Thesis entitled

HISTAMINE 2 RECEPTOR ANTAGONISM FOR PREVENTION OF ADVERSE CARDIAC REMODELLING IN CHRONIC PRESSURE OVERLOAD - A NOVEL THERAPEUTIC STRATEGY

Submitted by

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Name of the Guide

Name of the thesis examiner

CERTIFICATE

I**, Ajay Godwin Potnuri,** hereby certify that I had personally carried out the work depicted in the thesis entitled, **"Histamine 2 receptor antagonism for prevention of adverse cardiac remodelling in chronic pressure overload - A novel therapeutic strategy"** under the supervision of **Dr. R Renuka Nair,** except where external help was sought and acknowledged. No part of the thesis has been submitted for the award of any other degree or diploma prior to this date.

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Declaration by Guide

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This is to certify that Ajay Godwin Potnuri, in the Division of Cellular & Molecular Cardiology of this Institute, has fulfilled the requirements of the regulations relating to the nature and prescribed period of research for the PhD degree of the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum. The study entitled **"Histamine 2 receptor antagonism for prevention of adverse cardiac remodelling in chronic pressure overload - A novel therapeutic strategy"** was carried out under my direct supervision. No part of the thesis has been submitted for the award of any other degree or diploma prior to this date. Clearance was obtained from the Institutional Animal Ethics Committee for carrying out the study.

Date **Dr R. Renuka Nair**

CONTENTS

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Ajay Godwin Potnuri

List of Figures

ABBREVIATIONS

A wave	later filling wave
AMP	Adenosine Monophosphate
AMPK	AMP activated protein kinase
Ang II	Angiotensin II
ANOVA	Analysis of Variance
ANP	Atrial Natriuretic Peptide
BNP	Brain Natriuretic Peptide
BSA	Bovine serum albumin
CPCSE	Committee for the Purpose of Control and Supervision of
DBP	Diastolic blood pressure
DNPH	2,4-dinitrophenylhydrazine
E wave	early filling wave
EDV	end diastolic volume
EF	Ejection Fraction
ERK-MAPK	Extracellular signal-regulated kinases- Mitogen activated protein Kinase
ERR	Estrogen-related receptor
ESV	end systolic volume
ET-1	Endothelin 1
FS	Fractional Shortening
GLP-1	Glucagon-like peptide-1
GPCR	G protein coupled receptor
GSK3ß	Glycogen synthase kinase-3 ß

H2	Histamine 2
HCM	Hypertrophic cardiomyopathy
HHD	Hypertensive Heart Disease
HRP	Horse radish peroxidase
Hz	hertz
IGF-1	Insulin like growth factor-1
IVRT	isovolumetric relaxation time
IVSD	Inter ventricular septum thickness during diastole
IVSS	Inter ventricular septum thickness during systole
JNK-MAPK	Jun amino-terminal kinases Mitogen activated protein kinase
LDH	Lactate dehydrogenase
LVDD	Left ventricular internal diameter during diastole
LVH	Left ventricular hypertrophy
LVOT	left ventricular outflow tract
LVSD	Left ventricular internal diameter during systole
MAP	Mean Arterial pressure
MDA	Malonedialdehyde
MHC	Myosin heavy chain
MMPs	Matrix metalloproteinases
NADH+H+	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NFAT	Nuclear factor of activated T cells
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NRRE-1	Nuclear receptor response element-1

PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PI3K	Phosphoinositide 3-kinase
PKA	Protein Kinase A
PKC	Protein Kinase C
PKD	Protein Kinase D
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SDS	Sodium dodecyl sulphate
SHR	Spontaneously hypertensive rat
TBARS	Thiobarbituric acid reactive substances
TCA	Tricloro acetic acid
TEMED	Tetramethylethylenediamine

SYNOPSIS

Hypertensive Heart Disease (HHD) is a collective pathological condition of heart resulting from the uncontrolled blood pressure. Left ventricular Hypertrophy (LVH) is an important feature of HHD and considered to be a risk factor for stroke, myocardial ischemia and sudden cardiac death. It is an adaptive structural change in response to the uncontrolled chronic hypertension where the peripheral resistance escalates than the normal range and heart experiences an excess work load to pump the blood. Cardiac hypertrophy is hallmarked by increase in myocyte volume and conjoint extra cellular matrix deposition resulting in thickened left ventricular (LV) wall. Neovascularisation of this thickened LV wall occurs collectively in order to meet the increased myocardial blood demand. Factors that play a crucial role in initiating cardiac hypertrophy are reno-pulmonary and neuro-hormonal factors that are released in response to hypertension and the resultant oxidative stress.

The antecedent and crucial factors that starts concentrating in the blood stream, in response to the escalated blood pressure, are the catecholamines and the factors of renin-angiotensin – aldosterone system. These factors bind to their respective receptors on the myocardium and initiate the signal transduction pathways. In cardiac hypertrophy, the signal transduction pathways that gets activated by the catecholamines and factors of renin- angiotensin – aldosterone system usually promote the transcriptional level upregulation of the proteins that plays a major role in cytoskeleton mechanical transduction, mitochondrial biogenesis, fatty acid metabolism and calcium trafficking. The most interesting feature of this protein upregulation in cardiac hypertrophy is the reversion to the fetal gene program. The levels of adult isoforms of the chemico-mechanical transducers of motion called as alpha myosin heavy chain gets decreased and the fetal isoform, beta myosin heavy chain takes over. Expression levels of Atrial

Natriuretic Peptide (ANP) and Brain Natriuretic Peptide (BNP) increases and levels of SERCA2A and other proteins gets decreased as a part of fetal gene program.

This phase of cardiac hypertrophy with fetal gene expression and maintained systolic and diastolic function is termed as physiological hypertrophy. Later a stable phase of hypertrophy follows with no observable structural changes occurring with maintained cardiac function. At this point, heart remains functionally stable but, iin course of time can progress to cardiac failure. Even though uncontrolled hypertension stands as an obvious cause, the molecular factors that contribute to the shift of a stable hypertrophy to a pathological hypertrophy remains quite unidentified. Pathological hypertrophy is presented as a diastolic dysfunction followed by systolic impairment.

Apart from the factors discussed above, reports highlight the involvement of other elements such as serotonin, cortisol, aldosterone, endocannabinoids, deoxy corticosterone, osteopoitin and sex hormones in cardiac hypertrophy. Reports suggest that the sister compounds of biogenic monoamine family other than catecholamine and indolamines, do play a crucial role in cardiovascular physiology and pathology. Histamine [2-(4-imidazolyl) ethylamine] is a biogenic monoamine synthesized through decarboxylation of histidine. It is secreted majorly by mast cell degranulation in response to inflammatory incitement and to the minor extent by basophils, platelets, entero chromaffin like cells, endothelial cells, and neurons. Histamine plays a crucial role as a mediator of inflammation, vascular dilator and smooth muscle constrictor. Fascinating findings regarding the role of histamine in controlling gastric acid secretion and its availability in tissues undergoing growth or repair proposes additional physiological roles. Histamine elicits its biological activity through its family of receptors namely H1,

H2, H3 and H4. Myocardium expresses H1 and H2 receptors to a larger extent and H3 and H4 to minor extent. The presence of H1 receptor relatively higher in Reticular activating system of brain stem and peripheral blood vessels than in myocardium making it a poorer cardiovascular pharmacological target. Whereas H2 receptors being distributed largely in gastric and cardiovascular tissues and to a very minor extent in others can be a better pharmacological target for cardiovascular pathologies. The role of H3 and H4 receptors need to be elucidate further.

The presence of high levels of mast cells in the patients with ischemic and dilated cardiomyopathies indicate there involvement in the pathological process. The Levels of Mast cell as well as histamine in failing hearts were found to be higher. Pharmacological blockade of H2 receptor in experimental Myocardial Ischemia reperfusion in dogs was found to be cardio protective. Studies have shown that H2 blockers can protect heart independent of conventional beta blocker therapy indicating the importance of H2 receptor blockade. Retrospective and prospective randomized control trails showed a better clinical outcome in the heart failure patients with H2 Blocker therapy along with the conventional therapy when compared to their controls. Despite of this reports, the actual role of Histamine or the H2 receptor in chronic uncontrolled hypertension induced LVH is unknown and therapeutic importance of targeting Histamine 2 receptor in modulating LVH needs to be investigated. Presuming that Histamine 2 receptor expression is enhanced in conjunction with disease progression it is hypothesized that Histamine 2 receptor antagonism can prevent adverse cardiac remodelling in chronic pressure overload induced cardiac hypertrophy.

The study was designed with following objectives:

- To validate the suitability of the experimental model by screening for the presence of Histamine 2 receptor and myocardial histamine content in spontaneously hypertensive rat (SHR) and in its normotensive Wistar rat (WST)
- To compare the difference in Histamine 2 receptor expression and myocardial histamine content in spontaneously hypertensive rat (SHR) and normotensive Wistar rat (WST)
- Temporal variation in Histamine 2 receptor expression and myocardial histamine content in spontaneously hypertensive rat (SHR) and compare with normotensive Wistar rat (WST)
- To evaluate the effect of Histamine 2 receptor antagonism for prevention of LVH progression in SHR.
- Delineation of the mechanism of H2 receptor stimulation in initiation of cardiac hypertrophy

Spontaneously hypertensive rat (SHR), was used as the experimental model to study the temporal variation in Histamine 2 receptor expression and myocardial histamine content as well as the pharmacological outcome of Histamine 2 receptor antagonism. The SHR is a genetic rodent model of HHD and can mimic the clinical progression of hypertension in humans. Disease progression in SHR can be divided into early development of hypertension followed by a long stable phase of compensated cardiac hypertrophy and slow progression to heart failure. Before initiation of experimental studies, animals were screened for the essential features of cardiac hypertrophy like elevated blood pressure

and thickened myocardium were confirmed non-invasively with “Non-invasive blood pressure monitor” and “2D Echocardiography” respectively.

For validating the suitability of the experimental model 6 month old SHR was employed. For studying the temporal variation Histamine 2 receptor expression and myocardial histamine content, 1 month, 6 months and 12 months old SHR and WST were used (n=6). Histamine 2 receptor expression was quantified immunohistochemically and myocardial histamine content was estimated by O-phthalaldehyde based conjugation method.

Eighteen Adult SHR (6 months old) were randomly assigned into three groups of six rats each and received either distilled water or Famotidine (30mg/kg) (FAM) through oral gavage or Metoprolol (50mg/kg) (MET) through oral gavage for 60 days. They were compared with age and sex matched Wistar rat (WST). Famotidine is an H2 receptor antagonist and Metoprolol tartrate is a standard conventional beta blocker used for management of LVH. Blood pressure was measured non-invasively and 2D echocardiography was performed in both m mode and pulsed wave Doppler mode. Hypertrophy was measured from hypertrophy index [Heart weight / Tibia length ratio (mg: mm)], myocyte cross sectional area, myocardial and serum levels of Brain natriuretic peptide (BNP), and expression of Calcineurin A, myocardial fibrosis, Myocardial Hydroxyproline content, myocardial and serum levels of procollagen type 1 pro fibre levels (PCT1PF). Myocardial malonedialadehyde, Peroxiredoxin 3 Expression and Peroxiredoxin 3 Expression were used as the markers for myocardial oxidative stress. Myocardial stress was estimated by LDH activity. The rate of phosphorylation of Akt was also probed for estimation of adverse cardiac remodelling. Histamine 2 receptor

expression was quantified immunohistochemically and myocardial histamine content was estimated by O-phthalaldehyde based conjugation method. Commercially available ELISA kits were used for estimating BNP and PCT1PF and expression of Calcineurin A, Peroxiredoxin 3 and rate of phosphorylation of Akt were assessed by western blotting.

The difference in Histamine 2 receptor expression and myocardial histamine content in spontaneously hypertensive rat (SHR) and in its normotensive Wistar rat (WST)

The study has confirmed that Histamine 2 receptor expression and myocardial histamine content in 6 month old SHR was significantly higher than that of the age and sex matched Wistar.

Temporal variation in Histamine 2 receptor expression and myocardial histamine content in spontaneously hypertensive rat (SHR) and in its normotensive Wistar rat (WST)

Histamine 2 receptor expression in 1 month, 6 months and 12 months old SHR is significantly higher when compared to their age matched WST controls. There was a temporal increase in Histamine 2 receptor expression in SHR whereas it remained largely unaltered in WST.

The levels of Histamine in 1 month old SHR was found to be comparable with its age matched WST whereas the 6 month old and 12 month old SHR showed a significantly higher level of histamine content. There was an temporal increase in myocardial histamine content in SHR were as it remained unaltered in WST indicating the role of histamine in disease progression.

The effect of Histamine 2 receptor antagonism for prevention of LVH progression in SHR:

Administration of FAM and MET to the 6 month old SHR prevented progression of cardiac remodelling. Systolic and Diastolic blood pressures were normalised with MET treatment were as Systolic blood pressure was unaltered with FAM treatment and diastolic blood pressure was reduced. Both the treatments reduced cardiac hypertrophy as well as the oxidative stress. The diastolic function was improved upon treatments with not much difference in the systolic function. Intra cardiac pressure gradients were also significantly normalized. The effect of FAM on regression of LVH was found to be comparable with MET treatment.

Delineation of the mechanism of H2 receptor stimulation in initiation of cardiac hypertrophy

Exposure of H9c2 Cardiomyoblasts to graded doses of Amthamine (10µM and 5 µM) and 10 µM of Histamine for 42 hours had significantly increased the expression of calcineurin A and also caused an increase in rate of ERK phosphorylation. This effect was found to be dose dependent in nature ruling out the non-specificity. This indicates that H2 receptor stimulation may initiate cardiac hypertrophy by increasing calcineurin expression and by activating AkT and ERK MAPK pathway.

This study highlighted the possible association of Histamine with the progression of LVH in chronic pressure overload induced cardiac remodelling. It also indicated the probable involvement of Histamine 2 receptor by pharmacological blockade studies. Study also showed that stimulation of Histamine 2 receptor can cause dose dependent increase in calcineurin expression and phosphorylation of ERK, MAPK and AKT,

indicating the mechanism by which it can initiate cardiac hypertrophy. The above observations lead to the conclusion that, Histamine 2 receptor antagonism can prevent the progression of LVH in chronic pressure overload independent of beta blocker therapy. Study for the first time showed that H2 receptor antagonism a novel and a viable therapeutic option for the prevention of cardiac remodelling in chronic pressure overload.

I. INTRODUCTION

Hypertensive Heart Disease (HHD) is a collective pathological condition of heart resulting from the uncontrolled blood pressure. Left ventricular Hypertrophy (LVH) an important feature of HHD is a risk factor for stroke, myocardial ischemia and sudden cardiac death. LVH is hallmarked by increase in myocyte volume and simultaneous extra cellular matrix deposition resulting in thickened left ventricular (LV) wall. Neovascularisation occurs collectively in order to meet the increased myocardial blood demand. Catecholamines and the factors of renin- angiotensin – aldosterone system released in response to hypertension play a crucial role in initiating cardiac hypertrophy. These factors bind to their respective receptors on the myocardium and initiate the signal transduction pathways promoting the transcriptional level upregulation of the proteins that plays a major role in cytoskeleton mechanical transduction, mitochondrial biogenesis, fatty acid metabolism and calcium trafficking. The most remarkable feature of this protein upregulation in cardiac hypertrophy is the reversion to the fetal gene program with shift in isoforms of the chemico-mechanical transducers of motion.

In the early stages of LVH systolic and diastolic function are maintained followed by a stable phase of hypertrophy with no observable structural changes and maintained cardiac function. Upon persistent stimuli LVH shifts into a pathological process presented as a diastolic dysfunction followed by systolic impairment. Although the hypertension is considered as an obvious cause, the molecular factors that contribute to the shift of a stable hypertrophy to a pathological hypertrophy remains quite unidentified. Apart from the factors discussed above, reports highlight the involvement of other elements such as serotonin, cortisol, aldosterone, endocannabinoids, deoxy corticosterone, osteopoitin and sex hormones in cardiac hypertrophy. The sister compounds of biogenic monoamine family other than catecholamine and indolamines,

play a crucial role in cardiovascular physiology and pathology. Histamine [2-(4-imidazolyl) ethylamine] is a biogenic monoamine secreted majorly by mast cell degranulation in response to inflammatory incitement plays a crucial role as a mediator of inflammation, vascular dilator and smooth muscle constrictor. Apart from controlling gastric acid secretion histamine an additional role of growth and repair of tissues. Histamine 2 receptors, a subset of histamine receptor family is widely distributed largely in gastric and cardiovascular tissues.

The presence of high levels of mast cells in the patients with ischemic and dilated cardiomyopathies and in failing hearts indicate its role in cardiovascular pathology. Pharmacological blockade of H2 receptor in experimental Myocardial Ischemia reperfusion in dogs was found to be cardio protective independent of conventional beta blocker therapy. Stimulation of H2 receptor is known to exacerbate myocardial ischemia / reperfusion injury in rodent models. Knockout models of H2 receptor showed a slower attenuated rate of progression into failure with lesser degree of apoptosis and fibrosis. The theoretical evidence regarding usage of H2 receptor antagonists in management of cardiovascular diseases was reported by retrospective and prospective randomized study published in early 2006 in lesser number of subjects. In a recent Multi-Ethnic Study of Atherosclerosis (MESA), H2 receptor antagonist usage was found to be associated with less age-related changes in Left ventricular morphology and reduced risk for incident heart failure Despite of this reports, the actual role of Histamine or the H2 receptor in chronic uncontrolled hypertension induced LVH is unknown and therapeutic importance of targeting Histamine 2 receptor in modulating LVH needs to be investigated. Presuming that Histamine 2 receptor expression is enhanced in conjunction with disease

progression it is hypothesized that Histamine 2 receptor antagonism can prevent adverse cardiac remodelling in chronic pressure overload induced cardiac hypertrophy.

The study was designed with following objectives:

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- Delineation of the mechanism of H2 receptor stimulation in initiation of cardiac hypertrophy

Spontaneously hypertensive rat (SHR), was used as the experimental model to study the temporal variation in Histamine 2 receptor expression and myocardial histamine content as well as the pharmacological outcome of Histamine 2 receptor antagonism. Rats in the initial stable phase of compensated cardiac hypertrophy (6 months old) were chosen for pharmacological experimentations with Famotidine (30mg/kg) (FAM) through oral gavage or Metoprolol (50mg/kg) (MET) through oral gavage for 60 days. Whereas 1 month, 6 months and 12 months old SHR were used for analysing temporal variation.

Age and sex matched Wistar served as normotensive control. Animals were screened for essential features of cardiac hypertrophy like elevated blood pressure and thickened myocardium were confirmed non-invasively with “Non-invasive blood pressure monitor” and “2D Echocardiography” respectively. For delineation of the mechanism of H2 receptor stimulation in initiation of cardiac hypertrophy H9c2 cardiomyoblasts were used.

Blood pressure was measured non-invasively and 2D echocardiography was performed in both m mode and pulsed wave Doppler mode. Hypertrophy was measured from hypertrophy index [Heart weight / Tibia length ratio (mg: mm)], myocyte cross sectional area, myocardial and serum levels of Brain natriuretic peptide (BNP), and expression of Calcineurin A, myocardial fibrosis, Myocardial Hydroxyproline content, myocardial and serum levels of procollagen type 1 pro fibre levels (PCT1PF). Myocardial malonedialadehyde, Peroxiredoxin 3 Expression and Peroxiredoxin 3 Expression were used as the markers for myocardial oxidative stress. Myocardial stress was estimated by LDH activity. The rate of phosphorylation of Akt was also probed for estimation of adverse cardiac remodelling. Histamine 2 receptor expression was quantified immunohistochemically and myocardial histamine content was estimated by O-phthalaldehyde based conjugation method. Commercially available ELISA kits were used for estimating BNP and PCT1PF and expression of Calcineurin A, Peroxiredoxin 3 and rate of phosphorylation of Akt were assessed by western blotting.

A brief description of the functional, morphological and molecular changes associated with cardiac hypertrophy is given in the next chapter. Literature on the role of mast cells

and histamine in the cardiovascular diseases is also reviewed. A brief review was done regarding the management of blood pressure and novel therapeutic strategies.

The design of study and experimental methodology are given in the third chapter, in the fourth chapter, the results are presented and the findings discussed in the light of available information.

Salient observations of the study are listed in the fifth chapter. The conclusion and scope for further studies are also given.

The references cited in the text are listed in 'Bibliography'.

II. LITERATURE REVIEW

II.1 CARDIAC HYPERTROPHY

The heart being a mechanical organ capable of auto regulating and fine tuning its pump function to feedback the mechanical, biochemical and xenobiotic stress experienced during developmental, physiological and pathological conditions. During the postnatal growth, heart muscle increases its mass to counterbalance the functional load and aggregating wall stress resulting in cardiac hypertrophy. Cardiac hypertrophy (CH) is defined as the enlargement in the cardiac mass due to an increase in the size of terminally differentiated myocytes.

Even though cardiac hypertrophy is morphologically manifested as increase in the ventricular mass and relative wall thickness, there are several cellular, biochemical and molecular changes occur in course of time. The protein turnover of the ventricle increases in order to meet the necessities of enlarging myocardium. Re-induction of fetal cardiac gene programme is a key feature of cardiac hypertrophy where there will be an isoform shift and upregulation of various fetal genes that can support lesser chronotropy and higher inotrophy (Gupta, 2007). Substrate preference shift from fatty acid to glucose will be initiated(Ritchie and Delbridge, 2006), (Chatham and Young, 2012). Free radical stress an outcome as well as mediator of cellular actions of various cardiac hypertrophic stimuli, works in synergism by promoting myocyte growth and fibroblast proliferation. Extracellular matrix (ECM) deposition by the hyperplastic fibroblasts causes extensive transmural as well as perivascular fibrosis effecting the diastolic function of heart (Burlew and Weber, 2002). Functionally, in the initial stages, hypertrophied myocardium continues to maintain the cardiac output, designated as compensated phase. If the inciting stimuli continues to trigger myocardium, early stages of diastolic dysfunction

sets up followed by systolic dysfunction and global cardiac failure with dilated cardiac phenotype (Rossi and Carillo, 1991). This transition into failure marks cardiac hypertrophy as risk factor for cardiovascular mortality and morbidity.

II.1.1. **Classification of cardiac hypertrophy:**

It can be broadly classified into *pathological* or *physiological* hypertrophies depending on the nature of the stimulus, or else as *concentric* or *eccentric* based and the phenotype. Furthermore, CH is also classified as *left ventricular hypertrophy* (LVH) or *right ventricular Hypertrophy* (RVH) based on the regionalisation of the hypertrophy.

II.1.1.1. **Physiological Hypertrophy:**

Physiological hypertrophy is an adaptive response presented by the heart in order to counter the increasing biological needs. Conditions like chronic exercise or pregnancy leads to a uniform increase in the myocyte mass throughout the left ventricle while preserving the intra cardiac dimensions. This phenotype is achieved by increase in size and width of the myocytes more or less in an equal proportion. Despite of augmented size of the myocardium in physiological hypertrophy, the biochemical and molecular level mechanisms largely remain unaltered with higher rate of metabolism and increased collateral blood supply.

II.1.1.1.1. **Mediators of Physiological Hypertrophy:**

II.1.1.1.1.1 **Role of Insulin Growth Factor Receptor in Physiological Hypertrophy:**

Physiological hypertrophy is majorly mediated by peptide growth factors mediated PI3K/Akt pathway. Insulin growth factor (IGF) the major stimuli for physiological hypertrophy and perinatal cardiac growth, bind to its membrane receptor tyrosine kinases

followed by its dimerization, auto phosphorylation and activation PI3K dependent pathways (Duerr et al., 1995), (Siddle, 2011). Upon activation PI3K recruits Akt to the cell membrane by phosphorylating it at two different sites namely, Ser473 and Thr308 causing it to switch on the mammalian target of rapamycin (mTOR) mediated hypertrophic program (Siddle, 2011). Apart from activating the PI3K/Akt signalling IGFR is also known to activate the GRB2 SOS mediated Ras-Raf dependent ERK MAPK activation resulting in the switching on of hypertrophic gene program(Zhang et al., 2011).

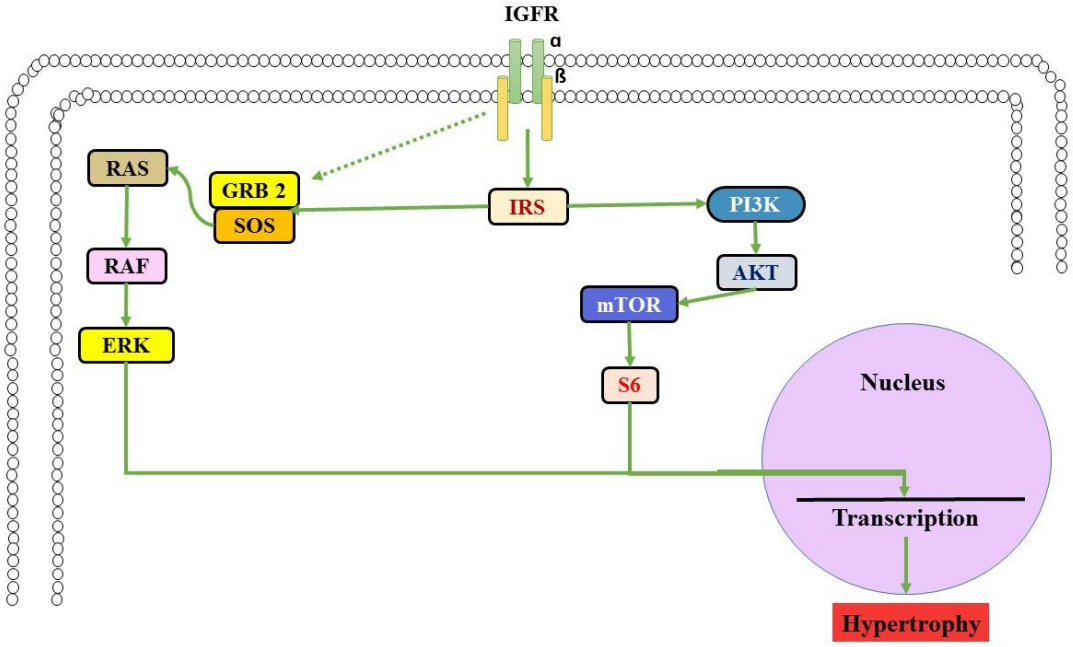

Figure no 1: Molecular mechanisms of Insulin growth factor receptor mediated physiological hypertrophy

II.1.1.1.1.2. **Athletes heart:**

The conventional interpretation of chronic exercised induced cardiac hypertrophy is to be favourable with increased myocardial mass, aerobic metabolism, and diastolic

remodelling, resulting in higher cardiac output. Nevertheless stamina based exercises like marathon running is concomitant with eccentric remodelling and strength based exercises like weight lifting and wrestling results in concentric remodelling. Especially in the case of stamina based exercises, the ventricular geometry resembles the early compensated chronic volume overload cardiac hypertrophy resultant of mitral regurgitation. This kind of hypertrophy is adaptive for long periods of time than the other types of cardiac hypertrophies before shifting into decompensation and failure (Grossman et al., 1975), (de Simone et al., 1987). Stamina based and strength based exercises defer in the type of stress they impose on myocardium, where stamina based endurance exercise causes loss of vascular resistance with intermittent pressure overload state while the other one increases vascular resistant with constant pressure overload state. But studies showed that this difference in duration of pressure overload stimuli cannot make a difference in morphology of the myocardium but can be altered by the nature of stimuli.

Accumulating evidence proves that prolonged exercise conditioning, at least with those activities that include a strength component (i.e., cycling and rowing, which combine strength and endurance), can mimic pathological hypertrophy and potentially lead to sudden cardiac death supported by the evidence that the ventricular dilation in these subjects is not reversed even after 5 years of retrieval from the sports field (Pelliccia et al., 2002). Even though all athletes are not prone to sudden cardiac deaths indicating the genetic susceptibility as the underlying reason for these puzzling problem. In agreement with this, many studies have reported that subjects who has an underlying inheritable genetic problem in the cardiac structural and metabolic genes and undergo endurance

activates are more prone to sudden cardiac death (Tin et al., 2002) (Crawford, 2007), (Myerson et al., 2012).

II.1.1.1.2. **Physio-morphological features and Cardiac hemodynamic of physiological hypertrophy:**

Cardiac hemodynamics of physiological hypertrophy when assessed by the trans-thoracic pulsed wave Doppler two dimensional echocardiography can be clearly distinguished from the normal heart by a shorter and intense aortic jets. Moreover the pressure volume loop analysis of the left ventricle of an exercise induced hypertrophied heart shows a higher stroke volume and shorter iso volumetric relaxation times indicating a healthy cardiac performance. The electrocardiographic analysis of the hypertrophied myocardium indicates slower heart rate with distinct and slightly wider QRS complex. Non symptomatic sinus bradycardia and junctional escape rhythm are quiet common in subjects with physiological hypertrophy.

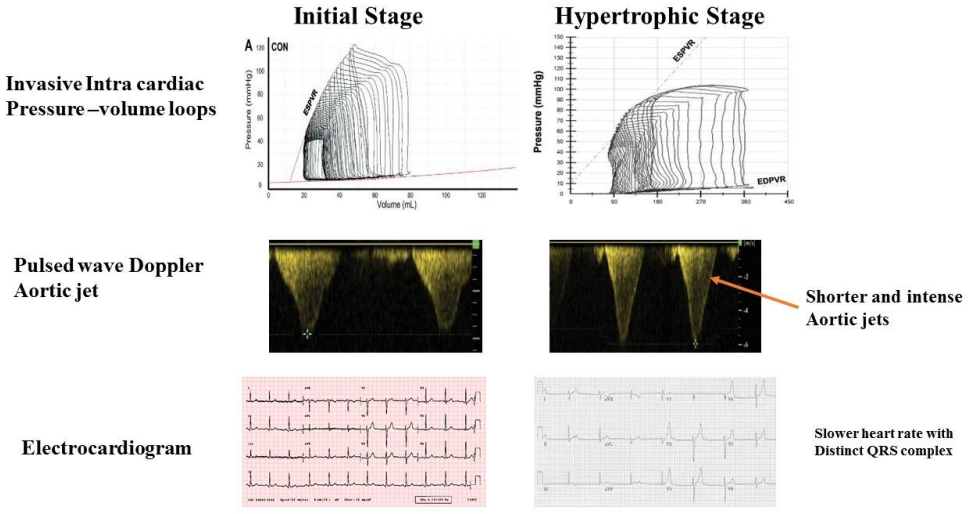

Figure no 2: Physio-morphological features and Cardiac hemodynamic of physiological hypertrophy

II.1.1.2. **Pathological Hypertrophy**:

The pathological hypertrophy a consequence to chronic external hemodynamic overload initiates as an adaptive response and ends in a maladaptive phase. Hemodynamic overloads broadly classified as volume overload and pressure overload are the most common triggers for pathological hypertrophy. Volume overload occurs in response to aortic or mitral regurgitation, valve prolapse and ventricular septal defects causing an increase in preload which results in eccentric cardiac hypertrophy(Mihl et al., 2008). Eccentric cardiac hypertrophy is marked with a dilated phenotype where, the sarcomeres are added in series such that the myocyte length is higher than that of width. In addition to this, the matrix metalloproteases (MMPs) gets activated in order to remodel the ECM to achieve the dilated phenotype(López et al., 2004).

On the other hand, pressure overload, a resultant pathological condition of uncontrolled hypertension or stenotic aortic valve causes increase in afterload which results in concentric cardiac hypertrophy(Rossi and Carillo, 1991). The sarcomeres are added in parallel to each other such that the myocyte width is higher than that of length. Extensive ECM deposition occurs due to hyperplasia of myo-fibroblasts(Conrad et al., 1995). The activity of MMPs in this type of pathological hypertrophy is observed to be either minimal or equal to physiological levels(López et al., 2004), (Díez, 2007).

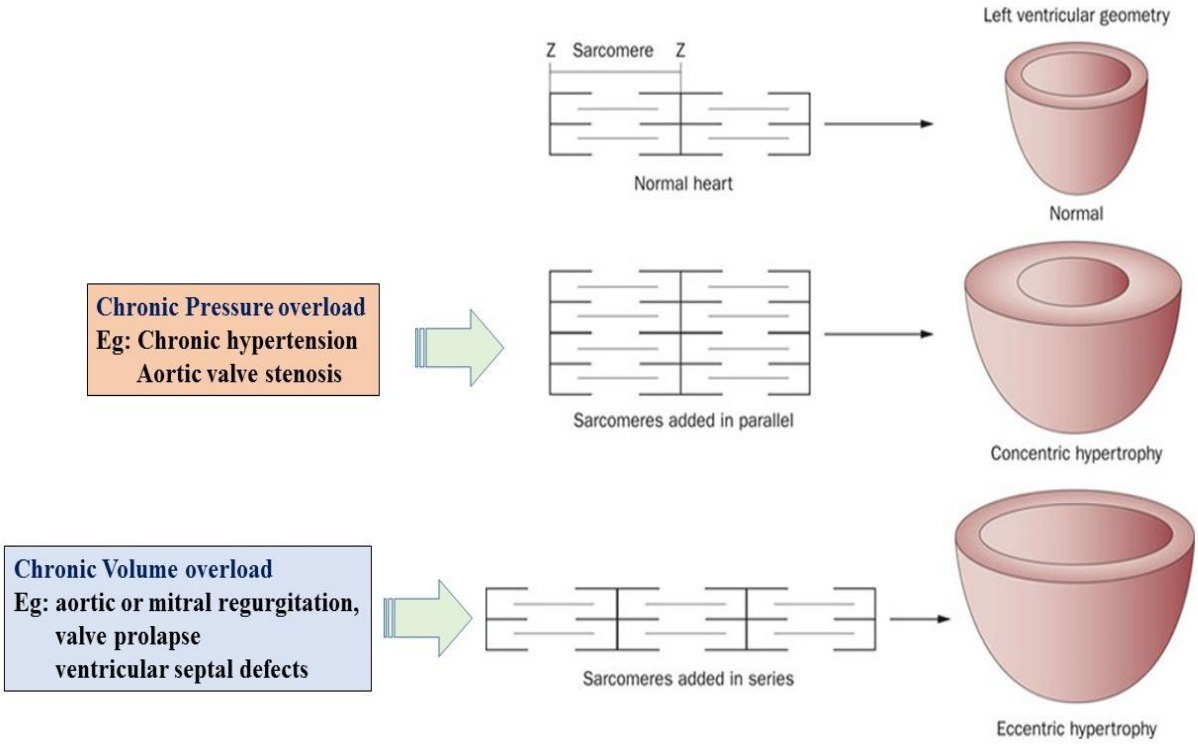

Figure no 3: Classification of cardiac hypertrophy into concentric and eccentric types illustrating the morphological differences between them.

