epinephrine and norepinephrine stimulation. The second classes of myocardial receptors are the cholinergic receptors, typically coupled to $G_{\alpha i}$, which are activated by acetylcholine. The third class of receptors, coupled primarily to $G_{\alpha q}$, includes angiotensin II, endothelin, and α-adrenergic receptors (proud C.G., 2004). Activation of these pathways is less important in modulating minute-by-minute cardiac function, but it is likely to play a major role in cardiac hypertrophic responses to pathological stimuli.Activation of Gaq–coupled receptors induces hypertrophy *in vitro* (Adams et al., 1998). Subsequent Studies utilizing *in vivo* approaches, including transgenic overexpression and conditional knockouts, have confirmed that Gq/G11 signalling is

both required for pressure overload hypertrophy as well as sufficient to provoke hypertrophy in the absence of hemodynamic stress (Akhter et al., 1998).

3.4.2 Low molecular weight GTPase (Ras, RhoA, Rac 1)

A number of in vitro and in vivo studies have implicated a Ras-dependent signaling pathway in the regulation of cardiac hypertrophy. Microinjection of activated Rasprotein into cultured cardiomyocytes increased both cell size and atrial natriuretic factor (ANF) expression. Transfection of expression vectors encoding activated *Raf-1* or *Ras* increased myocyte cell dimensions and augmented expression of hypertrophy-responsive promoters (Clerk and Sugden, 2000).

Ras activation can promote activation of Raf-1, PI3K, small GTPase Racl proteins, p120GAP, and p190GAP, leading to Rho activation. Ras is activated by a variety of Gq/G11 linked agonists, like angiotensin II, ET-1 and PE, each of which is sufficient to induce cardiac hypertrophy (Proud et al., 2000). In addition, Ras activity is known to result in activation of all three MAPK signaling branches (i.e. ERK 1 & 2, JNKs and p38) and Ras was also reported to activate the c-Jun N-terminal kinases (JNKs) in cardiomyocytes (Remirez et al., 1997). The Rho family of small G proteins, consisting of Rho, Rac, and Cd42 subfamilies, has been shown to both regulate the cytoskeletal organization of cardiomyocytes as well as to modulate cardiac growth via augmentation of hypertrophic gene expressionRaf-1 activation is associated only with ERK 1 and 2 activation (Hoshijima et al 1998; Charron et al., 2001). Whereas RhoA may plays only a limited role in the hypertrophic program of cardiomyocytes.

3.4.3 MAPK pathway

The MAPKs are elements of three-tiered protein kinase cascades and comprise basically three subfamilies, the extracellularly responsive kinases (ERKs), the c-Jun N terminal kinases (JNKs) and the p38 MAPKs. The ERKs are particularly implicated in growth-associated responses and the latter two groups are generally activated by cytotoxic stress factors (Clerk and Sugden, 2000).

3.4.4 Extracellular signal regulated Kinase (ERK) Pathway

Two separate ERK isoforms have been described, ERK1 and ERK2, that are coordinately phosphorylated and activated by a wide array of mitogenic stimuli. The major upstream activators of ERK1 and 2 MAPKs are two MAPK kinases (MAPKKs), MEK1 and MEK2, which directly phosphorylate the dual site in ERK1 and 2.In response to agonist stimulation or cell stretching, ERK1 and 2 become activated both in cultured cardiac myocytes and in isolated perfused hearts. These observations have implicated ERK1- and 2-signaling factors as regulators of the hypertrophic response (Clerk et al., 1998).

3.4.5 p38 MAPK pathway

Four separate p38 MAPK isoforms have been described including p38α, p38β,p38γ, and p38δ. p38 MAPKs are activated by a wide array of stress stimuli including chemical stress, physical stress, osmolar stress, radiation stress, and G protein–coupled receptor (GPCR) activation. The major upstream activators of p38 MAPKs are two MAPKKs, MKK3 and MKK6, which directly phosphorylate the dual site in p38 MAPKs. In cardiac myocytes, mechanical deformation, GPCR ligands (angiotensin II, endothelin-1, and PE), and mitogens are potent activators of p38 MAPKs (Clerk and Sugden, 2000). Activated p38 MAPKs directly phosphorylate serine and threonine residues in a wide array of cytoplasmic proteins and transcription factors to mediate stress responsive signaling, including MEF2, MAPKAPK2 and 3, ATF-2, ELK-1, Chop, and Max . p38α and p38β are thought to be the most important isoforms of p38 expressed in the human heart, whereas p38 γand p38δ are undetectable. The GPCR agonists PE and endothelin-1 are potent activators of p38 MAPKs in cardiomyocyte. In vivo, p38 MAPK activity is elevated by pressure overload hypertrophy in aortic-banded mice and in human hearts with failure secondary to advanced coronary artery disease (Wang et al., 1998). Pharmacologic inhibition of p38 kinase activity blocked agonist-stimulated cardiomyocyte hypertrophy in culture (Braz et al., 2003).

3.4.6 C-Jun NH2 TERMINAL Kinase

Three distinct JNK or SAPK (stress-activated protein kinase) genes have been identified in mammalian cells. Each is activated by the upstream MAPK kinases MKK4 and MKK7, which is turn are activated by MEKK1 or MEKK2. JNK isoforms become phosphorylated in response to stress stimuli (stretching) or GPCR activation. JNK activation has also been associated with load-induced cardiac hypertrophy in the rat, myocardial infarction, and human heart failure (Sopontammarak S 2005).

3.4.7 CALCINEURIN

Calcineurin is calmodulin dependent phosphatase that dephosphorylates transcription factors known as NFAT (nuclear factor of activated T cells) (Crabree and Olson, 2002) Calcineurin is a serine-threonine phosphatase that is uniquely activated by calcium-calmodulin. The calcineurin enzyme consists of a 59- to 61-kDa catalytic subunit termed calcineurin A and a 19-kDa calcium-binding EFhand domain containing protein. The catalytic subunit is encoded by three genes, calcineurinAα, calcineurin Aβ, and calcineurinAγ. In the adult human, rat, or mouse heart, both calcineurinAα and calcineurin Aβ gene products can be detected, but not calcineurinAγ.A conserved role for calcineurin-NFAT signaling was found in the heart.calcineurin has been shown to play a critical role in cardiomyocyte hypertrophy via that same principal pathway. Constitutive activation of calcineurin in mouse hearts by a transgenic strategy is sufficient to induce massive. Cardiac enlargement and heart failure (Molkentin et al, 1998).

3.4.8 PROTEIN KINASE C

PKC is a group of closely related serine– threonine protein kinases,which are Ca^{2+}activated, phospholipid-dependent enzyme that consists of 12 distinct isoforms, each containing a highly conserved carboxyl terminal kinase domain that includes an ATP-binding site. In the heart, the majority of PKC appears in the α and β forms , with the most dominant form expressed in the human heart being isoform α. Inactive PKC isoforms present in the cytosol, which is translocate to membranes in a Ca^{2+} dependent active form. Protein kinase C (PKC) classified as the (a) Classical PKCs (α, βI, βII, and γ), the diacylglycerol (DAG) and calcium-dependent enzymes,(b) The novel PKCs (θ, ε, δ, and η), which require DAG, but not calcium, for activity, and(c)

The atypical PKCs (ζ, λ), which are not stimulated by DAG or calcium, but are stimulated by other lipid-derived second messengers(Ruwhof and Laarse, 2000).

Different studies of myocardial hypertrophy or heart failure largely report similar overall findings that PKCα and PKCβ are upregulated, PKCε is either upregulated or preferentially activated, and levels of PKCδ and -λ/ζ. Various hormones are known to induce cardiac hypertrophy through the involvement of PKC activation and associated signal transduction mechanisms(Bishop at al., 1994).Isozyme-specific PKC activity has been shown to lead to ventricular hypertrophy. Increases in PKC activity and concentration, particularly of isozymes PKC-βI, PKC-βII and PKC-ε, during the development of left ventricular hypertrophy induced by pressure overload occurs in membrane and nuclear-cytoskeletal fractions. Another study showed an increase in PKC-δ isozyme, but not of PKC-α or PKC-ε, in volume-overload-induced left ventricular hypertrophy; however, PKC-α was found, along with PKC-δ, to have increased enzyme activity and protein expression in right ventricular hypertrophy(Dhalla and Muller, 2010).

PKCα. Although it is the most highly expressed of the myocardial PKC isoforms, PKCα is the least studied of the cardiac PKCs because, unlike PKCδ and -ε, it is not regulated in acute myocardial ischemia. Likewise, in contrast to PKCβ, PKCα is not regulated in diabetes. An initial comparative analysis of PKC isoforms using adenovirus-mediated transfection of wild type or dominant inhibitory forms of PKCα, -β2, -δ, and -ε in neonatal rat cardiomyocytes suggested that only PKCα was sufficient to stimulate cell hypertrophy and only inhibition of PKCα inhibited agonist mediated hypertrophy.

*PKC*β. There is an increase in PKC β in diabetes and human heart failure. Targeted overexpression of the PKC β isoform inhearts of transgenic mice results in left ventricular hypertrophy (LVH), cardiac myocyte necrosis and decreasedleft ventricular performance (Wakasaki et al. 1997; Fryer et al., 1998).

*PKC*δ. Although it has long been recognized as being activated in myocardial ischemia,relatively little is known about this PKC isoform in cardiac hypertrophy. A

cardiac PKCδ transgenic mouse model has not been described, and PKCδ-knockout mice have no basal cardiacphenotype. Translocation modification has confirmed that PKCδ is a critical mediator of post ischemic cardiomyocyte necrosis and contractile dysfunction in mice, rats, and pigs.

PKCε. The best-characterized PKC isoform in cardiac hypertrophy is PKCε. Implicated in hypertrophic signaling because it is activated by mechanical stress as well as genetic (Gq) and physiological (pressure overload) hypertrophic stimuli, PKCε was perceived to be a key mediator of maladaptive hypertrophy. However, both transgenic PKCε overexpression and translocation activation result in mice with normally functioning mildly enlarged hearts, i.e. adaptive hypertrophy. (Drown&Force,2005).

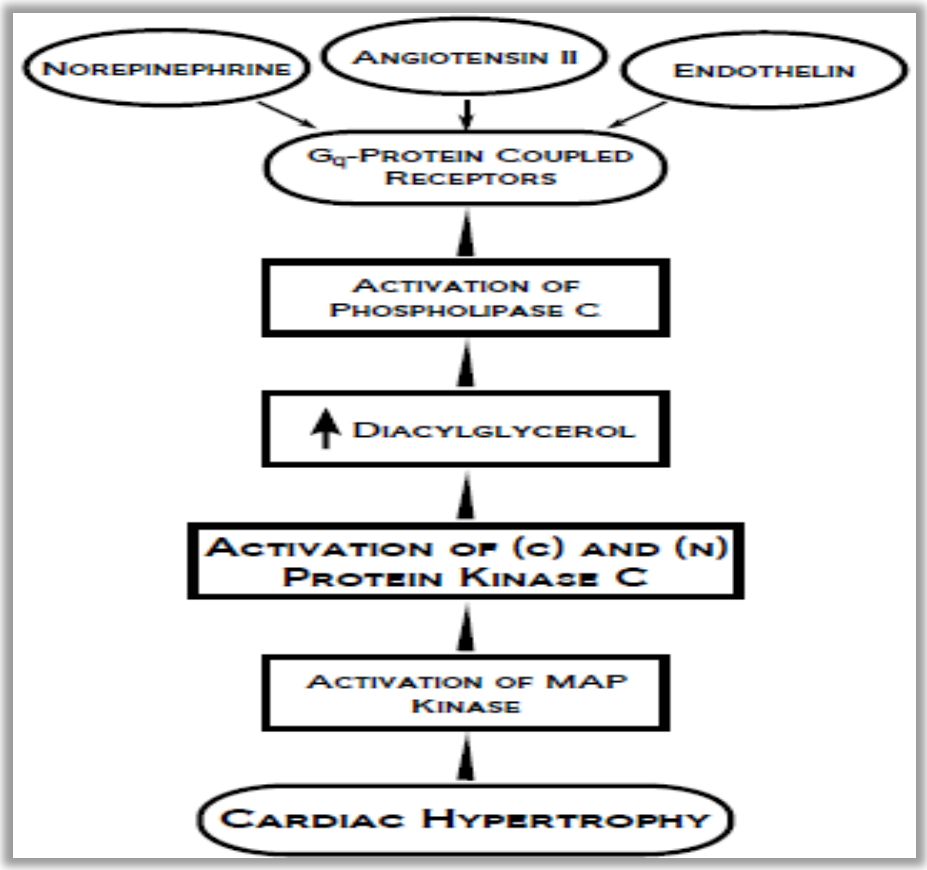


Figure 3: Signal transduction mechanisms involving the activation of conventional (c) and novel (n) protein kinase C and MAP kinase for the development of cardiac hypertrophy.

PKC inhibition has been shown to be beneficial in a number of ways, including preventing diabetes-induced abnormal cardiomyocyte mechanics and enhancing cardiac contractility. PKC-α and PKC-β isoforms in diabetic hearts showed increase expression which was associated with loss of contractility and subsequent HF due to their regulatory effect on cardiac contractility and Ca^{2+}handling in cardiomyocytes(Dhalla and Muller, 2010).Treatment with breviscapine decreased the activity ofPKC isoforms, and subsequently inhibited cardiomyocyte apoptosis induced via I/R injury and was shown to improve cardiac structure and function with satisfactory tolerance and safety (wang et al., 2009; cotter e al., 2002).Although beneficial effects of inhibiting PKC activation by angiotensin II have been illustrated, it has been shown that angiotensin II mediated activation of PKC can also limit infarct size in rabbit hearts due to MI (Liu et al., 1995). Ruboxistaurin, a PKC inhibitor, was also found to attenuate pathological fibrosis and impaired cardiac function after induced MI (Boyel et al., 2005).

3.5 GENE EXPRESSION IN THE HYPERTROPHIC CARDIOMYOCYTE

The intracellular signalling triggered by the different mediators induced by the hypertrophic stimulus are ultimately translated into messages that gain access to the cardiomyocyte nucleus, a process that, in turn, leads to changes in the transcription of numerous genes. It is known that, regardless of the species, the cardiomyocyte responds to the pathological hypertrophic stimulus with a characteristic succession of changes in gene expression. The first group of genes to be activated during mechanical stress are the early response genes, such as *c-fos*, *c-jun*, and *c-myc*, the transcription of which takes place 30 minutes later and peaks within the first hour (Komuro et al., 1990).

During the progression of pathological CH in animal and human models, the heart undergoes a change in metabolism: the major source of ATP generation, which, under normal conditions, is fatty acid oxidation, comes to be glucose utilization(Depre et al.,1998).The so-called PPAR (peroxisome proliferator-activated receptors) correspond to a group of transcription factors that have recently been the object of a great deal of interest, given their clinical importance and the central role they play in

the regulation of lipid metabolism in different species, including humans(Kiewer et al. 2001). The 3 known isoforms (α, β and γ) are expressed in the mammalian heart. They become activated in response to fatty acids, binding like heterodimers to the retinoic acid receptor (RXR) and to consensus sequences in the regulatory regions of a number of genes. Recent evidence has related the deactivation of the PPARα signalling pathway to the reduced gene expression of the enzymes involved in fatty acid oxidation in the hypertrophic heart (Barger et al., 2000). An interesting aspect is the fact that, in rats treated with fenofibrate, a PPARα agonist, an inhibition of CH secondary to pressure overload was observed, a finding that has been expanded on in studies in hypertensive patients with CH (Jamshidi et al., 2002).The γ isoform of PPAR, on the other hand, not only appears to play a protective role in CH, but also in hypertension-related vascular remodeling which, at least in part, would be mediated by an increased release of vasodilator substances, such as nitric oxide. The hypertrophic effect of PPARα and γ is probably related to the regulation of the fatty acids available for uptake by the myocardium and to an acceleration of their oxidation, which results in an increase in ATP synthesis(Kiec-Wilk et al., 2005).

