Chapter 7
Conclusion
CONCLUSIONS AND FUTURE PERSPECTIVES

Currently diabetic cardiomyopathy has heightened the attention due to its severity, increased intensity and a greatest cause of mortality worldwide. It imposes a long-term complications and its encroachment especially perceived into developed countries. The toxicity of existing drugs has shifted the attentiveness towards herbal remedies, which are also cost effective, safe and harmless alternative therapy. The molecular mechanisms of plants against diabetes related cardiovascular complications is poorly defined in literature, therefore in our study we tried to find out the potential targets of an antidiabetic plant *Syzygium cumini*, by screening ROS level, mitochondrial function, inflammatory and apoptotic pathways, matrix metalloproteinases activities. This plant is extensively known to suppress hyperglycemic conditions; however its cardioprotective potential is poorly defined. The extensive study on *Syzygium cumini* made during my doctorate thesis work reveals its cardioprotective potential under hyperglycemia induced stress, and proved it to be a potent cardiac stress reliever.

The entire study in the thesis was aimed to evaluate the antiglycation, antioxidative, cardioprotective and MMPs inhibition properties of *Syzygium cumini* in high glucose stress conditions. Firstly, *S. cumini* pulp and seed extracts were prepared with water and organic solvent extracts, which showed the highest number of phytoconstituents present in aqueous, ethanol and methanol pulp and seed extracts. These extracts were further examined for antiglycation and antioxidative potential. Various antioxidative assays such as DPPH, ABTS, NO, H$_2$O$_2$, O$_2^-$ and reducing power assay revealed the highest anti-glycoxidative potential of *S. cumini* methanol pulp (MPE) and seed extracts (MSE) as compared to other extracts. Further comparison between MPE and MSE concluded that MSE had the utmost anti-glycoxidative potential than that of MPE. To find out the reasons of maximum anti-glycoxidative potential in MSE; FTIR, GC-MS and HPLC studies were carried out with seed extracts. These techniques confirmed the highest intensities of functional groups contributing to polyphenols in MSE, presence of number of essential oils, hydrocarbons and enrichment of gallic acid in MSE as compared to other seed extracts. *Therefore we*
concluded that *S. cumini* methanol seed extract (MSE) is enriched with polyphenols, essential oils, hydrocarbons which contributes to its antiglycoxidation potential as compared to ethanol (ESE) and aqueous seed extracts (ASE) making it a potent extract for pharmacological studies.

Further study was carried out with *S. cumini* MSE to observe its effect on high glucose stressed H9C2 cardiac cells. The effect of MSE was compared with the purified gallic acid, which is a known cardioprotectant and is one of the components of *S. cumini* also. The doses of glucose (4.5 mg/ml), *S. cumini* MSE (9 μg/ml) and gallic acid (3.4 μg/ml) was selected by MTT assay. 4.5 mg/ml dose was sufficient for creating stress in H9C2 cells. Morphological analysis by various staining revealed the increase in cell size in glucose stressed cells whereas MSE treatment reduced the cell size. Gallic acid treatment on stressed cells showed the reversal in cell size lesser than MSE treated stressed cells. Nuclear morphology assays showed nuclear stress in glucose induced cells and MSE treatment lowers it significantly than gallic acid. MSE also has potential to suppress high glucose induced intracellular ROS overproduction, mitochondrial stress, loss of mitochondrial integrity and membrane potential. Inflammatory markers TNF and IL-6 expression was enhanced in stressed cells and MSE significantly reduce the expression than that of gallic acid. MSE downregulates the expression of apoptotic markers (Bax, caspase) and protects stressed cardiac cells from apoptosis as evident by TUNEL studies. *Hence we concluded that Syzygium cumini* methanol seed extract (MSE) exerts cardioprotective action by preventing the increase in cell size, reducing ROS generation, mitochondrial stress and suppressing the overexpression of proinflammatory cytokines and apoptotic markers in glucose induced H9C2 cells.

We further validated the cardioprotective effect of MSE on extracellular matrix remodeling during high glucose stress. We observed unregulated collagen content, increased MMP-9 activities and nuclear localization of NF-kB after glucose treatment and MSE/ gallic acid significantly suppress the ECM remodeling. *In silico* docking studies were conducted to determine the interaction of polyphenols with MMP-2 and 9 which showed that *S. cumini* polyphenols can serve as potent MMP inhibitors as their binding activities were comparable than that of FDA approved doxycycline. *The*
study concluded that *Syzygium cumini* methanol seed extract has cardioprotective phytomolecules that has ability to suppress MMP mediated glucose induced cardiac stress.

Also, a successful attempt has been made to synthesize silver nanoparticles by using *S. cumini* MSE (ScSNPs) as it has excellent reducing potential and its effect was tested for cardiac protection against glucose mediated stress. Green synthesized ScSNPs passed all the standard criteria as evident from UV-Vis spectroscopy, Zetasizer, FTIR, SEM and XRD studies. ScSNPs had good antioxidative as well as cardio protectant. *Therefore the study concluded that silver nanoparticles of Syzygium cumini methanol seed extract have the cardioprotective potential against glucose induced cardiac stress with no toxicity.*

While starting my thesis work, I had few questions in my mind (chapter 1) and designed my objectives accordingly and after completion of thesis, I suppose that I am able to answer most of them. Our study suggests that in order to develop therapeutic compounds, the bioactive molecules isolated from *S. cumini* may represent potential source of molecules of significant relevance for developing novel drugs, especially designed for treating and/or controlling the diabetic cardiomyopathy. Diabetes-induced cardiomyopathy is threat to millions of diabetics and a safer drug to decrease cardiac stress is urgently required. *S. cumini* seed extracts or its polyphenolic constituents may be suggested functional food for diabetic patients based on its inhibitory effect on high glucose-induced MMP-9 activity and cellular changes in cardiomyocytes.

**Future Perspectives**

Our study showed *S. cumini* has the potential to alienate the effects of cardiac stress in hyperglycemic condition. Novel therapeutic strategies can be designed in future to enhance its pharmacological potential as certain barriers like bioavailability and poor absorption may tend to limit its activity. Therefore, future studies are required:

- To validate the results from the present study on *in vivo* systems.
• To further understand the molecular mechanism of *S. cumini* as a natural matrix metalloproteinase-9 inhibitor in diabetic cardiomyopathy.

• To explore *S. cumini* polyphenols such as corilagin, quercetin, epigallocatechin gallate etc. as an MMP inhibitor.

• Extensive characterization of *S. cumini* silver nanoparticles.