Pathological hypertrophy, especially the pressure overload category, initiates as an adaptive response to the escalating and uncontrolled blood pressure, resulting in the early compensated phase of hypertrophy. Latter this shifts into a decompensated phase were the systolic and diastolic dysfunction sets up resulting in cardiac failure. Uncontrolled chronic hypertension leads to increased afterload in the left ventricle causing left ventricular Hypertrophy (LVH) with largely unaltered right ventricle.

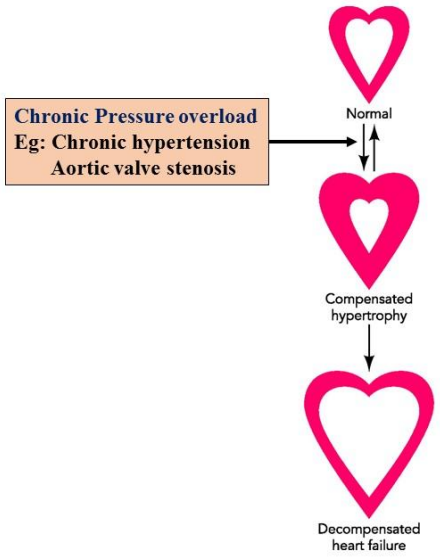

Figure no 4: Progression of pressure overload induced concentric hypertrophy from compensated to decompensated phases.

The left ventricular hypertrophy consequent to hypertension is considered as a risk factor for the myocardial ischemia (Brown et al., 2000), cardiac arrhythmias(Abraham and Hayes, 2003), stroke (Bots et al., 2002) and sudden cardiac death(Tin et al., 2002). Regression of the LVH is reduces the risk of heart failure and ventricular dysfunction independent of the treatment adopted (White et al., 1989), (George et al., 2010).

The significant features of pressure overload induced LVH are the increase in cardiomyocyte size, enhanced protein synthesis turnover, reactivation of fetal cardiac gene programme and reactivation of fetal cardiac metabolism: a shift in substrate preference from fatty acid to glucose. These alterations in myocardium are associated and initiated with an upsurge in reactive oxygen species (ROS) in a direct or indirect manner by instigating wide range of hypertrophic signal transduction pathways (Seddon et al., 2007).

II.1.1.2.1. **Mediators of pressure overload induced pathological hypertrophy:**

In a condition of uncontrolled hypertension, the prolonged wall stress experienced by the myocardium induces activation of various neuro-hormonal systems such as sympathetic nervous system, renin-angiotensin-aldosterone-system (RAAS) and endothelin dependent pathways. The catecholamines, angiotensin II, aldosterone, and endothelin 1 released into circulation in response to pressure overload promotes the development of pathological hypertrophy. Irrespective of the mediators and the signal transduction pathways that gets activated by them, the final molecular event in LVH is reactivation of fetal cardiac gene programme and metabolic shift which leads to myocyte hypertrophy.

II.1.1.2.1.1. **Sympathetic Nervous system and its role in pressure overload induced LVH:**

The Sympathetic Nervous system (SNS) controls the heart rate and contractility, reduces venous capacitance, and constricts resistance vessels. The cardiac SNS fibres are located sub-epicardially from base to apex in a gradient fashion regulates the cardiovascular reflexes (Pierpont et al., 1985). The cardiovascular low-threshold polymodal receptors and peripheral chemoreceptors activate the SNS were as aortic arch and carotid baroreceptors, cardiopulmonary baroreceptors deactivates (Malliani et al., 1983). Activation of SNS results in following cardio-vascular responses

1) positive chronotropy by right stellate ganglionic norepinephrine (NE) releasing neurons
2) Positive inotrophy and positive lusitrophy through left stellate ganglionic norepinephrine (NE) releasing neurons
3) Release of epinephrine (EPI) by the adrenal cortex into circulation
4) Local release of EPI and NE

These transmitters NE and EPI bind to 9 different 7 transmembrane spanning serpentine G protein coupled Adrenergic Receptors (ARs), namely, 3 alpha1-receptors (alpha1A, alpha1B, and alpha1D), 3 alpha2-receptors (alpha2A, alpha2B, and alpha2C), and 3 beta-receptors (beta1, beta2, and beta3). The human heart expresses all three types of beta adrenergic receptors with a predominant beta1and beta2 in a ratio of 70:30 and physiologically in active beta 3 receptor. Stimulation of cardiac beta1and beta2 receptors causes positive inotrophy, chronotropy, lusitropy and dromotropic effects. Being a G protein coupled receptors agonising Beta1-ARs activates Gs proteins and beta2-ARs activates both Gi and Gs proteins (Feldman et al., 2005). Gs signalling upon stimulation activates adenylyl cyclase mediated activation of cAMP-dependent protein kinase A, which in turn activates the L-type calcium channels (LTCC), cyclic nucleotide-gated channels and ryanodine receptors, causing increase in intra cellular calcium levels effecting the pacemaker activity (Ludwig et al., 1998). Increased intra cellular calcium levels causing activation of calmodulin dependent calcineurin pathway resulting in the Nuclear Translocation of Nuclear factor of activated T-cells (NFAT) a pro-hypertrophic transcriptional factor (Molkentin et al., 1998). The Gi signalling inhibits cAMP levels and activates mitogen-activated protein kinase dependent pathways causing activation of various nuclear transcriptional factors.

Myocardium also expresses alpha ARs relatively in a lower levels than the beta-ARs with a largely unknown physiological role. Being distributed densely in the coronaries, alpha ARs, primarily alpha 1 AR are reported to regulate the blood flow by vasoconstriction (Shannon and Chaudhry, 2006). Being coupled to the Gq family of heterotrimeric G proteins, upon agonising, alpha ARs cause activation of Phospho lipase c (PLC) mediated inositol Triphosphate (IP3) – Diacyl Glycerol (DAG) pathways.

Inositol Triphosphate causes spike in intra cellular calcium levels and DAG induces protein kinase C (PKC) and transient receptor potential channels.

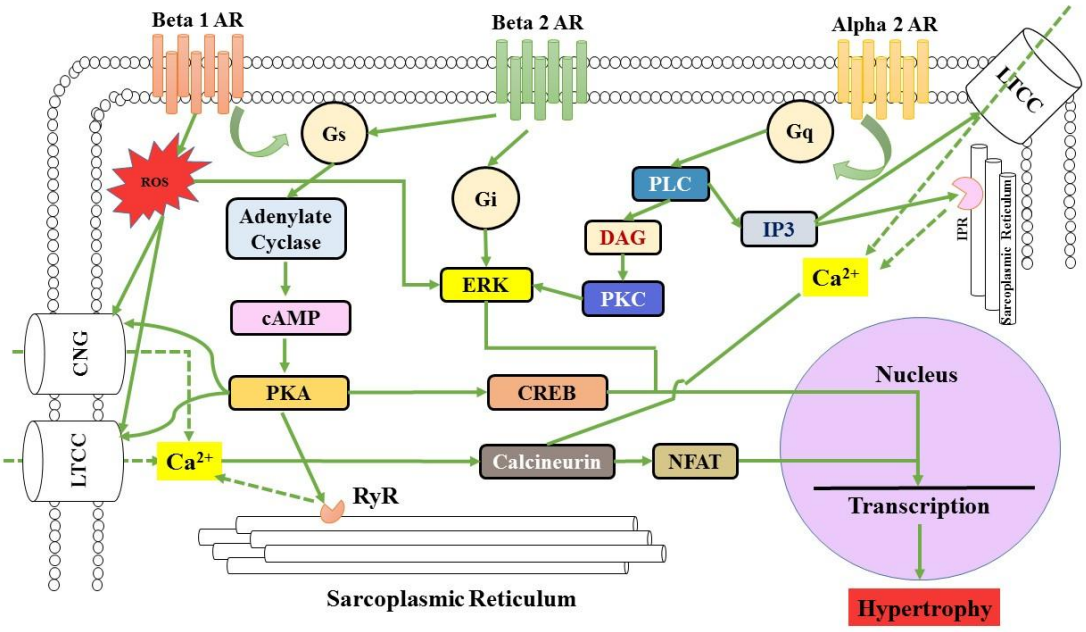

Figure no 5: Molecular mechanisms of adrenergic system induced cardiac hypertrophy

II.1.1.2.1.2. **Renin-Angiotensin-Aldosterone-System and its role in pressure overload induced LVH:**

In response to the increasing peripheral resistance the Renin-Angiotensin-Aldosterone system gets activated and secretes the angiotensin II and aldosterone into the circulation. These mediators bind to their respective receptors and initiate a cascade of signal transduction pathways. Angiotensin II binds to two different sub classes of receptors namely angiotensin II Type 1 receptor (AT1 receptor) and Angiotensin II type 2 receptor (AT2 receptor) out of which AT1 is the widely distributed in myocardium than the AT2 receptor. Being an Gq coupled receptor upon activation, AT1 receptor phosphorylates Phospho Lipase C (PLC) which cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) to

Inositol 1,4,5-trisphosphate (IP3) and Diacyl glycerol (DAG) (Harada et al., 1998). In addition to this, PLC also trans –activate ERK MAPK pathway in both DAG dependent and independent mechanisms. Inositol 1, 4, 5-trisphosphate binds to the IP3 receptor on sarcoplasmic reticulum and triggers stored calcium release by which calcium dependent pathways get activated. Furthermore AT1 receptor stimulates NADPH Oxidase triggering intracellular reactive oxygen species mediated activation of ERK, p38 and JNK MAPKs (Nickenig and Harrison, 2002). Reports even suggest the involvement of PKA mediated hypertrophic signalling by stimulating AT1 receptor in pressure overload induced cardiac hypertrophy (Guo et al., 2001). The involvement of AT1 receptor is further supported by the knock out mice studies where the mice were resistant to development of LVH upon subjection to aortic banding (Harada et al., 1998).

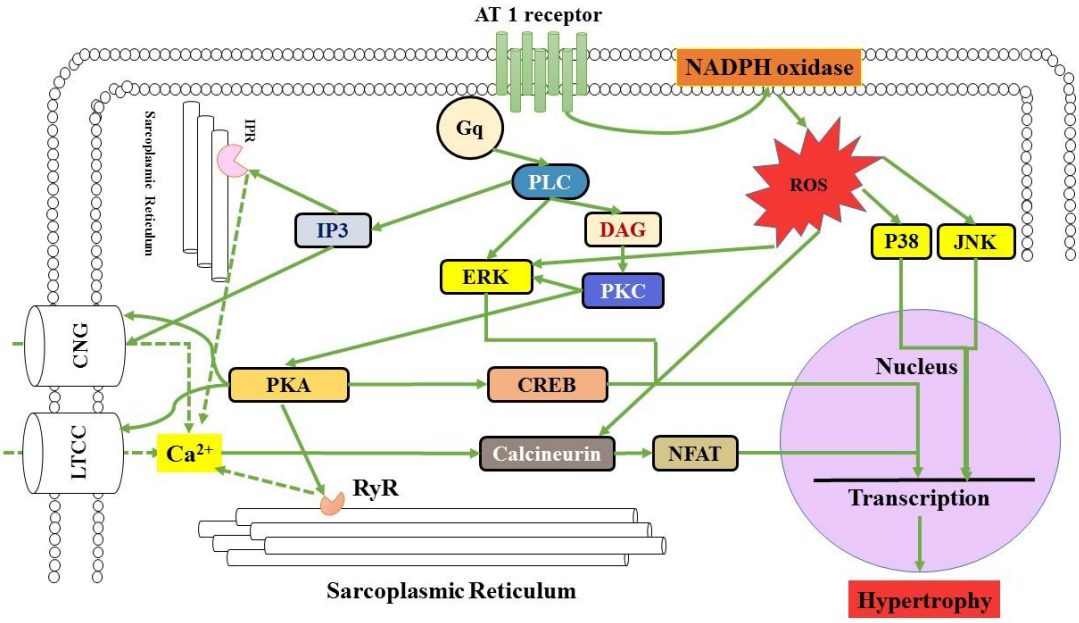

Figure no 6: Molecular mechanisms of angiotensin II induced cardiac hypertrophy

Aldosterone, and component of RAAS pathway also contributes to the development of cardiac hypertrophy. Circulating levels of aldosterone in patients with LVH are higher

when compared to their age and sex matched controls hinting the involvement of this biological factor in pathogenesis of cardiac hypertrophy. Being a mineralocorticoid, aldosterone binds to its intracellular mineralocorticoid receptor and stimulates the PLC mediated IP3-DAG pathways. This pathway leads to rise in Intracellular calcium levels by opening the LTCC and CNG channels as well as by calcium dependent calcium release mechanisms (Porter and Turner, 2009). Aldosterone is also known to trans-activate the Epithelial Growth Factor Receptor (EGFR) by phosphorylating it at Ser234 site which in turn switches on the GRB2-SOS mediated RAS-RAF pathway (Okoshi et al., 2004). This leads to activation of ERK and other MAPKs resulting in the upregulation of the hypertrophic genes. Aldosterone also activates intracellular ROS by which it modulates the activity of MMPs and secretion of collagen by the fibroblasts there by controlling the morphology of the myocardium (Rude et al., 2005). In agreement with these evidences the deficiency of aldosterone in circulation and blockade of mineralocorticoid receptor cardiac hypertrophy indicating its importance in the progression of the disease (Luther et al., 2012).

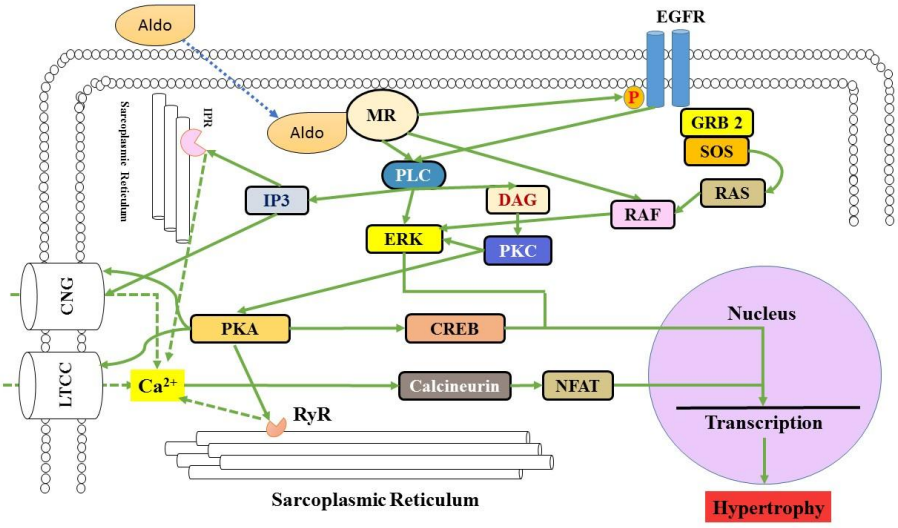

Figure no 7: Molecular mechanisms of aldosterone induced cardiac hypertrophy

II.1.1.2.1.3. **Endothelin Dependent pathways and its role in pressure overload induced LVH:**

Endothelins (ET) are the family of vasoconstrictive peptides released majorly form the endothelium in response to hypotension. There are three isoforms of endothelins identified to be crucial in controlling blood pressure, namely ET1, ET2 and ET3. They bind to the ETA and ETB receptors which are g protein coupled Trans membrane spanning serpentine receptors. These two types of ET receptors are found to be distributed in vasculature as well as on the myocardium (Zolk and Böhm, 2000). The other subtypes of ET receptors namely ETC and ETB2 are strictly restricted to vasculature and known to involve in controlling blood pressure (Lüscher and Barton, 2000). ETA receptors are coupled to Gq alpha sub unit and mediates PLC dependent pathways were as ETB receptor is coupled to Gs alpha sub unit and controls PKA mediated pathways. Blockade of these receptors with their respective blockers prevents progression of LVH indicating their pharmacological importance (Mishima et al., 2000).

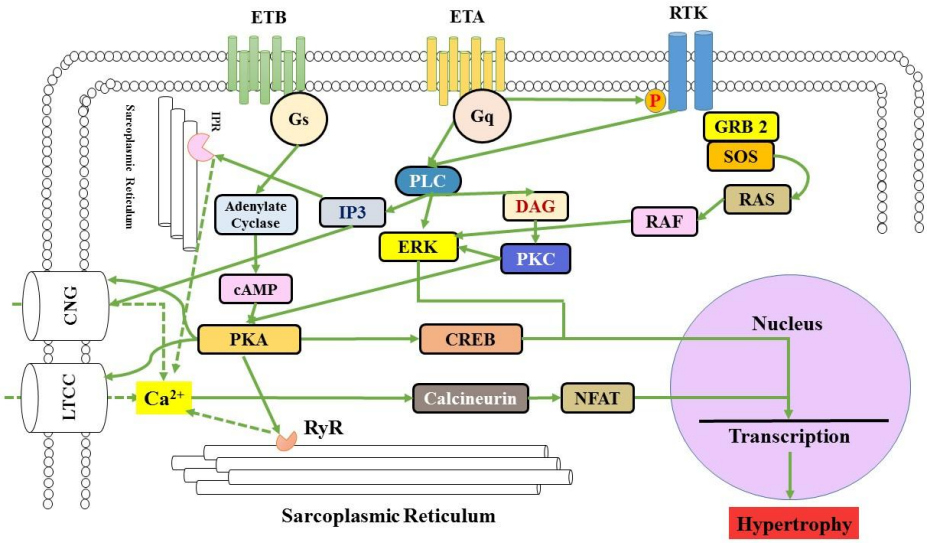

Figure no 8: Molecular mechanisms of Endothelin I induced cardiac hypertrophy

II.1.1.2.2. **Physio-morphological features and Cardiac hemodynamic of Pathological Hypertrophy:**

Cardiac hemodynamic of the pressure overload induced LVH when assessed by the trans-thoracic pulsed wave Doppler two dimensional echocardiography can be clearly distinguished from the normal heart by a larger and intense aortic jets. Moreover the pressure volume loop analysis of the left ventricle of a pressure overload induced hypertrophied heart shows a maintained stroke volume and higher iso volumetric relaxation times indicating an increased LV morphology with diastolic dysfunction. The electrocardiographic analysis of the hypertrophied myocardium indicates normal heart rate with distinct and slightly wider QRS complex. The R waves are usually spiked with the spiked S waves in RV leads. Slightly symptomatic sinus bradycardia and junctional escape rhythm are quiet common in subjects with pressure overload hypertrophy. Heart acendo-cresendo type pan S1-S2 murmur can be observed.

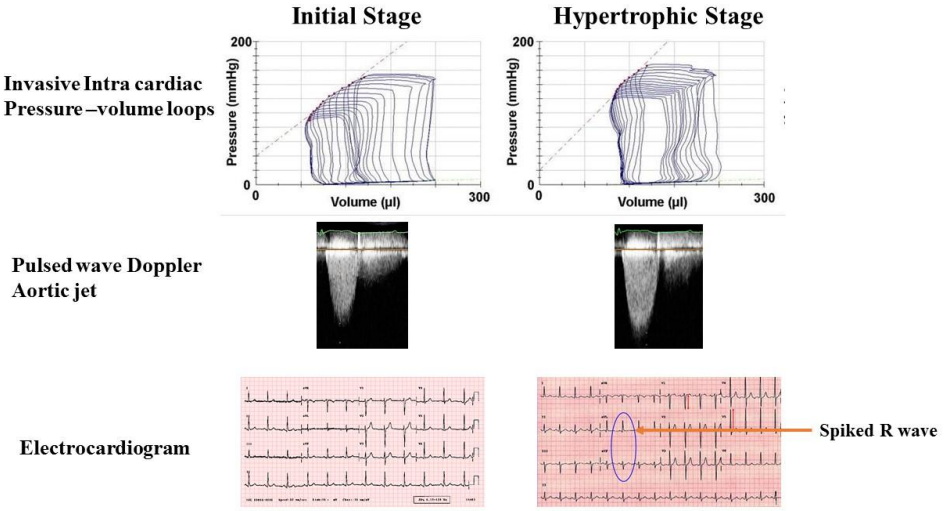

Figure no 9: Physio-morphological features and Cardiac hemodynamic of pressure overload induced LVH

Cardiac hemodynamic of the volume overload induced LVH when assessed by the trans-thoracic pulsed wave Doppler two dimensional echocardiography can be clearly distinguished from the normal heart by a steeper and intense aortic jets with mild to moderate regurgitation. Moreover the pressure volume loop analysis of the left ventricle of a volume overload induced hypertrophied heart shows a lesser stroke volume and lower iso volumetric relaxation times indicating an increased dilated morphology with systolic dysfunction. The electrocardiographic analysis of the hypertrophied myocardium indicates normal heart rate with wider QRS complex and pseudo ischemic pattern. The R waves are usually abnormal with the no or little S waves in RV leads. Slightly symptomatic sinus bradycardia and junctional escape rhythm are quiet common in subjects with volume overload hypertrophy. Heart acendo-cresendo type pan S3-S4 murmur with gallop can be observed.

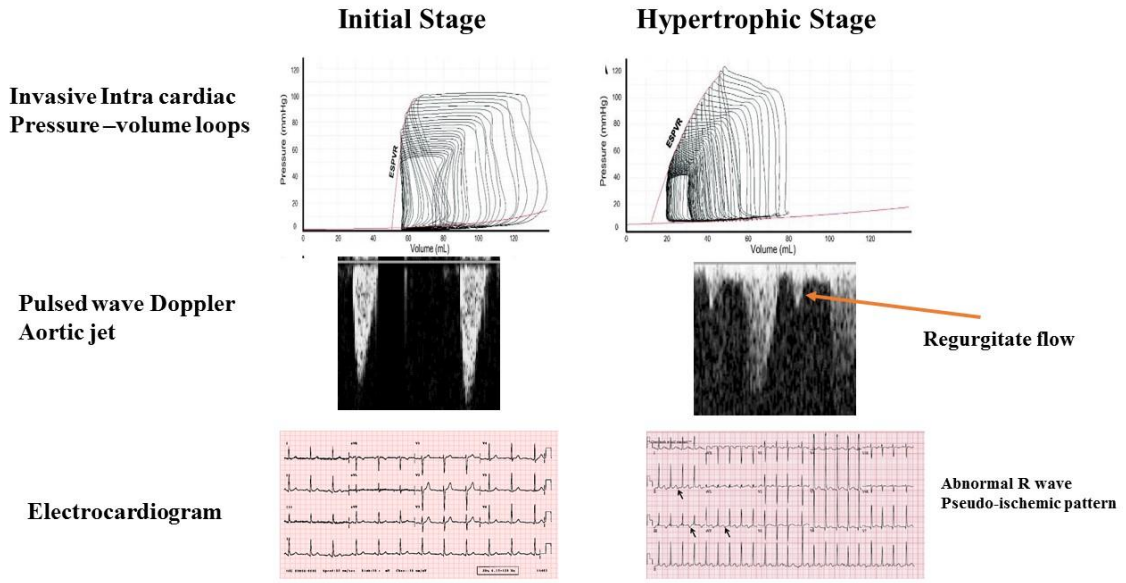

Figure no 10: Physio-morphological features and Cardiac hemodynamic of volume overload induced LVH

II.1.2. **Physiological Hypertrophy Vs Pathological Hypertrophy:**

Being an adaptive modification, irrespective of stimuli cardiac hypertrophy starts as an adaptive and compensated morphological alteration. But the physiological hypertrophy regresses upon withdrawal of the stimulus were as the pathological hypertrophy tends to progress into failure. Fetal gene expression a cardinal sign of pathological hypertrophy is not observed in physiological isotype, where it still runs the myocardium on adult form of proteins. Physiological hypertrophy depends on increased fatty acid oxidation due to PGC 1 Alpha mediated upregulation of mitochondrial biogenesis whereas pathological hypertrophy shifts its substrate preference from lipid to glucose (Riehle et al., 2011),(Pereira et al., 2014). Angiogenesis is another factor that differentiates physiological hypertrophy from pathological hypertrophy (Oka et al., 2014). A well synchronised neovascularisation is observed in the hypertrophied myocardium of exercise subjected rodents whereas the rate of neo vascularisation is far behind the rate of cardiac hypertrophy in pathological forms which is considered to be a major contributor for transition into failures (Shiojima et al., 2005). In addition pathological hypertrophy is accompanied with inflammation and apoptosis which in turn play a crucial role in myocardial remodelling and myocyte slippage (Empel and Windt, 2004). An extensive cardiac fibrosis is seen in pathological form of cardiac hypertrophy with minimal levels of MMP activity in initial stages followed by decreased fibroblast proliferation and increase MMP activity in later stages (Díez, 2007). In case of Physiological hypertrophy, the fibroblast proliferation will be well controlled a synchronous manner to have a well maintained myocardial stiffness a compliance(Porter and Turner, 2009).

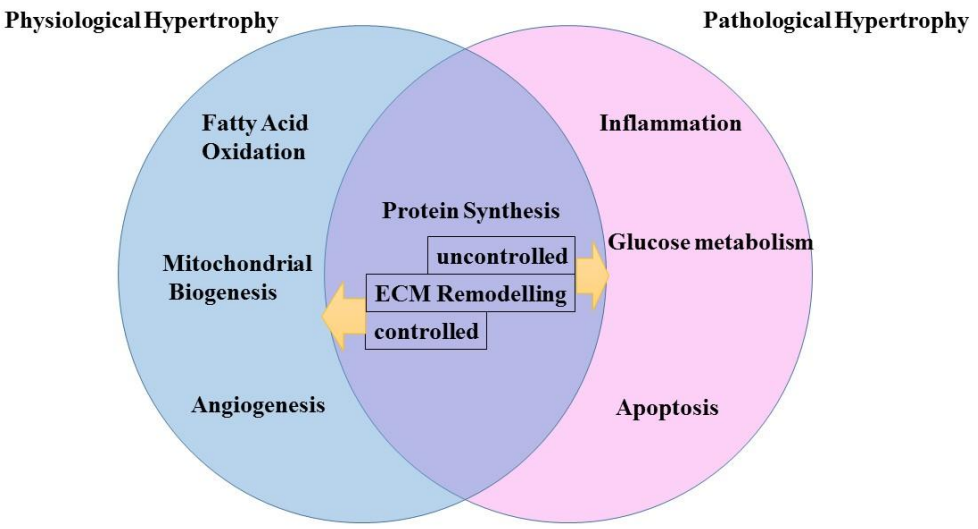

Figure no 11: Similarities and differences between physiological Hypertrophy and pathological Hypertrophy

II.1.3. **Transition from Physiological hypertrophy to Pathological hypertrophy:**

The transition of physiological hypertrophy into pathological hypertrophy is a puzzling process where the mechanisms behind it are poorly understood. In case of hypertensive heart disease, the early stages of LVH is considered to be an adaptive response to the escalating and uncontrolled blood pressure with compensation shifting into a decompensated stage with diastolic dysfunction. Sustained activation of the Akt is known to result in pathological LVH were as the intermittent and recurrent activation of Akt known to produce physiological phenotype of LVH (O'Neill and Abel, 2005), (Nagoshi et al., 2005). But the factor that induce the sustained activation of Akt which causes this shift are to be deciphered.

Additionally the involvement of mitochondrial remodelling as an undercurrent silent mechanism for this shift is being promoted as the major mechanism (Seddon et al., 2007), (Arcaro et al., 2016). Studies have shown that usage of mitochondrial specific antioxidants can prevent the progression of the LVH in rodent models (Q He et al., 2014). Overexpression of the mitochondria specific antioxidants like Peroxiredoxin 3 are known to be cardio protective and prevents the progression of LVH into failure (Matsushima et al., 2006).

Nevertheless, the actual mechanisms behind this shift are poorly understood as the knowledge regarding the mediators participating in the disease process needs to be reviewed and elaborated. Mechanisms by which mediators act on myocardium and there manner of action in consultation with various other factors should be schematically studied to have a better idea of this shift process. Mining the novel mediators for LVH will have a greater impact on this field of cardio vascular pathology and can help us in better management of hypertensive heart disease

II.1.4. **Novel Mediators of Left ventricular hypertrophy:**

Quest for the novel mediators can be advantageous in better management of LVH by finding the pharmacological targets that can prevent the progression of LVH. In recent studies, apart from the factor widely discusses in the sections I.1.3.1.1 many other factor are been found to be implicated actively in the disease progression of LVH.

Serotonin or 5 Hydroxytryptamine, a monoamine neurotransmitter primarily distributed in gastro intestinal tract, blood cells and central nervous system. They mediate their biological actions through 5HT receptors. Studies have found that serotonin mediates initiation and progression of cardiac hypertrophy in alliance with other mediators of

hypertrophy. Serotonin is also known to work in an interdependent manner with angiotensin receptor family in regulating the cardiac hypertrophy (Jaffré et al., 2009). Out the wide range of serotonin receptors, the 5HT2 subtype are well studied and found to be associated with progression of pressure overload induced cardiac hypertrophy. Serotonin binds to the 5HT2 receptors on cardiac fibroblast and induced there proliferation (Shyu, 2009). Pharmacological inhibition of the 5HT2 receptors regressed the pressure overload induced LVH indicating the role of serotonin in pathological remodelling (Jaffré et al., 2004).

Endocannabinoids are bioactive amides, esters, and ethers of long-chain polyunsaturated fatty acids. Endocannabinoid system is sensitive to the mood fluctuations resulting in the decreased circulating levels of endocannabinoids. In conditions like hypertension and chronic psychological stress, the levels of endogenous endocannabinoids decrease to minimal level compared to age and sex matched controls. Recent studies indicate that the endocannabinoid system plays a crucial role in regress of the cardiac hypertrophy. CB1 receptors, a major subset of endocannabinoid receptor family, prevent the cardiac dysfunction (Lu et al., 2014). Being widely expressed on myocardium CB1 receptor agonists can be a potent target for management of cardiac hypertrophy in hypertensive heart disease.

Histone deacetylase (HDAC) tightly control gene expression in the heart. In vitro studies indicates that pharmacological blockade of HDACs associated with suppressing hypertrophy (Ooi et al., 2015). Moreover, several studies showed that increased HDAC activity is linked with the fibroblast proliferation and cardiac fibrosis(Tao et al., 2014).

These findings concoct the concept of HDAC inhibition in management of hypertensive heart disease which needs to be tested in human subjects.

As the inflammation is an integral part of pathological hypertrophy, it creates a fervour to investigate the degree of association of inflammatory mediators in initiation and progression of pressure overload induced LVH. Mast cells are the major contributors of inflammation in neuro muscular system. Upon receiving the pro-inflammatory gestures from the tissues, in the form of cytokines, the resident mast cells gets degranulate and release a complex chemical cocktail of inflammatory mediators. Furthermore circulating cytokines also get recruited to the site of injury setting up further inflammation. Mast cells and their contribution to the cardiovascular pathology is an area that needs further research. This can help to find out novel strategies in managing the pathological remodelling in conjunction with the existing therapies to have a better therapeutic outcome.

II.2 MAST CELLS AND THEIR CONTRIBUTION TO CARDIOVASCULAR PATHOPHYSIOLOGY

Mast cells are derived from bone marrow and are involved in the pathogenesis of many diseases. They are reports indicating the presence of mast cells in the myocardium as well as in the failing hearts (Dvorak, 1986). Nevertheless, their role in cardiovascular diseases has been never gathered an interest notwithstanding the evidences demonstrating their possible contribution. Mast cells are the store houses for major inflammatory mediator, histamine and other proteases. They also release a wide range of cytokines and growth factors that are capable of intermediating tissue remodelling. Factors released from mast cells are can activate MMPs and remodel the collagen matrix

of the myocardium signifying their contribution in conditions such as myocardial infarction and heart failure. Alternatively, they are also been associated in the fibrotic remodelling of the myocardium in myocarditis. The pathologic importance of cardiac mast cells is little is known due to lack of mechanistic studies and studies focusing the activation and degranulation of mast cells.

II.2.1. **Mast cells activation:**

Notwithstanding the evidence of the presence and degranulation of the cardiac mast cells in myocardium in response to external stimuli, the dynamics of mast cell activation needs to be well deciphered. Our understanding about the secretagogue of cardiac mast cells and their impact on myocardium is also limited. Very few factors are known to activate the cardiac mast cells. This must become a central focus for understanding the myocardial remodelling process.