Another particularly important phenomenon involved in gene expression is histone acetylation. This reaction, mediated by histone acetyl transferase (HAT), modifies chromatin structure, which results in its decondensation and transcription activation. The reverse reaction is catalyzed by histone deacetylases (HDAC) which, through deacetylation, induce a compacting of the chromatin and repression of transcription. At least 9 mammalian HDAC genes have been described, and are grouped into two major categories: class I and class II. According to available evidence, these enzymes appear to play opposing roles in the hypertrophic process. Thus, whereas the role of HDAC I would be to inhibit antihypertrophic genes and, therefore, would favour myocyte growth, the action of HDAC II would be to “sequester” the transcription factor MEF-2 and, thus, would inhibit the hypertrophic phenomenon (Zhang C. L., 2002; Carreno et al., 2006).

3.6 FUNCTIONAL CHANGES IN CARDIAC HYPERTROPHY

A fundamental characteristic of cardiac remodeling is myocardial stiffness, which is associated with fibrosis, altered contractile and relaxation properties, and changes in cardiac cellularity (especially perivascular inflammation) is provided by a network of network of collagen(Caulfield and Borg et al., 1979;(Berk et al., 2007). Based on morphology, fibrillar collagen network can be subdivided into three components. The epimysium is located on the endocardial and epicardial surfaces of the myocardium, where it provides support for endothelial and mesothelial cells. The perimysium surrounds muscle fibers, and perimysial strands connect groups of muscle fibers together. The endomysium arises from the perimysium and surrounds individual muscle fibers. Struts of endomysium join muscle fibers together and function as the sites for connections to cardiomyocyte cytoskeletal proteins across the plasma membrane (e.g., laminin to dystroglycan; see below). The endomysium is also the source of ECM scaffolding for blood vessels. Morphologically, fibrotic tissue in the heart is visualized as perivascular fibrosis involving the intramural coronary arterial vasculature, interstitial fibrosis (accumulated perimysium), and microscopic scarring (Rossi e al., 1998).

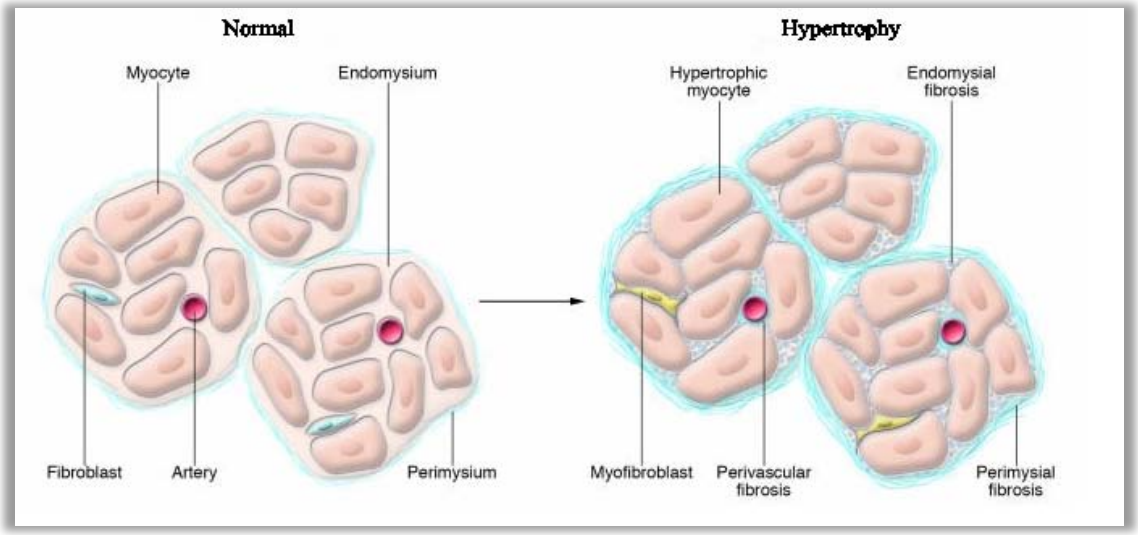


Figure 4: Morphology of the normal and hypertrophic cardiomyocyte

Changes in the collagen network present in hypertrophy impair both systolic and diastolic function (Janicki and Borwer, 2002). Collagen is a stable protein whose balanced turnover (synthesis and degradation) by cardiac fibroblasts is normally slow (estimated to be 80–120 days) (Shirwany and Weber, 2006). Collagen turnover is

primarily regulated by fibroblasts during normal physiology. However, under pathologic conditions like hypertrophy, morphologically distinct cells termed myofibroblasts appear. These cells are defined by their dual functions: fibroblast-like in terms of ECM synthesis and smooth muscle myocyte–like in terms of migration. Myofibroblast-mediated collagen turnover is regulated by autocrine and paracrine factors generated within the myocardium and by endocrine hormones derived from the circulation.

In hypertrophy, an increase in interstitial collagen is associated with diastolic heart failure, whereas degradation of endomysial and perimysial components of the collagen is accompanied by ventricular dilatation and systolic heart failure (Iwanaga et al, 2002). These suggest that the transition from compensated LVH to heart failure is associated with degradation of ECM. Loss of the collagen network might cause systolic dysfunction by at least three mechanisms (Janicki and Borwer, 2002).(i) Interruptions in the collagen matrix that provides support, geometric alignment, and coordination of contraction by cardiomyocyte bundles. (ii) The loss of the normal interactions between endomysial components such as laminin and collagen with their receptors (dystroglycans and integrins), which is required for contractile synchrony and long-term cardiomyocyte homeostasis. (iii) Displacement of cardiomyocytes, leading to a decrease in the number of muscular layers in the ventricular wall and to LV dilation.(Berk et al., 2007)

3.7 CARDIAC REMODELING

Cardiac remodeling may be defined as genome expression, molecular, cellular and interstitial changes that are manifested clinically as changes in size, shape and function of the heart after cardiac injury. The process of cardiac remodeling is influenced by hemodynamic load, neurohormonal activation and other factors still under investigation. The myocyte is the major cardiac cell involved in the remodeling process. Other components involved include the interstitium, fibroblasts, collagen and coronary vasculature; relevant processes also include ischemia, cell necrosis and apoptosis. Cardiac remodeling can be described as a physiologic and pathologic condition that may occur after myocardial infarction (MI), pressure overload (aortic stenosis, hypertension), inflammatory heart muscle disease (myocarditis), idiopathic dilated cardiomyopathy or volume overload (valvular regurgitation). Although the etiologies of these diseases are different, they share several pathways in terms of molecular, biochemical and mechanical events. Physiologic remodeling—compensatory changes in the proportions and function of the heart—is seen in athletes, but will not be discussed further in this paper.

3.7.1 The main components of cardiac remodeling

3.7.1A Cardiac myocytes

Myocytes and other cardiac cell types are believed to be fundamentally involved in the remodelling process. As the result of an insult, myocytes numbers decrease and surviving myocytes become elongated or hypertrophied as part of an initial compensatory process to maintain stroke volume after the loss of contractile tissue. The thickness of the ventricular wall also increases (Anversa et al., 1991; Cohn et al., 2000). Altered loading conditions stretch cell membranes and may play a role in inducing the expression of hypertrophy-associated genes. In cardiac myocytes, this may lead to the synthesis of new contractile proteins and the assembly of new sarcomeres. It is thought that the pattern in which these are laid down determines whether the cardiac myocytes elongate or increase their diameter (Francisand McDonald, 1992). Increased wall stress may precipitate energy imbalance and ischemia, which is one of the major determinants of myocardial oxygen demand. This is thought to lead to a vicious cycle of increased wall stress and wall thickness and further energy imbalance and ischemia (Dhalla et al.1996).

3.7.1B The role of fibroblast proliferation

Both fibroblasts and endothelial cells are activated in response to an ischemic insult. In human and animal models, fibroblast stimulation increases collagen synthesis and causes fibrosis of both the infracted and noninfracted regions of the ventricle, thus contributing to remodelling (Volders et al 1993; Cohn et al., 2000).

3.7.1C The role of collagen degradation

The myocardium consists of myocytes tethered and supported by a connective tissue network composed largely of fibrillar collagen, which is synthesized and degraded by interstitial fibroblasts. Myocardial collagenase is thought to be an important proenzyme present in the inactive form in the ventricle. Its activation after myocardial injury contributes to an increase in chamber dimension in response to the distending pressure that is thought to be a possible cause of myocyte slippage, which some consider one contributor to chamber remodelling (Gaudron et al., 1993; Cohn et al., 2000).

3.8 CURRENT TREATMENT OF CARDIAC HYPERTROPHY

However, there is no current therapy which reverse cardiac hypertrophy but goal of present medical therapies for treatment of cardiac hypertrophy is to improves heart function via suppression of neurohormonal activation (e.g. β-adrenergic receptor antagonists, angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists, and aldosterone receptor antagonists), reducing volume overload (diuretics), or hemodynamic symptoms (inotropic agents).

3.8.1 Beta blockers

β-blockers are the mainstay of treatment and the first choice, unless they are contra-indicated. Treatment with β-blockers improves ventricular relaxation, increases diastolic filling time and reduces susceptibility to ventricular and supra-ventricular arrhythmias.

3.8.2 Calcium channel blockers

Calcium channel blockers that do not have significant vasodilatory effects are also beneficial because of their negative inotropic and chronotropic effects as well as improvement of myocardial diastolic properties. They are used whenever β-blockers are not tolerated or in conjunction with the β-blockers.

3.8.3 ACE inhibitors

ACE inhibitors (lisinopril, trandolapril, enalapril, benazepril, fosinopril, perindopril, quinapril and captopril) decrease levels of circulating Ang II by inhibiting ACE. They cause vasodilation of blood vessels and reduced the work load of heart. ACE inhibitors may reduce central pressures and left ventricular afterload more effectively than antihypertensive drugs that do not directly interfere with the RAAS. Angiotensin converting enzyme (ACE) inhibitors reduce LV mass to a greater extent than other antihypertensive drug classes, when equivalent levels of blood pressure reduction are achieved.

3.8.4 Angiotensin receptor blockers

ARBs should, theoretically, be superior to ACEIs because they block the action of AII generated by both the classical and alternate pathways. Clinically important advantage of ARBs over the ACEIs are the much lower incidence of cough and angioedema compared with ACEIs, which make them safer and better-tolerated drugs.

3.8.5 Angiotnsin receptor blockers

Generally, losartan, valsartan and candesartan are used. ARBs have comparable effect of mortality and morbity to the ACE inhibitors.

3.9 ROLE OF ESTROGEN AND SERMs IN HEART

Estrogen effects are mediated by 2 different nuclear hormone receptors, ERα and ERβ, which are encoded by different genes and act as ligand-dependent transcription factors. Understanding the specific and physiological function of ERα and ERβ appears mandatory, because both receptors are expressed in cardiac myocytes, fibroblasts, and vascular cells, where they could exert either redundant, nonredundant, or even opposing biological effects (Pelzer et al., 2005). The biological effects of estrogens are transmitted by two different estrogen receptors, ERα and ERβ, which are encoded by different genes and mediate redundant, divergent or even opposing effects in different tissues (Pelzera et al., 2005).

3.9.1 Effect of estrogen and SERMs on serum lipids

Orally administered estrogen reduces LDL cholesterol levels and increases HDL cholesterol levels in postmenopausal women with normal or elevated baseline lipid levels (Granfone et al. 1992, Lobo RA 1991). Transdermally administered 17ß-estradiol has no effect on lipoprotein levels, suggesting that the hepatic effects of estrogen absorbed through the gut are important for changes in lipoprotein levels. The reduction in LDL cholesterol levels is probably a result of accelerated conversion of hepatic cholesterol to bile acids and increased expression of LDL receptors on cell surfaces (Windler 1980), resulting in augmented clearance of LDL from the plasma. The increase in HDL levels is due to increased production of apolipoprotein A-I and decreased hepatic lipase activity, effects that increase levels of HDL, the HDL subparticle considered the most active in reverse cholesterol transport. VLDL levels increase because of enhanced production of apolipoprotein B and triglycerides, but these particles may not be of atherogenic potential. Estrogen therapy has also been shown to reduce levels of lipoprotein(a), a lipoprotein with structural features of LDL and plasminogen, believed to be proatherogenic and antithrombolytic, that increases in plasma concentration after menopause (Soma et al., 1993).

In a randomized, controlled trial of healthy women who took 20 mg/day tamoxifen for 2 years, cholesterol concentrations decreased by 19% and ratios of total to high-density lipoprotein (HDL) cholesterol dropped by 11% (Grey et al., 1995). Further Clinical studies of raloxifene have examined different doses and durations of treatment in a trial of 601 women, those given 30 mg, 60 mg, or 120 mg/day

raloxifene showed statistically significant, dose-dependent improvements in total and LDL cholesterol v/s placebo after 2 years of treatment (Delmas et al., 1995).

3.9.2 Effects of estrogen and SERMs on the Endothelium

According to study by Arora et al., (1998) among postmenopausal women, clearly demonstrated a significant difference in both endothelium-dependent and independent vasodilatory response between women using estrogen replacement and those not using replacement. Women using hormone replacement had significantly higher serum levels of estradiol and had a much greater response to both acetylcholine and sodium nitroprusside. These findings suggest that estrogen positively affects both endothelium-dependent and endothelium-independent vasodilatation in the microcirculation (Arora et al., 1998). Various investigations with estrogens, both genomic and non genomic mechanisms have been described for SERMs in the endothelium. Mixed evidences have been obtained in studies of flow changes with SERMs. A study from Greece showed that raloxifene could significantly increase the ratio of NO to endothelin-1. Several other studies also showed that raloxifene could significantly improve flow-mediated endothelium dependent vasodilation in postmenopausal women to an extent similar to that of hormone replacement therapy, which demonstrates that SERMs can significantly improve endothelial function. (Cushmanet al., 2001; Delmas et al 1995; Walsh et al., 1998)

3.9.3 Effects of estrogen and SERMs on Coronary Artery Disease and Stroke

Estrogen may also inhibit atherogenesis by decreasing adhesion of monocytes to endothelial cells, which is important in formation of fatty streak either by directly inhibiting vascular cell adhesion molecule-1 expression or by increasing nitric oxide synthesis by endothelial cells or both (Khazaei et al., 2006). Animal study showed that raloxifene treatment could result in reduced lesion volume, enhanced mechanical stability of vascular calcification, and less inflamed lesions, characterized by less macrophage infiltration and reduced COX 2, MMP 1 and MCP 1 expression. Some clinical trials also demonstrated that SERMs gave some indirect indications favoring a protective effect on CAD or stroke. However, a study about the effect of raloxifene on stroke and venous thromboembolism according to subgroups in postmenopausal women, at increased risk of coronary heart disease, showed that the incidences of all

strokes did not differ between raloxifene and placebo treatment groups. However, there was a higher incidence of fatal strokes and venous thromboembolic events, which should be paid attention to by clinicians (Saitta et al., 2001, Vogelvang et al., 2002; Barrett-Connor 2002).

3.9.4 Effects of SERMs on Inflammation and Oxidative Stress

Some of the atheroprotective effects of estrogens are mediated through their interaction with the inflammatory process. Estrogens decrease the adhesion molecules, vascular cell and intracellular adhesion molecules (VCAM-1 and ICAM-1) and decrease the accumulation of leucocytes on the endothelium (Saltiki and Alevizaki, 2007).There are several studies that have demonstrated that SERMs have significant anti inflammatory effects. Long times, anti inflammatory actions of raloxifene in rat aorta have been observed, and inflammatory responses induced by lipopolysaccharide in mouse and rat microglial cells are weakened by SERMs. Data also shows that tamoxifen is associated with reductions of C reactive protein and the other inflammatory factors. Of late, increasing evidences demonstrated that SERMs such as raloxifene can protect endothelial cell function against oxidative stress and suppress oxidative stress induced endothelial cell apoptosis. However, the study has also shown that tamoxifen, another kind of SERMs, can induce oxidative stress and mitochondrial apoptosis via stimulating mitochondrial nitric oxide synthase, which means that it acts differently when suppressing oxidative stress for different kinds of SERMs. (Walsh et al., 2001; Mijatovic et al 1998; Shand 2002)

Further, studies on animal and human also indicate cardioprotective effect of estrogen. According to Node et al., (2000) estrogen attenuates on left ventricular hypertrophy (LVH), animal models have shown that the inhibition of cardiomyocyte hypertrophy by estrogen is correlated with a decrease in the expression of hypertrophy-associated genes, such as c-*fos*, in neonatal rat cardiomyocytes .Estrogen deficiency potentiates and estrogen replacement attenuates the development of both right and left ventricular hypertrophy in rat models (Farhat et al., 1993). The observation that female gender and sex hormones attenuate left ventricular hypertrophy in humans and in animal models has defined the myocardium as a direct target for estrogens (Carroll et al., 1992; Pelzera et al., 2005). Estrogen has been

previously proposed to significantly prevent cardiac hypertrophy. In ovariectomized mice, E2 administration prevents ventricular remodeling after myocardial infarction (Cavasin et al., 2003). Compared with postmenopausal women not taking sex hormone replacement (HR), sex steroid use results in a significant decrease in LV mass. HR also decreases significantly the left ventricular mass index in hypertensive women (Miya et al., 2002). Because blood pressure was not significantly affected in these studies, a direct action of sex steroids on the heart may have occurred. After myocardial infarction, heart failure and death occur less frequently in women taking HR (Pedram et al., 2008).

