SUMMARY

Substantial evidence suggests the involvement of oxidative stress in the pathophysiology of heart failure. Oxidative stress describes an imbalance between antioxidant defence and the production of reactive oxygen specie (ROS), which at high levels cause cell damage but at lower levels induce subtle changes in intracellular signaling pathways. The deleterious effects of ROS are mainly due to abilities of ROS to produce changes in subcellular organelles and induce intracellular Ca^{2+} overload. Various sources of intracellular ROS like xanthine oxidase, arachidonic acid metabolism, catecholamine and angiotensin-II also results in formation of highly reactive superoxide, hydrogen peroxide, hydroxyl and nitric oxide radical. Reports from several studies had shown beneficial effects of antioxidant therapy in hypertension, atherosclerosis, ischemic heart disease, cardiomyopathies and congestive heart failure. In Addition, epidemiological data as well as *in vitro* studies strongly suggest that functional food enriched with antioxidant phytochemicals have strong protective effects against major degenerative diseases including cancer and cardiovascular diseases.

Anthocyanin, a flavonoid from different sources are found to have a potential to reduce oxidative damage and apoptosis. RC had been widely studied for their antioxidant, anti-inflammatory, anticancer, hypolipidemic, cardioprotective along with antibacterial and antifungal activity. Hence, these supported us to investigate whether anthocyanin rich Red Cabbage (*Brassica olereacea* var. capitata F. Rubra; ARCE) extract can prevent the damage caused due to oxidative stress and protect cardiomyocytes from apoptosis.

In this study, presence of anthocyanin was confirmed by TLC, GC-MS and HPTLC analysis. TLC and HPTLC accounted the presence of cyanidin and

delphinidin monoglycoside in ARCE. GC-MS also accounted cyanidin-3-glucoside and delphinidin-3-glucoside from crude extract. Whereas, analysis of bands obtained from TLC showed presence of daughter ions of (epi) gallocatechin delphinidin, (epi) gallocatechin peonidin glucoside, peonidin glucoside in the first band and cyanidin, cyanidin-3(6“-acetyl glucoside), cyanidin dioxalyl glucoside, delphinidin-3(6“-acetyl glucoside) and delphinidin-3-glucoside in second band. Previous studies with RC anthocyanins had also shown the presence of cyanidin based anthocyanins which may or may not be acylated with acyl groups. Also, a detailed study on characterization and antioxidant potential of ARCE prompted to further look upon the potential of ARCE in regulating intracellular antioxidant status and improving cardiac function.