The role of endothelin in the extensive pressure overload induced left ventricular remodelling was discussed in the section II.1.1.2.1.3. Pharmacological antagonism of the ET-1 receptor apart from attenuating the MMP activation and ventricular dilatation, also stabilized the mast cell granulation indicating the role of endothelin in activating the resident mast cells (Fraccarollo et al., 2002). In another experiment, intravenous perfusion of endothelin caused extensive cardiac mast cell degranulation in the myocardium along with higher MMP-2 activity, collagen degradation with moderate dilatation (Murray et al., 2004). This pathological insult was prevented by the mast cell stabilizer, nedocromil (Murray et al., 2004). Endothelin is known to involve in mast cell maturation through ET-1 signalling supported by the studies with non-selective

endothelin receptor antagonist, bosentan which decreased the density of myocardial mast cells (Murray et al., 2008).

Reactive oxygen species underlie majority of disease either being a cause or consequence of the disease. Unexpectedly, the role of ROS in activation of mast cells is not clearly investigated. Studies in dogs showed that pollution can trigger mast cell recruitment into the hearts and can induce the degranulation with further accumulation of mononuclear cells, hinting the involvement of ROS in mast cell activation (Calderón-Garcidueñas et al., 2001). This hypothesis is further supported by invitro studies with mast cells incubated with Na2SO3 a potent pro oxidant resulting in a concentration-dependent histamine release confirming the role of ROS in mast cell degranulation (Meléndez et al., 2010). This pleiotropic effect of ROS on mast cells was prevented by anti-oxidants like ebselen and dyphenyleneiodinium. Interestingly mast cell degranulation releases TNF alpha which in turn causes spike in ROS during pre-conditioning of the ischemic heart which can be prevented by the superoxide dismutase mimetic, M40403 administration(Gilles et al., 2003), (Masini et al., 2002)..

Another reported recruiter of cardiac mast cell are neuropeptides, the important components of nervous system. The spatial arrangement of cardiac mast cells in a close proximity to the nerve endings provides a basic clue regarding the role of neuropeptides in mast cell activation (Silver et al., 2004). Neuropeptide are known to regulate vasculature and blood pressure with no information regarding their role in myocardial remodelling. Substance P is a tachykinin based neuropeptide, released from the afferent sympathetic fibres act on neurokinin receptors. Being a potent regulator of inflammation, it has been shown to induce degranulation of non-cardiac mast cells (Heaney et al.,

1995), (Guhl et al., 2005). Cardiac mast cells were initially thought to be relatively unresponsive to substance P, however, recent studies identified the artefacts in mast cell isolation which produced false negative results and confirmed the association of cardiac mast cells with substance P (Mihaylova et al., 2008). Neurotensin, similar to substance P, invoke non-cardiac mast cell degranulation (Zhao and Pothoulakis, 2006). Infusing Neurotensin to isolated hearts causes transient, release of histamine which can be prevented by compound 48/80, mast cell secretagogue and NT antagonist(Pang et al., 1998).

Cytokines which are released as a part of inflammation play a crucial role in cardiac pathophysiology. IL-33 a member of the IL-1 family binds to ST2 receptor and regulate the mast cell function to a level equal to that of the mast cell secretagogue, compound 48/80 (Saluja et al., 2015).

II.2.2. **Gender differences in Mast cells:**

The incidence of gender dissimilarities in cardiovascular disease are commonly observed in clinical studies. Women are less prone to cardiovascular diseases in their premenopausal state then compared to their age matched men. Factor that attribute the cardio protection other than the sex hormones can provide and answer to this disparity. Information regarding the differences in the density and activity of cardiac mast cells is extremely limited. However, in ovariectomized female rats received the mast cell secretagogue, compound 48/80, the hearts exhibited increased MMP-2 activity and reduced collagen volume fraction with moderate degree of ventricular dilatation in comparison with their age matched sham operated females (Chancey et al., 2005). Administration of oestrogen to ovariectomized female rats was able to reverse these

changes indicating the control of oestrogen over release of TNF alpha and other proteases(Kim et al., 2001). These findings could provide sufficient evidence regarding the existence of gender dissimilarities in myocardial mast cell population. Furthermore, that the oestrogen has a mast cell stabilising property which could be a key mechanisms behind its cardio protective action.

II.2.3. **Mast cells in hypertension:**

The presence of mast cells in the left ventricle of spontaneously hypertensive rats in correlation with collagen volume fraction was the first report establishing the relationship between mast cells and hypertension (Panizo et al., 1995). Isolated heart studies in 12 month old SHR showed that the cardiac mast cells induce NF-κB and IL-6 expression in the left ventricle. However, these studies could only able to establish a fundamental relationship between cardiac mast cells and cardiac fibrosis in the hypertensive heart without interpreting the molecular mechanism behind. Stabilizing the mast cells with nedocromil in SHR was to normalize cardiac fibrosis by preventing macrophage recruitment and attenuating the pro-inflammatory cytokines (Levick et al., 2009). Adult cardiac fibroblasts when exposed to tryptase proliferate and produce collagen further supporting the above findings. In inference, cardiac mast cells mediate cardiac fibrosis through inflammatory cell recruitment, cytokines and direct effects on fibroblasts. Despite of these reports regarding the relationship between mast cells and cardiac fibrosis, very little is known regarding their influence on cardiac myocytes.

II.3 HISTAMINE AND ITS ROLE IN CARDIAC BIOLOGY:

Pharmacological studies have provided convincing confirmations that histamine can be released in the mammalian heart by both immunological and non-immunological

mechanisms (Tucker et al., 1975). Infusion studies with histamine in dogs produced dilation of small resistance peripheral arterioles and a secondary hypotension associated with a constriction of the large capacitance arteries and veins which was compensated by amplified baroreflex mediated adrenergic activity upon long term administration (Vigorito et al., 1987). This effect observed in dogs was contradicted by the contrary observations in rodents and canines indicating the species variability and infusion rate (Haddy, 1960). Histamine exercises vasoactive effects on almost every vasculature in the body. But the physiological outcome would vary by type and location of blood vessel and determines by the route and the dose of histamine administered. In coronary arteries, histamine induces dilation and consequent increase in coronary blood flow as observed in dogs with a contrary and conflicting reports in rat and rabbits with decreased coronary blood flow.

Histamine augments the inotrophy, chronotropy and negative dromotropic effects with a mild to severe ventricular arrhythmias (Wolff and Levi, 1986). Yet again, these effects of histamine on the myocardium is strictly controlled by the dose and rate of histamine infusion, species and presence of anaesthesia. The effects of histamine at least in part, show a qualitative similarity to that of the effects produced by catecholamine. Though, cardiac stimulation induced by histamine remains unchanged with beta adrenergic receptor blockade indicating the independency of histaminic pathway from that of adrenergic mechanisms (Schellenberg et al., 1991). The differences in the sustainability of the cardiac responses between these two important mediators was proposed to be dependable on their ability to activate slow calcium channel in myocardial cells.

The arrhythmogenic action of histamine in isolated heart preparations is a major concern as it may be fatal in nature. Many hypotheses were postulated to explain the mechanism behind this idiosyncratic action of histamine. An increase in the slope of phase 4 spontaneous depolarization in the specialized cells of the sinoatrial node, atrium and ventricle probably as a result of altered permeability to potassium and calcium ions was believed to be the major mechanism behind histamine induced arrthymias (Baller and Huchzermeyer, 1989). This arrthythmogenic effect of histamine is amusingly not seen upon intravenous infusion or intracoronary infusion of histamine, but can be seen in cases where local histamine is released in response to anaphylactic shock or forceful inotrophy (Wolff and Levi, 1986). This highlights the probable involvement of histamine in sudden cardiac deaths which are majorly produced due to idiosyncratic ventricular arrhythmias.

II.4 HISTAMINE 2 RECEPTOR AND ITS IMPORTANCE IN CARDIOVASCULAR BIOLOGY:

The miscellany of histamine receptors was first discovered by the depressor studies in dogs where diphenhydramine could able to block the responses produced by moderate doses of histamine and could not block responses to larger doses of histamine suggesting that histamine might produce responses by interaction with minimum of two receptor sites (Black et al., 2010). These experiments provided the elemental evidence that two histamine receptors are present in the peripheral circulation. Confirming the classification of histamine receptors, studies on the histamine using isolated cardiac preparations of the guinea-pig showed that histamine increases the sinoatrial rate of not antagonized diphenhydramine. Latter the advent of histamine H2 receptor antagonists

further more justified the fact that a well-diversified histamine receptors exists. Studies found that the cardiovascular system expresses both types of histamine receptors, H1 and H2 receptors (Matsuda et al., 2004). The positive chronotropic effect and vasoconstrictor effect of histamine are mediated through the H2 receptor. The arrhythmogenic action of histamine in isolated heart preparations is also mediated by the H2 receptor subtype independent of beta adrenergic blockade (Schellenberg et al., 1991). These finding indicate the possible involvement of H2 receptor in the histamine mediated cardiovascular responses.

II.4.1. **Histamine 2 receptor biology: Its distribution, structure and signalling mechanisms:**

Development of highly selective H2 receptor agonists and antagonists has significantly enabled to characterize biological actions controlled by this amine receptor. Physiological actions controlled by H2 receptor include relaxation of airway, vascular smooth muscle contraction, chronotropic and inotropic regulation of heart, inhibition of basophil chemotactic responsiveness, inhibition of immunocyte proliferation and differentiation of promyelocytic leukemic cells (Parsons and Ganellin, 2006). Apart from these the crucial physiological role played by H2 receptor in the gastrointestinal tract is regulation of gastric acid secretion. This significant discovery made the H2 receptor antagonists to be used in the management of peptic ulcers and gastro oesophageal reflex disorder. Broadly the H2 receptor is found to be distributed in the Gastric parietal cells (oxyntic cells), Vascular smooth muscle, Neutrophils, Central nervous system, ventricles of heart and uterus (Hill et al., 1997).

In the face of these important interpretations, very little was known regarding the structure of this biogenic amine receptor sue to severe difficulties in purifying the receptor protein. However, the presumed structure of this receptor was recently achieved through molecular cloning techniques. Interestingly, two decades after the development of therapeutic antagonists of H2 receptor, the encoding gene was cloned in early 1990s helping the investigators to have a better understanding about this receptor(Gantz et al., 1991). A single genomic clone of H2 receptor contains 1,080-base pair open reading frame and a deduced amino acid sequence of 360 amino acids, conserved amino acid sequence within the NH2 terminus and COOH terminus in the third intracytoplasmic loop identical to that of the b2adrenergic receptor where they serves as the binding sites for the stimulatory G protein. These indications led to the discovery of the Gs coupling to H2 receptor and histamine mediated increase in the intracellular cAMP levels.

Binding of the agonists and antagonists to the H2 receptor was also well described by wang et al using x ray crystallography (Wang et al., 1998). They proposed that the Asp residue in the putative third-transmembrane domain (Asp98) binds with the positively charged NH^{3+} group of the ethyl ammonium side chain of histamine and the imidazole nitrogen atoms of histamine form hydrogen bonds with the Asp186 and Thr190 residues of the fifth-transmembrane domain of the H2 receptor. More over the ionization states of Asp186 and Thr190 are unidentified and assumed to serve as a proton donor or acceptor.

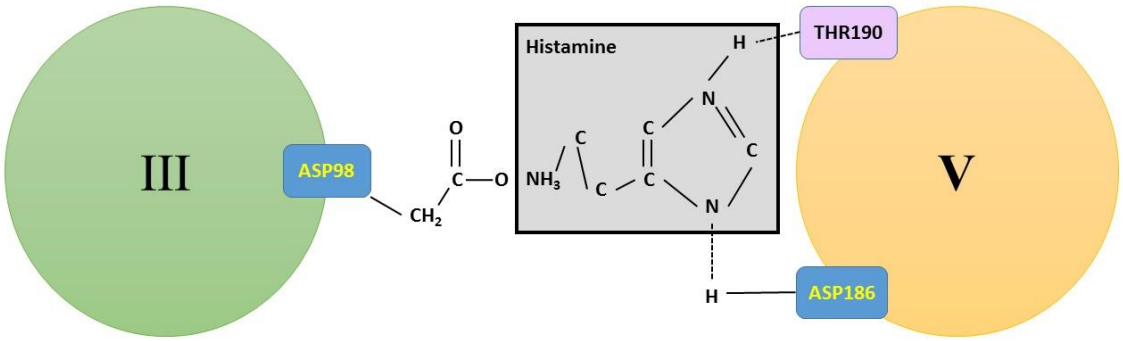

Figure no 12: Binding of Histamine to the III and V transmembrane domains of Histamine 2 receptor.

The non-pathological and physiological outcome of histamine in the biological systems is in general indorsed to the H2 receptor. Ligand mediated activation of H2 receptor causes guanine nucleotide-dependent, adenylate cyclase facilitated cAMP generation in wide range of mammalian tissues including cardiac myocytes and vascular smooth muscles (Hill, 1990). In addition to this, histamine H2 receptor controls mast cell degranulation by fuelling the phospholipid methylation (Tolone et al., 1982), upsurges the slow inward Ca^{2+} current in ventricular myocytes (Hill, 1990), inhibits the Cl^{2-} arbitrated K^{1+} conductance in hippocampus (Haas and Greene, 1986) and inhibition of phospholipase A2 (Hogan et al., 1995).

Though the results from preliminary experiments with cloned H2 receptor proposed the possible and independent coupling with both adenylate cyclase and phosphoinositide systems, the mechanism by which this receptor shows divergence in the signalling

pathways is not clear understood. Though discrepant, the studies aimed to illustrate the G proteins accountable for this rare phenomenon of dual signalling, splintered the riddle to some extent. Studies with pertussis toxin showed that the H2 receptor mediated cAMP generation and membrane phosphoinositide turnover were impervious ruling out the possible involvement of the members of the Gi/Go family of GTP-binding proteins (Wang et al., 1998). In disparity, the treatment with cholera toxin attenuated the H2 receptor arbitrated inositol phospholipid turnover and $[Ca^{2+}]_{I,}$ independent of adenylate cyclase activity (Delvalle et al., 1992). After a successions of experiments using immunoneutralisation techniques, the most probable candidate G proteins intricate with H2 receptor signalling (Gs and Gq) were discovered (Wellner-Kienitz et al., 2003). The histamine mediated stimulation of phospholipase C (PLC) is sensitive to cholera toxin, insensitive to pertussis toxin and dependent on GTP indicating that the H2 receptor activates adenylate cyclase and phosphoinositide signalling via separate GTP-dependent mechanisms. In Baculovirus-infected Sf9 cells and COS cells overexpressing H2 receptor, it was confirmed that the PLC activity was boosted with co-transfection with DNAs corresponding to a_q, a_{11}, a_{12} and a_{14} (Wellner-Kienitz et al., 2003). Though these findings compel one to believe the involvement of Gq mediated PLC activation, the sensitivity of the same signalling to cholera toxin remains puzzling and unclear.

Numerous central issues concerning the H2 receptor dual signalling continue to be uncertain as the structural conformations of the receptor vital in reigning the dual signalling are not fully interpreted. In this scenario, studies with synthetic receptor and b2-adrenergic/H2 chimeric receptors showed that COOH terminal tail plays a major role in signal transduction as it coordinates with the second and third intracellular loops in order to bind with separate G proteins (Wang et al., 1998), (Wang et al., 2000).

Interestingly the physiological importance of this dual signalling is unknown and is being proposed as a vital component of feedback mechanism and receptor desensitization.

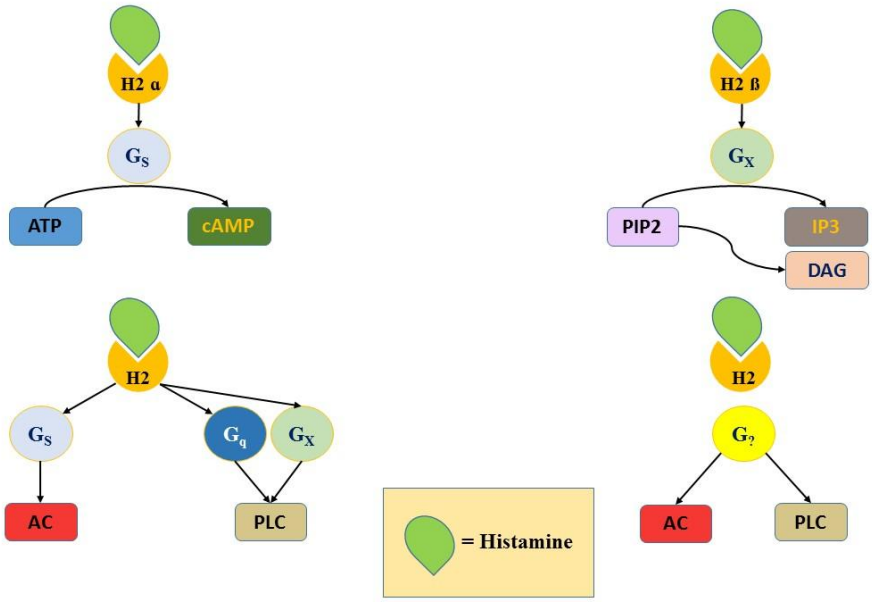

Figure no 13: Proposed mechanisms of H2 receptor mediated signal transduction pathways.

II.4.2. **Histamine 2 receptor and its role in cell growth:**

Although the H2 receptor arbitrated control over the cellular events has always been credited to adenylate cyclase system, the concurrent coupling of this receptor to the phosphoinositide system complements an additional and possible pathway by which some of the crucial biological actions are achieved. This rises an interesting question about the prospective role for PIP2 cascade in H2 receptor signalling as it can help us to find out the possible involvement of this receptor in cellular hypertrophy and hyperplasia (Seuwen and Pouysségur, 1992). Supporting this hypothesis, blocking H2 receptor with

ranitidine in human gastric carcinoma cell line MKN-45 prevented melanoma progression indicating that H2 receptor can play a crucial role in hyperplasia (Arima et al., 1991). Activation of H2 receptor was found to induce differentiation in human HL-60 promyelocytes indicating that the H2 receptor is involved in cellular growth and differentiation (Seifert et al., 1992). Further confirming this, H2 receptor over expressing HEK cell showed a higher degree of cellular growth and protein turn over upon exposure to histamine (Wang et al., 1997). Additionally, H2 receptor stimulation caused induction of the proto-oncogene c-fos independent of cAMP but sensitive to PKC activity. These findings conclude that histamine mediates cellular hypertrophy and hyperplasia via PKC mediated pathways.

II.4.3. **Histamine 2 receptor and inverse agonism:**

Recently certain GPCR model systems proved the existence of agonist independent basal activity which can be inhibited by certain antagonists but not others (Milligan et al., 1995). In receptor pharmacology, this phenomenon exhibited by a subset of GPCRs, is termed as inverse agonism, where the supposed to be an antagonist bind to the receptor and induces negative intrinsic activity exactly opposite to that of agonist. Based on this concept antagonist were reviewed and classified as inverse agonists and true antagonists. Certain universally used H2 antagonists like cimetidine and ranitidine, exhibit inverse agonism whereas the not observed with the prototype burimamide (Smit et al., 1996). In contradiction to the fundament concept of receptor biology that upon sustained activation a receptor gets downregulated, it has been observed that H2 receptor level inverse agonism caused an upregulation. The clinical implication of this phenomenal behaviour of H2 receptors needs further experimentation and translational testing.

II.4.4. **Studies on the role of Histamine 2 receptor in cardiovascular pathology:**

The role of mast cells in cardiovascular diseases is well supported by the presence of mast cells in the myocardium and increased levels of histamine in failing hearts (Panizo et al., 1995). Activation of H2 receptor is known to exacerbate myocardial ischemia / reperfusion injury in rodent models (Luo et al., 2013). This cardio protective effect of H_2 receptor blocker was reproduced in rapid ventricular pacing induced heart failure in dogs and found to be independent of β-blocker therapy (Takahama et al., 2010). Interference of H2 receptor with knockout techniques showed a slower rate of cardiac failure progression by plummeting the myocardial apoptosis and fibrosis (Zeng et al., 2014). Contradicting these findings, Histamine deficiency worsens myocardial infarction by compromising macrophage infiltration and augmenting the cardiomyocyte apoptosis cautioning the vulnerability concomitant with the usage of mast cell stabilizers in the management of acute myocardial infarction (Deng et al., 2015).

The first evidence on the helpfulness of H2 receptor antagonists in management of cardiovascular diseases was reported by retrospective and prospective randomized study published in early 2006 in lesser number of subjects (Kim et al., 2006). It found that inclusion of H2 receptor antagonists into the conventional therapy for congestive heart failure can have a better clinical outcomes when compared to their control. In a Multi-Ethnic Study of Atherosclerosis without clinical cardiovascular disease, H2 receptor antagonist usage was found to be associated with less age-related changes in Left ventricular morphology and reduced risk for incident heart failure (Leary et al., 2016).

The same study also reported the probable association between H2 receptor blockers and lesser RV mass and reduced RV end-diastolic volume (Leary et al., 2014).

II.4.5. **Histamine 2 receptor antagonists:**

The H2-histamine receptor blockers are widely used, and are available over the counter, that block the action of histamine at the H2 receptors of the parietal cells in the stomach there by declining the synthesis of gastric acid. They serve as a drug of choice for the treatment of dyspepsia and gastroesophageal reflux disease. Some H2 antagonists are inverse agonists rather than true antagonists as they block the constitutive activity of these receptors.

The first development was Nα-guanylyl histamine, a partial H2-receptor antagonist, which ultimately led to the development of burimamide, true H2 receptor antagonist, more potent than Nα-guanylhistamine. Nevertheless burimamide couldn't be marketed due to its poor bioavailability and severe GI side effects. Following this breakthrough, cimetidine, the archetypal H2 antagonist was developed by Sir James Black at Smith, Kline & French. The sister compounds were quickly developed by substituting the imidazole-ring of cimetidine with a furan-ring resulting in ranitidine, with lesser adverse effects, drug interactions and higher potency. This wonder drug was announced in 1981 and became the world's leading drug by 1988. Metiamide was an effective thiourea based H2 blocker associated with undesirable nephrotoxicity and agranulocytosis.

H2-antagonists are prescribed for the treatment of acid related gastrointestinal conditions, including; Peptic ulcer disease (PUD), Gastroesophageal reflux disease (GERD/GORD), Dyspepsia, Prevention of stress ulcer (a specific indication of ranitidine). Even though taken in combination with antacids, the H2-antagonists are

advantageous for having a sustained duration of action, superior efficacy, and ability to be used prophylactically ante cibum.

With cimetidine as an exception, H2 antagonists are generally well-tolerated with lesser adverse drug reactions (ADRs). Hypotension, headache, fatigue, diarrhoea, constipation, may be rarely presented in few individuals. The incidence of gynecomastia which was high with cimetidine occurs in 0.1% to 0.5 % of subjects with other H2 receptor antagonists. Cimetidine may also cause reversible hypo secretion associated dyspepsia, loss of libido, and impotency (Biron, 1979). Cimetidine exhibit a wide range of drug-drug and food-drug interactions. Whereas the novel H2-receptor antagonists are less probable to interfere with CYP metabolism. Ranitidine and famotidine have a feeble and insignificant CYP inhibitor activity than cimetidine.

II.5. MANAGEMENT OF PRESSURE OVERLOAD INDUCED LEFT VENTRICULAR HYPERTROPHY:

Management of pressure overload induced left ventricular hypertrophy depends on the primary cause. Correspondingly, controlling the elevated blood pressure significantly diminishes the expansion of LVH and its progression into failure. The incidence of LVH in the 48,000 hypertensive patients receiving standard antihypertensive therapy, decreased to 52% as evident from the meta-analysis of crucial hypertension trials over the past 20-year period (Moser and Frishman, 1998). Anti-hypertensives agents are used the set of drugs used to treat hypertension and to prevent its complications such as stroke and myocardial infarction. There are several classes of anti-hypertensives, but the most significant and clinically used classes of drugs are diuretics, calcium channel blockers, ACE inhibitors, angiotensin II receptor antagonists (ARBs), and beta blockers. Most of

these drugs are taken orally and can be administered for longer periods till the blood pressure gets normalised. Intra venous bolus dosage forms can be considered for perioperative circumstances as well as for the management of hypertensive crisis. The classification of the anti-hypertensive agents is summarized in figure

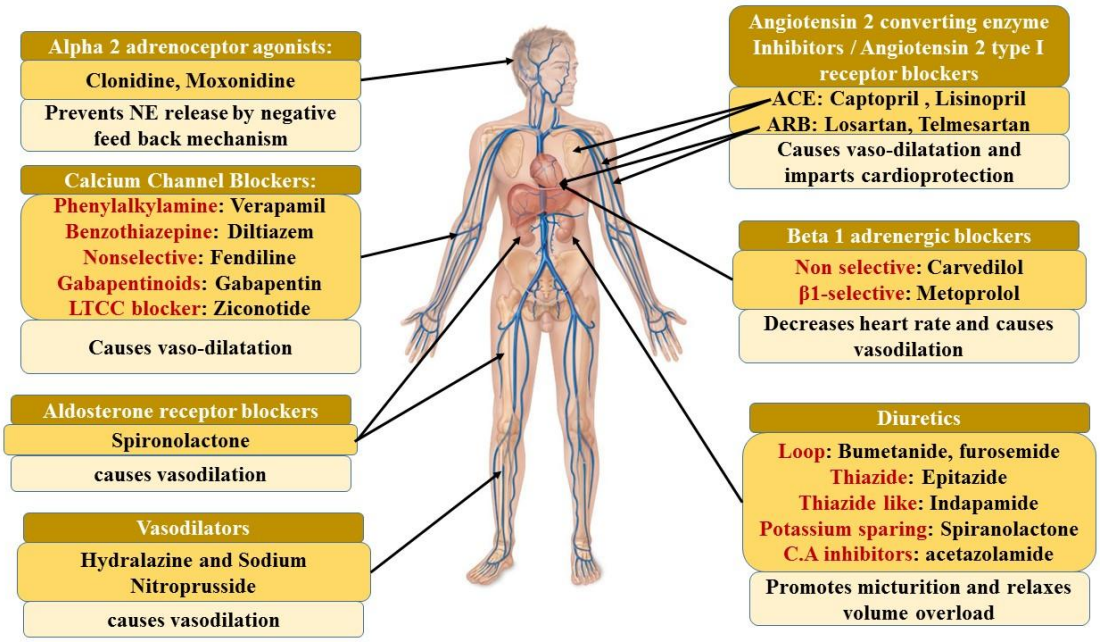

Figure no 14: Classification and mechanism of action of major classes of anti-hypertensive agents

Regression of the LVH is both conceivable and anticipated as an important goal in the management of hypertension. Being the preliminary cause for LVH, normalising the systolic BP is always associated with some degree of LVH regression. Changes in Left Ventricular Mass during treatment have been interrelated with BP attenuation predominantly when assessed by 24-hour monitoring. Though, this relationship is feeble, highlighting the influence of non-hemodynamic aspects, vasodilators like hydralazine, and minoxidil couldn't cause regression of LVH either in experimental models or in clinical trials despite lowering in blood pressure (Sen et al., 1974), (Gottdiener et al.,

1997). The NICE/BHS guidelines for management of hypertension is summarized in figure.

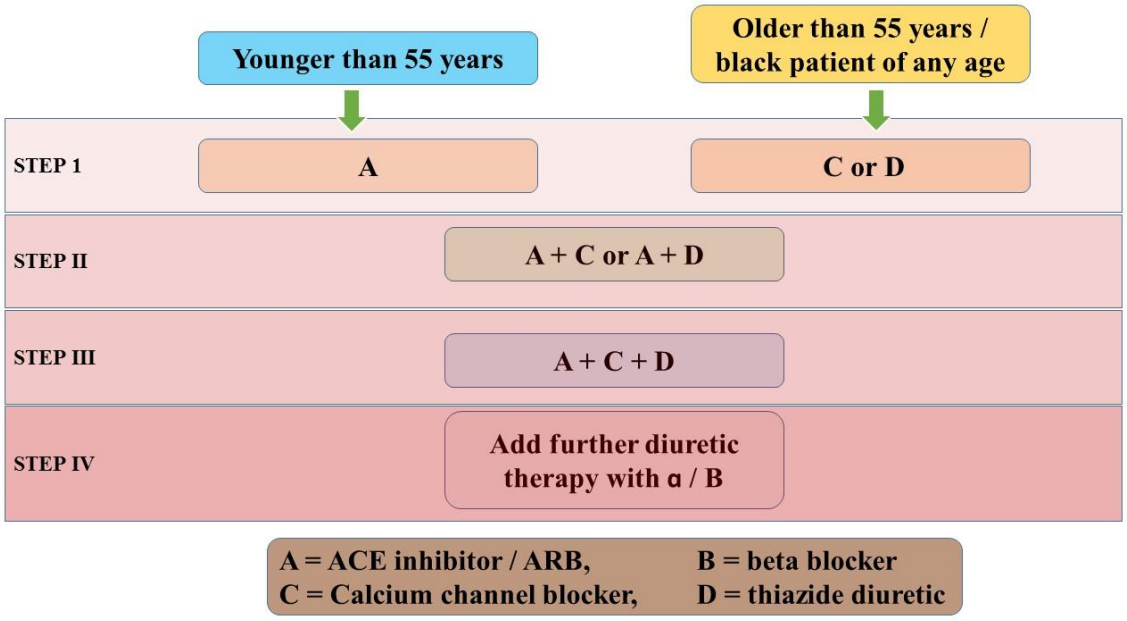

Figure no 15: NICE/BHS guidelines for drug treatment of hypertension

II.5.1. **Angiotensin II receptor type 1 blockers or Angiotensin Receptor Blockers:**

Despite of the preliminary contradictory reports regarding use of ARBs and chance of increase in adverse CV events such as myocardial infarction, successive systematic reviews and meta-analyses have disproved these findings (Bangalore et al., 2011). The landmark study, Losartan Intervention For Endpoint reduction in hypertension study (LIFE), a randomised trial against atenolol, the superiority of the ARBs was established over conventional beta blockers in greater degree of reduction in the composite end point of morbidity (Sica, 2002). Interestingly ARBs and Beta blockers had an identical extent of reduction on blood pressure.

Arterial remodelling forms the underlying pathophysiology for uncontrolled hypertension. The Candesartan Atenolol Carotid Haemodynamic Endpoint Trial (CACHET) investigated the comparative effects of ARBs with that of beta-blockers evaluating there potency to reverse the large artery remodelling. This clinical trial resulted in reduction in the intima-media thickness (IMT) and intima-media area (IMA) and increased distensibility to similar extents by both the treatments. However, in support to the LIFE trail, CACHET trail also reported the inferior ability of beta blockers in reduction of left ventricular mass index when compared to ARBs (Lincoff et al., 2002). Apart from these effects ARBs show a better improvement in stroke outcome as evident from the ACCESS trail (Schrader et al., 2007).

II.5.2. **Angiotensin Converting Enzyme Inhibitors (ACE inhibitors):**

Angiotensin converting enzyme inhibitors also seem to be advantageous to the patients with left ventricular hypertrophy as they augment the coronary endothelial function. This is achieved by the bradykinin facilitated release of nitric oxide and reduced myocardial oxygen consumption as the NO inhibits the rate of mitochondrial respiration (Zhang et al., 1997). Trandolapril Cardiac Evaluation (TRACE) Study showed that chronic ACE inhibitor treatment in subjects with LV compaction resultant of myocardial infarction significantly minimized risk of sudden cardiac death (Køber et al., 1995). The remarkable advantageous clinical outcomes of chronic ACE inhibitor therapy with reduced mortality was further demonstrated in the CONSENSUS, SOLVED and HeFT trials (Delahaye and de Gevigney, 2000).

Even though ACE inhibitors are found to be effective, Angioedema, an idiosyncratic life threatening adverse event is observed in few subjects. Elderly patients are in general

develop shortness of breath followed by swelling of the throat and tongue, the typical symptoms of anaphylactic shock. When starting an angiotensin-converting enzyme inhibitor, angiotensin II receptor blocker, or direct renin inhibitor, it is also important to screen for a change in renal function. Unrecognized renal artery stenosis with decreased glomerular filtration rate and elevated serum creatinine occurs in some subjects.

II.5.3. **Calcium Channel blockers:**

Calcium Channel Blockers follow ACE inhibitors and ARBs in swiftly facilitating the regression of left ventricular hypertrophy. These potent antihypertensive agents are used either in combination with ACE inhibitors or with the diuretics, serving as synergistic booster dosages. In addition to preventing the progression of LVH, CCBs improve coronary blood flow which in turn prevents the precipitation of ventricular tachyarrhythmia (Frishman et al., 1989). Long acting CCBs are safer and suitable for management of chronic hypertension induced LVH. Suitability of the sub categories of CCB depends on the patient's exercise tolerance. Subjects who present a hyper adrenergic response to exercise are usually assigned with phenylalkalyamines or benzothiazepines as these two chemical categories of CCBs temper the heart rate and blood pressure upon excersion. In disparity, conventional beta blockers only attenuate the heart rate by blocker beta adrenergic receptors on heart and causes transient to sub chronic heightening in the systolic blood pressure response due to unimpeded alpha constriction. Nevertheless, the pan adrenergic blockers like carvedilol and labetalol produce a response similar to phenylalkalyamines or benzothiazepines, relieving dyspnea and angina. Dihydropyridines are usually prescribed to older subjects with conduction abnormalities and slower heart rates.