3.10 Animal models of cardiac hypertrophy

3.10.1 Spontaneously hypertensive rats

The SHR is a commonly-used model of chronic hypertension (see above) which progresses to heart failure during the last six months of their lifespan of about 2 years (Mitchell et al., 1997). Thus, SHR should not be overlooked as a model to study the mechanisms of hypertension-induced hypertrophy as it progresses to heart failure. Electrophysiological studies have shown that action potentials from hypertrophied SHR left ventricular slabs or myocytes are prolonged. Apoptosis is observed in the hearts of 8 and 16 week old SHRs. Studies with multicellular SHR ventricular preparations usually show impaired contractility and responses to β-adrenoceptor stimulation whereas hypertrophied SHR myocytes have increased contractility.As hypertrophy in humans is usually associated with chronic hypertension, the SHR is a realistic model of human hypertrophy. This model also fulfills the other criteria in that it allows studies in chronic, stable disease, produces symptoms which are predictable and controllable, and allows measurement of relevant cardiac, biochemical and hemodynamic parameters.

3.10.2 Renal artery occlusion

Renovascular hypertension resulting from renal ischemia (see above) leads progressively to left ventricular hypertrophy and failure. Hypertension in the adult heart induces concentric ventricular hypertrophy, in which wall thickness increases without chamber enlargement. This hypertrophy is characterised by a lateral increase

in the size of the myocytes rather than an increase in the number of cells or average myocyte length. About 12 weeks after surgery, 2K1C rats show left ventricular heart failure with a 35% increase in left ventricular weight, increased left ventricular end-diastolic pressure and wall stress with reduced stroke volume and cardiac output together with marked ventricular fibrosis (Anversa et al., 1993; Doggrella and Brown, 1998).

3.10.3 **Pressure loading by outflow constriction**

Partial aortic constriction by aortic banding leads to a rapid increase in cardiac load and therefore cardiac hypertrophy. Under anaesthesia, a left thoracotomy is performed to expose the ascending aorta which is constricted to about 30% of the original cross-sectional area with a silver clip or a week hemoclip. Alternatively, a spirally cut polyethylene catheter tube is placed around the abdominal aorta to produce the aortic stenosis. This model has been used to define the changes occurring during hypertrophy, for example in cell size, myofibrils and myosin isoforms, incidence of apoptosis and altered adrenoceptor responsiveness. There is no evidence of chronic heart failure as liver enlargement or pleural or peritoneal effusions are not observed following short term banding. However, superimposition of streptozotocin-induced diabetes on the hypertension of abdominal aortic constriction led to ascites, liver and lung congestion and reduced heart noradrenaline levels as signs of heart failure. Outflow constriction by aortic banding is clearly a model of cardiac hypertrophy and not of heart failure, although several of the above studies have suggested it is a model of heart failure. The operative procedure is relatively easy and has a high success rate in generating cardiac hypertrophy in a short time. This model does not mimic a clinical condition and one cannot be certain that the hypertrophy associated with this short sharp pressure overload is similar to that observed with the more gradual process of essential hypertension in humans. Right ventricular hypertrophy independent of pulmonary hypertension can be produced by increasing right ventricular pressure following pulmonary artery banding. In 7 week old rats, banding to an internal diameter of 1.4 mm for twelve weeks led to right ventricular hypertrophy and an increased collagen fraction in the right ventricle. This model would appear to mimic a minor cause of right ventricular dysfunction in humans (Cutilletta et al, 1971, Doggrella and Brown, 1998).

3.10.4 Catecholamines

Heart failure is characterised by activation of the sympathetic nervous system leading to high circulating noradrenaline concentrations which correlate with cardiovascular morbidity. High noradrenaline concentrations are associated with β-adrenoceptor downregulation. Noradrenaline as the sympathetic neurotransmitter would seem to be the only relevant catecholamine for comparison with humans. Noradrenaline infusion leads to selective left ventricular hypertrophy; cAMP-mediated positive inotropic responses were reduced in the hypertrophied left ventricle. Unlike noradrenaline, isoprenaline is a nonselective β-adrenoceptor agonist producing no α-adrenoceptor-mediated vasoconstriction. The isoprenaline-infused rat has been used to study desensitization of the adenylatecyclase signalling pathway. Isoprenaline-treated rats developed myocardial necrosis and a progressive enlargement of the left ventricular cavity out of proportion to mass, as in humans with discrete myocardial infarction. These studies have not measured circulating noradrenaline or isoprenaline concentrations and therefore a comparison with levels in human heart failure is not possible. Further, there are no chronic studies of catecholamine-infused rats to determine whether these rats develop heart failure. Phaeochromocytomas produce very high catecholamine concentrations, usually much higher than in heart failure. Thus, rats with this transplantable tumour may be a suitable model for the pathological effects of chronic excess catecholamine concentrations but are unlikely to be relevant for chronic heart failure research. These rats develop cardiomyopathy and a functional desensitisation of β-adrenoceptor function but have not been further studied as models of heart failure (Teerlink et al, 1994;Mende et all 1992; Doggrella and Brown, 1998)

3.10.5 Transgenic rats

Transgenic techniques are now increasingly used since they offer the possibility of analysing responses by selected genes. The most commonly used species for transgenic experiments is mice but their small size limits their usefulness in cardiovascular research as few techniques are available for functional studies. Since the renin–angiotensin system is pivotal in controlling the cardiovascular system, the murine Ren-2 gene was chosen to generate transgenic rats. Male rats have a sustained

angiotensin II-dependent increase in blood pressure with low circulating renin levels thus providing convincing evidence for the physiological significance of tissue renin–angiotensin systems. At 12–14 weeks, male transgenic rats have concentric cardiac hypertrophy but no dilatation or any signs of heart failure, and downregulation of β_1-adrenoceptors. Female Ren-2 transgenic rats have been used to study the interplay between the renin–angiotensin system and oestrogen in the pathogenesis of hypertension (Paul et al., 1994; Doggrella and Brown, 1998)

3.11 TAMOXIFEN

Tamoxifen is competitive partial agonist inhibitor of estradiol at estrogen receptor. It is classified in the family of selective estrogen receptor modulators (SERMs)Tamoxifen is the current therapy of choice for patients with estrogen receptor negative breast cancer & prophylactic for women at high risk of developing the disease. Tamoxifen shows mechanism of action by competitively binding to estrogen receptors on tumors and other tissue targets, producing a nuclear complex that decreases DNA synthesis and inhibits estrogen effects.

Pharmacological Actions

Anti-estrogenic effect of tamoxifen includes inhibition of the proliferation of cultured human breast cancer cells and reduces tumor size and number in women. Tamoxifen binds to the high affinity estrogen receptor in the cytoplasm and the modified complex is the translocated in the nucleus. Tamoxifen competes for the binding site with the estradiol and by occupying receptor recedues the amount of receptor available for endogenous estradiol. It has depressant action on central nervous system. Estrogenic effects includes proliferation of uterus .tamoxifen may increase apolipoprotein A-I biosynthesis in liver through estrogen receptor mediated effects, resulting in a relative increase in HDL compared with LDL.(Micherone et al.,2005) also It is an inhibitor of sterol-8,7-isomerase (SD8I), which prevents the conversion of zymosterol into cholesterol (Gylling et al.,1998). Tamoxifen also has weak antioxidant properties, protecting LDL cholesterol from potentially harmful oxidation. (Wiseman et al., 1990) also Tamoxifen is likely to mediate anti-inflammatory effects, at least in part through the upregulation of the anti-inflammatory cytokine i.e. TGF-β.It also causes marked reduction in C-reactive protein (CRP) levels. (Bonanni at al., 2003).In women with an ovulatory infertility tamoxifen caused LH and FSH to rise at

about eight day of cycle. In men, by displacing estrogens from their hypohalamopituitary receptors, tamoxifen increase gonadotropin secretion and plasma level. Tamoxifen, by its antiestrogenic effects, decrease prolactin levels in patients with hyperprolactinemia.

Pharmacokinetics

Tamoxifen is well absorbed via oral route. It shows Peak concentrations occur 4-7 h after oral dosing. Peak concentrations after single oral doses of 20 mg are about 40 µ/l. It is well distributed in body from site of action. Tamoxifen is more than 99% protein-bound in serum predominantly to albumin. In patients with breast cancer, concentrations of tamoxifen and its metabolites in pleural, pericardial and peritoneal effusion fluid are between 20 and 100% of those in serum, but only trace amounts enter the cerebrospinal fluid. Concentrations in breast cancer tissue exceed those in serum. The volume of distribution is 50-60 l/kg. The elimination is biphasic, with an initial half-life of around 7 h and a terminal half-life of 7-11 days. It is metabolised by hepatic metabolism to 1-(4-ethanolyloxyphenyl)-1, 2-diphenylbut-1-ene (the primary alcohol), N-desmethyl tamoxifen, 4-hydroxy tamoxifen, 4-hydroxy-N-desmethyl tamoxifen, N-esdimethyl tamoxifen. The major excretory route is via the bile as metabolites and enterohepatic recirculation occurs. Less than 1% is excreted in the urine. (Buckley & Goa, 1989; Lien et al., 1989).

Uses

It is mainly used in adjuvant treatment of estrogen receptor positive & negative tumors in breast cancer in daily dose of 10-20 mg bid. Also it is indicated for Infertility in men and women at daily dose of 10-40mg bid. Currently it has completed phase II trial in treatment of brain tumors.

Side Effects

It includes hot flashes, vaginal bleeding, nausea, irregular menstrual periods, weight loss, and osteoporosis.

Contraindication

It is contraindicated in pregnancy because of the anti- estrogenic effects. Also in patients receiving anticoagulant therapy and patients having history of deep-vein thrombosis or pulmonary embolus.

3.12 CARDIAC EFFECTS OF TAMOXIFEN

Tamoxifen causes reversibly inhibition of PKC, 4 hydroxy Tamoxifen (metabolite of tamoxifen) permanently inactivated the enzyme by modifying the catalytic domain at lower concentrations. The *N*-demethyl tamoxifen, a metabolite of tamoxifen has also been shown to inhibit PKC in a reversible manner. The inhibition of PKC in the test tube requires higher concentrations of Tam (IC50 100 mM), while the ER-independent cell growth inhibition requires only 1–5 mM Tamoxifen (Gundimeda et al., 1996).In addition to its effect on estrogen receptors, tamoxifen have inhibitory effect on L- type Ca+2 Channels in vascular smooth muscle cells and reduced smooth muscle contractility which can be beneficial in cardiovascular disease. Tamoxifen may also be reducing contractility by inhibiting calmodulin activation of myosin light chainkinase. Other ion channel mechanisms may also be involved in the inhibitory effects of Tamoxifen. For example, Tamoxifen may also have potassium channel activating effects, similar to those recently demonstrated for estradiol on VSMC (Song et al, 1996).

According to McDonald et al. (1995) there was a significant reduction in risk of myocardial infraction in women receiving tamoxifen treatment. Rossner and Wallgren (1984) have demonstrated cardioprotective effect of tamoxifen. The rationale for study was based on data indicating that tamoxifen acts mainly as an estrogenic agonist in most tissues in postmenopausal women. They demonstrated that tamoxifen treatment resulted in changes in serum lipoproteins similar to those seen with estrogen replacement therapy. Two months after initiation of tamoxifen therapy, total serum cholesterol levels were significantly decreased, by approximately 15%, mainly as a result of decreased levels of low-density lipoprotein (LDL) cholesterol. These observations suggest that long-term treatment with tamoxifen in postmenopausal women should decrease morbidity from cardiac disease.

4. MATERIAL AND METHODS

4.1 PROTOCOL

The protocol of the experiment have been approved by institutional animal ethics committee as per the guidance of committee for the purpose of control and Supervision of experiments on animals (CPCSEA), Ministry of social justice and Empowerment, Government of India. (IPS/PCOL/MPH10-11/009, 17August 2011 and IPS/PCOL/MPH 10-11/2002, 14January 2011)

4.2 INDUCTION OF CARDIAC HYPERTROPHY

Adult Wistar rats of either sex of 6-8 week of age were choosen for the study and maintained under well-controlled conditions of temperature (22 ± 2°C), humidity (55 ± 5%) and 12h/12h light-dark cycle. Standard laboratory rat chew and UV-filtered water were provided *ad libitum.*

4.2.1 ISOPROTERENOL (ISO) INDUCED CARDIAC HYPERTROPHY

Adult wistar rats of either sex were used for the procedure. The rats were injected intraperitoneally (i.p.) with 5 mg/kg isoproterenol in 0.9% sodium chloride solution daily for 10 days. Control rats received equivalent amount of isotonic saline alone.

4.2.2 PARTIAL ABDOMINAL AORTIC CONSTRICTION (PAAC) INDUCED CARDIAC HYPERTROPHY

Adult wistar rats of either sex were used for procedure. Treatment of tamoxifen was started from 0^{th} day in sham treated and PAAC treated animals. Surgical procedure was done on 3^{rd} day in PAAC control and PAAC treated animal under anesthesia produced by ketamine (20mg/kg) and Xylazine (10mg/kg). Incision was made in abdominal wall to expose abdominal aorta. Abdominal aorta was ligated suprarenal with 4.0 silk suture along with 7-0mm blunt needle. Thereafter needle was removed to leave abdominal aorta partially constricted. Sham control and Sham treated animal underwent the same procedure except constriction of abdominal aorta.

4.3 TREATMENT PROTOCOL

The rats were then randomly divided into four groups as follows:

CON – Control animals (ISO group) or Sham Control (PAAC group).

TAM – Control animals treated with Tamoxifen (ISO group), Sham Control animals treated with Tamoxifen (PAAC group).

DIS – Hypertrophic control animals.

DIS+TAM – Hypertrophic animals treated with Tamoxifen (2 mg/kg/day, p.o.).

Tamoxifen was dissolved in saline and was administered orally (p.o.) at a dose of 2mg/kg/day, p.o. for 10 days and 4 weeks in ISO induced cardiac hypertrophy and PAAC induced cardiac hypertrophy respectively. Animals were maintained with free access to conventional dietary feed and water *ad libitum* through out experimental period. All animals were monitored regularly for changes in body weight and mortality throughout the course of study.

4.4 BLOOD SAMPLE COLLECTION AND SERUM ANALYSIS:

Blood samples were collected in clean dry centrifuge tubes as the end of experimental period from the retro orbital plexuses under light ether anesthesia and were allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 5000rpm for 20 min and stored at -20^0C until the analysis was carried out. Serum samples were analyzed for Total cholesterol, HDL- cholesterol, Triglycerides, LDL-cholesterol. C - Reactive protein (CRP), Lactate De-Hydrogenase (LDH), Creatinine Kinase (CK) spectrophotometrically (Shimadzu UV-1601, Japan) using biochemical diagnostic kits (Labcare Diagnostics Pvt. Ltd., India). Hemodynamic parameter viz. blood pressure, heart rate, rate of pressure development and decay were recorded by carotid artery cannulation using (LabscribSystem Inc., USA). After withdrawal of blood samples from retro-orbital plexus and recording hemodynamic parameters, animals were sacrificed, hearts were excised, extraneous tissues were separated and wet weight of the entire heart, left ventricle, right ventricle and femur length was noted down.

