Chapter 1

Introduction
INTRODUCTION

The global burden of diabetes is rapidly growing day by day and has become a serious concern not only in developing countries but also in the developed countries. In 2000, India was leading with highest number of people with type II diabetes (31.7 million), followed by China (20.8 million) and United States (17.7 million) and it is predicted to be get doubled in these countries by the end of 2030 [1, 2]. It is also observed that when diabetic population is associated with cardiovascular diseases, the chances of heart failure increases two to four times more as compared to non diabetic heart patients [3, 4]. Diabetes belongs to lifestyle disease or the disease of longevity/civilization [5]. People especially with Type II diabetes have higher rates of blood pressure which contributes to progression of cardiovascular diseases (CVD), a major cause of mortality and morbidity worldwide and almost 70% deaths occurred due to the severity of the disease [6, 7]. India is currently facing uncertain and risky future due to the potential burden of diabetes and its associated complications. To overcome these troubles, introduction of cost- effective novel/ advanced therapeutic strategies become the primary concern of the present day to provide a long term relief to the diabetic patients.

The increase in the blood glucose concentration leads to activation of alternative metabolic pathways and signaling events which contribute to the progression of various cardiac complications. These pathways stimulate the imbalance in the generation and the consumption of reactive oxygen species (ROS) and deplete substrates for the activity of certain important antioxidant enzymes. Also, increased intracellular glucose level leads to the formation of advanced glycation end products (AGEs), activation of protein kinase C (PKC), activation of Receptor for AGE (RAGE) [8, 9] and free fatty acids, which are responsible for structural and functional changes in blood vessels. Subsequently there is an alteration in the level of NO, H$_2$O$_2$ and other free radicals. The increased production of endothelin (ET-1), activation of transcription factors such as NF-$\kappa$B and AP-1, proinflammatory cytokines (TNF-$\alpha$, IL-6) and apoptotic markers (Bcl-2, Bax, caspases) are also involved in the ROS induced cardiac pathologies [10, 11]. However, the mechanisms underlying the
increased risk for diabetes-associated cardiovascular diseases need to be explored further.

Diabetic cardiomyopathy, most prevalent disease occurred due to diabetic complications, is characterized by abnormal myocardial structure and performance [12, 13]. Increases in free radicals generation, AGEs, lipid peroxides, mitochondrial dysfunction, activation of isoforms of protein kinase C, energy deficit, lipid accumulation, activation of peroxisome proliferators-activated receptors, imbalance in ATP/O² consumption ratio are the hallmark of diabetic cardiomyopathy (Figure 1.1) [14]. Currently available therapeutic drugs allow diabetic individuals to manage glucotoxicity and attain near-normal life expectancy, but may have the possibility of provoking other complications leading to multi-organ failure [15, 16]. To overcome these hurdles, designing of herbal therapies, targeting lipid and glucose level may prevent associated cardiac pathology.

Cardiac hypertrophy is found to be a critical feature of diabetic cardiomyopathy. Experimental and clinical studies have shown the involvement of pressure overload myocyte hypertrophy and left ventricular remodelling phenomenon in diabetes [17, 18]. Cardiac myocytes are terminally differentiated myocytes and loosing the tendency of proliferation after their birth [19]. Such pathological condition imposes overwork on the heart and postnatal cardiomyocytes undergo cardiac hypertrophy, characterized by an increase in the size of individual cardiac myocytes and whole organ enlargement. Although cardiac hypertrophy is initially compensatory, but once it prolonged may lead to deleterious effects owing to stroke, heart failure and sudden death [20, 21]. With regard to the differential gene expression induced by hypertrophic stimulation, it is well documented that cardiac transcription factors such as GATA, NFκB, NFAT, AP-1 play the leading role by regulating cardiac genes in hypertrophied myocardium [22-24]. Despite of that, increased cardiac fatty acid uptake, enhanced myocardial lipid storage, decline in cardiac efficiency, impaired mitochondrial energetics and Ca²⁺ handling contribute to cardiac hypertrophy [25, 26].
Hyperglycemia associated cardiac manifestations also contribute to the modulation of extracellular matrix, which is a dynamic entity of cell communication. Matrix metalloproteinases, a Zn and Ca dependent endopeptidases degrade the extracellular matrix components and lead to extracellular matrix (ECM) remodelling in diabetes. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are found to be critically involved in cardiac stress and their expression is enhanced under various pathophysiological conditions. The nuclear localization of NF-κB is reported to enhance during cardiac remodeling. Alteration in the level of other ECM components such as collagen, gelatin, integrin, laminin, fibronectin, and proteoglycans also contribute in ECM remodeling [27-30].