II.5.4. **Diuretics:**

Diuretics and CCBs are usually prescribed in combination with an ACE inhibitor or ARBs to attain a better reduction in blood pressure (Papademetriou, 1994). Amongst the wide range of available diuretics, thiazide-type diuretics followed by loop diuretics are widely employed in management of hypertension. Even though the preliminary goal of diuretics is to relieve the congestion and peripheral oedema, there are ambiguous and contradictory reports regarding their beneficial outcome. Idiosyncratically, Thiazide diuretics and to a lesser extent loop diuretics increase circulating LDL levels and can precipitate atherosclerotic events on long term usage (Weidmann et al., 1992). Indapamide being a lipid neutral compound is an exception to this regard an now is being promoted to replace the existing diuretics.

II.5.5. **Beta adrenergic blockers:**

Contemporary management protocols for hypertensive subjects with LVH do not precisely support inclusion of beta-blocker in the first line therapy as to avoid the intrinsic sympathomimetic activity of some members of this class causing increased risk of sudden cardiac death (Katholi and Couri, 2011). Nevertheless, some clinical trials underscore the heterogeneity of beta blockers and offer a strong foundation for favoured usage of carvedilol in these patients. Carvedilol being a unique molecule with combined pan adrenergic blocking properties also holds antioxidant potency which is substantially less with the other sister compounds of the family (Arumanayagam et al., 2001). Reduction of the LV mass and ECM remodelling by carvedilol was reported in both pre-clinical and clinical studies (Barone et al., 1998), (Hansson and Himmelmann, 1998) . Though there was a better reduction in these indices with carvedilol, the usage of

metoprolol at least in its tartrate form is more promoted due to its better bioavailability and higher potency. The correction of the early to late diastolic dysfunction with metoprolol was more evident than the carvedilol and found to be comparable with that of ACE inhibitors and ARBs (Poole-Wilson et al., 2003).

Studies using rodent models indicate that oxidative stress induces myocyte apoptosis which in turn plays a crucial role in transition of hypertrophy to failure (Empel and Windt, 2004). Both Metoprolol and Carvedilol being supplement with antioxidant properties may be a suitable option for the preclusion of the pathological remodelling by preventing apoptosis. This conception is strengthened by the favourable outcomes of MERIT-HF trail using metoprolol in reducing morbidity and mortality in patients presenting myocardial infarction (Wikstrand, 2000). Both LVH and hypercholerstemia are strong and interdependent predictors of cardiovascular morbidity and mortality. Conventionally, as evident from the GEMINI trail, the beta-blockers, with an exception to carvedilol and metoprolol, induce insulin resistance, with moderate to abnormal weight gain and increase levels of circulating triglycerides (Bakris et al., 2004).

II.5.6. **Patient compliance with anti-hypertensive drugs:**

Patient compliance is a major factor that determines the clinical outcome of a pharmacological intervention. In a landmark study by Bloom.B.S, the patient compliance data for the major categories of anti-hypertensive agents was studies (Bloom, 1998). Using logistic regression analysis, class of antihypertensive medication, patient age, and dosing frequency were identified as clinically key independent covariates that are predictive of persistence drug usage at 12 months. Though diuretics are frequently considered as first-line therapy for treating hypertensions, their usage is concomitant

with the poor patient compliance either alone or in combination with beta-blockers. Patient compliance is high with CCBs and ACE inhibitors, with the highest rate seen with the ARBs (Figure 16). Nevertheless, it is ambiguous whether these conclusions were drawn considering the aspects like drug tolerability, drug-drug interactions, drug-food interactions, financial incentives, drug availability, selection bias and other factors.

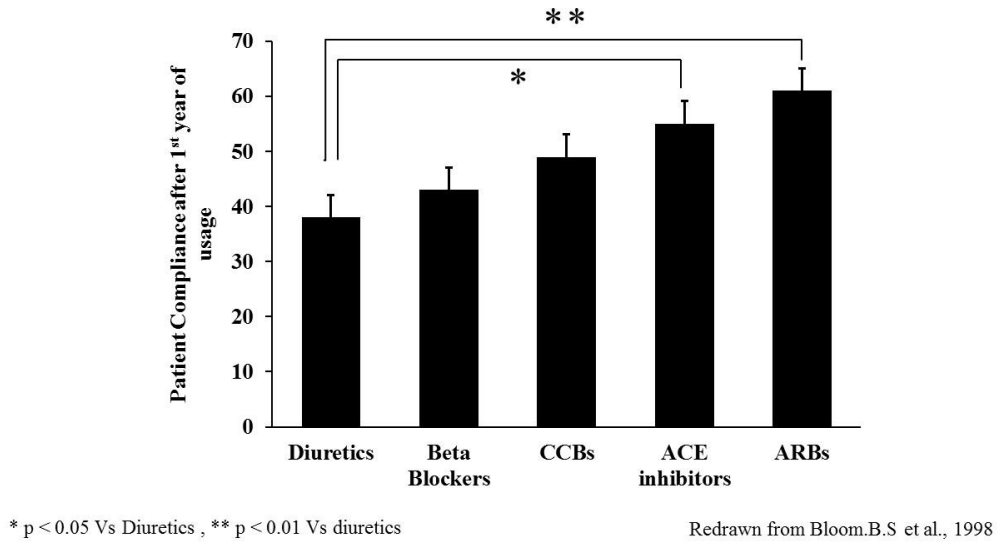

Figure no 16: Compliance at one year with antihypertensive treatment

II.5.7. **Future Methodologies for the management of LVH resultant of chronic hypertension**:

Multiple strategies are being worked out for proficient management of the blood pressure and regression of hypertrophy. A wide range of approaches are being tested ranging from surgical procedures to pharmacological interventions. Denervation of bilateral efferent and afferent renal sympathetic nerves is strongly advocated as the holistic solution for controlling hypertension (Sánchez-Álvarez et al., 2014). In a multi centric cohort study, the catheter-based renal denervation could able to reduce blood

pressure by an average of 27 mm of hg in 12 months period of time in 546 subjects with a preserved glomerular filtration rate (Krum et al., 2009). Carotid sinus stimulation with surgical implants causing baroreflex activation and reducing the systolic blood pressure was also found to be effective in management of hypertension (Bisognano et al., 2011). But the fact that LVH acts independent of blood pressure and tends to shift into failure curtails them from being a better solution for LVH management.

Pharmacologically, the applicability of statins in controlling blood pressure was an area of interest until the long term side effects of these potent HMG CO A reductase inhibitors where manifested (Feldstein, 2010). The ALLHAT trail conducted with an objective of evaluating the clinical outcomes of lipid lowering agents in controlling hypertension highlights the importance of statins (Antoniou et al., 2016). Nonetheless, weighting the good and evil can do more reasonableness will be the better way to endorse the annexation of these agents into anti-hypertensive therapy. (Katholi and Couri, 2011). Additionally mineralocorticoids antagonists (MRA) are also being tested for their usefulness in management of cardiac hypertrophy and persistent hypertension. Randomized ALdactone Evaluation Study (RALES) was the earliest randomized, double-blind, placebo-controlled trial on a MRAs in subjects with hypertension and presenting LVH with preserved ejection fraction. This trial indicated that subjects receiving sub chronic to chronic MRA treatment (Spironolactone) showed a better normalisation of systolic and mean blood pressures with a regression of LVH and circulating markers of apoptosis (Messaoudi et al., 2012). Reproducible results were obtained with the eplerenone, the next generation MRA with better reduction in LV mass and higher collateral coronary sprouting (Vizzardi et al., 2014).

Apart from these main stream cardiovascular drugs, unconventional systemic mediators of LVH are being targeted for developing novel therapeutic strategies. This approach can be helpful in management of barriers that arise during practising the existing treatment protocols. Studies are being conducted on Dopamine, Serotonin and Histamine to decipher their pathological participation and therapeutic targetability.

III. MATERIALS AND METHODS

III.1. DESIGN OF THE STUDY:

Left ventricular hypertrophy (LVH) is an adaptive and progressive consequence of uncontrolled hypertension. Despite this fact, LVH is an independent risk factor for cardiac failure and sudden cardiac death (Brown et al., 2000) (Tin et al., 2002). Activated sympathetic and renin angiotensin aldosterone systems, in response to uncontrolled hypertension are recognised as the major contributors for the cardiac hypertrophy. Additionally, higher mast cell count in hearts of patients with cardiac hypertrophy, dilated cardiomyopathy and myocardial infarction; and the increased circulating levels of the histamine in the heart failure subjects confirms the association of mast cells with cardiovascular pathophysiology (Dvorak, 1986), (Laine et al., 1999), (Hara et al., 2002). Histamine 2 receptors (H2R), widely distributed in the myocardium are known to arbitrate the cardiovascular effects of histamine. In a rodent model, disruption of H2R inhibited cardiac fibrosis and apoptosis precluding the failure (Zeng et al., 2014). Observations from a recent Multi-Ethnic Study point out that H2R blockers along with the conventional heart failure therapy reduce the risk for incidental heart failure attesting its importance in the disease pathology (Leary et al., 2016: 2). Nevertheless, the association of the myocardial histamine levels and H2R expression with the progression of hypertensive heart disease and the functional consequence of H2R antagonism are poorly understood. The H2R shares a unique structural homology with beta adrenoceptors (Del Valle and Gantz, 1997). Hence the equipotency of H2R antagonism with the conventional beta blocker therapy needs investigation. This study

was therefore designed to address the lacuna in the available information regarding the role of H2R antagonism in prevention of adverse cardiac remodelling. Spontaneously Hypertensive Rat (SHR) was selected as the experimental model, as it mimics the cardiovascular changes associated with chronic human hypertension (Berry et al., 2007). To examine whether the histamine levels and H2R expression increases with progressive cardiac remodelling, age associated changes in myocardial histamine levels was examined in one month, six month and twelve month old SHR. The functional consequence to treatment with H2R blocker (Famotidine) was studied in 6nmonth old SHR as it is the stable and adaptive phase of hypertrophy. Cardiovascular response to H2R antagonism was compared with that of a conventionally used beta blocker (metoprolol tartrate). This therapeutic strategy will be a novel approach to manage the hypertension-induced LVH. This therapeutic strategy will be a novel approach to manage the hypertension-induced LVH.

The study was designed with following objectives

i. To compare the expression of Histamine 2 receptor (H2R) and myocardial histamine content in 6 month old SHR and its age and sex matched Wistar.

ii. Analyse the age related changes in the expression of Histamine 2 receptor (H2R) and myocardial histamine content with the progression of disease.

iii. To study the cardiovascular response to H2R antagonist in stable phase of left ventricular hypertrophy.

iv. Compare the cardiovascular response to H2R antagonism with the conventional beta blocker therapy.

v. Delineation of the mechanism of H2 receptor stimulation in initiation of cardiac hypertrophy.

Prior to experimental studies, the SHR strain available in our institute was validated for the elevated blood pressure and presence of left ventricular hypertrophy. Adult (6 month) male SHR and its age and sex matched Wistar were chosen for the initial validation screening. Non-invasive Blood pressure monitoring and m-mode two dimensional transthoracic echocardiography was employed to assess blood pressure and the relative wall thickness.

To analyse the strain specific differences in the expression of Histamine 2 receptor (H2R) and myocardial levels of histamine, 6 month old SHR (n=6) and its age and sex matched Wistar were chosen. Expression of H2R in the myocardium was analysed by Immunohistochemistry and the myocardial levels of histamine was assessed by o-phthalaldehyde based flourimetric assay (Håkanson and Rönnberg, 1974).

The temporal variation in the expression of H2R and myocardial histamine content with the progression of disease was studied in 1 month, 6 month and 12 month old SHR and compared with its age and sex matched Wistar. Expression of H2R in the mid ventricular myocardium was analysed by Immunohistochemistry and the myocardial levels of histamine was assessed by o-phthalaldehyde based flourimetric assay (Håkanson and Rönnberg, 1974).

The cardiovascular response to the H2R antagonist was studied in 6 month old SHR (n=6). Famotidine, a H2R antagonist was selected and was orally administered to SHR for 60 days. Famotidine is an H2R antagonist, indicated for the efficient management of peptic ulcers. Unlike cimetidine, its proto molecule, famotidine has no effect on the cytochrome P450 enzyme system, has a very minimal drug- drug and drug-food interactions (Humphries and Merritt, 1999). Moreover famotidine is nine times more

potent than ranitidine and 32 times more potent than cimetidine, has a longer duration of action(Howard et al., 1985). The doses for this study was inspired from the previous in-vivo reports studying the various pharmacological actions of famotidine (Pradeepkumar Singh et al., 2007) (Ahmadi et al., 2011). The pharmacological consequence of H2R antagonism with famotidine in SHR was assessed by blood pressure measurement structural, histological and molecular markers of hypertrophy. The structural hypertrophy was assessed by m mode two dimensional transthoracic echocardiography and cardiac hypertrophy index. Myocyte cross-sectional area, a histological marker of cardiac hypertrophy and molecular level expression levels of calcineurin and serum and cardiac tissue lysate levels of b type natriuretic peptide was used to further confirm the pharmacological outcome.

The cardiovascular response exhibited by H2R antagonism in SHR was compared with the conventional beta blocker therapy to test the efficiency of the pharmacological outcome. Beta blockers are the first line drugs for the management of hypertension and the hypertension induced LVH. Metoprolol is a selective beta 1 receptor antagonist widely used in management of hypertension and its clinical outcome. It is known to regress hypertrophy and prevent its progression into failure (Corea et al., 1984), (Poole-Wilson et al., 2003). It is more efficient in regression of hypertrophy when compared with the partner molecules of the beta adrenoceptor antagonist making it a drug of choice in management of cardiovascular diseases (Poole-Wilson et al., 2003). The results from the landmark *African-American Heart Failure Trial* (A-HeFT) indicated the advantages of beta blockers over the competitive angiotensin receptor blockers in having long term benefits and lesser reoccurrence of adverse cardiovascular events (Ghali et al., 2007). For studying the equipotency and comparability of H2R antagonism with the beta

blocker therapy, 6 month old SHR was selected and treated with either Famotidine or metoprolol for 60 days and the effect on cardiac hypertrophy, cardiac fibrosis was assessed by functional, structural, biochemical and molecular variables.

III.2. *MATERIALS*

III.2.1. **Fine chemicals:**

1,1,3,3 tetra methoxy propane, 5′, 5′-dithiobis-(2-nitrobenzoic acid), acrylamide, agarose, ammonium per sulphate, bisacrylamide, Bovine Serum Albumin (BSA), chloramine T, colour burst electrophoresis marker, direct red, disodium hydrogen phosphate, DMEM High Glucose 1152, DMEM low glucose 5523, EDTA, Fetal Bovine Serum, glycine, nitrocellulose membrane (*Millipore USA*), o-phthalaldehyde , p–dimethylaminobenzaldehyde, per chloric acid, potassium chloride, potassium dihydrogen phosphate, potassium bicarbonate, protease inhibitor cocktail, Sodium dodecyl sulphate (SDS), N,N,N′,N′-Tetramethylethylenediamine (TEMED), Thiobarbituric acid, Tri Chloro Acetic acid (TCA), Triethanolamine, Tris–HCl, Per chloric acid, Trizma base and mercaptoethanol. All the fine chemicals were purchased from Sigma Aldrich, India unless mentioned in parenthesis.

III.2.2.**Routine Chemicals:**

Chloroform, ethanol, formalin, glycerol, Isopropanol, Methanol Hydrochloric acid, Phenol red, Propanol, Sodium chloride and Xylene. Routine chemicals were purchased from *Sisco Research Laboratories Limited India and Merck, India*.

III.2.3. **Drugs:**

Famotidine (European Pharmacopoeia (EP) – a kind gift from Intas pharma private limited, India), Ketamine (ketalar 50mg Inj, Parke Davis pharmaceuticals), Metoprolol tartrate (European Pharmacopoeia (EP) Reference Standard - M1830000 Sigma-Aldrich)

and Xylazine (Xylo B 20mg Inj, brilliant bio pharmaceuticals, India).

III.2.4.**Antibodies**:

Primary antibodies like Anti-AKT polyclonal antibody produced in rabbit (SAB4500800 Sigma Aldrich, India) , Anti-Calcineurin (α-Subunit) monoclonal antibody produced in mouse (C1956 Sigma Aldrich, India), Anti-Peroxiredoxin 3 polyclonal antibody produced in rabbit (ab73349, Abcam, India), Anti-Phospho-Akt (pSer124) antibody produced in rabbit (SAB4503853 Sigma Aldrich, India) and Anti-Rat H2 Histamine Receptor (extracellular) polyclonal antibody produced in rabbit (AHR-002 alomone labs, Israel) were used. Secondary anti bodies like Anti-Mouse IgG (whole molecule) Peroxidase antibody produced in rabbit (A9044 Sigma Aldrich, India) and Anti-Rabbit IgG (whole molecule) antibody produced in goat (R2004 Sigma Aldrich, India) were used.

III.2.5. **Kits**:

Coomassie protein assay reagent, Lactate Dehydrogenase Activity Assay Kit (MAK066 Sigma Aldrich, India), Rat BNP ELISA Assay Kit (KT-8580, Kamiya), Rat procollagen I N-terminal Peptide ELISA Kit (CSB-E12774r, Cusabio) and Super signal West Femto Substrate kit (*Thermo scientific USA*).

III.2.6. **Instruments**:

Deep freezer -20^{O}C (*Vest frost*), Deep freezer -80^{O}C (*Sanyo*), EASY pure UV/UF compact reagent grade water system (*Barnstead, USA*), Electrophoresis unit (*Bio-Rad laboratories, USA*), ELISA reader (*Bio-Tek instruments, USA*), Eppendorf centrifuge 5415 R, GE Vivid I Echocardiography machine with 10mHz Micro convex neonatal sector array ultrasound, Homogenizer (*IKA, Labortechnik, Germany*), Hot air oven (*Tempo, India*), Ice machine (*Hoshizaki, Japan*), Incubators (*Beston India; Kemi, India*),

Low speed magnetic stirrer (*Remi, India*), Microwave oven (IFB), pH meter (*Lab India*), Small Animal Non-invasive Blood Pressure System (NIBP200A) (*Biopac Systems, USA*) with BIOPAC Data Acquisition Unit (MP35/MP30), Steam distillation unit (*Beston*), UV- visible Spectrophotometer (*Shimadzu*), Water bath (*LKB, Sweden*) and Weighing balance (*Sartorius, USA and Ohaus*),

III.2.7**. Softwares:**

BSL PRO for NIBP (*Biopac*), Echo Pac Clinical workstation software, ImageJ (*NIH*) and *Image-Pro plus 5.1* for image analysis (*Media Cybernetics*).

III.3. COMPOSITION OF REAGENTS AND BUFFERS

III.3.1. **Phosphate buffered saline (PBS) –pH 7.4:**

Chemical	**Concentration**
NaCl	137mM,
KCl	2.7mM,
KH_2PO_4	1.76mM,
Na_2HPO_4	10.14mM.

III.3.2. **10% Buffered formalin:**

Chemical	**Quantity (For 1 L)**
NaH_2PO_4 (anhydrous)	3.5g
Na_2HPO_4 (anhydrous)	6.5g
Formalin	100ml
Distilled water	900ml

III.3.3. **Harrison's Haematoxylin**:

Chemical	Quantity for 1 L
Alum	100g
Haematoxylin	5g
Ethanol	50mL
Mercuric Oxide	2.5g
Distilled water	q.s for 1 L

III.3.4.**Eosin Solution**:

Chemical	Quantity for 100mL
Eosin	1gm
Propanol	q.s for 100 mL

III.3.5.**Picro-Sirius Red staining solution**:

Chemical	Quantity for 100mL
Sirius red or Direct red	0.1gm
Saturated picric acid	q.s for 100 mL

III.3.6. **RIPA Buffer**:

Chemical	Quantity for 30mL
Tris HCL	234mg

NaCl	261mg
EDTA	11.6mg
1 % Triton X 100	3mL
1 % Sodium Deoxy Cholate	3mL
Deionised water	q.s for 30mL

III.3.7. **10 X Running buffer (pH 8.3) for SDS–polyacrylamide gel electrophoresis (SDS- PAGE):**

Chemical	**Quantity for 1 L**
Tris base	30gm
Glycine	144gm
SDS	10gm
Deionised water	q.s for 30mL

Buffer was stored at room temperature and 1 X buffer was prepared just before use.

III.3.8. **SDS gel-loading buffer (6X):**

Chemical	**Quantity for 100mL**
SDS	9gm
Bromo phenol blue	30mg
Beta mercapto ethanol	9mL
Glycerol	50mL

1M Tris HCl- pH 6.8	q.s for 100mL

III.3.9. **Resolving gel buffer (pH 8.8):**

Chemical	**Quantity for 100mL**
Tris Base	18.16gm
deionised water	q.s for 100mL

III.3.10. **Stacking gel buffer (pH 6.8)**:

Chemical	**Quantity for 100mL**
Tris base	6.5gm
deionised water	q.s for 100mL

III.3.11. **30 % acrylamide – bisacrylamide solution**:

Chemical	**Quantity for 100mL**
Acrylamide	30gm
N'N'-bis-methylene-acrylamide	0.8gm
deionised water	q.s for 100mL

Solution was prepared in amber coloured bottled and stored at 4^0C.

III.3.12. **10 % APS solution**:

1gm of APS was dissolved in 10mL of distilled water in an amber coloured vial and stored at 4^0C.

III.3.13. **10% SDS solution**:

1gm of SDS was dissolved in 10mL of distilled water and stored at room temperature.

III.3.14. **Blocking buffer**:

Chemical	**Quantity for 10mL**
Skimmed milk	500mg
1 X TBST – Tween 20	10mL

III.3.15. **Transfer buffer (Towbin's buffer):**

Chemical	**Quantity for 1L**
Tris base	3.27gm
Glycine	14.4gm
Methanol	200mL
Deionized water	800mL

III.3.16. **Tris-buffered saline (TBS) (10X, pH 7.6):**

Chemical	**Quantity for 10mL**
Tris base	24.2 gm

Sodium chloride	80gm
Deionized water	q.s for 1L

For preparing 1 X TBS the 10 X stock was diluted with deionised water and 500µL of Tween 20 was added.

III.3.17. **Erhlich Reagent**:

Chemical	**Quantity for 100mL**
Para di amino benzaldehyde	2gm
95 % ethanol	50mL
Concentrated HCl	50mL

III.3.18. **Ellman reagent**:

Chemical	**Quantity for 10mL**
5,5'-dithiobis-(2-nitrobenzoic acid)	40mg
PBS	10mL

III.3.19. **Acetate- Citrate buffer**:

Chemical	Quantity for 10mL
Sodium Acetate	8.023gm
Citric acid	19.21gm

III.3.20. **DMEM High Glucose Medium**:

Chemical	Quantity for 1000mL
DMEM High Glucose powder (D1152)	17.3gm
Sodium Bicarbonate	3.7gm
Penicillin	280µL
Gentamycin	1.26mL

III.3.21. **DMEM Low Glucose Medium**:

Chemical	Quantity for 1000mL
DMEM Low Glucose powder (D5523)	10gm
Sodium Bicarbonate	3.7gm
Penicillin	280µL

Gentamycin	1.26mL

III.4. EXPERIMENTAL STUDIES:

Spontaneously hypertensive rat (SHR) was used as the in vivo experimental model of left ventricular hypertrophy (LVH). It mimics the clinical progression of hypertension induced left ventricular hypertrophy by developing hypertension at 1 month of age and left ventricular hypertrophy by 2 months. A stable phase of hypertrophy is seen in SHR from 6month to 12 months followed by progression into failure.

Spontaneously hypertensive rat purchased from Animal Resource Centre, Australia and the Wistar rats were maintained in the Division of Laboratory Animal Science of the Institute (SCTIMST). The animals were housed at 22±2°C in 55±10% relative humidity in individually ventilated cages. Light levels measured at 1 meter height were less than 300 Lux and a 12:12 hour dark: light pattern was maintained. The rats were fed with standard pellet feed and drinking water *ad libitum.*

III.4.1. **Comparison of differences in the expression of H2R and myocardial histamine content in adult SHR and Wistar**.

For establishing the strain specific variation in the expression of H2R and myocardial histamine content, 6 month old SHR which is known to be an early stable phase of LVH was selected and the immuno-histochemical probing was performed for mid ventricular H2R expression. The myocardial histamine content was analysed flourimetrically and the results were compared with age and sex matched Wistar.

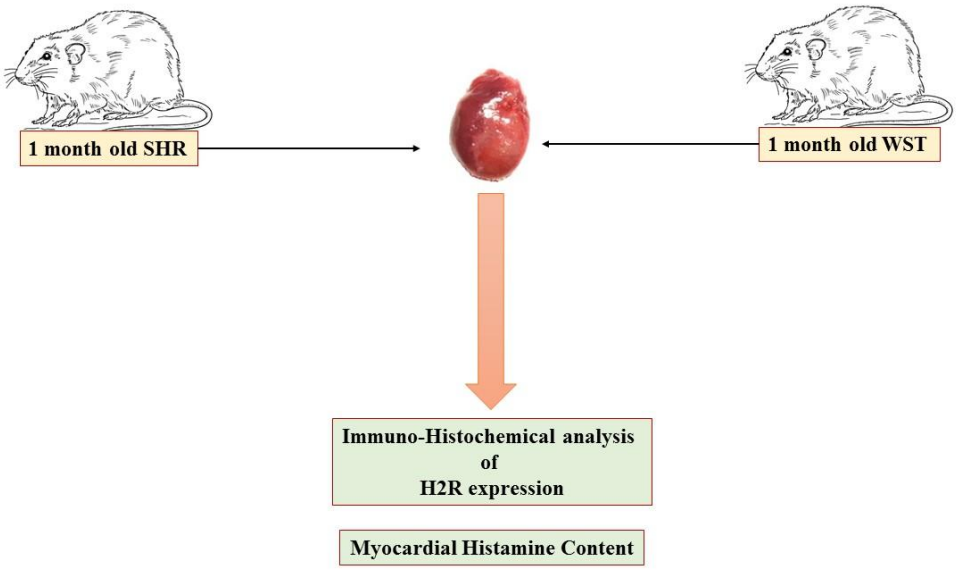

Figure no 17: Study plan for Comparison of differences in the expression of H2R and myocardial histamine content in adult SHR and Wistar.

III.4.2. **Age related changes in the expression of Histamine 2 receptor (H2R) and myocardial histamine content with the progression of disease.**

The temporal variation in the expression of Histamine 2 receptor (H2R) and myocardial histamine content was analysed in different age groups of SHR. One month old SHR, were the oxidative stress is established with no hypertension, six month old SHR were the oxidative stress and hypertension are seen with an evident and stable LVH and twelve month SHR with initial signs of transition into failure were considered for studying the temporal variations. The expression of Histamine 2 receptor (H2R) and myocardial histamine content was assessed as described in the previous section (III.4.1).

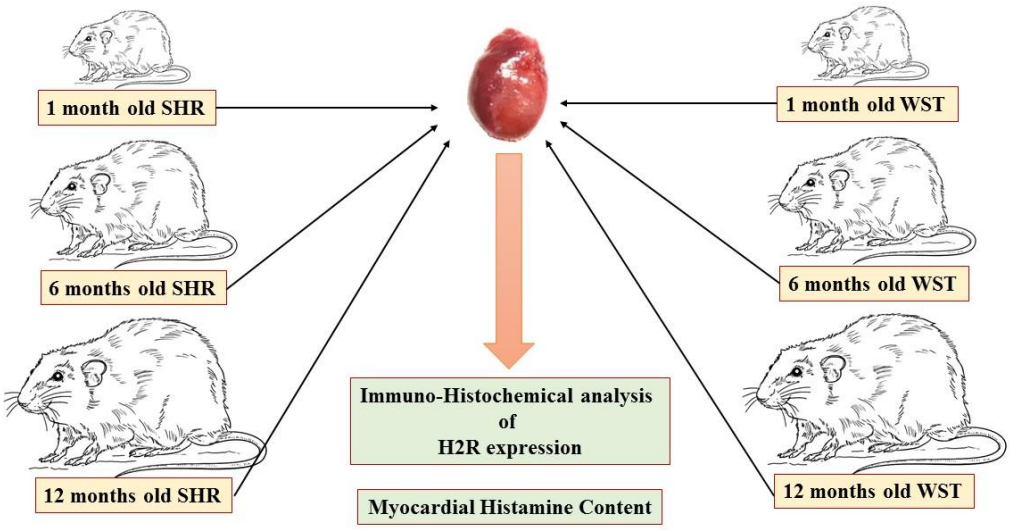

Figure no 18: Study Plan for assessing the age related changes in the expression of Histamine 2 receptor (H2R) and myocardial histamine content with the progression of disease.

III.4.3. **To study the cardiovascular response to H2R antagonist in stable phase of left ventricular hypertrophy.**

The cardiovascular response to the Famotidine, a H2R antagonist was studied in 6 month old SHR (n=6). The drug was dissolved in the distilled water and a dose of 30 mg/kg was administered to the rats through oral gavage, once daily, for 60 days. The pharmacological consequence of H2R antagonism with famotidine in SHR was assessed by blood pressure measurement structural, histological and molecular markers of hypertrophy. The structural hypertrophy was assessed by m mode two dimensional transthoracic echocardiography and represented as relative wall thickness and LV mass. Cardiac hypertrophy was morphologically assessed by cardiac hypertrophy index. Myocyte cross-sectional area, a histological marker of cardiac hypertrophy and molecular level expression levels of calcineurin and serum and cardiac tissue lysate

levels of b type natriuretic peptide was used to further confirm the pharmacological outcome.

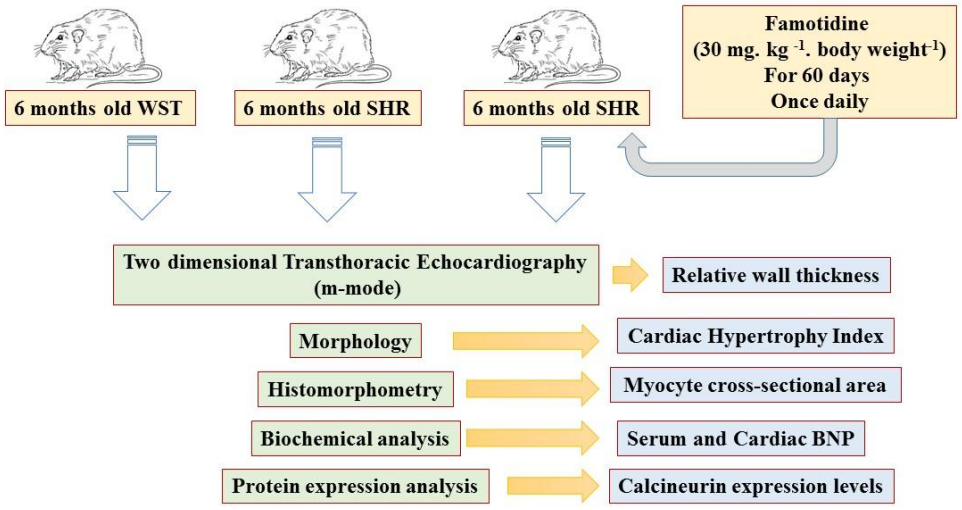

Figure no 19: Study Plan for assessing the cardiovascular response to H2R antagonist in stable phase of left ventricular hypertrophy**.**

III.4.4. **Comparison of the cardiovascular response to H2R antagonism with the conventional beta blocker therapy.**

The cardiovascular response elicited by Famotidine in SHR was compared with the conventionally used first line cardio protective beta blocker to test the efficiency of the pharmacological consequence. For studying the equipotency and comparability of H2R antagonism with the beta blocker therapy, 6 month old SHR was selected and treated with either Famotidine or metoprolol for 60 days through oral gavage at a dose 30 mg $^{-1}$. Kg $^{-1}$. Body weight $^{-1}$ and 30 mg $^{-1}$. Kg $^{-1}$. Body weight $^{-1}$ respectively. The effect of the drug treatments on cardiac hypertrophy was assessed by morphological, histolomorphometrical, biochemical and molecular markers with a special emphasis on cardiac fibrosis. The functional outcome was evaluated by two dimensional transthoracic

echocardiography using both the m mode as well as pulsed wave Doppler mode. The effect of treatments on the intra cardiac pressure gradients was studying and analysed in depth to further examine the improvement of performance based cardiac fluid dynamics. The results from each drug treated group were compared with their normotensive and hypertensive controls as well as compared with each other to arbiter the effectiveness of H2R antagonism.