4.5 ESTIMATION OF VARIOUS SERUM BIOCHEMICAL PARAMETERS:

4.5.1 SERUM BIOCHEMICAL ESTIMATIONS

Collection of serum:

The blood samples were withdrawn from retro-orbital plexus under light ether anesthesia without any anticoagulant and allowed to clot for 10 minutes at room temperature. It was centrifuged at 5000 rpm for 20 minutes. The serum obtained was kept at -20ºC until used.

4.5.1.1 LIPID PROFILE

- **Estimation of Total cholesterol:**

Principle

Cholesterol esters are hydrolyzed by Cholesterol esterase to produce cholesterol. Hydrogen Peroxide is then produced from oxidation of cholesterol by cholesterol oxidase. The indicator quinoneimine is formed from hydrogen peroxide and 4 - aminoantipyrine in the presence of phenol and peroxide. The absorption of the red quinoneimine dye is proportional to the concentration of cholesterol in the sample.

Procedure:

Reagents and samples were pipetted out as shown below:

	Blank	Standard	Serum sample
Sample	-	-	10 µl
Standard	-	10 µl	-
Reagent	1000 µl	1000 µl	1000 µl

The contents were mixed and incubated for 30 min. at R.T. and absorbance of sample (AT) and standard (AS) against reagent blank was measured at 505 nm.

Calculations:

Total Cholesterol (mg/dl) = Abs. of TC / Abs. of Std. X 200 mg/dl

➢ **Estimation of LDL Cholesterol:**

Principle:

Direct determination of serum LDL-C (low density lipoprotein cholesterol) levels without the need for any pre-treatment of centrifugation steps. The assay takes place in two steps.

-1^{o} elimination of lipoprotein non-LDL

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone + } H_2O_2$$

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

-2^{o} measurement of LDL-C

$$\text{Cholesterol esters} \longrightarrow \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } O_2 \longrightarrow \text{4-Cholestenone + } H_2O_2$$

$$2H_2O_2 + \text{TOOS + 4-AA} \longrightarrow 2H_2O + O_2$$

The intensity of color formed is proportional to the LDL-C concentration in the sample.

Procedure:

Reagents and samples were pipetted out as shown below:

	Blank	Standard	Serum sample
R1 (µl)	375	375	375
Standard (µl)	-	5	-
Sample (µl)	-	-	5

Mixed and incubated for 5 mins at 37°C

R2 (µl)	125	125	125

The contents were mixed and incubated for 30 min. at R.T. and absorbance of sample (AT) and standard (AS) against reagent blank was measured at 546 nm.

Calculations:

LDL-C (mg/dl) = Abs. of sample / Abs. of Calibrator X Calibrator conc.

- **Estimation of Triglycerides:**

Principle:

Triglycerides are determined after enzymatic hydrolysis with lipases. The quinonemine indicator is formed from hydrogen peroxide, 4-aminophenazone, and 4-chlorophenol under the catalytic influence of peroxidase.

Procedure:

Reagents and samples were pipetted out as shown below:

	Blank	Standard	Serum sample
Sample	-	-	10 µl
Standard	-	10 µl	-
Reagent	1000 µl	1000 µl	1000 µl

The contents were mixed and incubated for 30 min. at R.T. and absorbance of sample (AT) and standard (AS) against reagent blank was measured at 505 nm.

Calculations:

Triglycerides (mg/dl) = Abs. of TG / Abs. of Std. X 200 mg/dl

- **Estimation of HDL Cholesterol:**

Principle:

Direct determination of serum HDL-C (High Density Lipoprotein Cholesterol) levels without the need for any pre-treatment or centrifugation of the sample. The method depends on the properties of a detergent which solubilizes only the HDL so that HDL-C is released to react with the cholesterol esterase, cholesterol oxidase and chromogens to give colour. The non HDL lipoprotein LDL, VLDL and chylomicrons are inhibited from reacting with the enzymes due to abruption of the detergents on their surfaces. The intensity of the color formed is proportional to the HDL-C concentration in the sample.

Procedure:

Reagents and samples were pipetted out as shown below:

	Blank	Standard	Serum sample
R1 (µl)	750	750	750
Standard (µl)	-	10	-
Sample (µl)	-	-	10

Mixed and incubated for 5 mins at 37°C. Read absorbance A1.

R2 (µl)	250	250	250

The contents were mixed and incubated for 30 min. at R.T. and absorbance of sample (AT) and standard (AS) against reagent blank was measured at 505 nm.

Calculations:

HDL-C (mg/dl) = ΔA sample / ΔA Calibrator X Calibrator conc.

4.5.1.2 CARDIAC BIOMARKERS:

- **Estimation of creatine kinase:**

In vitro quantitative measurement of CKMB concentration in serum was done by using diagnostic kit (Lab Care Diagnostics, India Limited.).

Principle-

This procedure involves measurement of Creatine kinase (CK) activity in the presence of an antibody to CK-M monomer. This antibody completely inhibits the activity of CK-MM and half of CKMB while not affecting the B subunit of CK-MB and CK-BB. Than the CK method is used to quantitatively determine CK-BB activity. CK catalyses the reaction between creatine phosphate and ADP, giving creatine and ATP. ATP and glucose in the presence of G6PDH oxidises, and reduces NAD to NADH. The rate of NADH formation is determined photometrically at 340 nm & is directly proportional to CK-BB activity. The CK-MB activity is calculated by multiplying CKBB x 2.

Clinical significance-

CK-MB is an enzyme formed by the association of two subunits from muscle (M) and nerve cells (B). CK-MB is usually present in serum at low concentration; it is increases after an acute infarct of myocardium and later descends at normal levels. Also is increased, rarely, in skeletal muscle damage.

Procedure

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Sample (CKMB) as shown below:

Working reagent (Rw)-Mix 4 ml of Enzyme Reagent I with 1 ml of Enzyme Reagent II.

	Blank	Standard	Sample
Rw (µl)	1000	1000	1000
Standard (µl)	-	50	-
Sample (µl)	-	-	50

Mix well and after 10 min. at 37°C. Measure the change in absorbance. Repeat readings every minute for next 5 minutes. Calculate Δ A/min at 340 nm.

Calculation-

Δ A/min. x 3376 = U/l CKBB

CKMB = CKBB x 2

- **Estimation of Lactate dehydrogenase:**

In vitro quantitative measurement of LDH concentration in serum was done by using diagnostic kit (Lab Care Diagnostics, India Limited.).

Principle-

Lactate is oxidised to Pyruvate in the presence of NAD by the action of lactate dehydrogenase. The rate of formation of NADH is directly proportional to LDH concentration.

Procedure

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Sample (LDH) as shown below:

Working reagent (Rw)-Mix 9 ml of Buffer reagent with 1 ml of Enzyme reagent.

	Blank	Standard	Sample
Rw (µl)	1000	1000	1000
Standard (µl)	-	25	-
Sample (µl)	-	-	25

Mix well and after 1 min incubation, measure the change in absorbance per min. (Δ A/min.) for next 2 minutes at 340 nm.

Calculations:

Activity (U/L) = Δ A/min x 6592

➢ **Estimation of CRP-Turbilatex:**

In vitro quantitative measurement of CRP-Turbilatex concentration in serum was done by using diagnostic kit (Lab Care Diagnostics, India Limited.).

Principle-

The CRP-Turbilatex is a quantitative turbidimetric test for the measurement of C-reactive protein (CRP) in human serum or plasma. Latex particles coated with specific anti-human CRP are agglutinated when mixed with samples containing CRP. The agglutination cause an absorbance change, dependent upon the CRP content of the patient sample that can be quantified by comparison from a calibrator of known CRP concentration. CRP is an acute-phase protein present in normal serum, which increases significantly after most forms of tissue, bacterial and virus infections, inflammation and malignant neoplasia. During tissue necrosis and inflammation resulting from microbial infections, the CRP concentration can rise up to 300 mg/L in 12-24 hours.

Procedure-

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Sample (CRP) as shown below:

Working reagent (Rw)-Mix 1 ml Latex Reagent + 9 ml Diluent

	Blank	Standard	Sample
Rw (µl)	1000	1000	1000
Standard (µl)	-	5	-
Sample (µl)	-	-	5

Adjust the instrument to zero with distilled water. Mix and read the absorbance at 540 nm after 10 Seconds (A1) and after 2 minutes (A2) of the sample addition.

Calculation-

(A2-A1) sample __ x Calibrator concentration =mg/L CRP
(A2-A1) calibrator

4.5.2 HYPERTROPHIC PARAMETER

After measurement of hemodynamic parameters, animals were sacrificed. The skin was quickly incised at the midline over the sternum and the heart was exposed by cutting the pericardium. Heart was isolated from the body, blotted with filter paper to remove excess of blood, remaining extraneous tissues were removed and weight of the heart was noted down to calculate the index of cardiac hypertrophy as wet heart weight to femur length ratio and weight of the left ventricles was noted down to calculate the index of left ventricular hypertrophy as wet left ventricle weight to wet heart weight ratio. Also left ventricular weight to right ventricular weight ratio was estimated. Left ventricular wall thickness (screw micrometer) was also calculated.

4.5.3 HEMODYNAMIC PARAMETERS

The animals were anaesthetized by Ketamine (20 mg/kg, i.p.) + Xylazine (10 mg/kg, i.m.). The body temperature was maintained at 37 ± 1 °C during the experiment. The carotid artery behind the trachea was exposed and cannulated for the measurement of hemodynamic parameters using a transducer (BP 100) and Labscrib Systems (IWORX, New Hampshire, USA). The hemodynamic parameters observed were systolic (SBP), diastolic (DBP) and mean arterial blood pressure (MAP), heart rate, rate of pressure development (dp/dt_{max}) and rate of pressure decay (dp/dt_{min}). All the data were analyzed using Labscrib software (Version 118).

4.5.4 Estimation of LV collagen content

Estimation of LV collagen was performed according to method described by Drobnik et al., 2009.

Preparation of hydrolysate of sample:-

The tissue for collagen measurement was hydrolyzed with 6 N Hydrochloric acid at 110º C for 12 hrs. And after 12 hrs hydrolysate obtain was used for estimation.

Reagents:

1. 6 N Hydrochloric acid
2. Citrate buffer : For 10 ml, mixture of 8.2 ml of sodium citrate (294.1 mg/ 10ml) and 1.8 ml of Citric acid (210.14mg/10ml)
3. Chloramin T: 1.4% in citrate buffer
4. Ehrlich Reagent: 2.4 g paradimethylaminobenzaldehyde (PDAB) in 8 ml of Ethanol (95%) + 0.54 ml Concentrated H_2SO_4.

Procedure:-

Blank	**Sample**
200µl of Distilled water	200µl of tissue hydrolysate
200µl of Ethanol (95%)	200µl of Ethanol (95%)
200µl of Chloramine T	200µl of Chloramine T
Oxidised for 20 min at RT	
400µl of Ehrlich reagent	400µl of Ehrlich reagent
Incubate for 15 min at 60º C	

After incubation, absorbance was read against blank at 573 nm. The collagen level was calculated using standard cureve which was plotted using standard Hydroxyproline (Sigma chemicals, USA).

4.5.5 OXIDATIVE STRESS PARAMETERS

Heart, kept in cold conditions (precooled in inverted petridish on ice) was removed. It was cross chopped with surgical scalpel into fine slices and was chilled in the cold 0.25 M sucrose, quickly blotted on a filter paper. The tissue was minced and homogenized in Phosphate buffer saline, pH 7.4 with 25 strokes of tight teflon pestle of glass homogenizer at a speed of 2500 rpm. The clear supernatant was used for other enzymes assays.

4.5.6.1 Superoxide Dismutase (SOD)

SOD was estimated by the method of **Mishra and Fridovich, 1972**.

Principle:

The $O_2^{\cdot -}$ substrate for SOD is generated indirectly in the oxidation of epinephrine at alkaline pH by the action of oxygen on epinephrine. As $O_2^{\cdot}$ builds in the solution, the formation of adrenochromeaccelerates because $O_2^{\cdot}$ also reacts with epinephrine to form adrenochrome. Toward the end of there action, when the epinephrine is consumed, the adrenochrome formation slows down. If observed for long times, the adrenochrome disappears and brown, insoluble products form in the solution.

SOD reacts with the $O2^{\cdot}$ formed during the epinephrine oxidation and therefore slows down the rate of formation of the adrenochrome as well as the amount that is formed. Because of this slowing process, SOD is said to inhibit the oxidation of epinephrine.

Reagent:

1. EDTA 0.0001 M (9.3 mg/250 ml)
2. Carbonate buffer pH 9.7 (8.4 gm $NaHCO_3$ + 10.6 gm Na_2CO_3 in 500 ml)
3. Epinephrine – 0.003 M (50 mg/100 ml in 2 pH HCL and cover with aluminium foil.)

Procedure:

Blank	**Test**
0.2 ml of D.W.	0.2 ml of supernatant
0.1 ml EDTA	0.1 ml EDTA
0.5 ml Carbonate buffer	0.5 ml Carbonate buffer
1 ml epinephrine	1 ml epinephrine

All reagents were mixed well and absorbance was read against blank at an interval of 30 sec. for 3 min. at 480 nm. The SOD level was calculated using standard cureve which was plotted using standard SOD (MP Bio, USA).

4.5.6.2 Reduced Glutathione :-

Reduced of glutathione (GSH) was estimated by the method of Moran **et. al., 1979.**

Principle:-

Glutathione present in RBC consist of sulfhydryl groups. 5,5 dithiobis 2- nitro benzoic acid (DTNB), A disulphide compound, gets readily attacked by these sulfhydryl groups and forms a yellow coloured anion which measured colorimetrically at 412 nm.

Reagents:-

1. Trichloroacetic acid (TCA) (10%):-10 gm of TCA in 100 ml of distilled water.

2. 0.3 M Na_2HPO_4 :-4.26 gm of Na_2HPO_4 in distilled water.

3. DTNB (Dithiobis nitro benzoic acid) (Fresh):- 40 mg in 100 ml of 1% Sodium citrate & cover with aluminium foil.

Procedure:-

Blank	**Test**

0.2 ml of D.W.	0.2 ml of supernatant
1 ml of TCA (10%)	1 ml of TCA (10%)
Keep in ice bath for 30 min & Centrifuge for 10 min at 4˚C at 3000 RPM, take 0.5 ml of supernatant	
0.5 ml of Supernatant	0.5 ml of Supernatant
2 ml di-sodium hydrogen phosphate	2 ml di- sodium hydrogen phosphate
0.25 ml DTNB(Cover with aluminium foil)	0.25 ml DTNB(Cover with aluminium foil)

All reagents were mixed well absorbance was read against blank at 412 nm. The GSH level was calculated using standard cureve which was plotted using standard GSH (Sigma Chemicals, USA).

4.5.6.3 Total Protein estimation:-

Total Protein was estimated by the method of **Lowry et al, 1951.**

Principle:-

The phenolic group of tyrosine and trytophan residues (amino acid) in a protein will produce a blue purple color complex, with maximum absorption in the region of 660 nm wavelength, with Folin- Ciocalteau reagent which consists of sodium tungstate molybdate and phosphate. Thus the intensity of color depends on the amount of these aromatic amino acids present and will thus vary for different proteins.

Reagents:-

1. Reagent A:-2 % sodium carbonate in 0.1 N NaOH.

2. Reagent B:-0.5 % copper sulphate ($CuSO_4.5\ H_2O$) in 1 % potassium sodium tartrate.

3.Reagent C :-Alkaline copper solution (Mix 50 ml of solution A & 1 ml of solution B prior to use)

4. Reagent D:-Folin- Ciocalteau reagent.

Procedure:-

Blank	**Test**
0.2 ml of D.W.	0.2 ml of supernatant
Diluted up to 1 ml with Tris HCL	Diluted up to 1 ml with Tris HCL
5 ml Reagent C	5 ml Reagent C
Allowed it for 10 minutes	
0.5 ml Reagent D	0.5 ml Reagent D

All reagents were mixed well and kept at room temperature for 30 min. in dark place and absorbance was read against blank at 600 nm. The protein level was calculated using standard cureve which was plotted using standard albumin (Sigma chemicals, USA).