![Image](image1.png)

**Figure 1.1:** Mechanism of hyperglycemia inducing diabetic cardiomyopathy. The pathological conditions induced by ROS, which corresponds to diabetic cardiomyopathy. ROS majorly involves in lipid peroxidation, extracellular matrix accumulation, inflammation and apoptosis [14]

Presently the drugs often prescribed for patients with diabetes have a limited ability to cure and can create more problems in the long run in different organs like eyes, kidneys, nerves and cardiovascular system [31]. Metformin, pioglitazone are well known antidiabetic drugs, but have the cardiotoxicity issues with an increase in slow
heart rate and leading to cardiac arrhythmia [32, 33]. Recent studies have shown that the drugs from plant compounds with combined antioxidative and antiglycative potential are more effective in treating diabetes mellitus [34-36].

Herbal remedies are nowadays in trend for the treatment of various ailments due to the side effects and toxicity of drugs. India is enriched with medicinal plants and at present research is being conducted to identify the novel phytoconstituents for the formation of herbal drugs. The research team of our laboratory is also working with the screening of therapeutic potential of various plants and analyzing their antiglycation, antioxidant, anti-inflammatory, antiapoptotic and cardioprotective activities. We have investigated the therapeutic properties of some plants such as *Camellia sinensis*, *Allium sativum*, *Curcuma longa* and *Syzygium cumini*. A list of some well known antidiabetic plants with known therapeutic potency is shown in table 1.1. Many commonly consumed plant polyphenols have been shown to have specific and potent health-promoting benefits. However, very few are explored extensively. Medicinal plant such as *Terminalia arjuna* failed in clinical trials, though it is reported to have cardioprotective potential. Therefore more study with other plants need to be carried out to explore the cardiac protection.

Among all these plants, *Syzygium cumini* was chosen for analysis of cardioprotective potential against glucose induced stress. *Syzygium cumini* (*Eugenia jambolana*), a well known antidiabetic plant from myrtaceae family is an evergreen tropical tree, native to India, Pakistan and Indonesia. The plant has traditional medicinal uses and wide therapeutic approaches. The dark purple coloured fruits are riped in 2-3 months and found to be a rich source of vitamins and other natural antioxidants etc [37, 38]. It is a good source of neutraceuticals because of its medicinal values. The plant is well known for its antidiabetic activity, however unexplored for its cardioprotective properties. The different parts of the plant have been used in the treatment of cholera, blisters in mouth, cancer, colic, pimples and stomachache and various diseases, by various traditional practitioners in India [39, 40].
Research is also being conducted to increase the bioavailability and better absorption. In this regard, nanotechnology has wide scope in pharmacotherapeutics and found to have significant uses in medicinal fields [41, 42].