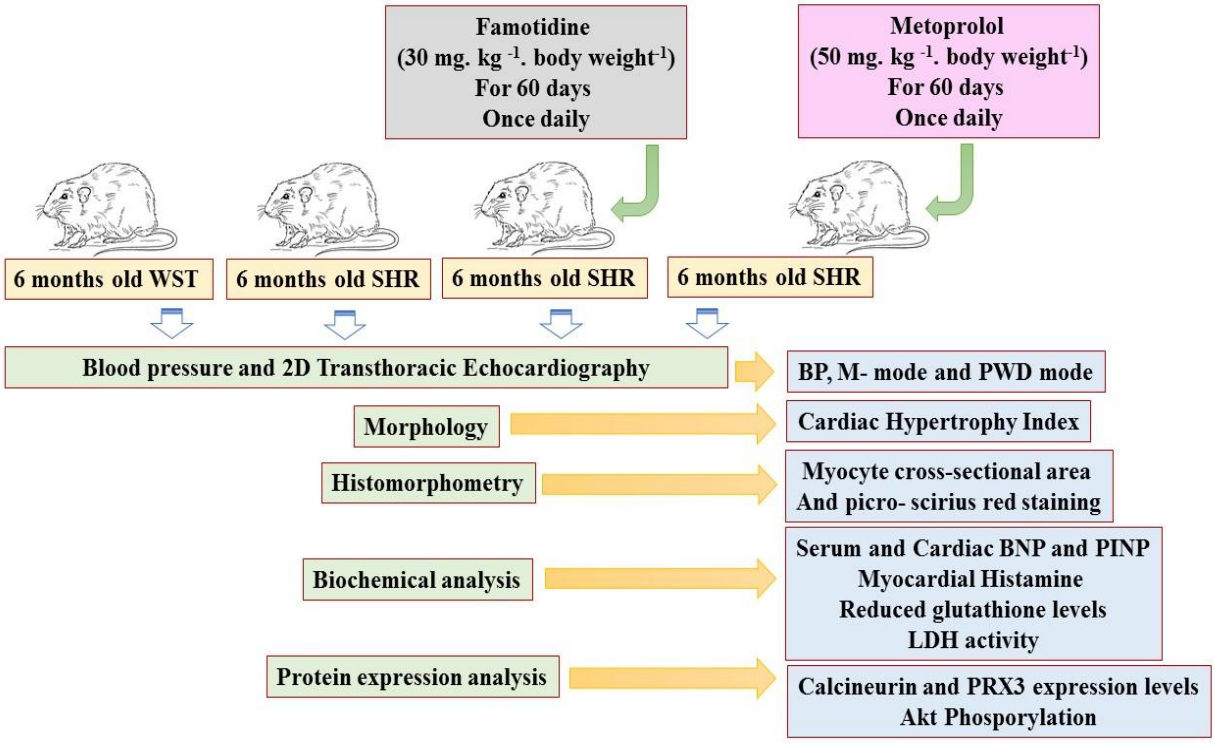

Figure no 20: Study Plan for comparing of the cardiovascular response to H2R antagonism with the conventional beta blocker therapy.

III.4.5. Delineation of the mechanism of H2 receptor stimulation in initiation of

cardiac hypertrophy.

For Delineation of the mechanism of H2 receptor stimulation in initiation of cardiac hypertrophy, H9c2 cardiomyoblasts were employed. H9c2 cardiomyoblasts were procured from NCCS Pune and were cultured in DMEM High glucose media supplemented with Sodium bicarbonate, penicillin, gentamycin and 10 % fetal Bovine serum. Cells were subjected to total serum deprivation for 24 hours prior to experimentations with DMEM low glucose medium supplemented with Sodium bicarbonate, penicillin and gentamycin and latter exposed to either Amthamine (5µM) or (10µM) or histamine (10µM) for 48 hours. Cells were then harvested after thorough PBS wash and protein was isolated and used for Immunoblotting experiments. Expression of calcineurin in response to treatments and degree of ERK and AkT phosphorylation are analysed.

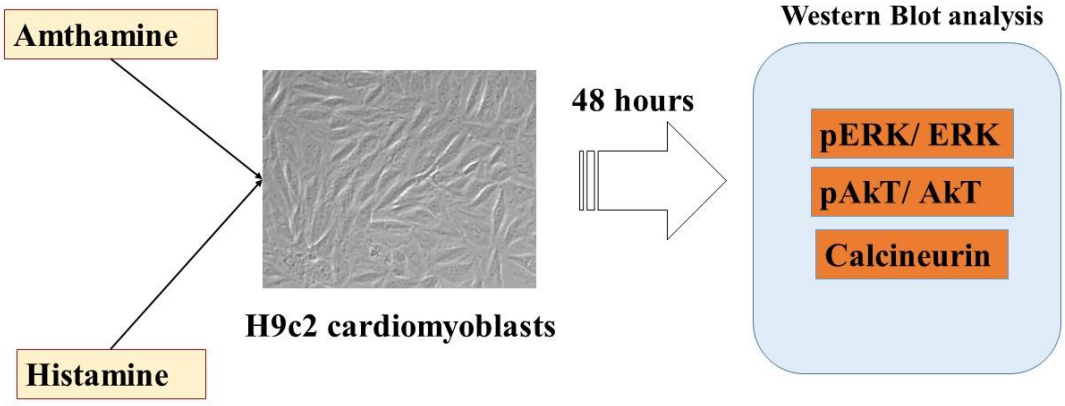

Figure no 21: Study Plan for Delineation of the mechanism of H2 receptor stimulation in initiation of cardiac hypertrophy.

III.5. METHODOLOGY

III.5.1.**Cell culture:**

III.5.1.1. Maintenance of Cell lines:

H9c2 cardiomyoblasts were procured from NCCS Pune and were cultured in DMEM High glucose media supplemented with Sodium bicarbonate, penicillin, gentamycin and 10 % fetal Bovine serum.

III.5.1.2. Experimentation with Cell lines:

Cells were subjected to total serum deprivation for 24 hours prior to experimentations with DMEM low glucose medium supplemented with Sodium bicarbonate, penicillin and gentamycin and latter exposed to either Amthamine (5µM) or (10µM) or histamine (10µM) for 48 hours. Cells were then harvested after thorough PBS wash and protein was isolated and used for experiments.

III.5.2.**Measurement of Blood Pressure**

The Blood pressure was measured using Non-invasive Blood Pressure Monitoring System for Small Animals (BIOPAC Systems.Inc) using an in built tail-cuff sphygmomanometer. For accurate measurement of non-invasive blood pressure, the tail of the animal was pre-warmed to 32^{O}C. After restraining the animal, the tail cuff was placed proximal to the base of the tail to occlude the blood flow. Upon deflation of the cuff, the blood pressure was determined which accorded with the reinstatement of caudal artery pulse recorded by the piezo electric pulse transducer placed distal to the cuff. Six consecutive determinations were made in each session of blood pressure measurement and the average was obtained. The systolic blood pressure value coincided with the point of appearance of the first pulse when the cuff is deflated and the point of the first

maximum peak corresponding to the diastolic blood pressure value.

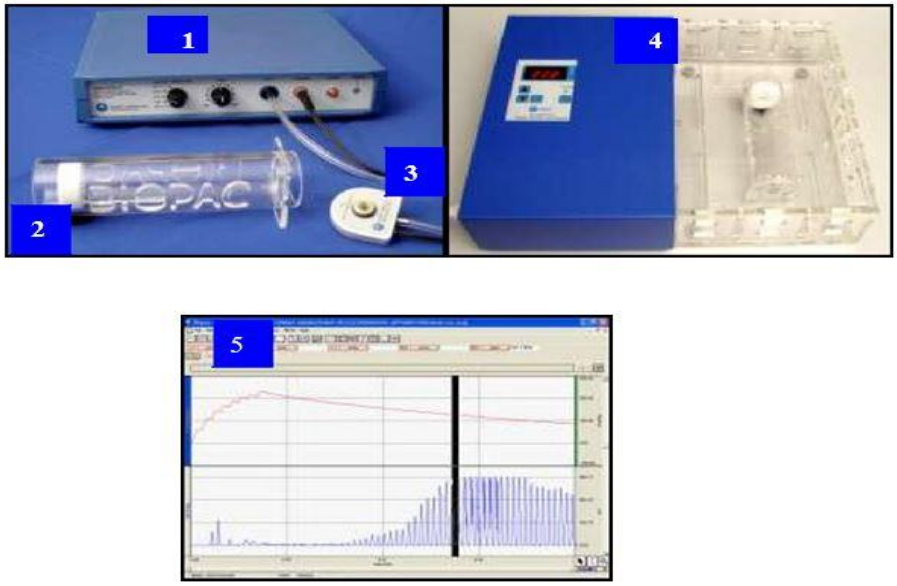

Figure no 22: Set up for blood pressure measurement. Where 1. NIBP200A Front Panel, 2. Restrainer, 3.Piezo electric pulse transducer (distal to cuff), 4.Animal heating chamber and 5. A typical recording using BSL PRO software for NIBP

III.5.3. **Two Dimnentional Echocardiography**:

Transthoracic echocardiography was performed to evaluate the left ventricle function following standard protocol. After anaesthetizing with ketamine (80 $mg.kg^{-1}$ body weight) and xylazine (20 $mg.kg^{-1}$ body weight) animals were placed in left lateral decubitus position and echocardiographic measurements were performed using M mode, 2D and Doppler imaging using *GE Vivid i* with 10 MHz linear transducer. Anaesthetized animals were allowed to breath spontaneously with oxygen supplementation through nose cone. The ultrasound transducer probe was placed to obtain short- and long-axis and four-chamber (ventricles and atria) and apical cardiac views. Left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), posterior wall thickness (PW) and septal wall thickness (IVS) during diastole were measured

using M mode according to the American Society of Echocardiography guidelines. LV mass (LVM), Shortening fraction (SF), and Relative wall thickness were calculated following standard protocol. Left ventricular end-systolic and end-diastolic areas were traced in a single-plane apical 4-chamber view and ejection fraction was calculated with the use of inbuilt software using the modified single-plane method (Simpson's rule). Mitral flow was recorded at the tip of the mitral valve from an apical view using Doppler imaging. Maximal velocities of the E and A waves were recorded and E/A ratio calculated. Isovolumic relaxation time (IVRT) was measured as the interval between the aortic closure click and the start of mitral flow. The Tei index was calculated on the same apical view.

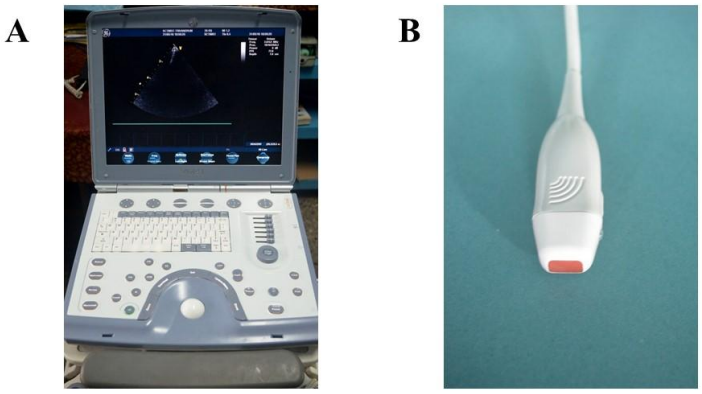

Figure no 23: Set up for two dimentional echocardiography. Where A. two dimentional echocardiography machine and B. Probe

III.5.4. **Dissection of heart and preparation of Serum and tissue lysate for biochemical and molecular biology analysis:**

Blood was collected from lateral tail vein and serum was separated by centrifugation and stored at -80^{0}C till further experimentation. Hearts were excised immediately and rinsed

in ice-cold normal saline. Excess fluids from the hearts were gently removed using a tissue paper and weighed. Hearts were then sampled for further investigations. Mid ventricular sections for Histomorphometry were fixed in 10 % neutral buffered formalin. Tissue samples for western blot analysis were snap freezed in liquid nitrogen and preserved at -80^{0}C till further experimentation.

Tissue lysates were prepared by adding 10 parts of RIPA buffer to 1 part of tissue and homogenising with tissue homogenizer. The homogenate was incubated on ice for 30 min and centrifuged at 5000g for 10 min at 4^{0}C and the supernatant was used for the estimation of various biochemical and immunoblotting estimations. Protein concentrations of tissue lysates were estimated by Bradford method.

III.5.5. **Myocardial Histamine content:**

Myocardial histamine content was assessed in tissue lysates as per modified Shores method. Briefly the histamine from tissue lysates was extracted with n-butanol and alkalinized perchloric acid followed by condensation with o-phthalaldehyde (OPT) to yield a product with strong and stable fluorescence. The fluorescence was measured in a spectrofluorometer (excitation 360nm, emission 450nm). Levels of histamine were quantified using a standard graph prepared and normalised with protein concentration.

III.5.6. **Immunohistochemistry:**

The ventricular sections were deparaffinized for thrice in xylene for 15min each followed by rehydration with descending grades of alcohols. The hydrated sections were then blocked for endogenous peroxidases by incubating 3% hydrogen peroxide in methanol for 3 mins. The target antigen was retrived using 0.01M freshly prepared sodium citrate buffer (pH-6.0) at 95^0 C for 20 minutes. The sections were blocked with 3% BSA and incubated with primary antibody at 4^{o}C overnight. The dilutions of the

primary antibodies were as follows- Dystrophin- 1:50 and CD36 and 3-nitro tyrosine- 1:100. After washing with PBS, the sections were treated with HRP conjugated secondary antibody (dilution 1:200) for an hour at room temperature. After washing off unbound secondary antibody, the sections were treated with the chromogen, diaminobenzidine (DAB) (Sigma).

III.5.7. **Myocardial Lipid peroxidation assay**:

Myocardial lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS). In a fresh glass tube, to 1mL of the tissue homogenate two milliliter of TBA-TCA-HCI reagent was added and vortexed thoroughly. This mixture was then boiled at 95^0C for 15 minutes and cooled under running tap water. Contents of the tubes were centrifuged for 10 minutes and the supernatant was collected. The absorbance of supernatant was read at 535 nm against reagent blank. The concentration of TBARS in the samples were calculated using a standard graph prepared with 1,1,3,3 tetra methoxy propane and normalised with protein concentration.

III.5.8. **Enzyme Linked Immuno Sorbant Assay for Myocardial and serum B type Natriuretic Peptide (BNP) levels:**

For measuring the BNP levels in the myocardium and in the serum, a commercially available kit from *kamiya biomedical company, USA* was employed. The assay was conducted according to the instructions provied along with the kit. In principle, the kit works by an competitive inhibition enzyme immunoassay technique. This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between BNP concentration in the sample and the assay signal intensity. Sample or calibrator (biotin labeled rat BNP) is added to the microplate precoated with monoclonal antibody specific for rat BNP followed incubation and washing of the

unbound conjugate. An avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and futhur incubated followed by addition of the substrate solution. The intensity of color developed is reverse proportional to the concentration of BNP in the sample.

III.5.89 **Enzyme Linked Immuno Sorbant Assay for Myocardial and serum Procollagen I N-terminal peptide (PINP) levels**:

For measuring the PINP levels in the myocardium and in the serum, a commercially available kit from *CusaBio life Sciences Ltd, China* was employed. The assay was conducted according to the instructions provied along with the kit. This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for PINP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any PINP present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for PINP is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of PINP bound in the initial step. The color development is stopped and the intensity of the color is measured.

III.5.10. **Lactate Dehydrogenase (LDH) activity assay:**

Lactate Dehydrogenase (LDH) activity in serum and cardiac tissue lysates were determined using by Lactate Dehydrogenase Activity Assay Kit from *Sigma Aldrich, India,* as per manufacturers guidelines and results were expressed as $mU.mL^{-1}$. This LDH Activity Assay kit quantifies LDH activity by using a principle of NAD reduction

to NADH by LDH, which is precisely detected colorimetrically at 450 nm.

III.5.11. **Myocardial Hydroxyproline Content**:

Myocardial hydroxyproline concentration was assessed according to the method of Reddy et al with minor modifications [36]. Myocardial lysate from pre weighed tissue was hydrolysed in hydrochloride acid (6 M) for 4 hours at 110°C and oxidized with the chloramine T in acetate-citrate buffer, pH 6.0. Then the mixture was incubated for 20 minutes at room temperature and the reaction was stopped by adding 20 volumes of Ehrlich's reagent solution. The samples were further incubated at 65°C for 15 minutes, and absorbance was measured spectrophotometrically at 550 nm. The results were determined as $mg.g^{-1}$ of wet tissue weight.

III.5.12. **Reduced Gluthione assay:**

Reduced glutathione content in myocardial tissue lysate was assayed by the method developed by Beutler et al [35]. Optical density of stable yellow color chromophoric product resulting from the reaction of 5′, 5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) with tissue lysate was determined at 412 nm. Amount of reduced glutathione was quantified using a standard graph and normalised to protein content.

III.5.13. **Heamatoxylin and Eosin staining:**

Tissue sections were rehydrated in descending grades of alcohol and were stained with haematoxylin for 3 minutes followed by differentiation in acid alcohol for 30 seconds. Bluing was done in slow running tap water and the sections were then stained with Eosin for one minute. Sections were dehydrated in ascending order of alcohols cleared with xylene and were then mounted with DPX.

III.5.14. **Picro-Sirius red staining:**

The extent of myocardial fibrosis was determined by Picro - Sirius red staining. Briefly,

the deparaffinised tissue sections were rehydrated in descending grades of alcohol and were stained with 1% Sirius red in saturated aqueous solution of picric acid for 90 minutes followed by wash with two changes of acidified water. Sections were dehydrated in ascending order of alcohols cleared with xylene and were then mounted with DPX. Fibrosis was expressed as the percentage of stained area in a particular microscopic field. Perivascular and gap areas were excluded from the measurement of interstitial fibrosis. For the determination of Perivascular fibrosis percentage of stained area were normalized by vessel area and quantified by ImageJ.

III.5.15. **Western Blot analysis:**

Western blot analysis was carried out following the procedure described by Maniatis et al (1982). 100mg of tissue samples were homogenized in 1ml of RIPA buffer containing protease and phosphatase inhibitor cocktail (Sigma). The extracts were then kept on ice with intermittent vortexing for 60min and centrifuged at 10,000rpm for 30 minutes at 4^0C. The supernatant was collected and protein concentration was determined using Coomassie protein assay reagent (Sigma Aldrich). 40 µg of total protein was processed with the gel loading dye and further fractionated on 10% SDS-polyacrylamide gels at 100 V. Separated proteins were then electro-transferred on to a nitrocellulose membrane. The membranes were then taken out and washed with deionized water and reversibly stained with Ponceau S to ensure the transfer efficiency. The membranes were then washed twice with Tris-buffered saline containing 0.1% Tween-20 (TBS-T) for 5' to de stain and the nonspecific binding sites were blocked with 5% non-fat milk in TBS-T at room temperature. After blocking, the membrane was incubated with primary antibody solution (with respective antibody specific dilution) for overnight at 4^0C in a shaker. The membranes were then washed thrice in TBS-T and incubated with horseradish

peroxidase-conjugated secondary antibody at room temperature for 1 hour.

Bands of the target protein were visualized using chemiluminescence detection kit and luminesce was capture on to a radiographic photo film. The membranes were further re-probed for anti-β actin, which served as loading control. The images of the photo films that are developed using the developer – fixer solution was taken using a *Bio-Rad gel doc system, USA*. The intensity of target protein bands was quantified using the image J software and was normalised with the intensity of their respective β actins. The data was represented as relative fold change.

III.6. STATISTICAL ANALYSIS:

Values are expressed as mean ± SD. Variation between groups was determined by using one-way analysis of variance (ANOVA) followed by comparisons between treated and control groups by using Bonferroni post hoc test. $P < 0.05$ was considered to be statistically significant difference.

IV. RESULTS

IV.1. **Comparison of differences in the expression of H2R and myocardial histamine content in adult SHR and Wistar**.

IV.1.1. **Differences in the expression of H2R in adult SHR and Wistar.**

The myocardial expression of H2R in six months old SHR was compared with age matched normotensive Wistar rat. Expression of H2R as assessed by immunohistochemistry showed a higher degree of expression in SHR than that of the Wistar rat were significantly higher ($p<0.01$). The higher degree of H2R expression in SHR may be an indicative of involvement of this receptor in the pathophysiology of hypertension induced LVH.

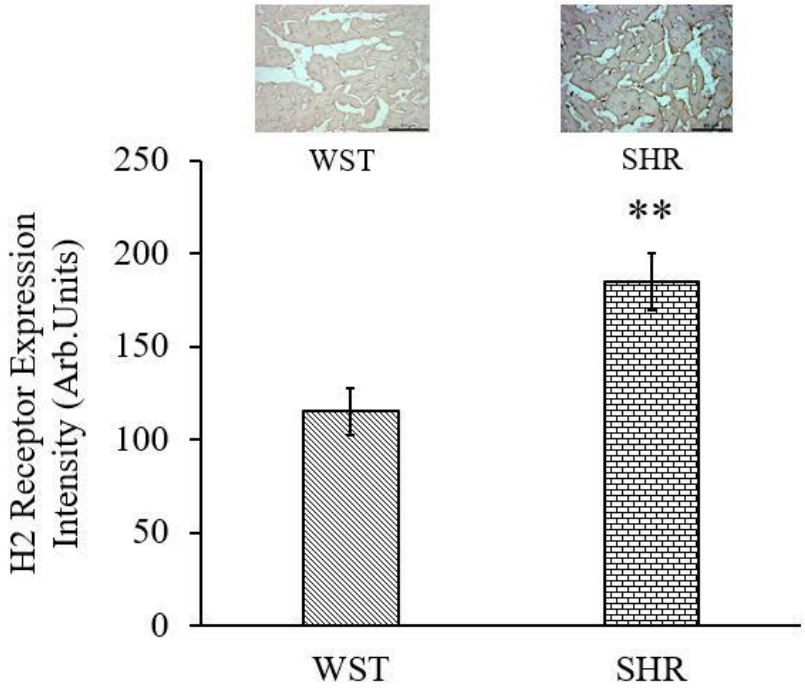

Figure no 24: Differences in the expression of H2R in 6 month old adult SHR and Wistar (WST). Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs age matched WST and ANOVA $p<0.01$.

IV.1.2. **Differences in the myocardial histamine content in adult SHR and Wistar.**

The myocardial histamine content in six months old SHR was assessed flourimetrically by o-phthalaldehyde based condensation method and compared with age matched normotensive Wistar rat. Myocardial levels of histamine content in SHR were found to be higher than that of the Wistar rat ($p<0.01$). This gesture of the presence of higher content of myocardial histamine in SHR is an indicative of involvement of this mediator in the pathophysiology of hypertension induced LVH.

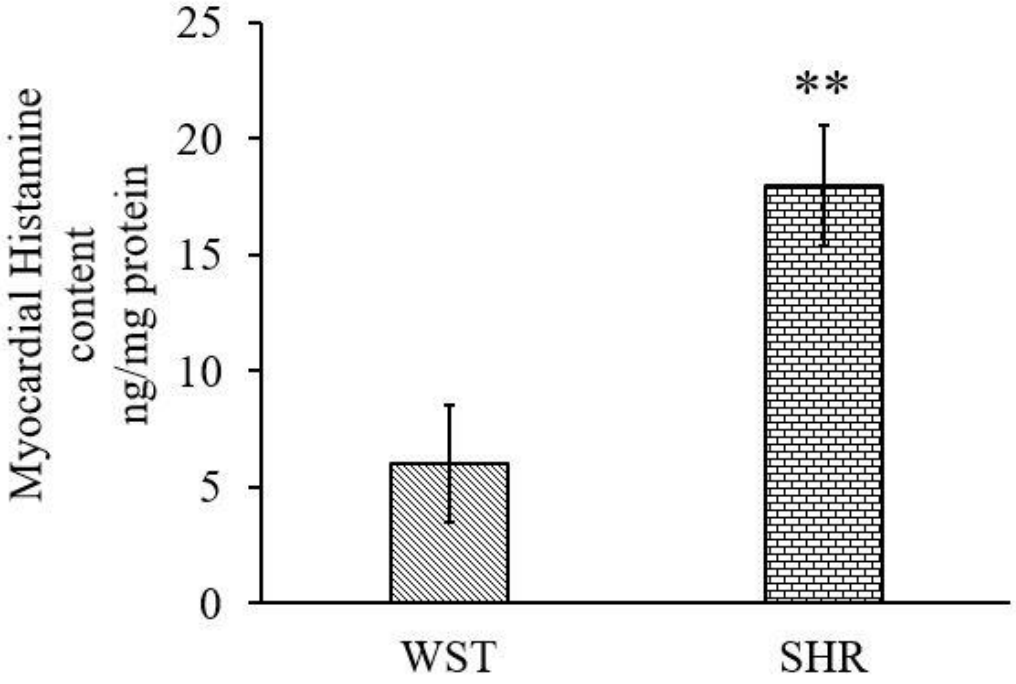

Figure no 25: Differences in the myocardial histamine content in adult SHR and Wistar (WST). Data is represented as mean ± SD. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs age matched WST and ANOVA $p<0.01$.

IV.2. **Age related changes in the expression of Histamine 2 receptor (H2R) and myocardial histamine content with the progression of disease.**

IV.2.1. **Age related changes in the expression of H2R in adult SHR and Wistar.**

The myocardial expression of H2R in one, six and twelve months old SHR was compared with respective age matched normotensive Wistar rat. Expression of H2R as assessed by immunohistochemistry showed a higher degree of expression in SHR than that of the Wistar rat were significantly higher ($p<0.01$ and $p < 0.001$). The expression of H2R in SHR changed significantly ($p<0.01$) with age were as it was unaltered in WST.

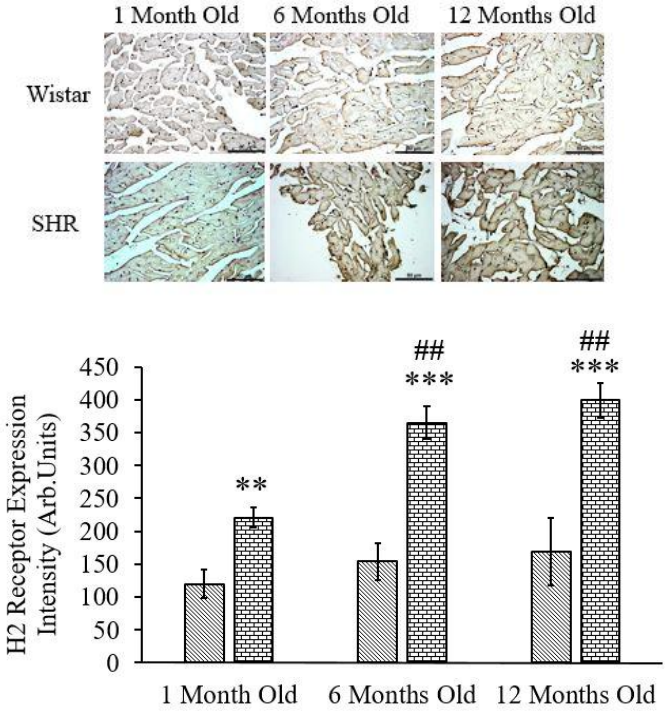

Figure no 26: Age related changes in the expression of H2R in adult SHR and Wistar (WST). Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs age matched WST, *** $p<0.001$ Vs age matched WST, ## $p<0.01$ Vs one month old SHR and ANOVA $p<0.01$.

IV.1.2. **Age related changes in the myocardial histamine content in adult SHR and Wistar.**

The myocardial histamine content in one, six and twelve months old SHR was assessed flourimetrically by o-phthalaldehyde based condensation method and compared with age matched normotensive Wistar rat. Myocardial levels of histamine content in one month SHR and WST were found to comparable but at the ages of six and twelve the myocardial levels of histamine in SHR are higher than that of the Wistar rat ($p<0.01$ and $p < 0.001$). The myocardial levels of histamine in SHR has significantly increased in six and twelve month old SHR when compared to one month old ($p < 0.05$ & $p < 0.001$) were unaltered in the WST.

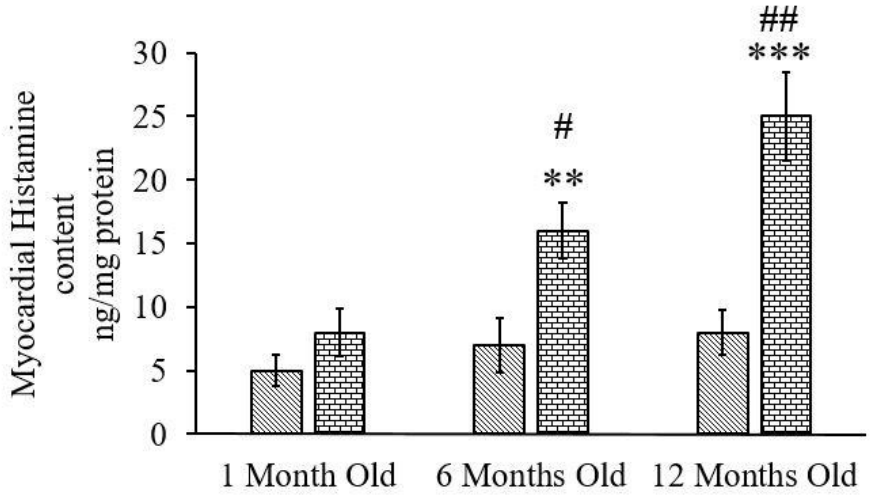

Figure no 27: Age related changes in the myocardial histamine content in adult SHR and Wistar (WST). Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs age matched WST, *** $p<0.001$ Vs age matched WST, # $p<0.05$ Vs one month old SHR ## $p<0.01$ Vs one month old SHR and ANOVA $p<0.01$.

IV.3. **Cardiovascular response to H2R antagonist in stable phase of left ventricular hypertrophy.**

IV.3.1. **Effect of famotidine treatment on Blood pressure:**

Evaluation of blood pressure by non-invasive tail cuff sphygmomanometer showed that SBP DBP and MAP of the SHR were significantly higher ($p<0.01$) than that of the Wistar rat. The DBP and MAP were significantly attenuated with 60 days of sub chronic famotidine treatment whereas the SBP remained unaltered with a non-significant reduction. ($p<0.05$) (Fig.).

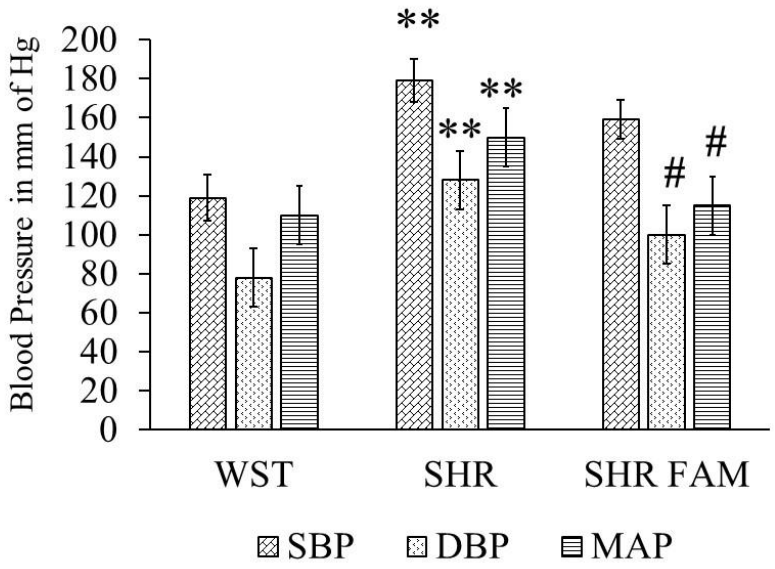

Figure no 28: Effect of famotidine treatment on blood pressure in SHR. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and # $p<0.05$ Vs SHR. ANOVA $p<0.01$. Where SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure and MAP: Mean Arterial Pressure.

IV.3.2. **Effect of famotidine treatment on Left Ventricular Mass as assessed by m mode two dimensional trans-thoracic echocardiography:**

Increased Left ventricular Mass is a key indicator for the presence of pressure overload induced cardiac hypertrophy. In accordance with the elevated blood pressures as shown in IV.3.1. the LV Mass in SHR as evaluated by m mode of two dimensional trans-thoracic echocardiography was significantly higher when compared to that of the Wistar rat indicating the presence of left ventricular hypertrophy ($p<0.01$). Sub chronic famotidine treatment for 60 days significantly attenuated LV mass indicating the reduction in the left ventricular hypertrophy. ($p<0.01$) (Fig.).

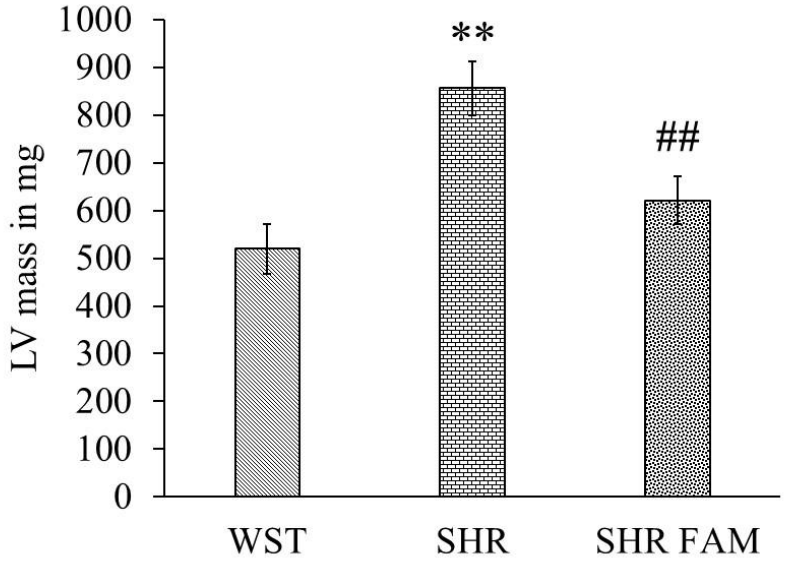

Figure no 29: Effect of famotidine treatment on LV Mass in SHR. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and ## $p<0.01$ Vs SHR. ANOVA $p<0.01$.

IV.3.3. **Effect of famotidine treatment on Relative wall thickness as assessed by m mode two dimensional trans-thoracic echocardiography:**

Relative wall thickness is an arbitral way of expressing the degree of increase in Left ventricular Mass a key indicator for the presence of pressure overload induced cardiac hypertrophy. In accordance with the findings in IV.3.1. and IV.3.2., the RWT in SHR as was significantly higher when compared to that of the Wistar rat indicating the presence of left ventricular hypertrophy ($p<0.01$). Sub chronic famotidine treatment for 60 days significantly attenuated the RWT indicating the reduction in the left ventricular hypertrophy. ($p<0.01$) (Fig.).