4.5.6.4 **Lipid peroxidation: (MDA) :-**

Malondialdehyde formation (MDA) was estimated by the method of Ohkawa et al., 1979.

Principle:-

The method estimates Malondialdehyde (MDA), a product of lipid peroxidation process. One molecule of MDA reacts with two molecules of thiobarbituric acid (TBA) under mildly acidic conditions to form a pink colouredchromogen, whose intensity was measured colorimetrically at 535 nm.

Reagents:-

1. **Sodium lauryl Sulphate (SLS) (8%):-**8 gm of SLS in 100 ml of distilled water.

2. **Acetic acid (20 %):-**Prepared in 0.27 M hydrochloric acid (2.29 ml HCL in 100 ml water)

3. **Thiobarbituric acid (TBA) (1% in Tris hydrochloride, pH 7): (Freshly prepared):-**

1 gm of thiobarbituric acid in 100 ml of Tris hydrochloride buffer pH 7.

Procedure:-

Blank	**Test**
0.2 ml of D.W.	0.2 ml of Homogenate
0.2 ml of SLS	0.2 ml of SLS
1.5 ml acetic acid in HCl	1.5 ml acetic acid in HCl
1.5 ml TBA	1.5 ml TBA
0.6 ml DW	0.6 ml DW
Heated for 45 min in water bath at 95^0C and cool	
5 ml mixture of n-butanol:pyridine(15:1)	5 ml mixture of n-butanol:pyridine(15:1)

All reagents were mixed well and pink colour developed in upper organic layer, the absorbance of which was read against blank at 532 nm. Malondialdehyde level was calculated using molar exitinction coefficient of malondialdehyde.

4.6 FIXATION AND PROCESSING OF TISSUES FOR HISTOLOGICAL STUDIES

Liver and aorta were collected after the rats were sacrificed. After blotting free of blood and tissue fluids, were fixed in 10% formalin solution. After 24 hours the tissues were washed thouroughly in repeated changes of 70% alcohol and then dehydrated in ascending grades of alcohol (70-100%). Dehydration in absolute alcohol was followed by treatment of tissues with toluene: xylene (50:50) followed by 10%, 50%, 70%, 90% paraffin wax in toluene and finally 2 changes in 100% wax (paraffin wax, 60-62ºC) followed by embedding of tissue in wax.

5-15µm thick section were serially cut on a leitz microtome in horizontal plane and mounted in glass slide with the help of egg albumin in glycerin solution (50% v/v). The sections were deparafinned in xylene and downgraded through 100%, 90%, 70%, 50%, & 30% alcohol and finally in water. They were then stained with 10% hematoxylin for 3-5 minutes and the staining was intensified by placing in running water. The hematoxylin stained sections were stained with 10% eosin for 2 minutes and were then quickly passed through ascending grades of alcohol and finally treated with xylene followed by mounting in DPX.

The sections were observed and desired areas were photographed in an Olympus photomicroscope for morphometric studies of different cells. The sections were viewed under 40X and 100X magnification.

4.7 STASTICAL ANALYSIS:

All the values are expressed as mean ± S.E.M. Statistics was applied using SPSS software version 17.0 (USA). Statistical significance between normal control and disease control groups and between disease control and disease treated group was tested using student's t-test. Differences were considered to be statistically significant when $p < 0.05$.

5. RESULTS

5.1 SERUM LIPID PROFILE

Effect of Tamoxifen on Serum Cholesterol, LDL, Triglyceride and HDL level

Isoproterenol control and PAAC control rats exhibited significantly ($p < 0.05$) increase level of serum total cholesterol, LDL and triglyceride and significantly ($p < 0.05$) decreased levels of serum HDL as compare to control rats. Treatment with tamoxifen (2mg/kg/day, p.o.) showed significant ($p < 0.05$) reduction in serum cholesterol ad serum LDL levels but did not produce any significant ($p < 0.05$) effect on serum cholesterol and HDL levels. Further treatment with tamoxifen (2mg/kg/day, p.o.) did not produce any significant ($p < 0.05$) change in serum cholesterol and serum LDL levels in control rats. (Figure 5.1a, 5.1b, 5.1c, and 5.1d)

Figure 5.1a: Effect of Tamoxifen on Total Cholesterol level

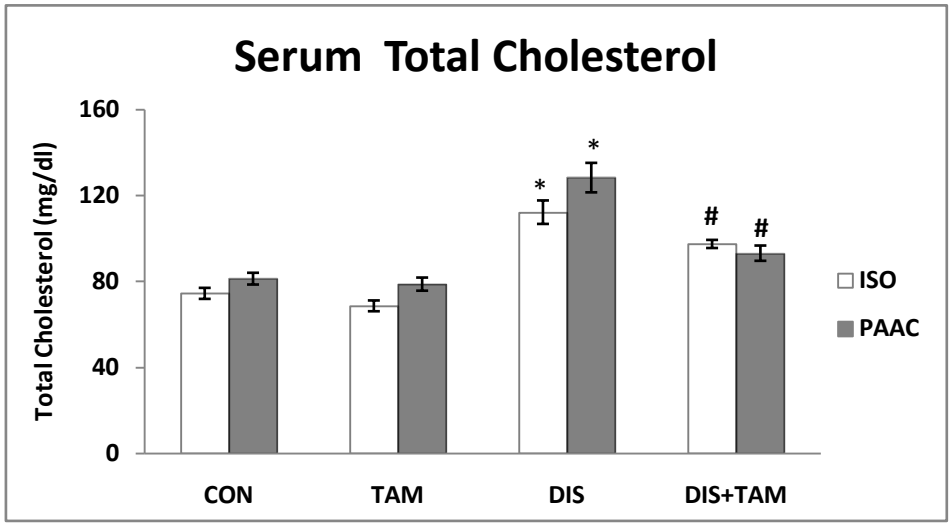
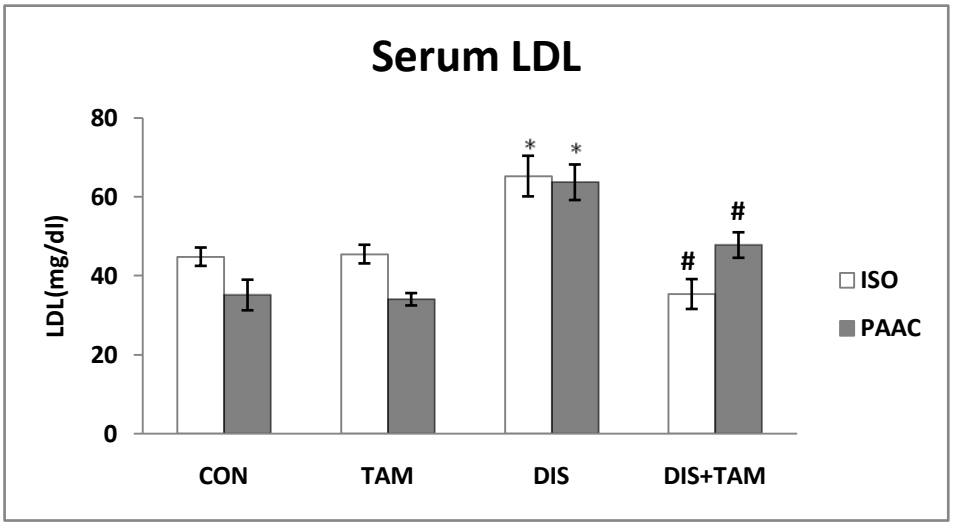


Figure 5.1b: Effect of Tamoxifen on serum LDL level



* Significantly different from normal group (p<0.05, t Test).
# Different from Disease induced group (p<0.05, t Test).

Values are expressed as Mean ± SEM of 6 rats.

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PAAC- Partial Abdominal Aortic Constriction induced cardiac hypertrophy.

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TAM – Control animals treated with tamoxifen (ISO group)/ #
Sham control animals treated with tamoxifen (PAAC group).
DIS – Hypertrophic control animals.
DIS+TAM – Hypertrophic animals treated with tamoxifen (2mg/kg/day, p.o.)

Figure 5.1c: Effect of Tamoxifen on serum Triglyceride level

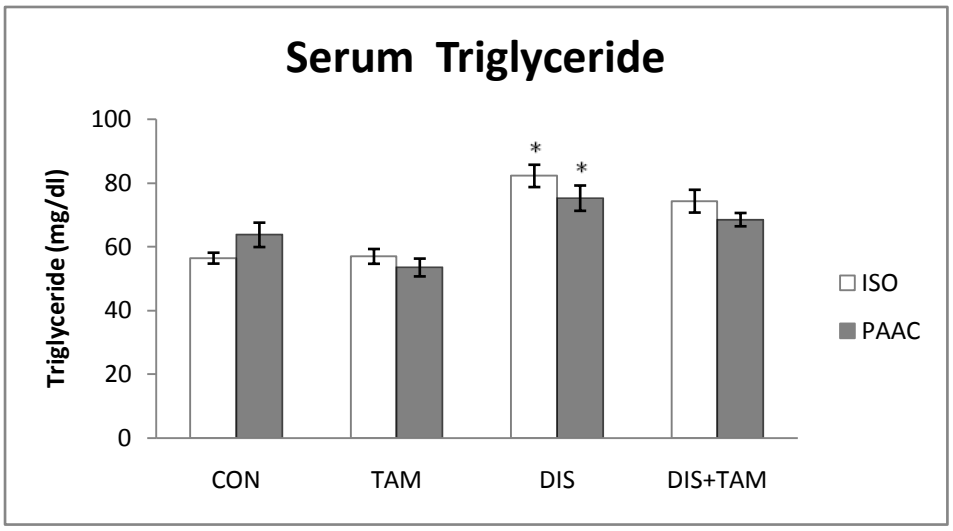
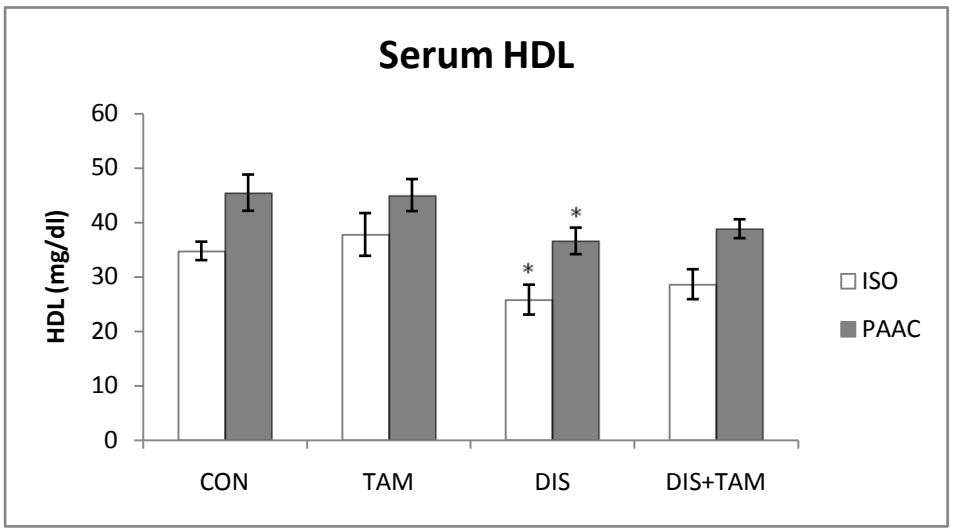


Figure 5.1d: Effect of Tamoxifen on serum HDL level



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DIS+TAM – Hypertrophic animals treated with tamoxifen (2mg/kg/day, p.o.)

5.2 SERUM CARDIAC MARKERS

Effect of Tamoxifen on serum Creatinine Kinase-MB, Lactate dehydrogenase and C reactive protein level.

Isoproterenol control and PAAC control rats produced significant ($p<0.05$) increase in serum Creatinine Kinase-MB and Lactate dehydrogenase level as compare to control rats. Treatment with tamoxifen (2 mg/Kg/day, p.o.) showed significant ($p<0.05$) reduction in both Creatinine Kinase- MB and Lactate dehydrogenase levels. (Figure 5.2a,5.2b). Further hypertrophic control rats showed significant ($p<0.05$) increase in C - reactive protein levels as compare to control rats, treatment with tamoxifen (2mg/kg/day, p.o.) in hypertrophic treated rats showed significant ($p<0.05$) reduction in C reactive protein levels (Figure 5.2c). However, treatment with tamoxifen (2mg/kg/day, p.o.) in control rats does not produce any significant ($p < 0.05$) change in creatinine kinase –MB, lactate dehydrogenase and CRP.

Figure 5.2a: Effect of Tamoxifen on serum LDH level

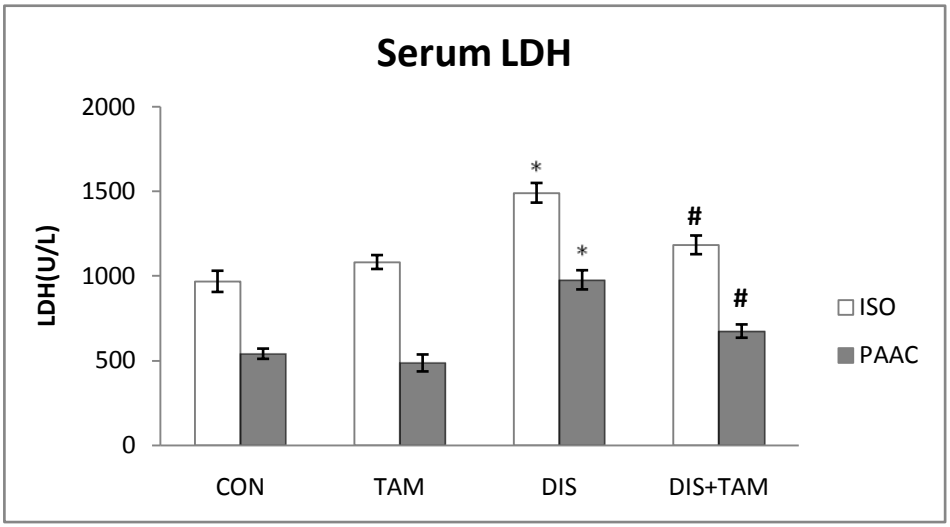

Figure 5.2b: Effect of Tamoxifen on serum CK-MB level

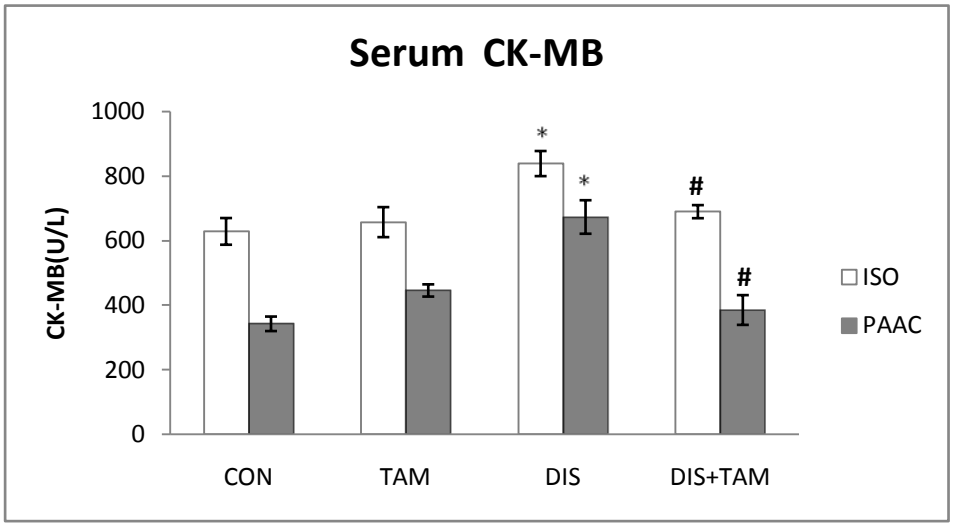

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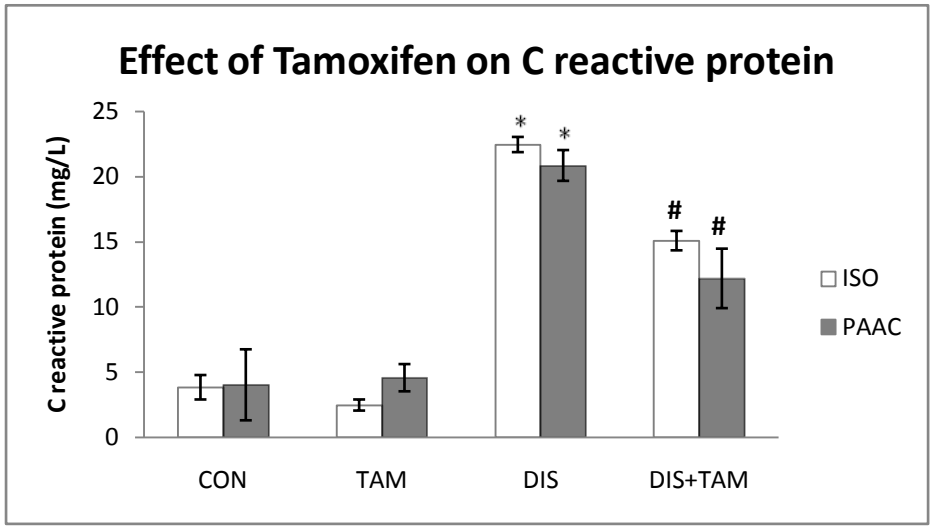
Figure 5.2c: Effect of Tamoxifen on serum C Reactive Protein level



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DIS – Hypertrophic control animals.
DIS+TAM – Hypertrophic animals treated with tamoxifen (2mg/kg/day, p.o.)