**The relevance and expected outcome of the proposed study**

Proposed work aims to study the screening of cardioprotectants from traditionally used Indian medicinal plant, *Syzygium cumini* and to understand the mechanism of cardio protection, which would help to develop therapeutic antioxidative and cardioprotective strategies against diabetic cardiomyopathies, a major cause of mortality worldwide.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Plants (Botanical Name)</th>
<th>Main Phytomolecules</th>
<th>Antidiabetic activity</th>
<th>Cardioprotective activity during diabetic stress</th>
<th>Anti Apoptotic activity</th>
<th>Anti matrix metallo proteinase activity</th>
<th>Molecular mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bitter gourd (Momordica charantia)</td>
<td>Flavonoids</td>
<td>Yes</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>2</td>
<td>Tulsi (Ocimum sanctum)</td>
<td>Flavonoids, Polyphenols</td>
<td>Yes</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>Pomegranate (Punica granatum)</td>
<td>Anthocynins, Flavonoids, Tannins</td>
<td>Yes</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>4</td>
<td>Raimunia (Lantana camara)</td>
<td>Alkaloids, Terpenoids, Phenolics</td>
<td>Yes</td>
<td>X</td>
<td>X</td>
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<td>5</td>
<td>Barbados nut (Jatropha curcas)</td>
<td>Phenolics, Flavonoids, Saponins</td>
<td>Yes</td>
<td>X</td>
<td>X</td>
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<td>6</td>
<td>Geley (Tinospora cordifolia)</td>
<td>Alkaloidal constituents</td>
<td>Yes</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>7</td>
<td>Chinese Aloe (Aloe vera)</td>
<td>Aloe emodin, Aloin</td>
<td>Yes</td>
<td>X</td>
<td>X</td>
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<tr>
<td>8</td>
<td>Amaltas (Cassia fistula)</td>
<td>Xanthenes, Flavans, Flavonols</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>9</td>
<td>*Haldi (Curcuma longa)</td>
<td>Curcuminoïds, curcumin</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Origin of the proposed work

Diabetes is found to be commonly associated with cardiovascular diseases causing significant morbidity and mortality. Many antidiabetic drugs have cardiovascular side effects too. Cardiac toxicity believed to be a multi-factorial process and leads to cardiomyocytes death as terminal downstream events. In diabetes, the levels of free radicals increase drastically, thereby disturbing the equilibrium between free radical productions and antioxidant capability which ultimately lead to cardiac failure. Based on the growing demands of natural products and considering the disadvantages associated with synthetic drugs, the study stimulates the usage of natural products.
Reports have demonstrated that anti-oxidant natural substances including herbal medicines could inhibit the diabetic cardiomyopathies by inhibition of ROS generation. Hence *S. cumini* as an antidiabetic plant, may represent a promising source for protecting cardiac cells against hyperglycemic stress. We also propose that the synergistic effects of phytochemicals present in extracts exert better effect than the purified phytoconstitutent. To take into account the increasing diabetic population and associated cardiac malfestations, there is an upsurge to develop a safe and less toxic therapy for long-term relief. The proposed study is therefore designed to investigate the effect of *S. cumini* against glucose induced cardiac stress.

**Key Questions**

The key questions of the proposed studies were-

1. What are the various bioactive components in *Syzygium cumini*?
2. Which is the most enriched extract of *Syzygium cumini*?
3. What is the optimised glucose dose and time for generating stress on cardiac myocytes?
4. What is the safe *Syzygium cumini* dose for treatment of stressed cardiomyocytes?
5. Is the well known antidiabetic plant *S. cumini*, a persuasive cardioprotectant too?
6. Is there any effect of *Syzygium cumini* on extracellular matrix components?
7. Does *S. cumini* act as a therapeutic target for MMP inhibition under glucose induced cardiac stress?
8. What is the effect of *S. cumini* on inflammatory cytokines and apoptotic proteins?
9. Can we increase the bioavailability of most enriched *Syzygium cumini* extract?
10. Do silver nanoparticles synthesised using *S. cumini* methanol seed extract efficiently suppress glucose induced stress on cardiac myocytes?

In order to answer these questions, the present study was designed to characterize *S. cumini* for the presence of phytochemicals and to analyze its cardioprotective potential under glucose induced stress. The main objectives of the study were-
Objective 1: Screening of Syzygium cumini pulp and seed extracts for their anti-glycoxidative potential and detailed characterization of selected S. cumini extracts.

Objective 2: To investigate the effect of S. cumini methanol seed extract (MSE) on glucose induced cardiac stress.

Objective 3: To evaluate the effect of S. cumini methanol seed extract (MSE) on extracellular matrix components.

Objective 4: Characterization of S. cumini silver nanoparticles (ScSNPs) and analyzing their cardioprotective potential on glucose induced stress.