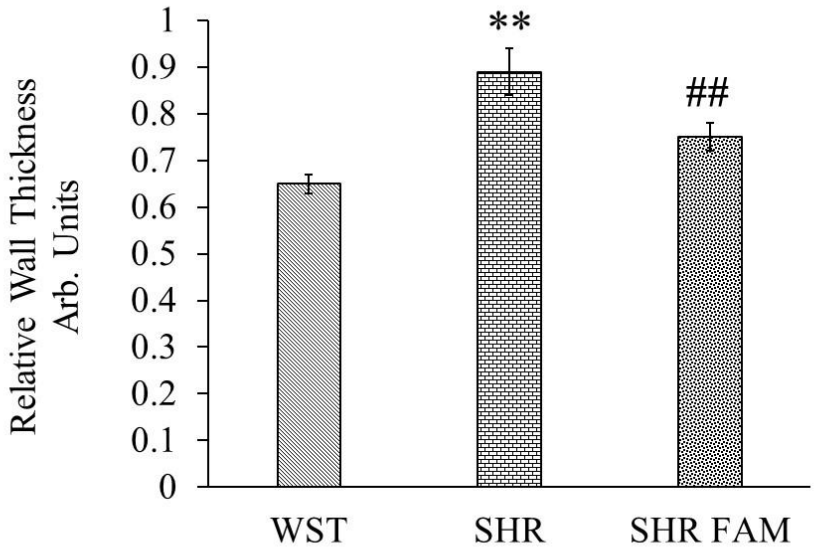

Figure no 30: Effect of famotidine treatment on RWT in SHR. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and ## $p<0.01$ Vs SHR. ANOVA $p<0.01$.

IV.3.4. **Effect of famotidine treatment on cardiac hypertrophy index:**

Cardiac Hypertrophy Index is the ratio between the Heart weights to the tibia length, which stands as a morphological indicator for the presence of pressure overload induced cardiac hypertrophy. In accordance with the findings in previous sections the cardiac hypertrophy index in SHR as was significantly higher when compared to that of the Wistar rat indicating the presence of left ventricular hypertrophy ($p<0.01$). Sub chronic famotidine treatment for 60 days significantly attenuated Cardiac Hypertrophy Index indicating the reduction in the left ventricular hypertrophy. ($p<0.01$) (Fig.).

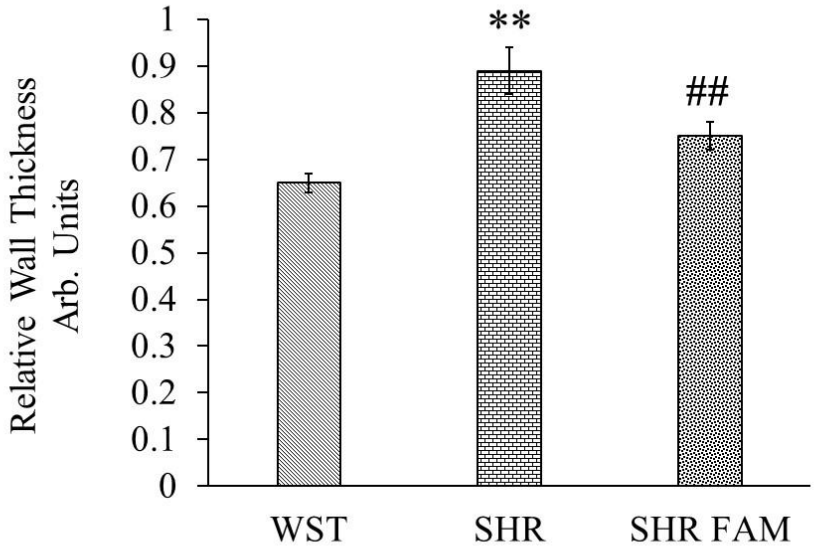

Figure no 31: Effect of famotidine treatment on Cardiac Hypertrophy Index in SHR. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and ## $p<0.01$ Vs SHR. ANOVA $p<0.01$.

IV.3.5. **Effect of famotidine treatment on Myocyte cross-sectional area:**

Myocyte cross-sectional area is the cross sectional area of each myocyte measured from a histology slide stained with haematoxylin and eosin. It is a histological indicator for the presence of pressure overload induced cardiac hypertrophy. In accordance with the findings in previous sections the Myocyte cross-sectional area in SHR as was significantly higher when compared to that of the Wistar rat indicating the presence of left ventricular hypertrophy ($p<0.01$). Sub chronic famotidine treatment for 60 days significantly attenuated Myocyte cross-sectional area indicating the reduction in the left ventricular hypertrophy. ($p<0.01$) (Fig.).

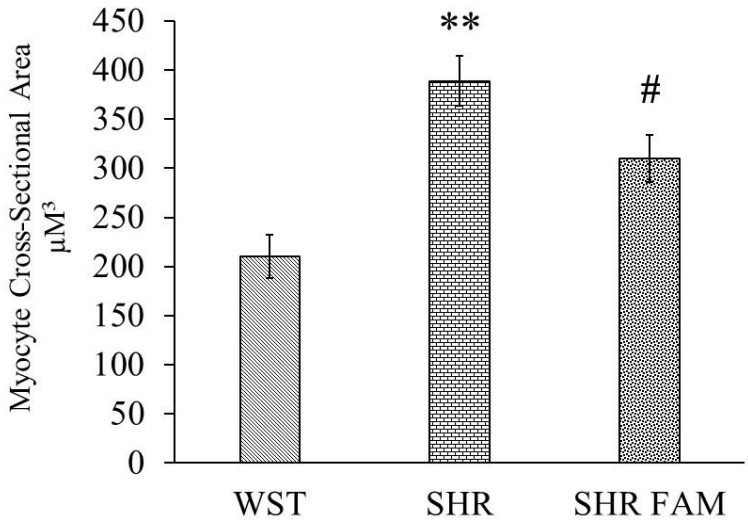

Figure no 32: Effect of famotidine treatment on Myocyte cross-sectional area in SHR. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and ## $p<0.01$ Vs SHR. ANOVA $p<0.01$.

IV.3.6. **Effect of famotidine treatment on Serum and myocardial B type natriuretic peptide levels:**

B type natriuretic peptide is an indicator for the fetal gene expression and considered to be an indicator for the presence of pressure overload induced cardiac hypertrophy. In accordance with the findings in previous sections the serum and myocardial B type natriuretic peptide levels in SHR as was significantly higher when compared to that of the Wistar rat indicating the presence of left ventricular hypertrophy ($p<0.01$). Sub chronic famotidine treatment for 60 days significantly attenuated both serum and myocardial B type natriuretic peptide levels indicating the reduction in the left ventricular hypertrophy. ($p<0.01$) (Fig.).

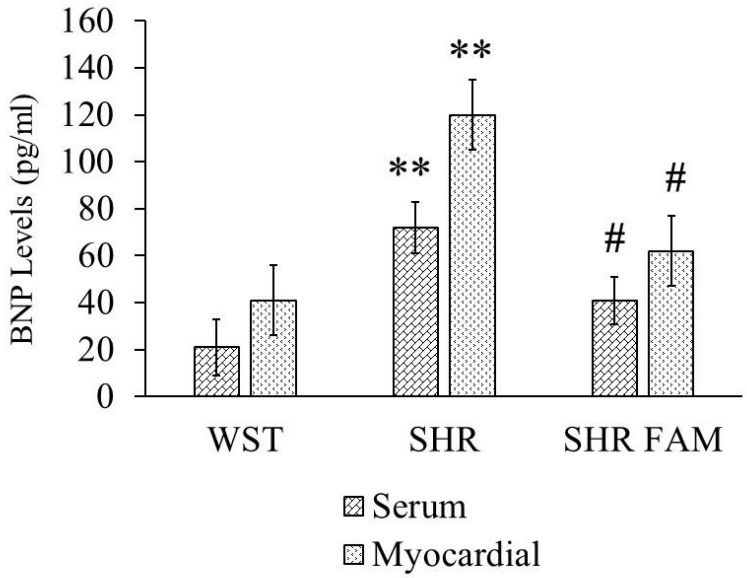

Figure no 33: Effect of famotidine treatment on serum and myocardial B type natriuretic peptide levels in SHR. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and ## $p<0.01$ Vs SHR. ANOVA $p<0.01$.

IV.4. **Comparison of the cardiovascular response elicited by H2R antagonism with the conventional beta blocker therapy.**

IV.4.1. **Comparison of the anti-hypertensive response elicited by Treatments.**

IV.4.1.1. **Comparison of the effect of famotidine treatment on Blood pressure with the metoprolol:**

Evaluation of blood pressure by non-invasive tail cuff sphygmomanometer showed that SBP DBP and MAP of the SHR were significantly higher ($p<0.01$) than that of the Wistar rat. The DBP and MAP were identically attenuated with 60 days of sub chronic treatment with famotidine and metoprolol treatments, whereas the SBP remained unaltered with a non-significant reduction. ($p<0.05$) (Fig.).

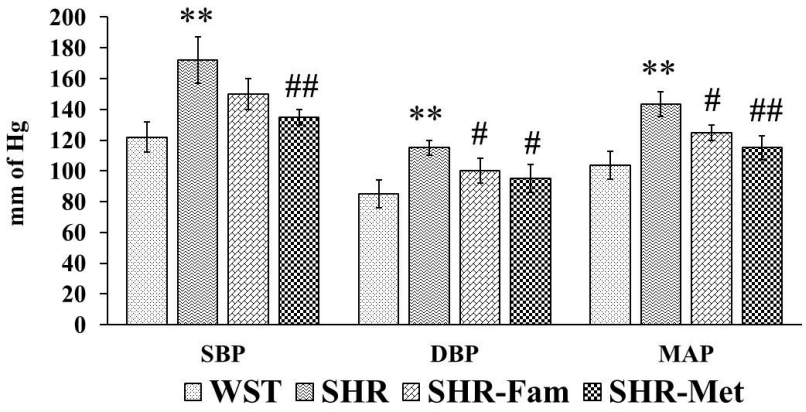

Figure no 34: Comparison of the effect of famotidine treatment on Blood pressure with the metoprolol. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST, # $p<0.05$ Vs SHR and ## $p<0.01$ Vs SHR. ANOVA $p<0.01$. Where SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure and MAP: Mean Arterial Pressure.

IV.4.2. **Comparison of the anti-hypertrophic response elicited by Treatments.**

IV.4.2.1. **Comparison of the effect of famotidine treatment on LV mass as evaluated by m mode two dimensional trans-thoracic echocardiography with the metoprolol:**

Evaluation of LV mass by m mode two dimensional trans-thoracic echocardiography showed that LV mass of the SHR were significantly higher ($p<0.01$) than that of the Wistar rat. The LV mass was identically attenuated with 60 days of sub chronic treatment with famotidine and metoprolol treatments. ($p<0.01$) (Fig.).

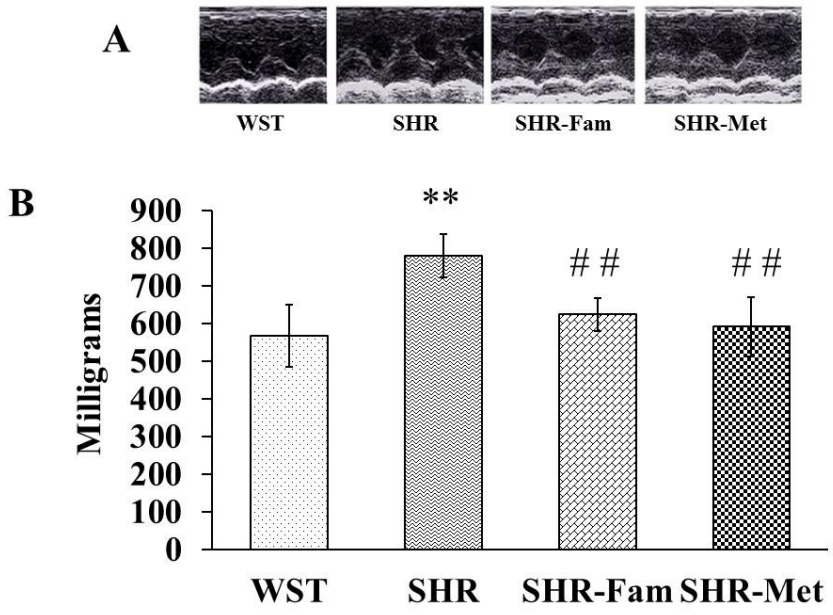

Figure no 35: Comparison of the effect of famotidine treatment on LV mass as evaluated by m mode two dimensional trans-thoracic echocardiography with the metoprolol. Where A is the pictorial representation of the M mode acquisition and B is the graphical representation of the LV mass in milligrams. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and ## $p<0.01$ Vs SHR. ANOVA $p<0.01$.

IV.4.2.2. **Comparison of the effect of famotidine treatment on Relative wall thickness as evaluated by m mode two dimensional trans-thoracic echocardiography with the metoprolol:**

Evaluation of RWT by m mode two dimensional trans-thoracic echocardiography showed that RWT of the SHR were significantly higher ($p<0.01$) than that of the Wistar rat indicating the presence of cardiac hypertrophy. The RWT was identically attenuated with 60 days of sub chronic treatment with famotidine and metoprolol treatments. ($p<0.01$) (Fig.).

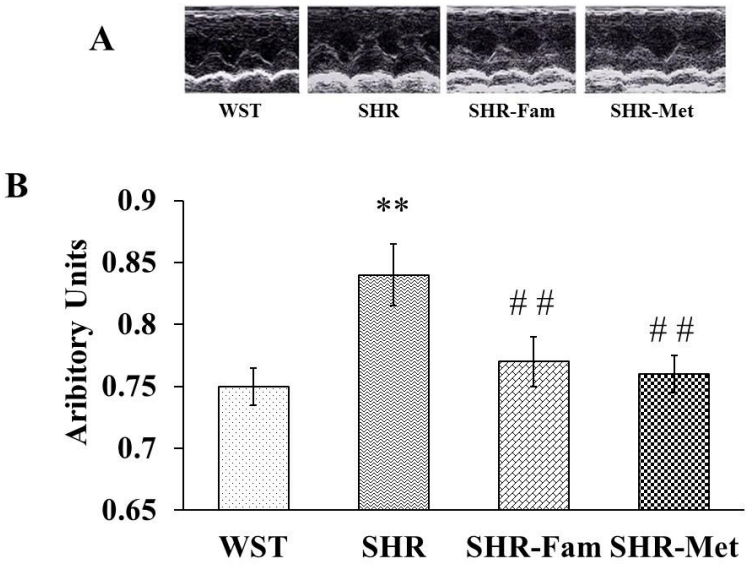

Figure no 36: Comparison of the effect of famotidine treatment on relative wall thickness as evaluated by m mode two dimensional trans-thoracic echocardiography with the metoprolol. Where A is the pictorial representation of the M mode acquisition and B is the graphical representation of the RWT in arbitory units. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and ## $p<0.01$ Vs SHR. ANOVA $p<0.01$.

IV.4.2.3. **Comparison of the effect of famotidine treatment with metoprolol on intra-cardiac dimensions as evaluated by m mode two dimensional echocardiography:**

Intra-Cardiac dimensions indicate the cardiac morphology and changes produced in response to pressure overload. Left ventricular diameter during systole and diastole as well as the septal wall thickness during systole and diastole was assessed to analyse the cardiac hypertrophy as well as degree of diastolic failure. Left ventricular diameter during systole and diastole were lower in SHR ($p<0.01$) than that of the Wistar rat in accordance to the increased wall thickness and lv mass as indicated in sections IV.4.2.1 and IV.4.2.2. Both famotidine and metoprolol treatments increased the Left ventricular diameter during systole and diastole in a similar fashion indicating the regression of hypertrophy. ($p<0.01$ Vs SHR) (Fig.).

Septal thickness was found to be higher in SHR ($p<0.01$ Vs WST) indicating the presence of the cardiac hypertrophy and as well as ruling out the involvement of hypertrophic genetic cardiomyopathy where the septal thickness remains unaltered. Septal thickness during systole was normalised identically with both the treatments ($p<0.05$ and $p<0.01$ respectively). Surprisingly the effect of treatments on septal thickness during diastole was not uniform. Metoprolol treatment ($p<0.01$) attenuated the Septal thickness during diastole whereas the famotidine treatment was not able to restore the wall morphology during diastole.

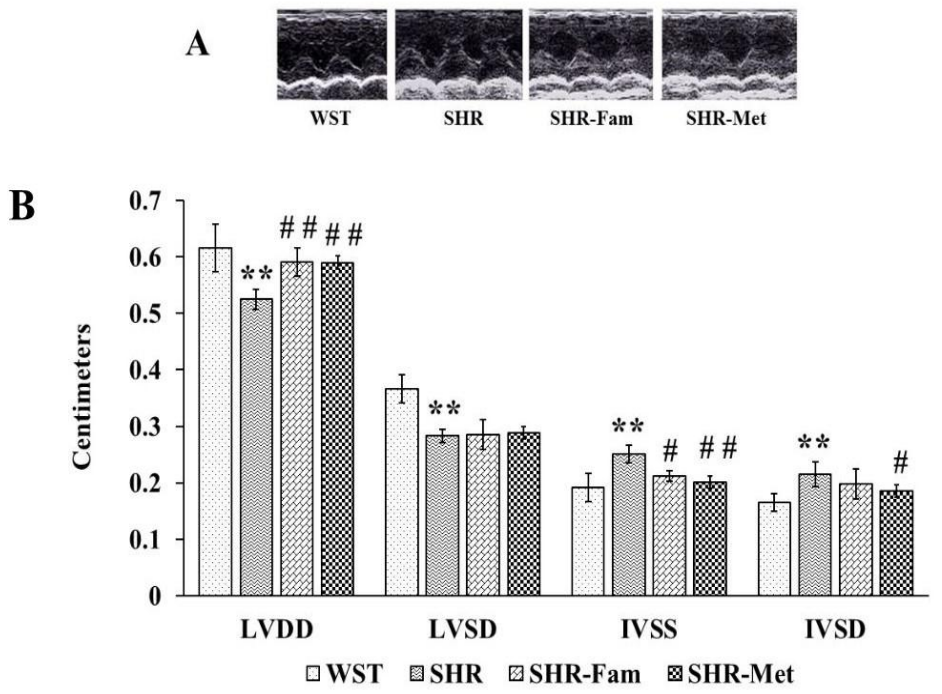

Figure no 37: Comparison of the effect of famotidine treatment with metoprolol on intra-cardiac dimensions as evaluated by m mode two dimensional Trans-Thoracic echocardiography. Where A is the pictorial representation of the m mode Trans-Thoracic two dimensional echocardiography and B is the graphical representation of intra-cardiac dimensions expressed as centimetres. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST, # $p<0.05$ Vs SHR and ## $p<0.01$ Vs SHR. ANOVA $p<0.01$. LVDD: Left ventricular Diameter during Diastole, LVSD: Left ventricular Diameter during Systole, IVSS: Inter Ventricular Septal Thickness during Systole and IVSD: Inter Ventricular Septal Thickness during Systole.

IV.4.2.4. **Comparison of the effect of famotidine treatment on cardiac hypertrophy index as evaluated by Heart weight by tibia length ratio with the metoprolol:**

Evaluation of cardiac hypertrophy index indicated the presence of cardiac hypertrophy in the SHR ($p<0.01$) when compared to that of the Wistar rat. An identical reduction in the cardiac hypertrophy index was elicited by the 60 days of sub chronic treatment with famotidine and metoprolol treatments. ($p<0.01$) (Fig.).

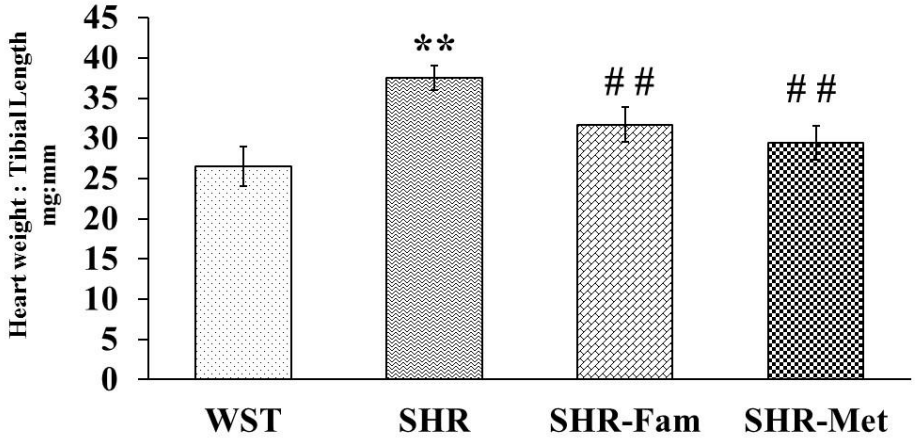

Figure no 38: Comparison of the effect of famotidine treatment on cardiac hypertrophy index as evaluated by Heart weight by tibia length ratio with the metoprolol represented as mg: mm. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and ## $p<0.01$ Vs SHR. ANOVA $p<0.01$.

IV.4.2.5. **Comparison of the effect of famotidine treatment with metoprolol treatment on Myocyte cross-sectional area as evaluated by histomorphometric analysis of haematoxylin and eosin stained tissue sections:**

Evaluation of myocyte cross-sectional area by histomorphometric analysis of haematoxylin and eosin stained tissue sections showed higher degree of hypertrophied myocytes in SHR ($p<0.01$) than that of the Wistar rat indicating the presence of cardiac hypertrophy. The myocyte cross-sectional area was identically normalised with sub chronic treatment with famotidine and metoprolol treatments. ($p<0.01$) (Fig.).

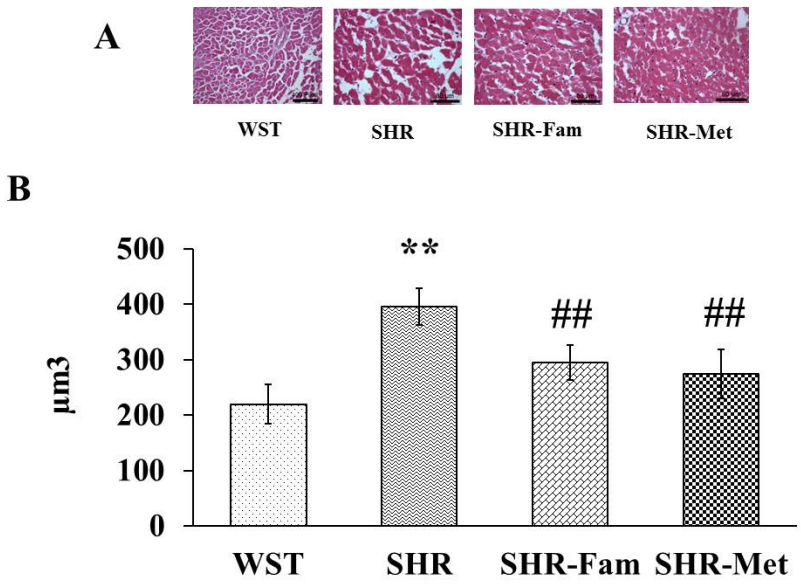

Figure no 39: Comparison of the effect of famotidine treatment on Myocyte cross-sectional area as evaluated by histomorphometric analysis of haematoxylin and eosin stained tissue sections with the metoprolol. Where A is the pictorial representation of the haematoxylin and eosin stained tissue sections and B is the graphical representation of the Myocyte cross-sectional area as μM^3. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and ## $p<0.01$ Vs SHR. ANOVA $p<0.01$.

IV.4.2.6. **Comparison of the effect of famotidine treatment with metoprolol treatment on Serum and myocardial levels of B type natriuretic peptide as evaluated with ELISA technique:**

Evaluation of circulating and myocardial BNP levels indicated the presence of hypertrophy in SHR ($p<0.01$) than that of the Wistar rat with higher levels in SHR. The myocyte cross-sectional area was identically normalised with sub chronic treatment with famotidine and metoprolol treatments. ($p<0.01$) (Fig.).

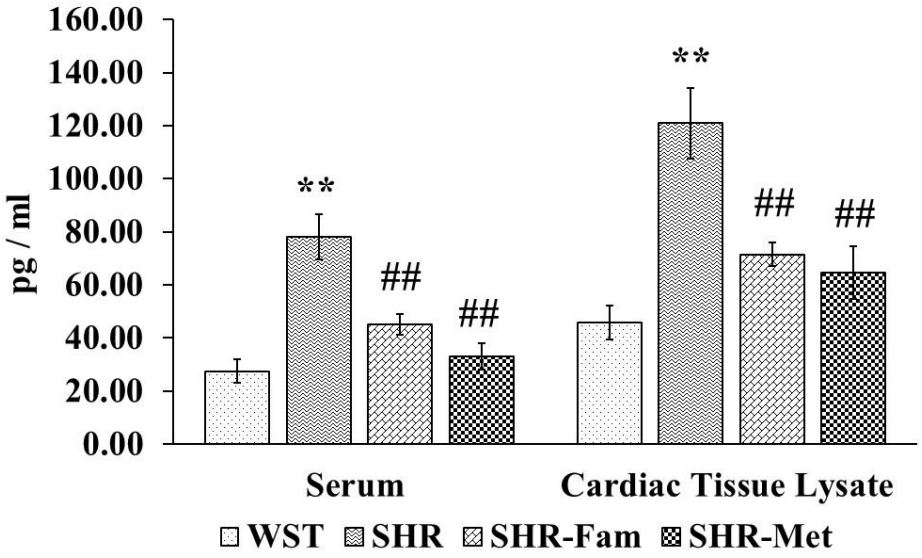

Figure no 40: Comparison of the effect of famotidine treatment on Serum and myocardial levels of B type natriuretic peptide as evaluated with ELISA technique with the metoprolol. Where A is the pictorial representation of the haematoxylin and eosin stained tissue sections and B is the graphical representation of the Myocyte cross-sectional area as μM^3. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and ## $p<0.01$ Vs SHR. ANOVA $p<0.01$.

IV.4.2.7. **Comparison of the effect of famotidine treatment on myocardial calcineurin A expression as evaluated by western blotting with metoprolol:**

Calcineurin A being a marker for cardiac hypertrophy, promotes fetal gene expression by NFAT translocation. Levels of calcineurin A expression are higher in SHR ($p<0.01$) than that of the Wistar rat indicating the active signalling cascade. Upon sub chronic treatment with famotidine and metoprolol treatments there was a reduction in the levels of expression, which was found to be comparable. ($p<0.01$ Vs SHR) (Fig.).

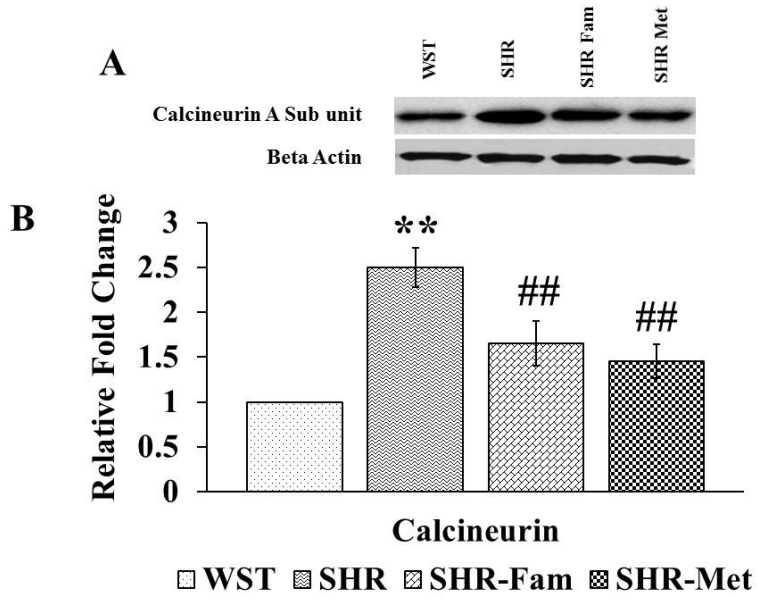

Figure no 41: Comparison of the effect of famotidine treatment with myocardial calcineurin A expression as evaluated by western blotting with metoprolol. Where A is the pictorial representation of the immunoblotted protein bands of calcineurin A as developed by electro chemiluminescence and B is the graphical representation of the myocardial calcineurin A expression represented as relative fold change. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and ## $p<0.01$ Vs SHR. ANOVA $p<0.01$.

IV.4.3. **Comparison of the Effect of treatments on cardiac systolic function.**

IV.4.3.1. **Comparison of the effect of famotidine treatment with metoprolol on End diastolic and systolic volumes as evaluated by m mode two dimensional trans-thoracic echocardiography:**

Left ventricular end diastolic and systolic volumes, the amount of blood remaining after diastole and systole respectively are the indicators of systolic function. They were mathematically calculated from the intra cardiac dimensions obtained from the M mode two dimensional trans-thoracic echocardiography.

The left ventricular End diastolic volume in SHR was significantly lower than that of WST ($p<0.01$) in agreement with the findings reported in sections IV.4.2.1, IV.4.2.2 and IV.4.2.3. This drop in the left ventricular End diastolic volume indicate the setting up of early stages of diastolic dysfunction with loss of compliance. An identical attenuation in the left ventricular end diastolic volume was observed with sub chronic treatment with famotidine and metoprolol treatments. ($p<0.01$) (Fig.).

An unaltered left ventricular end systolic volume is a hallmark feature of the pressure overload induced cardiac hypertrophy in its early stages of diastolic dysfunction indicating proper tensile strength in the myocardium. In agreement with this fact the m mode analysis of the left ventricular End systolic volume in the SHR was found to be comparable with WST. Treatment with famotidine and metoprolol could not alter the left ventricular End systolic volume.

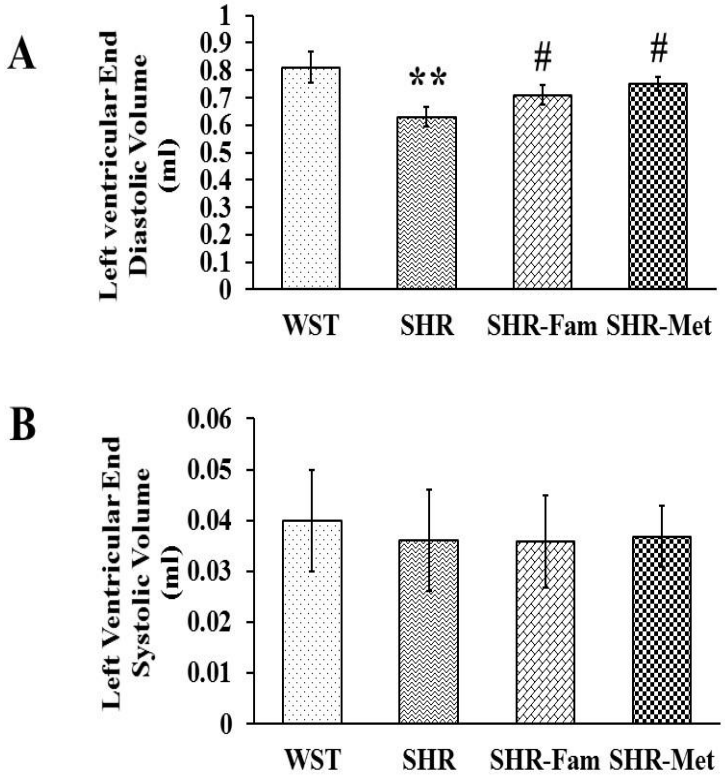

Figure no 42: Comparison of the effect of famotidine treatment with metoprolol on LVEDV and LVESV as evaluated by m mode two dimensional trans-thoracic echocardiography. Where A is the Graphical representation of the effect of famotidine treatment in comparison with metoprolol on LVEDV represented as millilitres and B is the Graphical representation of the effect of famotidine treatment with metoprolol on LVESV represented as millilitres. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and # $p<0.05$ Vs SHR. ANOVA $p<0.01$.

IV.4.3.2. **Comparison of the effect of famotidine treatment with metoprolol on Fractional Shortening and Ejection Fraction as evaluated and deducted by m mode two dimensional trans-thoracic echocardiography:**

Similar to that of left ventricular end systolic volume, an unaltered ejection fraction is a hallmark feature of the pressure overload induced cardiac hypertrophy. In agreement with this fact EF in the SHR was found to be comparable with WST and was unaltered with treatments. The fractional shortening in SHR was significantly higher than that of WST ($p<0.05$) in indicating the diastolic dysfunction. This increase in FS was unaltered with sub chronic treatment with famotidine and metoprolol treatments.

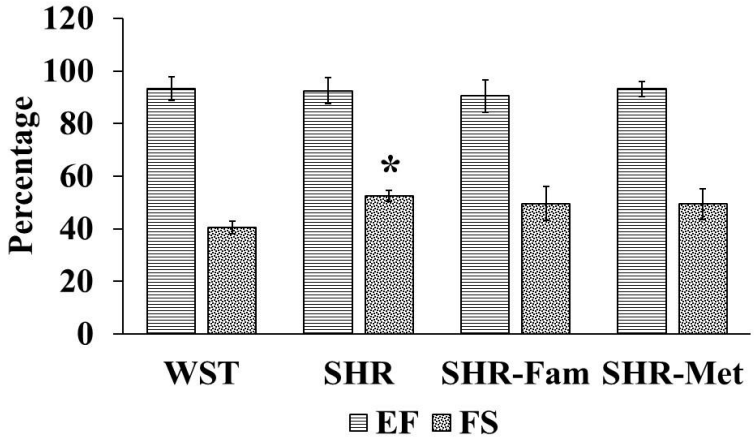

Figure no 43: Comparison of the effect of famotidine treatment with metoprolol on EF and FS as evaluated by m mode two dimensional trans-thoracic echocardiography represented as percentage. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. * $p<0.01$ Vs WST ANOVA $p<0.01$.