5.3 HYPERTROPHIC PARAMETERS

Effect of Tamoxifen on Cardiac hypertrophy index, Left ventricular hypertrophy index.

Isoproterenol control and PAAC control rats exhibited significantly ($p<0.05$) increased in Cardiac hypertrophy index, Left ventricular hypertrophy index, LVW/RVW, LV wall thickness level as compare to control group. Treatment with tamoxifen (2 mg/Kg/day, p.o.) in hypertrophic treated group showed significant ($p<0.05$) reduction in Cardiac hypertrophy index, Left ventricular hypertrophy index. (Figure 5.3a, 5.3b).

Figure 5.3a: Effect of Tamoxifen on Cardiac hypertrophic index

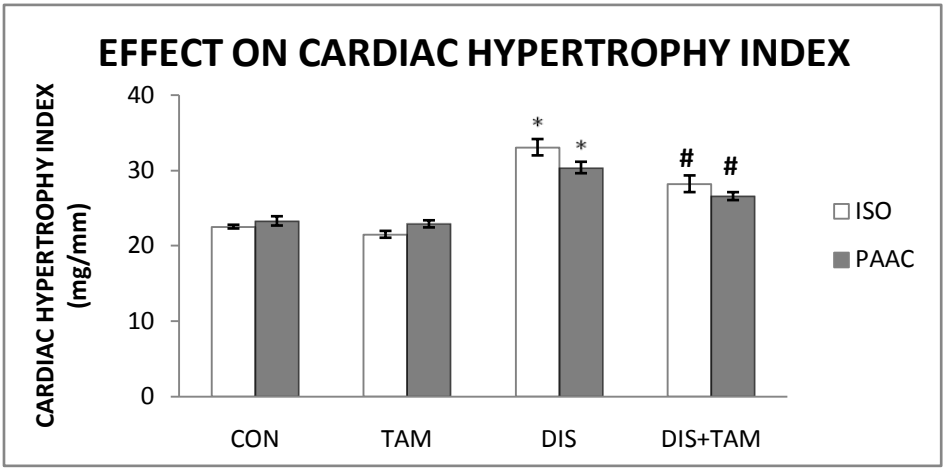
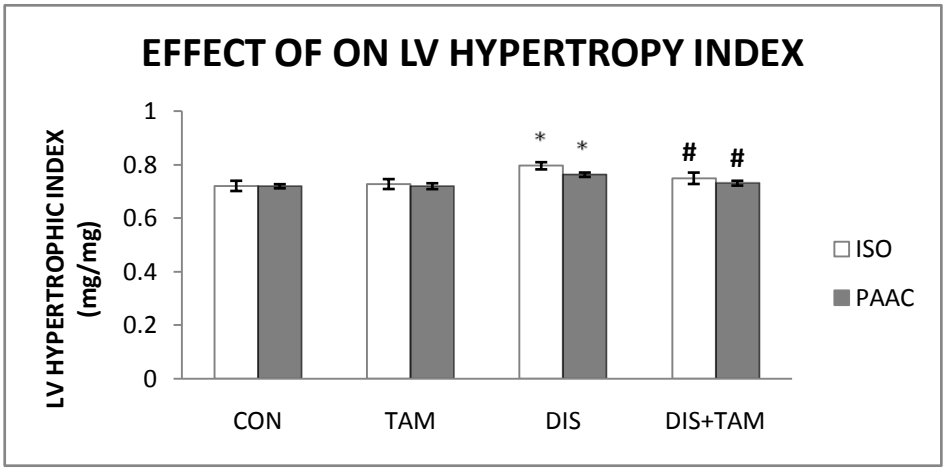


Figure 5.3b: Effect of Tamoxifen LV hypertrophy index



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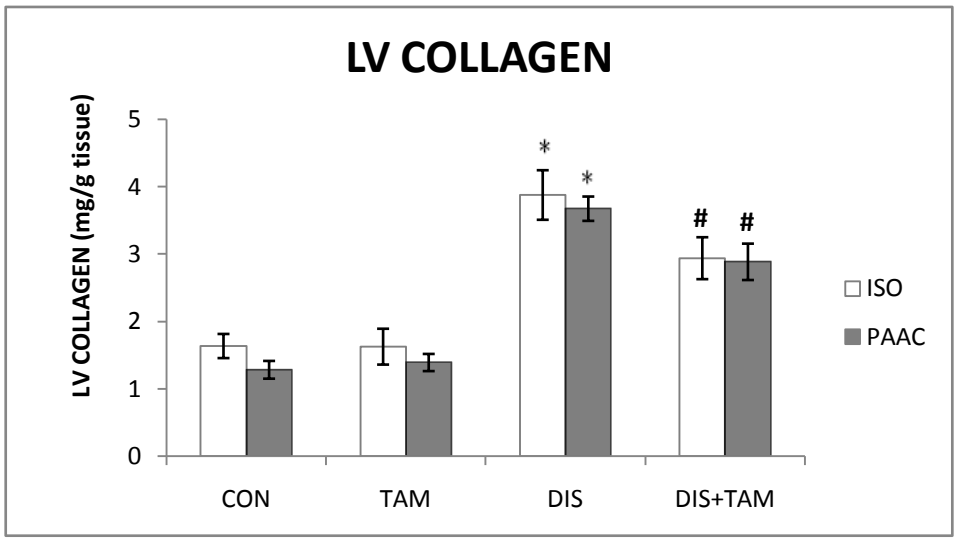
5.4 Effect of Tamoxifen on Cardiac hypertrophy index, Left ventricular hypertrophy index.

Figure 5.4a: Effect of Tamoxifen on LV Collage levels



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DIS+TAM – Hypertrophic animals treated with tamoxifen (2mg/kg/day, p.o.)

5.5 HEMODYNAMIC PARAMETERS

Effect of Tamoxifen on Blood pressure, Heart rate, Rate of pressure development and decay.

Isoproterenol control and PAAC control rats showed significant ($p<0.05$) increased in blood pressure and significant ($p<0.05$) reduction in heart rate as compare to control rats. Treatment with Tamoxifen (2 mg/Kg/day, p.o.) in hypertrophic treated group did not produce significant ($p<0.05$) change in blood pressure and heart rate. (Figure 5.5a, 5.5b). Further, hypertrophic control rats showed a significant ($p<0.05$) reduction in rate of pressure development and decay as compare to control rats. Treatment with Tamoxifen (2 mg/Kg/day, p.o.) in hypertrophic treated group produced significantly ($p<0.05$) increased in rate of pressure development and decay (Figure 5.5c, 5.5d). Further, Tamoxifen (2mg/kg/day, p.o.) treatment did not produced significant ($p<0.05$) change in blood pressure, heart rate and rate of pressure development and decay in control rats.

Figure 5.5a: Effect of Tamoxifen on Blood pressure

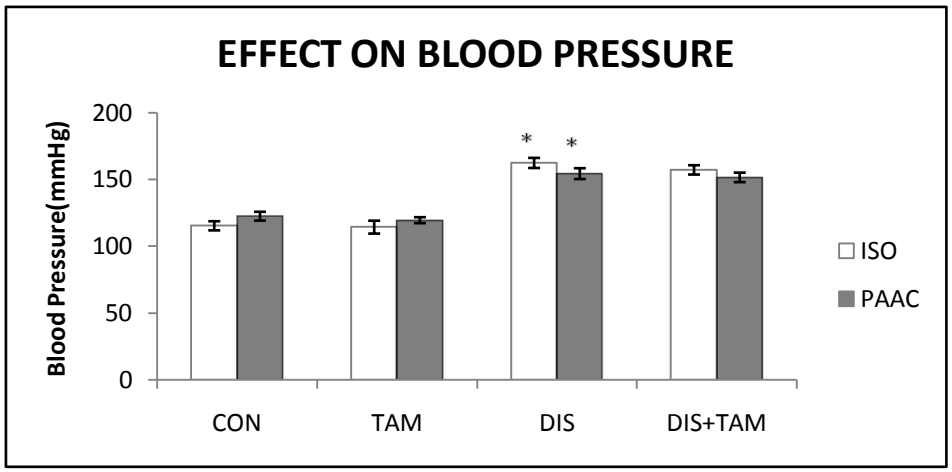
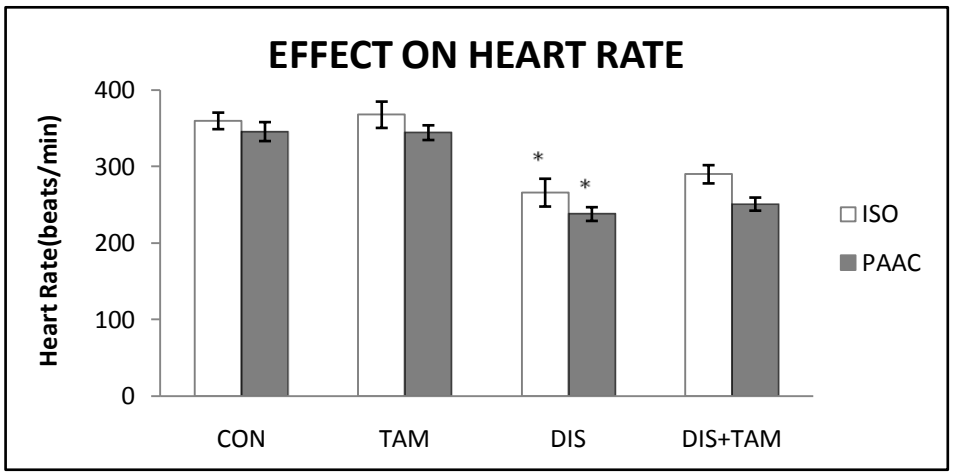


Figure 5.5b: Effect of Tamoxifen on Heart rate



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DIS+TAM – Hypertrophic animals treated with tamoxifen (2mg/kg/day, p.o.)

Figure 5.5c: Effect of Tamoxifen on Rate of Pressure development

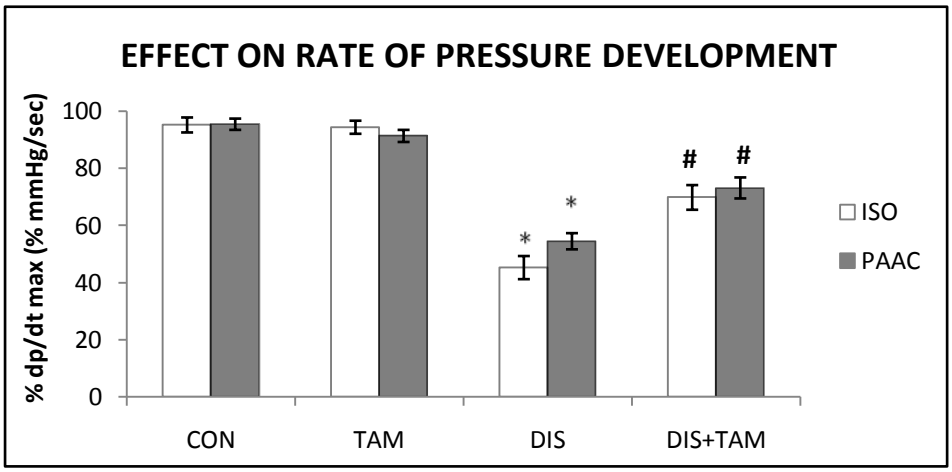
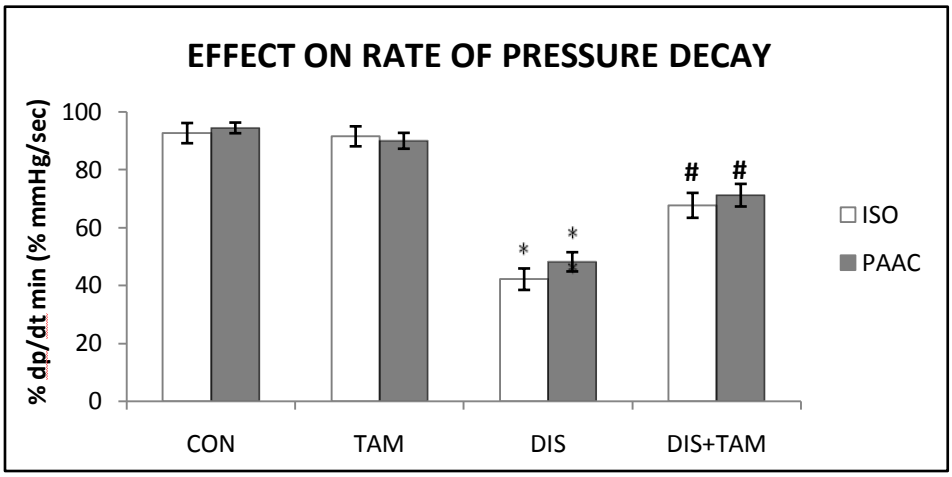


Figure 5.5d: Effect of Tamoxifen on Rate of Pressure decay



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5.6 ANTIOXIDANT PARAMETERS

Effect of Tamoxifen on LV Superoxide Dismutase (SOD), Glutathione (GSH), Protein, Malondialdehyde (MDA) levels

Isoproterenol control and PAAC control rats showed significant ($p<0.05$) reduction in left ventricular SOD, Glutathione levels as compare to control rats. Treatment with Tamoxifen (2 mg/kg/day, p.o.) in hypertrophic treated rats showed significant ($p<0.05$) increase in Left Ventricular SOD, Glutathione levels (Figure 5.6a, 5.6b). Also, hypertrophic control rats showed a significantly ($p<0.05$) increased left ventricular Protein and MDA levels as compare to control rats. Treatment with Tamoxifen (2 mg/Kg/day, p.o.) in hypertrophic treated group showed significant ($p<0.05$) reduction in left ventricular Protein and MDA levels (Figure 5.6c, 5.4d). Howerver, treatment of Tamoxifen (2mg/kg/day, p.o.) did not produced significant ($p<0.05$) change in LV superoxide dismutase, Glutathion, Protein, Malondialdehyde levels in control rats.