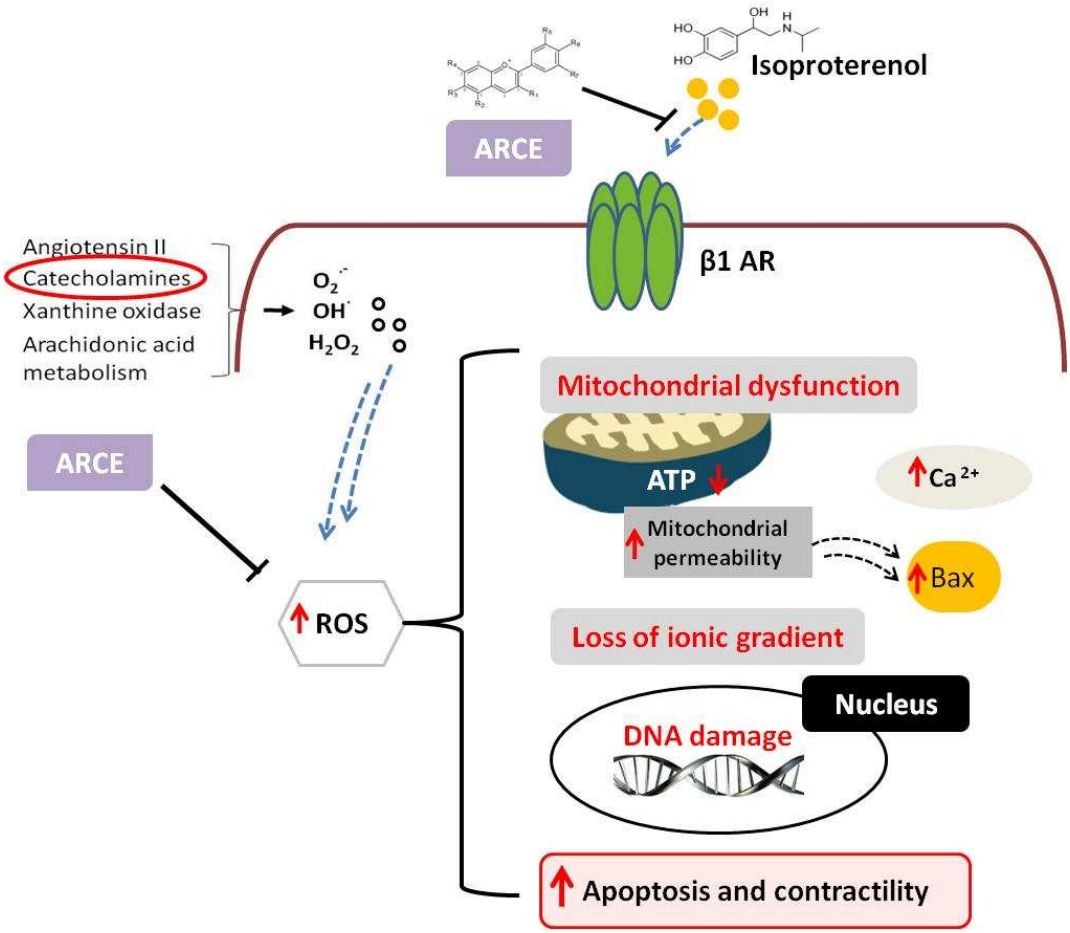

Figure 6.1: Schematic representation of proposed study. Explaining the ROS mediated intracellular changes as reported in several studies and mechanism of ARCE mediated prevention.

In *in vitro* study, pre-treatment with ARCE for 24 h was found to be more effective then 12 h and 24 h pre-treatment was used for further analysis. This study was designed to investigate the protective effects of ARCE against H_2O_2 induced apoptosis in H9c2 cells. H_2O_2 induced oxidative stress and cellular damage is widely used model to study therapeutic potential *in vitro*. FACs analysis and mRNA study of *bax* and *bcl-2* showed preventive effect of ARCE against H_2O_2 induced cell death by preventing the exhaustion of intracellular enzymatic antioxidants (*sod* and *catalase*). ARCE mediated prevention of H_2O_2 induced intracellular oxidative stress, loss in mitochondrial membrane potential and apoptosis in H9c2 cells were further supported by DCF-DA, RHO-123 and DAPI staining. Also, *caveolin-3* specific to the cardiac membrane and involved in signaling was downregualted in H_2O_2 treated group. However, ARCE+H_2O_2 group recorded improved levels of *caveolin-3* similar as control group.

Explaining the role of ARCE in preventing the H_2O_2 induced exhaustion of intracellular enzymatic antioxidants, stabilizing the membrane potential and preventing apoptosis in H9c2 cells.

These results were further validated by isoproterenol (ISO) induced model for myocardial infarction (MI). Male Charles foster rats were fed with ARCE before induction of MI. Increased circulating levels of CK-MB and cardiosomatic index were observed in ISO treated rats. However, reduced circulating levels of CK-MB and regulated level of cardiosomatic index were observed in ARCE pre-treated rats. ARCE pretreatment increased the levels of intracellular enzymatic antioxidants (*sod* and *catalase*) and prevented ISO induced damage in histoarchitecture of cardiac tissue as observed in TTC, HXE and picrosirus red staining. Increase in *ANKRD1* and *bax* with decrease in *bcl-2, caveolin-3* and *SERCA2a* levels had been reported by several

studies in cardaic tissue of ISO induced MI model. Alleviation of ISO induced decrease in *caveolin-3* and *SERCA2a* levels of cardiac tissue by ARCE explains its role in regulating membrane integrity and intracellular calcium signaling. Also, ARCE was instrumental in regulating the levels of *ANKRD1, bax* and *bcl-2,* which are involved in regulation of mechanosensing machinery and apoptosis in cardiac tissue.

These findings suggest that, the reported antioxidant potential of ARCE have significant potential to reduce intracellular oxidative stress, prevent membrane damage, regulate intracellular calcium homeostasis, prevent apoptosis and imparts overall cytoprotection to cardiac tissue.

Result obtained in above study prompted to further look insight the interaction of anthocyanin with β1AR, which is involved in ISO induced modulations in cardaic tissue. In this study cyanidin-3-glucoside, cyanidin-3,5-diglucoside, delphinidin-3-glucoside and isoproterenol were docked with β1AR to determine the possibilities of its interaction with receptor and preventing the binding of isoproterenol. Cyanidin-3-glucoside and delphinidin-3-glucoside recorded less free energy than cyanidin-3,5-diglucoside required to interact with β1AR. Subsequently ligplot analysis showed strong hydrophobic interaction and hydrogen binding of cyanidin-3-glucoside and delphinidin-3-glucoside then cyanidin-3,5-diglucoside and isoproterenol. Ligplot analysis recorded that cyanidin-3-glucoside and isoproterenol interact with similar amino acid residue in the active site of β1AR, hence binding of cyanidin-3-glucoside can inhibit the binding of isoproterenol to the active site of β1AR and can prevent the isoproterenol induced overstimulation of β1AR.

This study explains that along with antioxidant potential, membrane stabilizing potential, ARCE is instrumental in regulating intracellular homeostasis and

preventing further cardiac damage. This mechanism was further attributed to antioxidant activity of anthocyanin and its ability to prevent the binding of catecholamine at β1AR. Thus, further can prevent the formation of intarcellualr ROS and other pathobiological changes in cardiac tissue.