IV.4.4. **Comparison of the Effect of treatments on cardiac diastolic function.**

IV.4.4.1. **Comparison of the effect of famotidine treatment with metoprolol on Mitral valve kinetics:**

Pulsed Wave Doppler analysis of the mitral valve kinetics showed a decreased e wave pattern and an increased a wave pattern in SHR when compared to that of WST ($p<0.01$) indicating the presence of diastolic dysfunction. Treatment with Famotidine and metoprolol couldn't alter the e wave pattern when compared to that of SHR were as they have reduced the wave pattern in an identical manner ($p<0.05$ for FAM and $p<0.01$ for Met both compared to SHR).

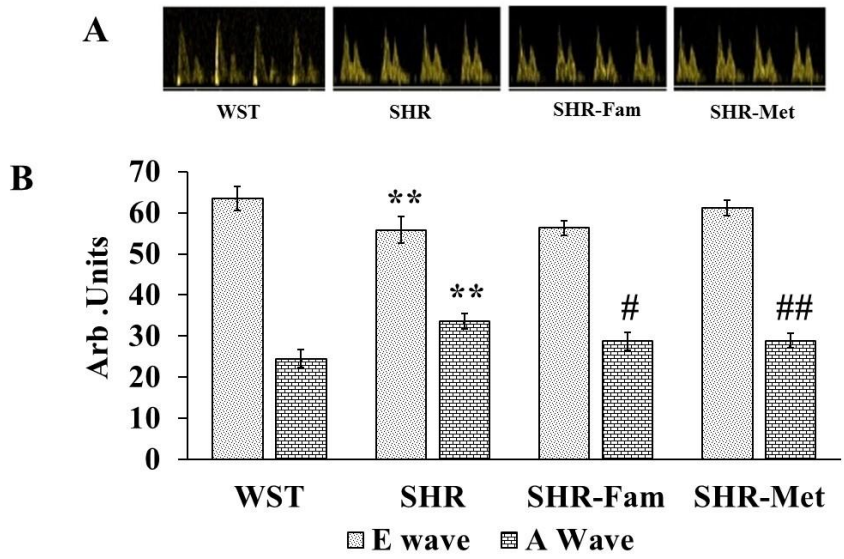

Figure no 44: Comparison of the effect of famotidine treatment with metoprolol on e wave and a wave Where A is the pictorial representation of pulsed wave Doppler and B is the Graphical representation of the effect of famotidine treatment in comparison with metoprolol on e and a waves represented as Arb units. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and # $p<0.05$ Vs SHR. ANOVA $p<0.01$.

IV.4.4.2. **Comparison of the effect of famotidine treatment with metoprolol on e/a ratio:**

Pulsed Wave Doppler analysis of the mitral valve kinetics can be represented as e/a ratio which gives a better understanding than analysing them individually. A decrease in this ratio indicates the setting up of diastolic dysfunction with and enlarged left ventricular chamber. Accordingly in SHR the e/a ratio was lower than that of WST ($p<0.01$). Treatment with Famotidine and metoprolol showed an identical increase in the e/a ratio when compared to that of SHR ($p<0.05$ for both treatments compared to SHR).

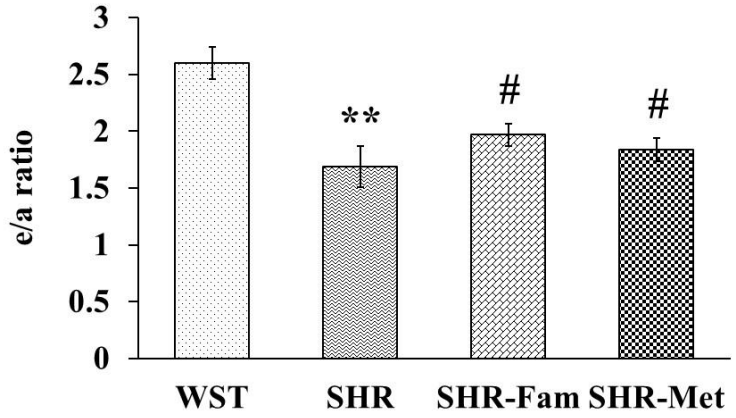

Figure no 45: Comparison of the effect of famotidine treatment with metoprolol on e/a ratio as assessed by the pulsed wave Doppler mode of two dimensional echocardiography. Represented as Arb units of ratio. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and # $p<0.05$ Vs SHR. ANOVA $p<0.01$.

IV.4.4.3. **Comparison of the effect of famotidine treatment with metoprolol on iso - volumetric relaxation time:**

Iso Volumetric relaxation time a key indication for the diastolic function is analysed from the IVRT was measured from the start of the aortic valve closure signal to the start of the mitral valve opening signal. An increase in this relaxation time indicates the loss of LV compliance with setting up of diastolic dysfunction and enlarged left ventricular chamber. Accordingly in SHR the IVRT was higher than that of WST ($p<0.01$). Treatment with Famotidine and metoprolol showed an identical increase in the e/a ratio when compared to that of SHR manner ($p<0.05$ for FAM and $p<0.01$ for Met both compared to SHR).

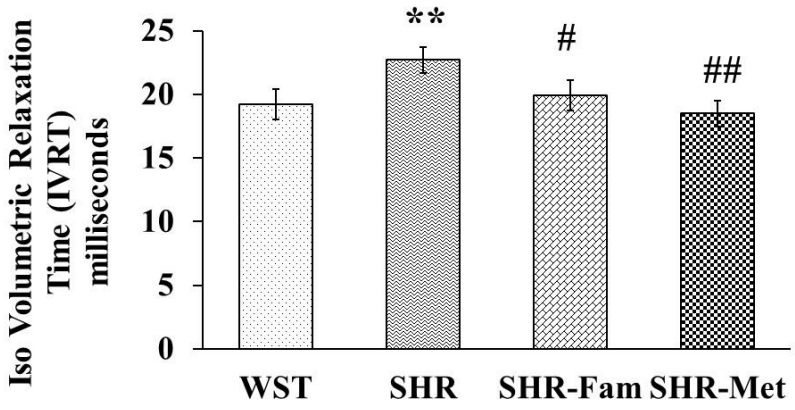

Figure no 46: Comparison of the effect of famotidine treatment with metoprolol on IVRT and represented as milli seconds. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST, # $p<0.05$ Vs SHR and ## $p<0.01$ Vs SHR. ANOVA $p<0.01$.

IV.4.5. **Comparison of the Effect of treatments on cardiac global function.**

IV.4.5.1. **Comparison of the effect of famotidine treatment with metoprolol on Tei Index:**

Tei Index is a global parameter of cardiac function, combining information of both systole and diastole and it is defined as the ratio of total isovolumic time divided by ejection time (ET). An increase in this index indicates the excessive stress the LV experiences with an augmented wall tension in response to the escalating peripheral resistance. The tei Index in SHR was higher than that of WST ($p<0.01$) indicating the augmented wall tension and higher peripheral resistance. Treatment with Famotidine and metoprolol showed an identical decrease in the Tei index when compared to that of SHR manner ($p<0.05$ for both treatments compared to SHR).

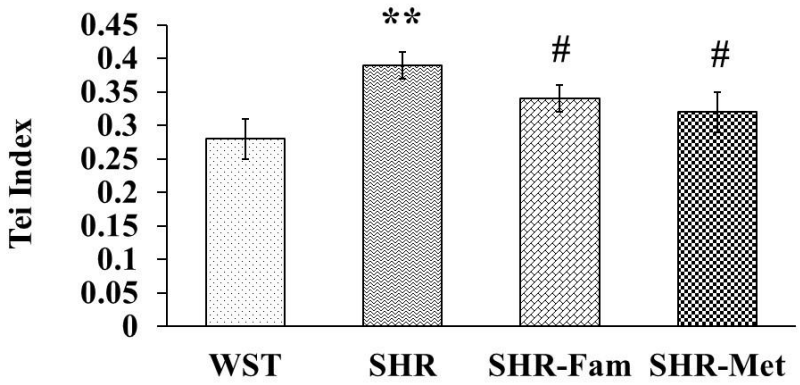

Figure no 47: Comparison of the effect of famotidine treatment with metoprolol on Tei Index and represented as Arb Units. Data is represented as mean ± SD and n=6 for each

group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and # $p<0.05$ Vs SHR. ANOVA $p<0.01$.

IV.4.5. **Comparison of the Effect of treatments on Intra cardiac Blood Dynamics.**

IV.4.5.1. **Comparison of the effect of famotidine treatment with metoprolol on Intra cardiac Pressure gradients:**

Both the Trans mitral and Trans aortic pressure gradients were analysed to find out Mean and maximum pressures. The LVOT, M mean and max of SHR were found to higher than that of WST indicating the hypertension and left ventricular hypertrophy. This was normalised with treatments. Metoprolol showed a better effect on LVOT PG mean and max indices and M PG max when compared to famotidine ($p<0.05$).

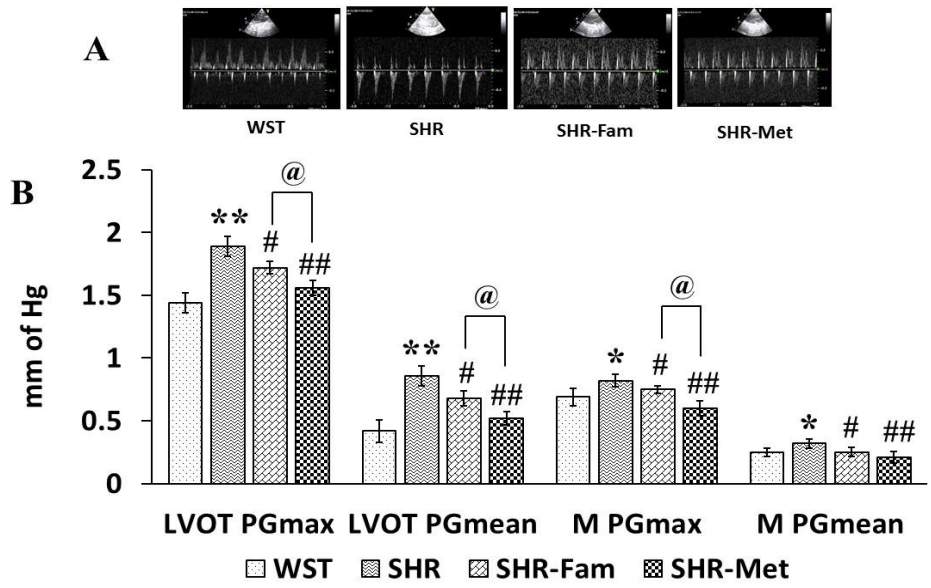

Figure no 48: Comparison of the effect of famotidine treatment with metoprolol on Intra cardiac Pressure gradients represented as mm of Hg. Where A is the graphical representation of Trans aortic pressure gradient and B is the effect of famotidine treatment with metoprolol on Intra cardiac Pressure gradients. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA

followed by Bonferroni post hoc Test. * $p<0.05$ Vs WST, ** $p<0.01$ Vs WST, # $p<0.05$ Vs SHR, ## $p<0.01$ Vs SHR and @ $p<0.05$ Vs SHR-Fam. ANOVA $p<0.01$.

IV.4.5.1. **Comparison of the effect of famotidine treatment with metoprolol on Intra cardiac Pressure velocity time integrals:**

A velocity time integral is the integral of all of the velocities during the time of flow across a valve or in a vessel. Increase in Trans-aortic velocity time integrals indicates presence of hypertension and reduction in Trans-mitral velocity time integrals indicates presence of diastolic dysfunction. In SHR LVOT V mean and max were found to higher than that of WST and M V max and mean were lower than that of WST. This was normalised with treatments. Metoprolol showed a better effect on LVOT V max when compared to famotidine ($p<0.05$).

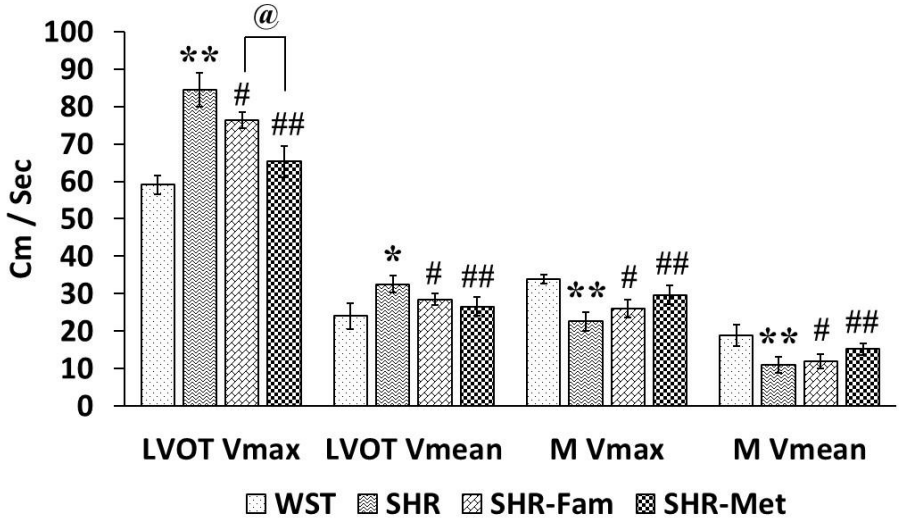

Figure no 49: Comparison of the effect of famotidine treatment with metoprolol on Intra cardiac Pressure velocity time integrals represented as Cm/Sec. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA

followed by Bonferroni post hoc Test. * p<0.05 Vs WST, ** p<0.01 Vs WST, # p<0.05 Vs SHR, ## p<0.01 Vs SHR and @ p<0.05 Vs SHR-Fam. ANOVA p<0.01.

IV.4.6. **Comparison of the Effect of treatments on Cardiac Stress marker.**

IV.4.6.1. **Comparison of the effect of famotidine treatment with metoprolol on Myocardial and serum levels of Lactate dehydrogenase activity:**

Lactate dehydrogenase activity is an indicator for the tissue stress and was known to get elevated in the left ventricular hypertrophic condition. Both the serum and cardiac tissue lysate levels of LDH were higher in SHR when compared to WST (p<0.01). An attenuation in the activity levels of LDH was observed upon treatment with famotidine and metoprolol in an identical fashion.

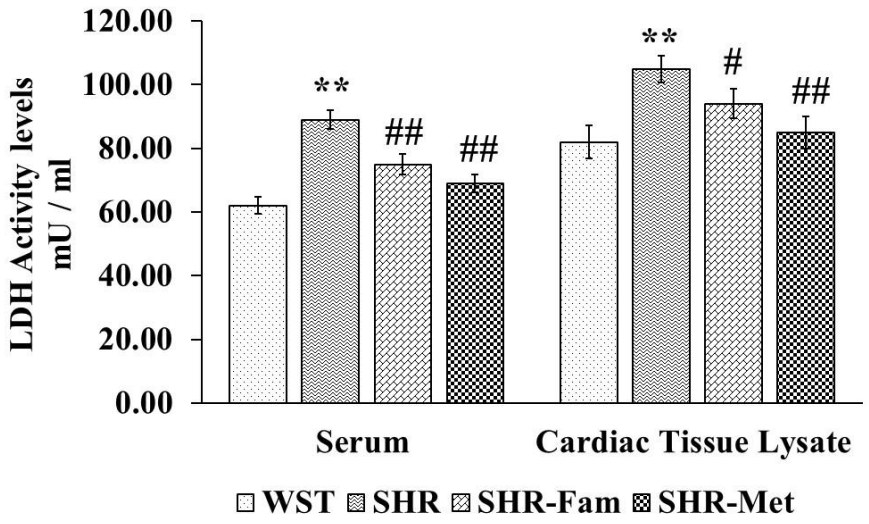

Figure no 50: Comparison of the effect of famotidine treatment with metoprolol on lactate dehydrogenase activity represented as mU/ml. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by

Bonferroni post hoc Test. ** p<0.01 Vs WST, # p<0.05 Vs SHR and ## p<0.01 Vs SHR ANOVA p<0.01

IV.4.7. **Comparison of the Effect of treatments on Cardiac Fibrosis.**

IV.4.7.1. **Comparison of the effect of famotidine treatment with metoprolol on myocardial hydroxyproline content:**

Hydroxyproline being largely restricted to collagen makes it an indicator of collagen content. The cardiac tissue lysate levels of hydroxyproline were higher in SHR when compared to WST (p<0.01) and were attenuated upon sub chronic treatment with famotidine and metoprolol in an identical fashion (p<0.01 for both Vs SHR).

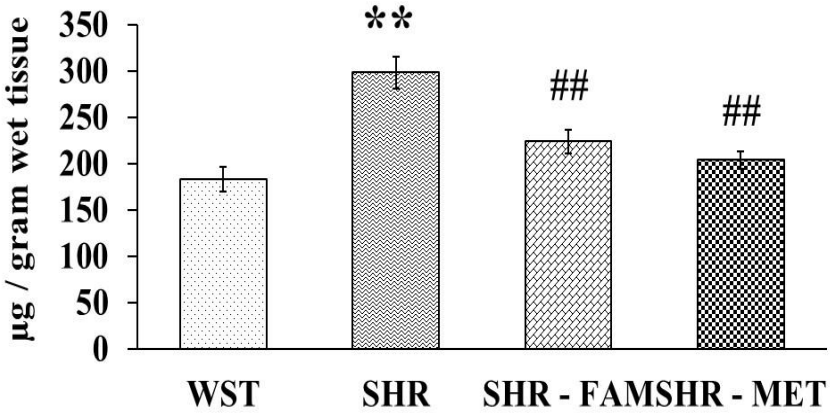

Figure no 51: Comparison of the effect of famotidine treatment with metoprolol on myocardial hydroxyproline content represented as µg/Gram wet tissue. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way

ANOVA followed by Bonferroni post hoc Test. ** p<0.01 Vs WST and ## p<0.01 Vs SHR ANOVA p<0.01.

IV.4.7.2. **Comparison of the effect of famotidine treatment with metoprolol on myocardial and serum levels of N terminal of procollagen type 1 pro fibre:**

N terminal of Procollagen type 1 pro fibre being precursor to collagen stands as an indicator of collagen turn over. The Serum and cardiac tissue lysate levels of PINP were higher in SHR when compared to WST (p<0.01) and were attenuated upon sub chronic treatment with famotidine and metoprolol with a better outcome with metoprolol treatment (p<0.05 SHR -Fam Vs SHR-Met).

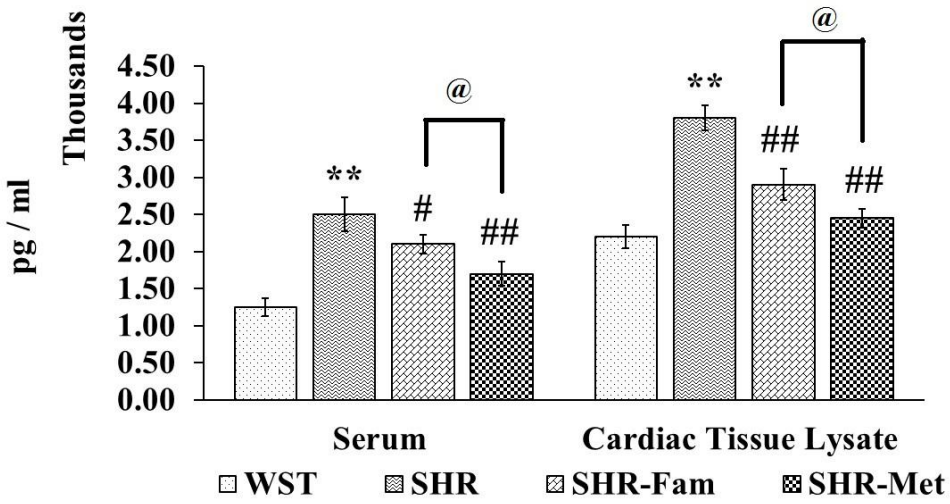

Figure no 52: Comparison of the effect of famotidine treatment with metoprolol on myocardial and serum levels of N terminal of procollagen type 1 pro fibre represented as pg/ml. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** p<0.01 Vs WST, #

p<0.05 Vs SHR, ## p<0.01 Vs SHR and @ p<0.05 SHR-Fam Vs SHR-Met ANOVA p<0.01.

IV.4.7.3. **Comparison of the effect of famotidine treatment with metoprolol on perivascular fibrosis as stained with picro-scirius red stain:**

Picro-Scirius red staining of histological sections indicated the presence of higher degree of perivascular fibrosis in SHR when compared to WST (p<0.01) and was identically attenuated upon sub chronic treatment with famotidine and metoprolol (p<0.01 for both Vs SHR).

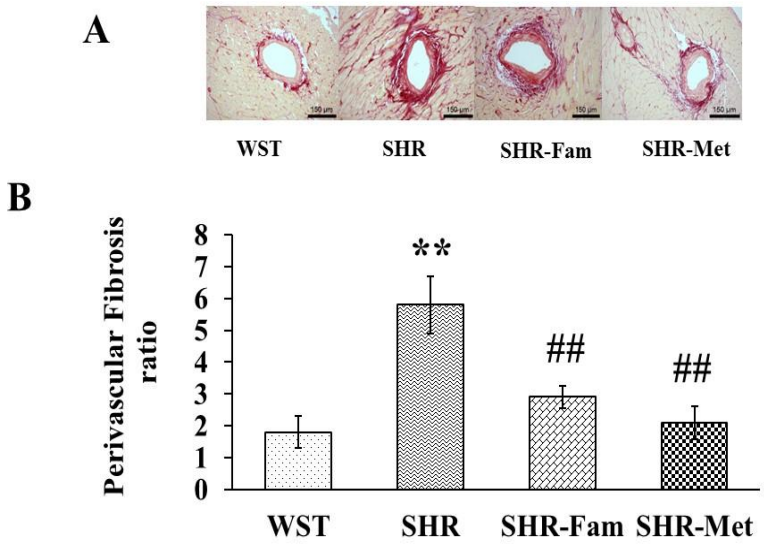

Figure no 53: Comparison of the effect of famotidine treatment with metoprolol on perivascular fibrosis as stained with picro-scirius red stain represented as Perivascular fibrosis ratio. Where A is the pictorial representation of the picro-scirius red stained histological sections and B is the graphical representation of effect of famotidine treatment with metoprolol on perivascular fibrosis. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by

Bonferroni post hoc Test. ** p<0.01 Vs WST, # p<0.05 Vs SHR and ## p<0.01 Vs SHR. ANOVA p<0.01.

IV.4.7.4. **Comparison of the effect of famotidine treatment with metoprolol on Transmural fibrosis as stained with picro-scirius red stain:**

Picro-Scirius red staining of histological sections indicated the presence of higher degree of transmural fibrosis in SHR when compared to WST (p<0.01) and was identically attenuated upon sub chronic treatment with famotidine and metoprolol (p<0.01 for both Vs SHR).

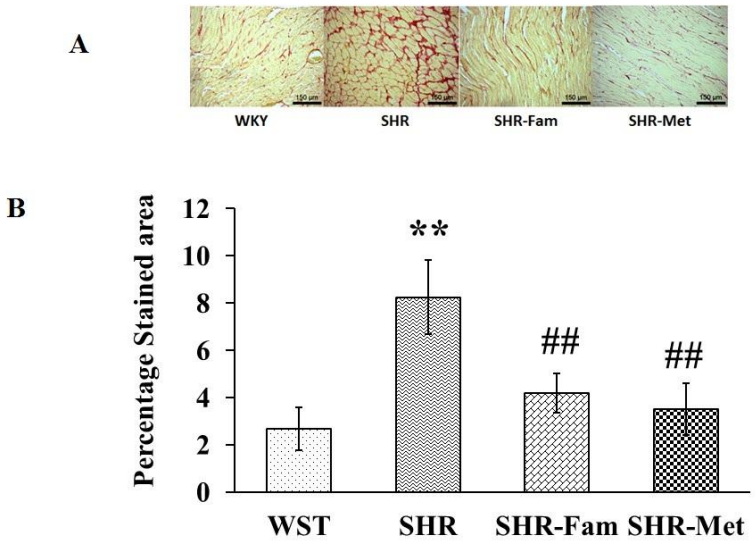

Figure no 54: Comparison of the effect of famotidine treatment with metoprolol on Transmural fibrosis as stained with picro-scirius red stain represented as Percentage stained area. Where A is the pictorial representation of the picro-scirius red stained histological sections and B is the graphical representation of effect of famotidine treatment with metoprolol on Transmural fibrosis. Data is represented as mean ± SD and

n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** p<0.01 Vs WST, # p<0.05 Vs SHR and ## p<0.01 Vs SHR. ANOVA p<0.01.

IV.4.8. **Comparison of the Effect of treatments on Cardiac Oxidative stress and Anti-Oxidant status.**

IV.4.8.1. **Comparison of the effect of famotidine treatment with metoprolol on myocardial lipid peroxidation:**

Myocardial Lipid peroxidation an indicator of oxidative stress was represented as thiobarbituric acid reacting species. The levels of the TBARS in the myocardial lysates of the SHR were found to be significantly higher when compared to WST (p<0.01). The sub chronic treatment with famotidine and metoprolol attenuated the levels of TBARS in an identical fashion (p<0.01 for both Vs SHR).

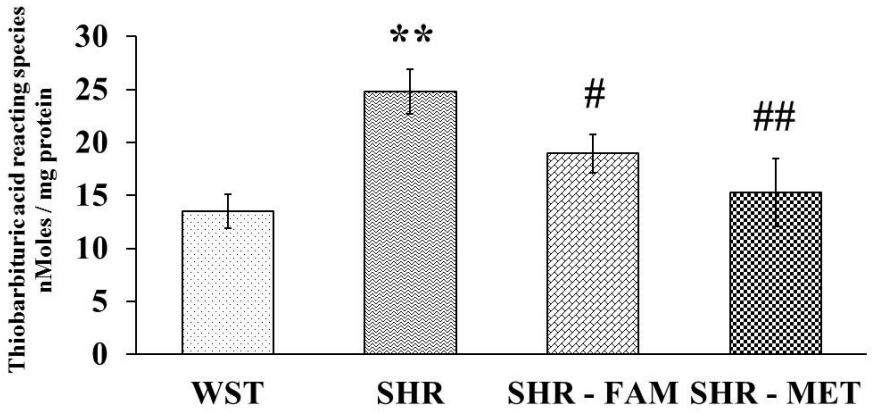

Figure no 55: Comparison of the effect of famotidine treatment with metoprolol on myocardial Lipid peroxidation content represented as nMoles of Thiobarbituric acid

reacting species / mg of protein. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST, # $p<0.05$ Vs SHR and ## $p<0.01$ Vs SHR ANOVA $p<0.01$.

IV.4.8.2. **Comparison of the effect of famotidine treatment with metoprolol on myocardial reduced glutathione levels:**

Myocardial reduced glutathione levels stands as an indicator of anti-oxidant potential of the tissue. The levels of the reduced glutathione levels in the myocardial lysates of the SHR were found to be significantly lower when compared to WST ($p<0.01$) indicating the presence of oxidative stress and decreased antioxidant reserves. The sub chronic treatment with famotidine and metoprolol upregulated the levels of Myocardial reduced glutathione levels in an identical fashion ($p<0.01$ for both Vs SHR).

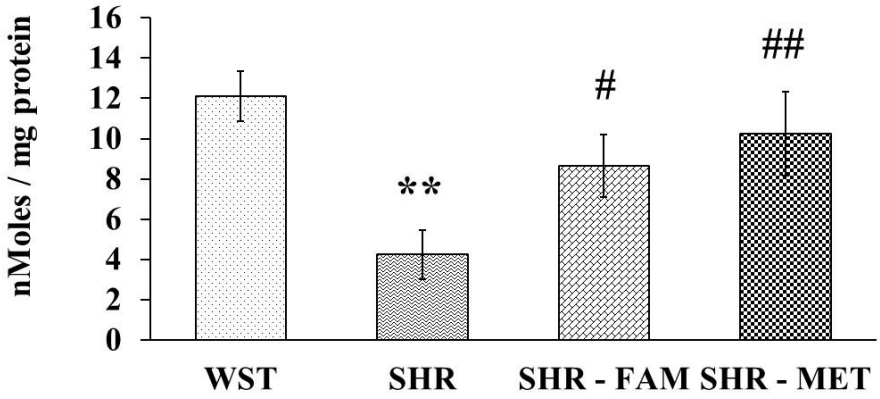

Figure no 56: Comparison of the effect of famotidine treatment with metoprolol on myocardial reduced glutathione content represented as nMoles / mg of protein. Data is

represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** p<0.01 Vs WST, # p<0.05 Vs SHR and ## p<0.01 Vs SHR ANOVA p<0.01.

IV.4.8.3. **Comparison of the effect of famotidine treatment with metoprolol on myocardial expression of Peroxiredoxin 3:**

The levels of Peroxiredoxin 3 in the myocardial lysates of the SHR were found to be significantly lower when compared to WST (p<0.01) indicating the presence of oxidative stress and decreased mitochondrial antioxidant reserves. The sub chronic treatment with famotidine and metoprolol upregulated the levels of Myocardial Peroxiredoxin 3 levels in an identical fashion (p<0.01 for both Vs SHR).

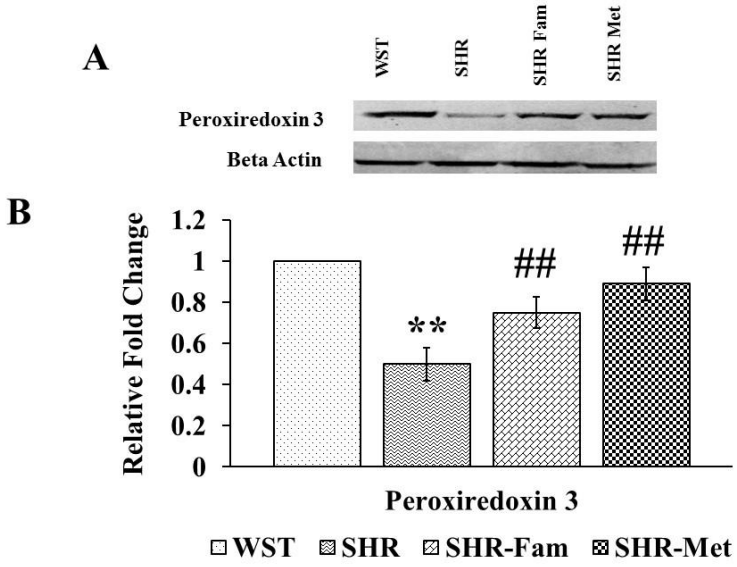

Figure no 57: Comparison of the effect of famotidine treatment with metoprolol on myocardial Peroxiredoxin 3 levels represented as relative fold change. Where A is the pictorial representation of Immunoblot analysis of Peroxiredoxin 3 and B is the graphical representation of effect of famotidine treatment with metoprolol on myocardial

Peroxiredoxin 3 levels. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and ## $p<0.01$ Vs SHR ANOVA $p<0.01$.

IV.4.9. **Comparison of the Effect of treatments on Cardiac Oxidative stress and mediators of cardiac hypertrophy.**

IV.4.9.1. **Comparison of the effect of famotidine treatment with metoprolol on myocardial Akt phosphorylation ratio:**

The rate of Akt phosphorylation in the myocardial lysates of the SHR were found to be significantly lower when compared to WST ($p<0.01$) indicating the process of active cardiac remodelling. Treatment with famotidine and metoprolol normalised the rate of Akt phosphorylation in an identical fashion ($p<0.01$ for both Vs SHR).

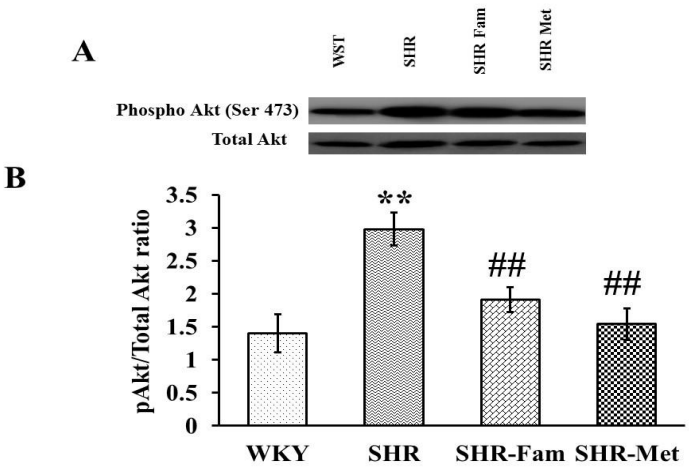

Figure no 58: Comparison of the effect of famotidine treatment with metoprolol on myocardial rate of Akt phosphorylation represented as pAKt/Total Akt ratio. Where A is the pictorial representation of Immunoblot analysis of total and phosphorylated AkT and B is the graphical representation of effect of famotidine treatment with metoprolol on

myocardial rate of Akt phosphorylation. Data is represented as mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs WST and ## $p<0.01$ Vs SHR ANOVA $p<0.01$.