Figure 5.6a: Effect of Tamoxifen on LV SOD levels

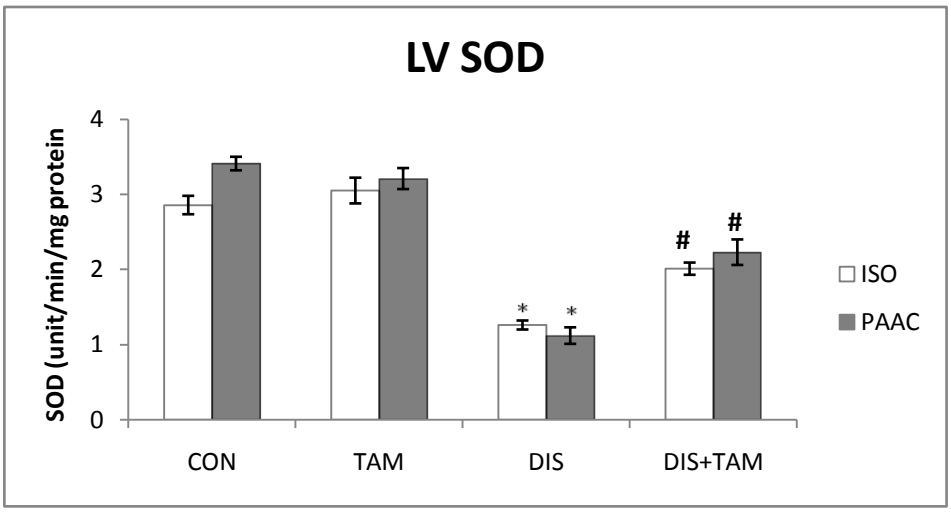

Figure 5.6b: Effect of Tamoxifen on LV GSH levels

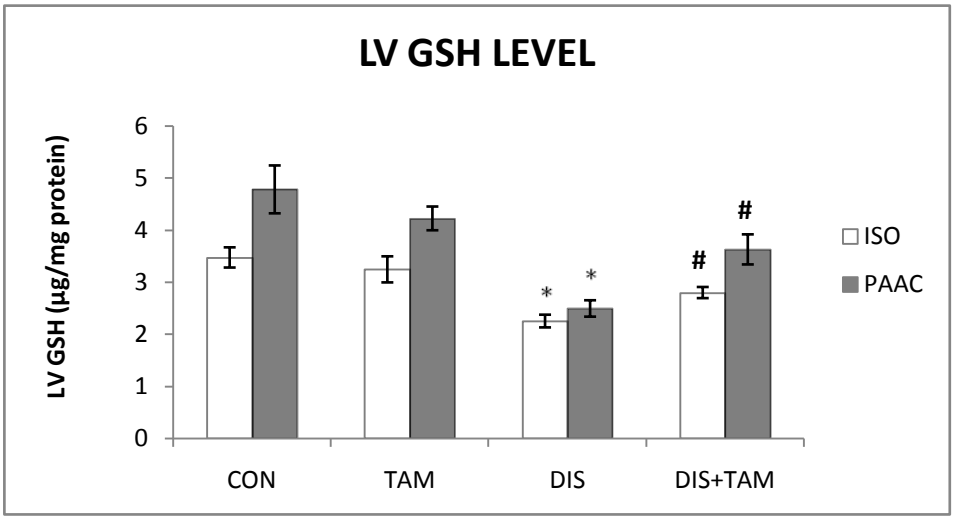

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Figure 5.6c: Effect of Tamoxifen on LV Protein levels

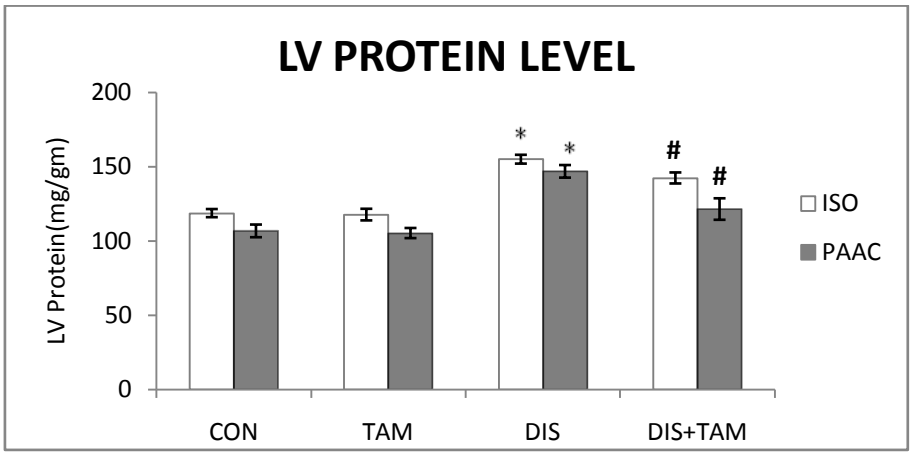
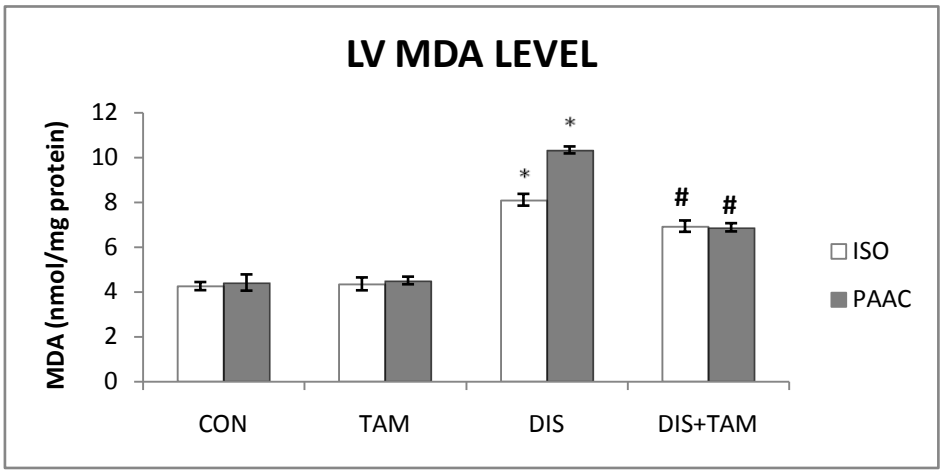


Figure 5.6d: Effect of Tamoxifen on LV MDA Levels



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5.7 HISTOPATHOLOGICAL STUDIES

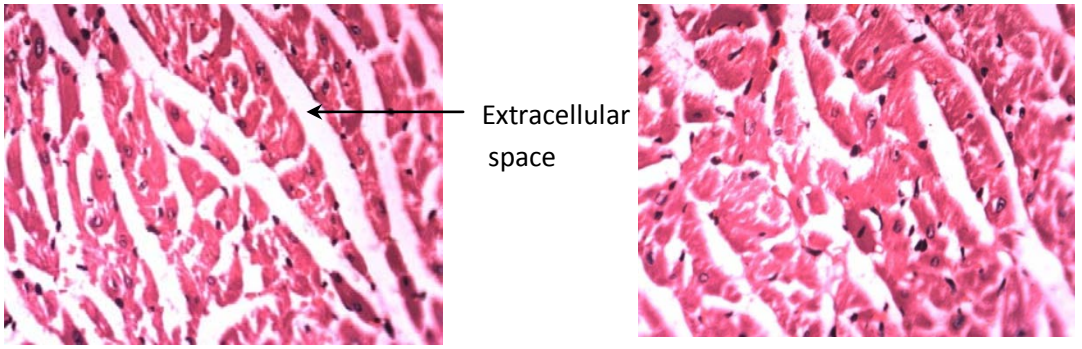
Microscopically, control rats showed no pathological changes in the LV myocardiocyte (Figure 5.7.1.A_1, 5.7.1.A_2) or in LV myocardial fibres (Figure 5.7.2.A_1, 5.7.2.A_2).

Histopathological examination of sections of LV fibres from untreated hypertrophic rat (Figure 5.7.1.C_1, 5.7.1.C_2, 5.7.2.C_1, 5.7.2.C_2) showed marked microscopic changes like reduction in extracellular space, increase in cardiomyocyte diameter, intense fibrosis and fibre disarray.

Tamoxifen treated section showed less reduction in extracellular space (Figure 5.7.2.D_1, 5.7.2.D_2) and less increase in cardiomyocyte diameter (Figure 5.7.1.D_1, 5.7.1.D_2). The extent of fibrosis and cardiac fibre disarray was less.

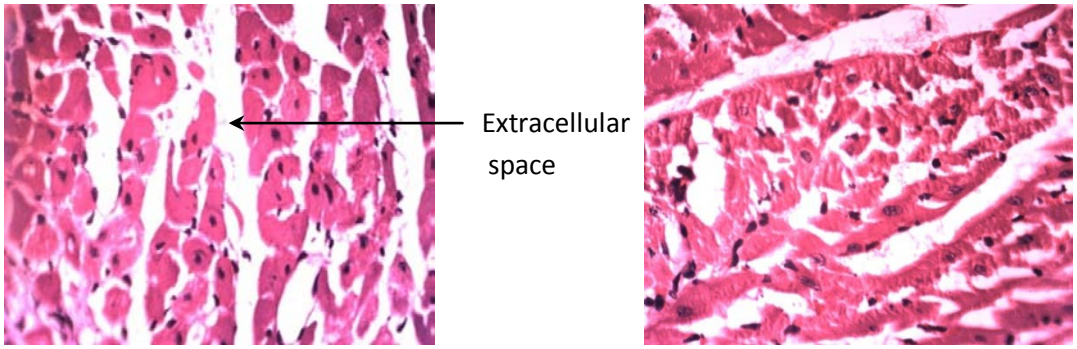
The control treated LV myocardial cells (Figure 5.7.1.B_1, 5.7.1.B_2) and LV fibres (Figure 5.7.2.B_1, 5.7.2.B_2) myocardial didn't show any histological alterations.

Figure 5.7.1 Histopathological sections of LV showing cardiac myocytes



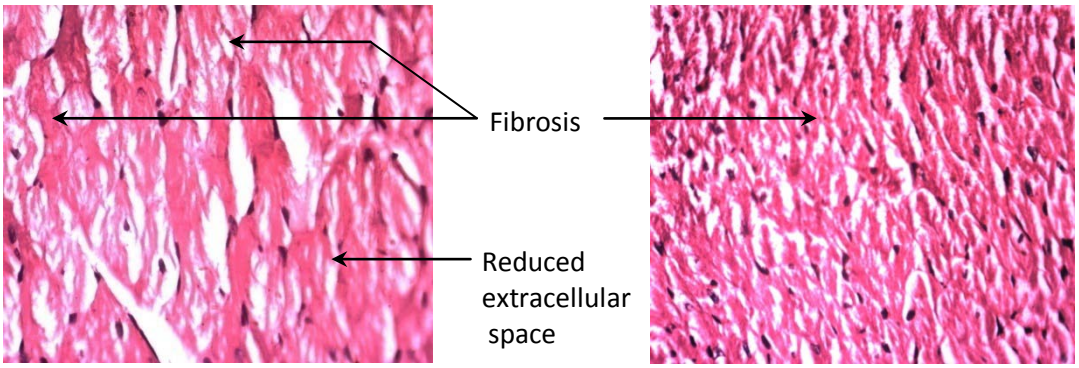
5.7.1. A $_1$: Control

5.7.1. A $_2$: Sham Control



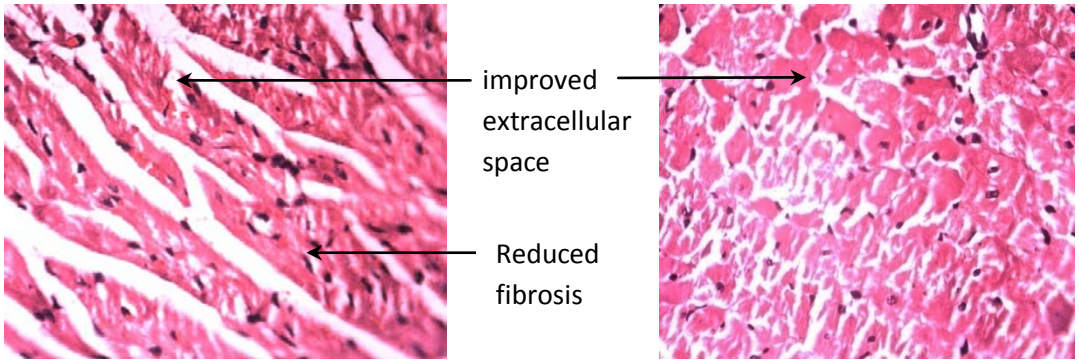
5.7.1. B $_1$: Control Treated

5.7.1. B $_2$: Sham treated



5.7.1. C $_1$: Hypertrophic control (ISO)

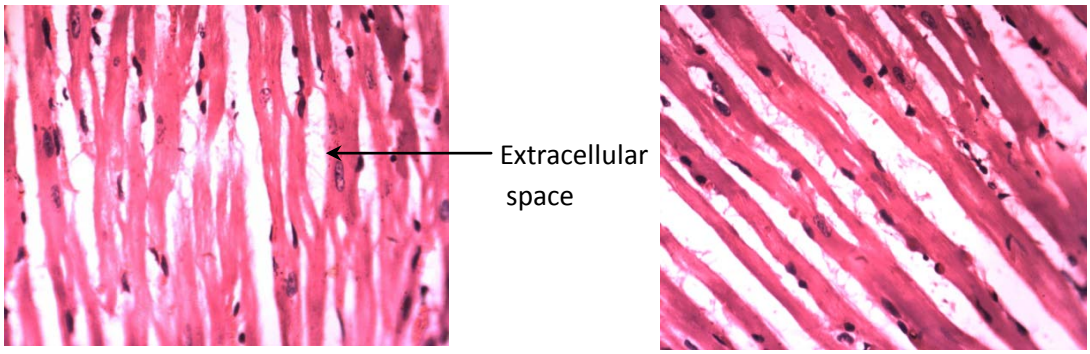
5.7.1. C_2: Hypertrophic control (PAAC)



5.7.1. D $_1$: Hypertrophic Treated (ISO)

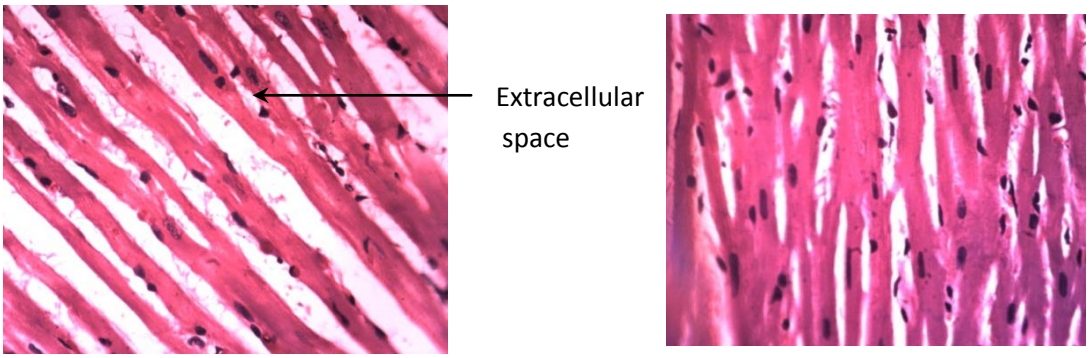
5.7.1. D $_2$: Hypertrophic Treated (PAAC)

Figure 5.7.2 Histopathological sections of LV showing cardiac fibers



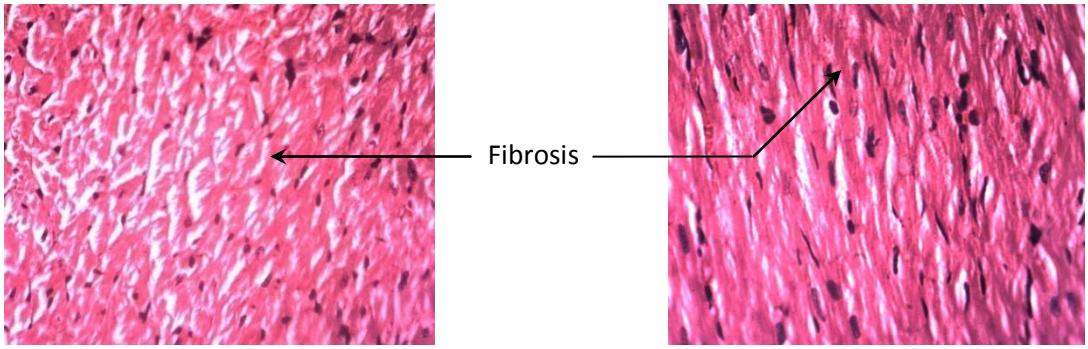
5.7.2. A $_1$: Control

5.7.2. A $_2$: Sham Control



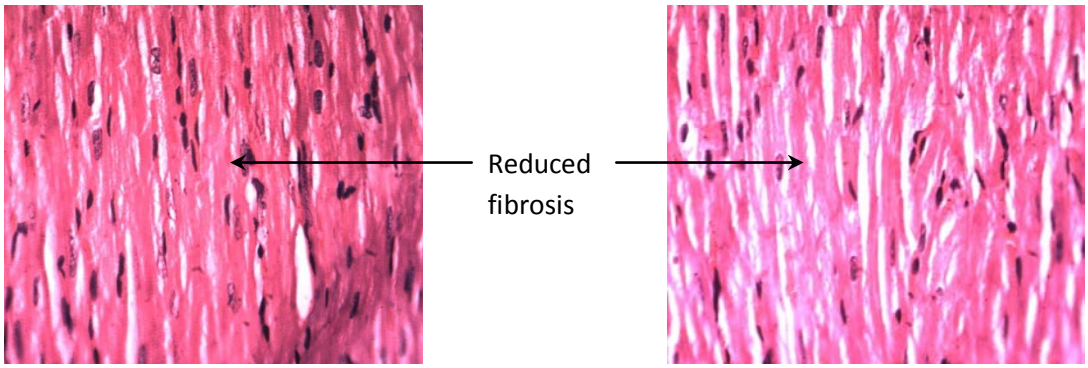
5.7.2. B $_1$: Control Treated

5.7.2. B $_2$: Sham treated



5.7.2. C $_1$: Hypertrophic control (ISO)

5.7.2. C$_2$: Hypertrophic control (PAAC)



5.7.2. D $_1$: Hypertrophic Treated (ISO)

5.7.2. D $_2$: Hypertrophic Treated (PAAC)

6. DISCUSSION

Cardiac hypertrophy is a fundamental process of adaptation to an increased workload due to hemodynamic overload. Development of cardiac hypertrophy is initially beneficial since it augments the number of contractile units and reduces ventricular wall stress to normal levels according to the law of Laplace. However, the adaptation has its limits and heart failure may ensue. Furthermore, arrhythmias and ischemic heart disease may develop which increase the risk of sudden death. (Ruwhof and Laarse 2000) Pathological cardiac hypertrophy is associated with fibrosis, sarcomere disarrangement, fetal gene expression and apoptotic cell death. (Balakumar and Singh 2006; Kang et al. 2004)

Present investigation was carried out using 2 models of cardiac hypertrophy viz chemical induced hypertrophy using isoproterenol (ISO) and pressure overload induced hypertrophy produced by partial abdominal aortic constriction (PAAC). The effects of ISO on heart are mediated through β_1 and β_2 adrenoceptors. Which, mediate the positive inotropic and chronotropic effects to β adrenoceptor agonists. Thus, ISO produces relative ischemia or hypoxia due to myocardial hyperactivity and coronary hypotension. Additionally, ISO causes myocardial damage due to excessive production of free radicals resulting from oxidative metabolism of catecholamines and also enhances protein synthesis and is responsible for abnormal lipid metabolism (Iams and Yeager 1981; Singal et al.,1983). Greenwood et al. (2001) has reported adrenergic overactivation also contributes to hypertrophy in hypertensive patients. Also it has been reported that patients with left ventricular hypertrophy demonstrate increased plasma norepinephrine concentration and enhanced sympathetic nerve activity compared with subjects without hypertrophy. Pressure overload hypertrophy results in thickening of ventricular walls and of cardiac myocytes and increased sarcomeric protein content with expression of fetal cardiac genes and gene products (Baker and Aceto, 1990; Sakata et al., 1998). Many studies have demonstrated that hemodynamic overload activates the tissue RAS in the heart mRNA and/or protein levels of renin, ACE, angiotensinogen and Ang II receptors have been reported to be increased in hypertrophied hearts. Also, mechanical stretch of cultured cardiac myocytes activates the phosphorylation cascade of protein kinases, induces the

expression of immediate early genes and fetal-type genes, and increases the protein synthesis rate. (Dostal et al, 1990; Schunkert et al., 1990; Harada, et al., 1998).

Dyslipidaemia is one of the most important modifiable risk factors for CHD. It has been characterized by increased levels and altered composition of VLDL-C and reduced levels and altered composition of HDL-C (Goldschmid et al. 1994). ISO administration has been reported to stimulate adenylate cyclase activity resulting in enhanced cAMP formation which enhances lipid biosynthesis resulting in hyperlipidaemia. In present study, increase in serum total cholesterol, serum LDL levels, serum triglyceride level and decrease in serum HDL level was found in hypertrophic control rat. Treatment with tamoxifen significantly reduces elevated serum total cholesterol and LDL levels. However, tamoxifen did not produce any serum triglyceride and HDL levels. It has been reported that tamoxifen produces inhibition of sterol-Δ8,7-isomerase (SD8I), which prevents the conversion of zymosterol into cholesterol and It also affects cholesterol esterification by inhibiting Acetyl-Coenzyme A acetyltransferase (ACAT) (Grainger and Schofield, 2005). Moreover, tamoxifen stimulates the expression and activity of the LDL receptor (Suarez et al. 2004).Thus these could be possible mechanism of tamoxifen in reduction in LDL and triglyceride levels. Our reports are consistence with other reports. Vanitha et al (1997) have reported that tamoxifen decreases cholesterol level in atherosclerosis suffering animals. Hozumi et al (1998) also reported tamoxifen decreases the concentration of total and low-density lipoprotein cholesterol and increases in the concentration of triglycerides and HDL cholesterol in patients with breast cancer of postmenopausal onset. Thus, above finding provides evident of beneficial effect of tamoxifen in coronary heart disease.

CK–MB is one of three separate forms of the enzyme creatinine kinase. CK–MB is found mostly in heart muscle. It rises when there is any disease or damage to heart muscle cells (Mullner et al., 1996). Concentrations of CK-MB have been found to be significantly higher in heart muscle of experimental animals and human myocardium with coronary artery disease, aortic stenosis, or heart failure, compared to normal (Hakan et al., 2002). A number of studies have shown that the concentration of CK-MB is higher in ventricular myocardial tissue in animal models of hypertrophy or

ischemia and in humans with a variety of cardiac conditions, compared to controls or young individuals without cardiac disease (Welsh et al., 2002; Mudersa et al. 2001). In present study, also there is significant increase in serum CK-MB level in hypertrophic rats, and treatment with tamoxifen significantly reduced CK-MB level indicating decrease in myocardial damage as well as reduction in cardiac hypertrophy. Lactate dehydrogenase (LDH) catalyses the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+. It converts pyruvate, the final product of glycolysis to lactate when oxygen is absent or in short supply (Kumar et al, 2003). A significant increase level of LDH is found in case of myocardial damage due to hypertrophy (Peppes et al. 2008; Zhang et al. 1998). In present study, we found significant increase in serum LDH level in both animal models. Treatment with Tamoxifen significantly reduced serum LDH level indicative of reduction in myocardial damage. It has been reported that oxalate-induced PKC activation in LLC-PK_1 cells is responsible for LDH release (Thamilselvan et al., 2009). Thus, tamoxifen being PKC inhibitor, the decrease in LDH levels is justified.

C-reactive protein (CRP) is a sensitive, nonspecific systemic marker of inflammation, elevated serum CRP levels are associated with traditional cardiovascular risk factors and obesity (Buckley et al. 2009). Tissue necrosis is a potent acute-phase stimulus, and following myocardial infarction, there is a major CRP response, the magnitude of which reflects the extent of myocardial necrosis. Furthermore, the peak CRP values at around 48 h after the onset, powerfully predict outcome after myocardial infarction. Importantly, CRP is deposited within all acute myocardial infarcts and compelling experimental evidence now suggests that the CRP response not only reflects tissue damage in this context, but may also contribute significantly to the severity of ischemic myocardial injury (Hirschfield and Pepys, 2003). In present study, we found elevated CRP level in hypertrophic rat while treatment with tamoxifen significantly lower level of CRP in hypertrophic rats. Oral estrogen replacement therapy and combined hormone replacement therapy (HT) are reported to cause a sustained increase in CRP in postmenopausal women which may be due to direct stimulation of synthesis of CRP by estrogen (Vongpatanasin et al., 2003; Montecucco and Mach, 2009). Our study is consistent with report made previously by Bonanni et al (2003).

They have shown that tamoxifen reduced CRP level at lower doses than satandard dose (20mg/day) in healthy hysterectoamized women.

Collagen deposition in the myocardium has two effects on the structure and function of the heart; increased collagen deposition is a prerequisite to prevent dilation of the infarcted area, yet its excessive accumulation in the infarcted and non-infarcted areas of the myocardium leads to tissue stiffness, increasing the incidence of arrhythmias and adverse effects on the elasticity of the myocardium which results in ventricular systolic and diastolic dysfunction (Jack et al., 2002; Lodi et al., 2007). Leenen et al. (2001) have reported increase in total collagen level in LV and RV by isoproterenol through AT_2 receptor stimulation on fibroblast. Also it has been reported that collagen level increases significantly in PAAC induced cardiac hypertrophy (Gautam et al., 2009). In present study, there also found significant increase in LV collagen level in hypertrophic control rats. Treatment with tamoxifen significantly reduced LV collagen content in hypertrophic treated rats. Neugarten et al (2000) has reported inhibition of type I and IV collagen by tamoxifen and other selective estrogen receptor modulators which is mediated through inhibition of COL4A1 gene transcription and type IV collagen protein synthesis inhibition. . εPKC inhibition in hypertension-induced HF leads to the suppression of pathological remodelling such as myocardial fibrosis, vasculopathy and inflammation that correlated with improvement of myocardial function (Palaniyandi et al., 2008). Moreover, hemodynamic overload may stimulate cardiac hypertrophy and induce cardiac injury fibrosis through protein kinase C activation (komuro et al., 1991). So, tamoxifen causes inhibition of protein kinase C, which might be possible mechanism of inhibition of collagen deposition.

Fibrosis is which is caused by the accumulation of extracellular matrix (ECM) proteins, such as collagens plays important role in cardiac remodelling and dysfunction. Cardiac remodelling is generally characterized by changes in gene expression, molecular mechanisms and cellular structures which are clinically evident as a result of alterations in cardiac size, shape and function after pressure overload and volume overload (Babick and Dhalla, 2007). Although accumulation of ECM in heart may be adaptive (physiological) initially, excess accumulation leads to decreases in tissue compliance and function (Sasamura, Shimizu-Murata & Saruta

2005). It has been reported that PKC activation is one of the regulator of isoproterenol induced cardiac hypertrophy (Choudhary et al., 2006). Further, Bayer et al. (2003) and Braun et al. (2002) have reported PKC upregulation in pressure overload cardiac hypertrophy. The Isoproterenol induced cardiac hypertrophy and partial abdominal aortic constriction induced cardiac hypertrophy models employed in the present study have depicted cardiac hypertrophy index, LV hypertrophic index, LV wall thickness, LV protein content suggesting the development of cardiac hypertrophy (Reddy et al. 1996). Treatment with Tamoxifen in treated group significantly reduced cardiac hypertrophy. Protein kinase C is responsible for the hypertrophic gene regulation in cardiac hypertrophy, thus Tamoxifen probably produces attenuates cardiac hypertrophy.

The abdominal aortic constriction may be initially responsible to increase mean arterial blood pressure, which has been observed to return to normal value after about one and a half-hour of partial abdominal aortic constriction. However, the marked increase in mean arterial blood pressure in the partial abdominal aortic constriction model may be due to pathological cardiac hypertrophy (Li et al. 2007). Isoproterenol and partial abdominal aortic constriction-induced increase in mean arterial blood pressure and decrease in heart rate have been noted in the present study. Treatment with tamoxifen did not produce significant change in mean arterial blood pressure and heart rate. Maximal rate of pressure development to the instantaneous pressure were used as indices of systolic function. The left ventricular dysfunction has been associated with decrease in rate of pressure development (dp/dt_{max}) and rate of pressure decay (dp/dt_{min}). Further, various studies have demonstrated that increase in left ventricular collagen content may produce cardiac stiffness and fibrosis resulting that ultimately disrupts coordination of myocardial excitation-contraction coupling in both diastole and systole (Balakumar and Singh, 2006; Chintalgattu et al., 2009). In the present study, the left ventricular dysfunction assessed in terms of dp/dt_{max} and dp/dt_{min}, which was found to be decrease in hypertrophic rats. Treatment with tamoxifen significantly increased dp/dt_{max} and dp/dt_{min}. Further, Chintalgattu et al (2009) reported PKC-δ association in AngII induced ERK activation in fibroblasts. Activation of PKC by phorbol ester in the heart leads to significant loss of contractile function (Buenaventura et al., 1995).

Reactive oxygen species are highly toxic by-products of aerobic metabolism, react unfavourably with adjoining macromolecules resulting in severe cell and tissue damage. ISO causes myocardial ischemia due to excessive production of free radicals resulting from oxidative metabolism of catecholamine (Balakumar and Singh, 2006). Elevation of lipid peroxides in ISO-induced rats could be attributed to the accumulation of lipids in the heart and the irreversible damage to the myocardial membranes. ROS aggravate cardiac hypertrophy in pressure overload induced cardiac hypertrophy (Han et al., 2009; Tsujimoto et al., 2005). In present study, in both models of cardiac hypertrophy, we found increase in oxidative stress, which was evident by increase in MDA level and decrease in total antioxidant level includes reduced glutathione and superoxide dismutase which was measured in heart tissues. Treatment with tamoxifen significantly prevented this oxidative stress by reducing MDA levels and significantly increasing glutathione and superoxide dismutase levels. The study reported by Thangaraju et al. (1994) showed a significantly decreased concentration of malondialdehyde, an end product of lipid peroxidation and remarkably increased levels of enzymic and nonenzymic antioxidants in tamoxifen-treated patients. Also, Inhibition of lipid peroxidation and antioxidant effect of tamoxifen and its metabolites (Wiseman et al., 1990). Tamoxifen enhanced MnSOD expression responsible for its cardioprotective against ADR-induced cardiomyocytes injury (Daosukho et al., 2005). Thus Tamoxifen prevents oxidative stress and thereby preserves cardiovascular function.

7. SUMMARY AND CONCLUSION

In summary, our data suggests that treatment with Tamoxifen,

- Produced significant decrease in serum cholesterol and LDL levels but not alter serum triglyceride and HDL levels.
- Significantly reduced serum cardiac markers viz CK-MB, LDH and significantly reduced inflammatory marker i.e. CRP levels
- Produce significant decrease in hypertrophic parameters like cardiac hypertrophic index, LV hypertrophic index, LVW/RVW ratio, LV wall thickness and cardiomyocyte diameter
- Did not alter blood pressure and increase in heart rate, but significantly increased in rate of pressure development and decay.
- Significantly reduced LV collagen level and significantly increased in LV Na^{+}-K^{+} ATPase activity
- Significantly increased antioxidant enzyme like Superoxide dismutase and reduced glutathione and reduced prooxidant MDA levels in LV.

In conclusion, our data suggests that tamoxifen produces beneficial effects on cardiac hypertrophy as evident specially from reduction in hypertrophic parameters including collagen levels and thereby preserves LV systolic and diastolic dysfunction which may thereby prevent heart failure.

APPENDIX

LIST OF INSTRUMENTS

INSTRUMENTS	SOURCE
UV Spectrophotometer	ELICO SL- 164
Homogeniser	Remi Motors Pvt. Ltd
Centrifuge	Remi Motors Pvt. Ltd.
Electronic Balance	Lab Series C-3, Roy Electronics
pH Meter	LI 127, Elico Ltd.
Micropipette	Accupette
Hot Air Oven	Elico Ltd.
Microtips	Tarsons Ltd.
Aluminium foils	Nice Chemicals Ltd.

LIST OF CHEMICALS

CHEMICAL	SOURCE
Isoproterenol	MP biomedicals ltd
Rat Diet	PranavAgro, Pune
Diagnostic kits	Lab Care Dianostics Pvt. Ltd.
Dithiobisnitro benzoic acid	Sisco Research Lab.

All other chemicals were purchased from CDH Ltd., New Delhi.

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