IV.4.10. **Comparison of the Effect of treatments on Cardiac Histamine levels and Histamine 2 receptor expression.**

IV.4.10.1. **Comparison of the effect of famotidine treatment with metoprolol on myocardial Histamine levels:**

The myocardial histamine content in SHR were found to be higher than that of the Wistar rat ($p<0.01$). The sub chronic treatment with famotidine and metoprolol decreased the levels of Myocardial histamine in an identical fashion ($p<0.01$ for both Vs SHR).

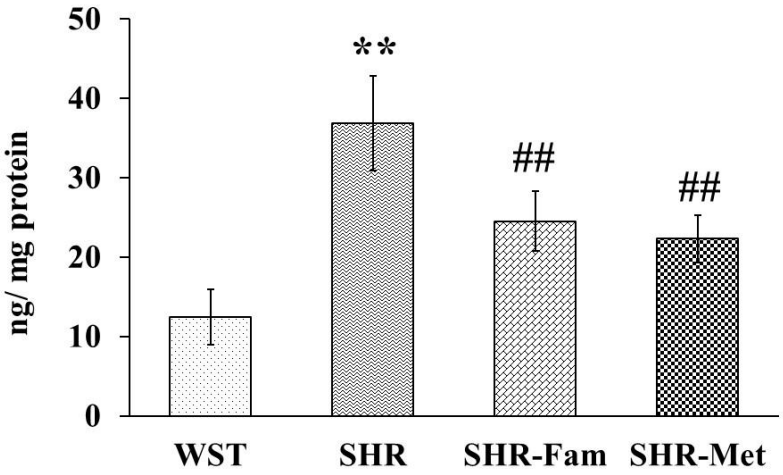

Figure no 59: Comparison of the effect of famotidine treatment with metoprolol on myocardial Histamine content represented as ng / mg of protein. Data is represented as

mean ± SD and n=6 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** p<0.01 Vs WST and ## p<0.01 Vs SHR **ANOVA p<0.01.**

IV.4.10.2. **Comparison of the effect of famotidine treatment with metoprolol on myocardial Histamine 2 receptor expression levels:**

The myocardial histamine 2 receptor expression levels in SHR were found to be higher than that of the Wistar rat (p<0.01). The sub chronic treatment with decreased the levels of Myocardial histamine 2 receptor expression levels and the metoprolol treatment couldn't alter the expression patter when compared to the SHR (p<0.01 for SHR FAM Vs SHR).

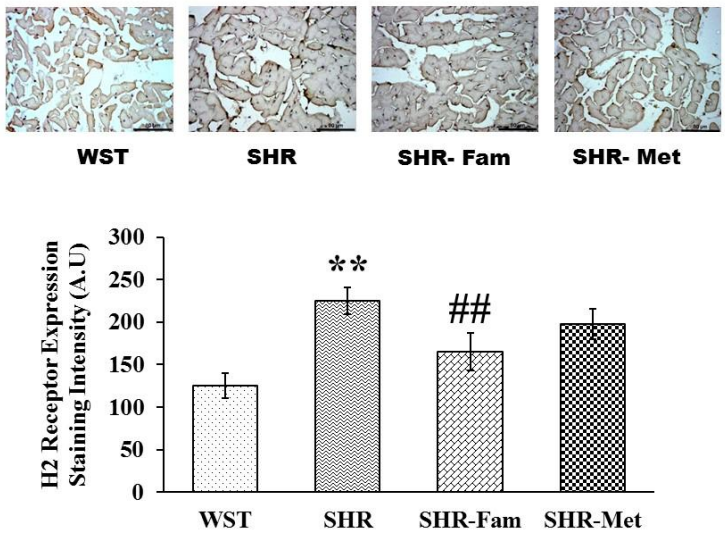

Figure no 60: Comparison of the effect of famotidine treatment with metoprolol on Histamine 2 receptor expression levels content represented as Arbiter units of Histamine 2 receptor expression staining intensity. Data is represented as mean ± SD and n=6 for

each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** p<0.01 Vs WST and ## p<0.01 Vs SHR ANOVA p<0.01

IV.5. **Delineation of the mechanism of H2 receptor stimulation in initiation of cardiac hypertrophy**.

IV.5.1. Effect of H2 receptor stimulation on ERK phosphorylation in H9c2 cardiomyoblasts:

The rate of ERK phosphorylation in H9c2 cardiomyoblasts upon exposure to Amthamine 5µM and 10 µM was found to be higher than that of the untreated (p<0.01). A similar effect was observed with the exposure to histamine 10 µM and found to be comparable with that of amthamine.

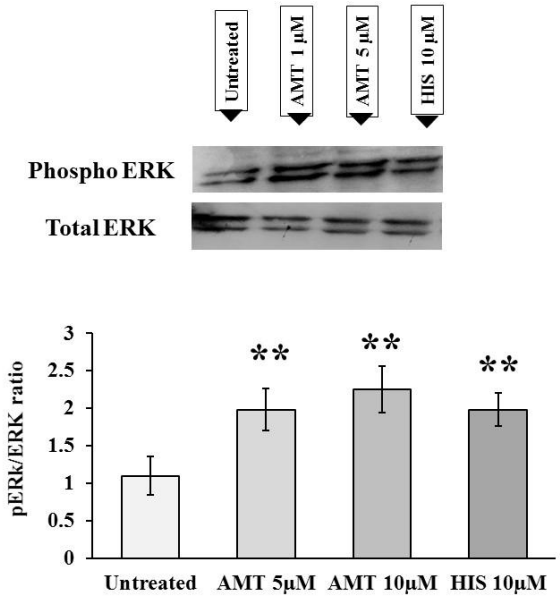

Figure no 61: Effect of H2 receptor stimulation on ERK phosphorylation in H9c2 cardiomyoblasts represented as ratio between phosphorylated and total forms of ERK. Data is represented as mean ± SD and n=3 for each group. Variation was analysed by

one way ANOVA followed by Bonferroni post hoc Test. ** p<0.01 Vs Untreated ANOVA p<0.01.

IV.5.2. Effect of H2 receptor stimulation on calcineurin A expression in H9c2 cardiomyoblasts:

The degree of calcineurin A expression in H9c2 cardiomyoblasts upon exposure to Amthamine 5µM and 10 µM was found to be higher than that of the untreated (p<0.01). A similar effect was observed with the exposure to histamine 10 µM and found to be comparable with that of amthamine.

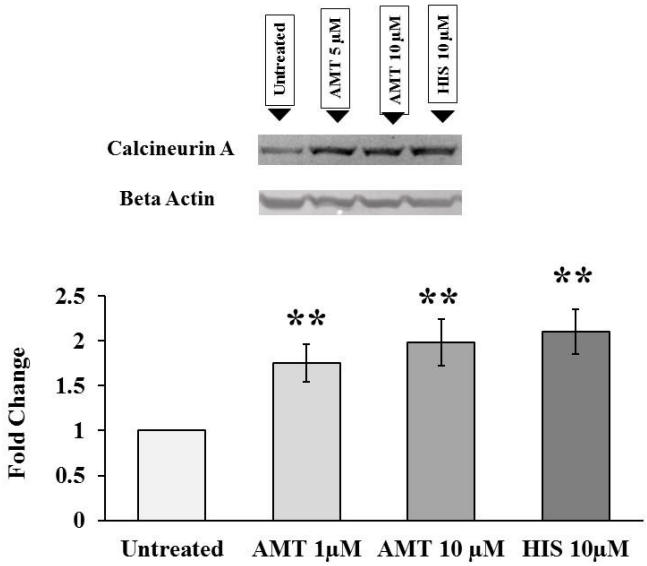

Figure no 62: Effect of H2 receptor stimulation on degree of calcineurin A expression in H9c2 cardiomyoblasts represented as fold change after normalisation with beta actin. Data is represented as mean ± SD and n=3 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** p<0.01 Vs Untreated ANOVA p<0.01.

IV.5.2. Effect of H2 receptor stimulation on rate of AkT phosphorylation in H9c2 cardiomyoblasts:

The rate of AkT phosphorylation in H9c2 cardiomyoblasts upon exposure to Amthamine 5µM and 10 µM was found to be higher than that of the untreated ($p<0.01$). A similar effect was observed with the exposure to histamine 10 µM and found to be comparable with that of amthamine.

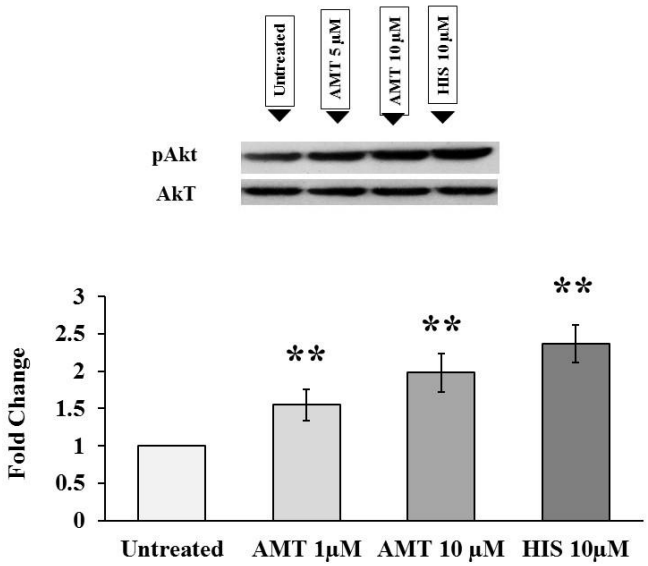

Figure no 63: Effect of H2 receptor stimulation on AkT phosphorylation in H9c2 cardiomyoblasts represented as ratio between phosphorylated and total forms of AkT. Data is represented as mean ± SD and n=3 for each group. Variation was analysed by one way ANOVA followed by Bonferroni post hoc Test. ** $p<0.01$ Vs Untreated ANOVA $p<0.01$.

V. DISCUSSION

Persistent hypertension leads to cardiac remodelling characterised by myocyte hypertrophy and fibroblast proliferation with excessive collagen deposition. Despite of the general perception of LVH as an adaptive response, it has gained an ample attention by its virtue to become an independent risk factor for adverse cardiovascular events. Presence of LVH can increase the risk of stroke, cardiac arrhythmias, coronary artery disease and sudden cardiac death (Bots et al., 2002), (Tin et al., 2002). Studies focussing on the participation of unique mediators of LVH can aid in developing novel drug targets. The role of mast cells in cardiovascular diseases is well supported by the presence of mast cells in the myocardium and increased levels of histamine in failing hearts (Panizo et al., 1995). Histamine is implicated in progression of congestive heart failure and suspected to have a role in HHD (Hara et al., 2002), (Laine et al., 1999). (Panizo et al., 1995). Stimulation of H2 receptor is known to exacerbate myocardial ischemia / reperfusion injury in rodent models (Luo et al., 2013). Pharmacological blockade of H2 receptor showed cardio protective and prevented failure independent of β-blocker therapy (Takahama et al., 2010). Knockout models of H2 receptor showed a slower attenuated rate of progression into failure with lesser degree of apoptosis and fibrosis (Zeng et al., 2014).

The theoretical evidence regarding usage of H2 receptor antagonists in management of cardiovascular diseases was reported by retrospective and prospective randomized study published in early 2006 in lesser number of subjects (Kim et al., 2006). It found that inclusion of H2 receptor antagonists into the conventional therapy for congestive heart failure can have a better clinical outcomes when compared to their control. In a Multi-Ethnic Study of Atherosclerosis (MESA), H2 receptor antagonist usage was found to be associated with less age-related changes in Left ventricular morphology and reduced risk

for incident heart failure (Leary et al., 2016). With this inadequate information regarding the role of H2 receptor in LVH, The present study is aimed to establish the possible association between histamine and H2 receptor with the progression of adverse cardiac remodelling while investigating the outcome of Histamine 2 receptor antagonism in precluding the LVH progression using famotidine, a histamine 2 receptor antagonist. The equipotency of this novel therapeutic strategy was compared with the conventional beta blocker therapy.

Spontaneously Hypertensive Rat (SHR) is a genetic model of LVH which mimics the progression of human essential hypertension (Berry et al., 2007). The pathological process of the LVH progression in SHR can be divided into a log phase from 2-6 months followed by a plateau or stable phase from 6-12 months and a rapid decompensation phase resulting in failure (Purushothaman et al., 2011). The log phase of SHR mimics the adaptive cardiac remodelling with a preserved ejection fraction, whereas the stable phase undergoes rapid molecular adaptations with minimal incidence of morphological and structural changes. Studies have shown that the rapid molecular alterations that undertake in this stable phase of LVH may be key for its progression into failure (Berry et al., 2007). This stands as the rationale behind selecting the stable phase SHR (6months old) for performing the pharmacological testing.

V.1. **Association between the myocardial histamine and H2 receptor expression with the progression of LVH resultant of chronic pressure overload:**

In agreement with the clinical findings inferring the possible association, the study reported that levels of myocardial histamine in SHR was significantly higher than that of WST (Figure no 25). Correspondingly, the degree of H2 receptor expression was also

found to be higher in SHR (Figure no 24) indicating the possible association of both Histamine and H2 receptor with hypertension induced LVH. Further confirming this association there was an age related increase in the levels of Histamine and H2 receptor expression in SHR while these indices remained unaltered in WST (Figure no 26) and (Figure no 27).

V.2. **Cardiovascular response to H2R antagonist in stable phase of left ventricular hypertrophy:**

Increased myocyte mass and reactivation of the fetal gene program, enabling adaptive remodelling are the characteristic features of the cardiac hypertrophy. Brain Natriuretic Peptide (BNP), a key consequence of fetal genes activation, is released in response to stretch and cleaved into an N-terminal peptide fragment and the active peptide. The active form of BNP bind to the natriuretic peptide receptor B and causes vasodilation and natriuresis. Plasma and myocardial levels of natriuretic peptides are higher in concentric hypertrophy and correlate with its geometry and progression into failure (Kohno et al., 1995), (Nishikimi et al., 1996). Morphological and histological markers of LVH namely Hypertrophic index and myocyte cross sectional area can be employed for assessing the degree of hypertrophy (Schubert et al., 2001).

In an endeavour to evaluate the cardiovascular response to H2R antagonist in stable phase of left ventricular hypertrophy, it was observed that the Mean arterial blood pressure and the diastolic blood pressures were normalised with an unaltered systolic blood pressure (Figure no 28). This stabilisation of the blood pressure was paralleled as attenuation in the left ventricular mass and relative wall thickness as evaluated by 2D echocardiography (Figure no 29), (Figure no 30). Further endorsing these findings the

cardiac hypertrophy index, myocyte cross sectional area and the serum and myocardial levels of BNP were also found to be normalised with the famotidine treatment (Figure no 31),(Figure no 32) and (Figure no 33). These cluster of inter-dependable observations confirmed the association of myocardial histamine with the LVH progression. Attenuation of the LVH with the famotidine treatment in SHR, indicates that the hypertrophic responses of histamine are mediated through Histamine 2 receptor.

V.3. **Comparison of the cardiovascular response elicited by H2R antagonism with the conventional beta blocker therapy:**

The cardiovascular vascular effects produced by famotidine in the stable phase of hypertrophy were then compared with the metoprolol treatment in another set of experiment with an objective of evaluating the equipotency of this novel therapeutic strategy. Receptor structural homology and similarities in the signal transduction patterns between the Beta adrenoceptor and histamine 2 receptor, stands as an rationale behind comparing the treatment outcome with beta blocker therapy (Gantz et al., 1991), (Del Valle and Gantz, 1997) (Kooistra et al., 2013). Unlike in the previous experiment were only the fundamental markers of LVH were analysed, this experiment was designed to study crucial aspects of LVH with the analysis of functional outcome.

Metoprolol, being an antihypertensive agent, could able to normalise the three components of blood pressure. In agreement with the results produced in the section V.1., famotidine reduced the mean and diastolic blood pressures without altering the systolic pressure identical to that of metoprolol treatment (Figure no 34). The antihypertrophic effect of famotidine was found to be similar to that of metoprolol as evident from the left ventricular mass, relative wall thickness, cardiac hypertrophy index,

myocyte cross sectional area and BNP levels (Figure no 35), (Figure no 36),(Figure no 38), (Figure no 39) and (Figure no 40). In addition to these variables, the effect of the treatments on calcineurin A expression was also evaluated. Calcineurin A is a calcium and calmodulin dependent serine/threonine protein phosphatase which promotes the nuclear translocation of nuclear factor of activated T cell (NFAT) by dephosphorylation. This in turns switches on the fetal gene program causing the LVH progression. Multiple preclinical models including, exercise, hypertension, mineralocorticoid and neuroendocrine mediated LVH were each associated with increased cardiac calcineurin activity (Molkentin et al., 1998) and (Eto et al., 2000). Stimulation of neonatal rat cardiac myocytes with isoproterenol caused an increase calcineurin A activity with higher rate of NFAT nuclear translocation. This effect was blocked with metoprolol administration in both invitro and in vivo models (Morimoto et al., 2001). This phenomenon was also observed in your study with a lesser degree of calcineurin A expression and interestingly reproduced by antagonising H2 receptor with famotidine in an identical fashion (Figure no 41). This observation indicates the ability of H2 receptor antagonists similar to beta blockers in normalising the calcium overload induced calcineurin A expression and activation by which they prevent the LVH induction and progression. The indecorous intra-cardiac dimensions as seen in SHR were also normalised with beta blocker therapy and H2 receptor antagonism in an undistinguishable manner (Figure 37).

The systolic function in hypertensive heart disease remains unaltered until the decompensation and dilatation succeeds. Similarly, the EF, and ESV were found to be identical and was unaltered with the treatments (Figure no 42) and (Figure no 43). Nonetheless the FS was higher in SHR when compared to WST and remained

unresponsive to the treatments (Figure no 43). The diastolic function is the key aspect to be reviewed in the subjects with hypertension and LVH. Although LVH starts as an adaptive modification with a preserved systolic and diastolic functions, the extensive cardiac fibrosis persuades the diastolic dysfunction due to loss of LV compliance (Burlew and Weber, 2002). End diastolic volume is the crucial determinant of diastolic function which was found to be hampered in SHR indicating the compromised LV dilation. This reduction in EDV can be further assessed by the early and late (E, A) mitral inflow velocities. The E wave corresponds to the blood entering into the LV during the diastole and A wave represents the late filling atrial kick. Significant differences were observed in E, A velocities and E/A ratios in SHR when compared to WST signifying the presence of diastolic dysfunction. Although E velocity values were comparable the decrease in A velocities and increase in E/A ratio in Metoprolol and Famotidine treated animals suggests improvement in diastolic function (Figure no 43) (Figure no 45). The Hypertension Genetic Epidemiology Network (HyperGEN) Study considered the Iso Volumetric relaxation time as a crucial determinant for analysing diastolic function (Bella et al., 2001). Impairment in IVRT (Longer) indicate the presence of diastolic dysfunction and compromised relaxation. In agreement with the presence of excessive cardiac fibrosis as discussed above, this study found that SHR presents a higher IVRT indicating the compromised relaxation which was rectified identically by the famotidine and metoprolol treatments further supports improvement in diastolic function (Figure no 46).

Concocting the systolic and diastolic functions, the myocardial performance index (Tei index) stands as the indicator for the global cardiac function independently of heart rate. Although the Tei index corresponds to the proficient functioning of the heart, it also

indicates the degree of mechanical stress the myocardium undergoing (Masugata et al., 2009). The higher Tei index values observed in SHR indicates the normal systolic function and the strenuous mechanical stress experienced by the myocardium due to the heightened peripheral resistance. Upon treatment with Famotidine and metoprolol the reduction in Tei index suggests the possibility of reduced stiffness, improved relaxation and improved global myocardial performance with the treatments (Figure no 47).

Heart guides the blood through the atria to ventricles and pumps it into the aorta, fleeting through various valves, with a well synchronised mechanical contraction. This course of intra-cardiac blood flow causes development of intra-cardiac pressures and incorporates the pressure gradients while transiting through the valves. Even though, a little is known regarding the contribution of these pressure gradients, reports specify that they can play a very crucial role in cardiovascular pathology. In the instance, the extreme aortic jet resultant of elevated trans aortic pressure gradient causes the aortic dissection and fibrotic coronary ostium (Movsowitz et al., 2000). In cardiovascular diseases like HHD with LVH, where the pressure inside the blood vessels as well as in the cardiac chambers are higher than the normal levels, examining the intra cardiac pressure gradients is defensible. The mean and max trans-aortic and trans-mitral pressure gradients in SHR where higher than the WST indicating the existence on intra cardiac stress. Treatment with metoprolol and famotidine has significantly tempered the elevated pressures gradients with a better outcome with beta blocker (Figure no 48). A velocity time integral (VTi) is the integral of all of the velocities during the time of flow across a valve or in a vessel denoting the speed with which the blood travels. This variable is sensitive to diastolic performance as well as the volume of blood in the atria. Higher trans-aortic Vmax and V mean as seen in SHR denotes the presence of higher peripheral resistance

while the lower trans-mitral Vmax and V mean denotes the impaired diastolic relaxation. Metoprolol showed a better recover in trans-aortic Vmax than famotidine, while the other variables were normalised to similar extent by the treatments (Figure no 49).

Cardiac fibrosis is an integral feature of hypertrophy, characterised by excessive deposition of collagen by fibroblasts. Excessive ECM deposition by the rapidly dividing fibroblasts leads to loss of LV compliance resulting in diastolic dysfunction (Burlew and Weber, 2002). Therapeutic strategies targeting the cardiac fibrosis may be useful in prevention of diastolic dysfunction and LVH progression into failure (Díez, 2007), (Fan et al., 2012) and (Tao et al., 2014). Despite of discrepant reports regarding the ability of metoprolol in reducing cardiac fibrosis, Chan et al observed that, chronic metoprolol administration to SHR was associated with decreased cardiac fibrosis(Chan et al., 2011). Appreciating the prominence of cardiac fibrosis in LVH, the collagen content of myocardium was assessed by measurement of myocardial and serum levels of myocardial hydroxyproline content, of pro collagen type 1 fibre levels were analysed and confirmed histologically by Picro-Sirius red staining. An attenuation in these markers of cardiac fibrosis was observed with the famotidine treatment similar to that of metoprolol indicating the involvement of histamine in the stromal expansion and excessive collagen deposition (Figure no 51), (Figure no 52), (Figure no 53) and (Figure no 54).

The experimental models of hypertrophy have reported increased activity of lactate dehydrogenase (LDH) as well as elevated efflux of lactate from the hypertrophied myocardium(York et al., 1976). In support to this, experiments with the Langendorff perfused rat heart showed that epinephrine increases the release of the lactate dehydrogenase (LDH) and induces myocardial cellular damage (Wheatley et al., 1985).

Studies have shown the involvement of apoptosis in myocardial hypertrophy as a process of transition into failure, which causes the myocyte slippage and transformation into dilated phenotype (Empel and Windt, 2004). Studies have found the coexistence of apoptosis with the elevated levels of LDH activity in the preclinical models of cardiac hypertrophy (Sheng et al., 2007) and (Fu et al., 2007). Goteborg Metoprolol Trial showed that acute MI subjects with metoprolol treatment showed a better normalisation of LDH activity when compared to the diuretics and ACE inhibitors (Herlitz et al., 1983) and (Herlitz et al., 1984). In accordance with these findings, this study also found an elevated levels of LDH activity in SHR which was identically normalised with the metoprolol and famotidine treatments.

Substantial evidences suggest the involvement of oxidative stress in mediating cardiac hypertrophy, including upregulation of fetal genes, hyperplasia of cardiac fibroblasts and increased collagen deposition (Seddon et al., 2007). Oxidative stress activates calcineurin and suppresses 5' AMP-activated protein kinase mediated cytoprotective pathways (Choudhary et al., 2006), (H He et al., 2014),(Jun et al., 2011). It is also reported that oxidative stress is implicated in PI3K/Akt pathway mediated myocyte apoptosis (Jun et al., 2011). In this study the oxidative Stress in SHR was higher when compared to WST and treatment with famotidine and metoprolol significantly attenuated lipid peroxides and preserved reduced glutathione levels (Figure no 55), (Figure no 56). The observations of this study are in agreement with the previous findings, supporting the antioxidant potential of metoprolol (Rizzi et al., 2014), (Arumanayagam et al., 2001). The antioxidant potential of famotidine was reported in non-cardiac tissues like liver, brain and gastric mucosa (Ahmadi et al., 2011), (Kesiova et al., 2006). The present study

found a significant attenuation in myocardial oxidative stress upon treatment with famotidine comparable to that of metoprolol.

Peroxiredoxins are thioredoxin based antioxidant proteins, are widely present in myocardium. Peroxiredoxin 3 is mitochondria specific and studies with transgenic mice overexpressing Peroxiredoxin 3 showed the lessened tendency of LV remodelling and progression into failure (Matsushima et al., 2006). Peroxiredoxin 3 levels in SHR are reported to be lower than that of Wistar rat, and is found to be involved in enhancing oxidative stress (Tanito et al., 2004). In accordance to this this study also found that a significant reduction in Peroxiredoxin 3 levels in SHR, which was recovered upon treatment with Metoprolol and famotidine (Figure no 57). This cardiac vascular effects of famotidine might be the at least in a part a consequence of reduction in oxidative stress in the hypertrophied myocardium and may be the key determinant for its pharmacological action.

Mediators of hypertrophy trigger a cascade of signalling pathways, which actively turns on the hypertrophic gene expression. Phosphatidylinositol 3-kinase (PI3K)/Akt (PKB) pathway is one of the prominent pathways by which hypertrophic mediators induce hypertrophy. Phosphatidylinositol 3-kinase (PI3K)/Akt pathway upon activation leads to cardiac hypertrophy and increased collagen deposition (Taniyama et al., 2005). Even though Akt signalling pathway is supposed to impart resistance against apoptosis and myocyte death (Fujio et al., 2000), sustained activation of Akt pathway is associated with maladaptive hypertrophy and failure (Taniyama et al., 2005). Elevated levels of Akt phosphorylation was found to be associated with adverse outcome in several models of experimental and genetic hypertension (Soesanto et al., 2009). In a Hypertrophied heart,

myocyte experiences beta adrenergic receptor mediated activation of calcineurin, one of the key mediators of cardiac hypertrophy (Fu et al., 1999), (Lunde et al., 2011). This study has shown that the levels of Akt phosphorylation in SHR are significantly elevated when compared to normotensive controls, indicating their role in modulating hypertrophy and fibrosis. The increase in Akt phosphorylation and calcineurin levels were significantly attenuated by metoprolol and Famotidine treatments suggesting the possible involvement of phosphatidylinositol 3-kinase (PI3K)/Akt mediated pathways (Figure no 58).

The myocardial levels of histamine and the degree of histamine 2 receptor expression post treatment was also analysed. The objective behind estimating myocardial levels after the treatment was to ensure the prevention of Histamine 1 receptor mediated arrhythmias due to accumulating histamine (Wolff and Levi, 1986). The elevated levels of histamine in SHR were brought down by famotidine and metoprolol treatments in a comparable manner. The autoregulation of the mast cell degranulation by the H2 receptor is well reported and antagonising the H2 receptor is linked up with mast cell stabilization and prevention of degranulation (Hogan et al., 1995). As a consequence of this, the myocardial histamine content was found to be lesser post treatment in famotidine group. Interestingly the levels of myocardial histamine were also found to be lesser with metoprolol treatment which might be consequent to reduction in the oxidative stress which can cause the lipid peroxidation of mast cells and induces degranulation (Figure no 59) (Meléndez et al., 2010). The unique feature of H2 receptor antagonists is to upregulate the receptor density in the early phases of exposure followed by the downregulation and internalisation (Del Valle and Gantz, 1997). In agreement with this

concept, the H2 receptor expression was also found to be decreased upon treatment with famotidine and was unaltered with metoprolol (Figure no 60).

V.4. **Delineation of the mechanism of H2 receptor stimulation in initiation of cardiac hypertrophy:**

As previously discussed calcineurin plays a crucial role in pathological remodelling by promoting NFAT nuclear translocation. The other major factor that contributed for promoting the LVH is ERK MAPK pathway. Catecholamine are known to promote ERK activation in a calcineurin dependent manner establishing a cross talk between these two crucial pathways (Zou, Hiroi, et al., 2001), (Zou, Yao, et al., 2001). More over activation of calcineurin mediated pathways in the mast cells as a part of histamine mediated mast cell degranulation also points out at the role of histamine in activating the calcineurin dependent pathways (Harrison et al., 2007). Both Calcineurin and ERK MAPK pathways are known to cross activate Akt dependent pathways in cardiac hypertrophy setup (O'Neill and Abel, 2005). In the light of these findings when the H9c2 cardiomyoblasts were exposed to H2 receptor specific agonist amthamine at a dose of 5 and 10 µM and Histamine a dose of 10 µM produced an dose dependent increase in the expression of calcineurin (Figure no 62). The rate of phosphorylation of ERK as well as AkT were also found be increased dose dependently upon stimulation of H2 receptor in agreement with the expression patterns of calcineurin (Figure no 61) (Figure no 63). These finding indicate the active participation of calcineurin, AkT and ERK MAPK pathways in histamine 2 receptor mediated cardiac hypertrophy. These pathways may be contributing in an independent manner or they may be working in coordination to promote the cardiac hypertrophy.

VI. SUMMARY AND CONCLUSION

Chronic hypertension leads to adverse cardiac remodelling characterised by myocyte hypertrophy and fibroblast proliferation with excessive collagen deposition. Despite the general perception of LVH as an adaptive response, it has gained ample attention as an independent risk factor for adverse cardiovascular events. Studies focussing on identification of unique mediators of LVH can aid in developing novel drug targets. The role of mast cells in cardiovascular diseases is supported by the presence of mast cells in the myocardium and increased levels of histamine in failing hearts. Histamine is implicated in progression of congestive heart failure and is suspected to have a role in HHD and stimulation of H2 receptor is known to exacerbate myocardial ischemia. Pharmacological blockade of H2 receptor showed cardio protective effect and prevented failure independent of β-blocker therapy. Knockout models of H2 receptor showed a slower attenuated rate of progression into failure with lesser degree of apoptosis and fibrosis.

The advantage of using H2 receptor antagonists in management of cardiovascular diseases was reported by retrospective and prospective randomized study and the Multi-Ethnic Study of Atherosclerosis (MESA), where H2 receptor antagonist usage was associated with less age-related changes in Left ventricular morphology and reduced risk for incidental heart failure. With this background information regarding the role of H2 receptor in LVH, the present study aimed to establish the possible association between histamine and H2 receptor with the progression of adverse cardiac remodelling while investigating the outcome of Histamine 2 receptor antagonism in precluding the LVH progression using famotidine, a histamine 2 receptor antagonist. The equipotency of this novel therapeutic strategy was compared with the conventional beta blocker therapy.

Spontaneously Hypertensive Rat (SHR) is a genetic model of LVH which mimics the progression of human essential hypertension. The pathological process of the LVH progression in SHR can be divided into a log phase from 2-6 months followed by a plateau or stable phase from 6-12 months and a rapid decompensation phase resulting in failure. The log phase of SHR mimics the adaptive cardiac remodelling with preserved ejection fraction, whereas the stable phase undergoes rapid molecular adaptations with minimal incidence of morphological and structural changes. Studies have shown that the rapid molecular alterations that prevail in this stable phase of LVH may be key for its progression into failure. This stands as the rationale behind selecting the stable phase of SHR (6months old) for performing the pharmacological testing.

The significant findings of the present study are

1. H2R expression and histamine levels in the myocardium were found to be more in SHR when compared to WST.
2. H2R expression, histamine levels and TNF alpha expression were found to increase with age in SHR where as it remained unaltered in WST.
3. Stimulation of H2R with AMT induced AkT and ERK phosphorylation and Calcineurin A expression.
4. Improvement in the cardiovascular function was observed in SHR with FAM treatment with rectified intra cardiac pressure gradients.
5. Morphological regression of hypertrophy was evident from cardiac hypertrophy index, LV mass and relative wall thickness in SHR treated with FAM
6. Histological indications of regression of hypertrophy was evident from Myocyte cross-sectional area, cardiac fibrosis in SHR treated with FAM

In conclusion, targeting histamine-2 receptor for prevention of cardiac remodelling in chronic pressure overload is an unexplored area. The observations of this study indicate that histamine-2 antagonist famotidine reduces cardiac hypertrophy and preserves cardiac function in spontaneously hypertensive rat. This provides a viable option for preventing cardiac remodelling, significantly in patients where beta blocker is contraindicated. It can also be used as an adjuvant medication along with conventional therapies.

The highlight of the study is repurposing the use of H2receptor antagonist as a cardioprotective agent

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VIII. PUBLICATIONS

Publications:

1. Ajay Godwin Potnuri, Lingesh Allakonda, Arulvelan Appavoo, Sherin Saheera, Renuka R. Nair, Targeting histamine-2 receptor for prevention of cardiac remodelling in chronic pressure overload, Int. J. Card, January 1, 2016Volume 202, Pages 831–833.
2. Famotidine prevents hypertension induced Histamine-2 receptor mediated progressive cardiac remodelling as effectively as metoprolol – communicated.

Conference proceedings:

1. Prevention of progressive cardiac remodelling by histamine 2 receptor antagonism – A novel approach, Abstract page no 51, Indo Canadian Symposium on Heart Failure, at Rajiv Gandhi centre for Biotechnology, Thiruvananthapuram, Kerala. Held on March 2015.