Summary and Conclusion

Diabetes is associated with most known risk factors for cardiovascular diseases in overall population including obesity, dyslipidemia, thrombosis, infarction, hypertension, activation of multiple hormone and cytokine systems, autonomic neuropathy, endothelial dysfunction and coronary artery disease. In light of these common contributing pathologies it remains uncertain whether diabetic cardiomyopathy is a distinct disease. The factors responsible for the overall incidence of heart failure in diabetic patients are also not clear. A great many cardiovascular drugs have the potential to induce adverse reactions. The prevalence of such reactions is not known, many are asymptomatic and therefore are believed to go unreported. As more drugs are marketed and the population includes an increasing number of elderly, the number of drug prescriptions is also expected to increase. Accordingly, it can be predicted that the occurrence of adverse drug reactions may continue to increase.

Complementary and alternative medicine plays a significant role in many aspects of healthcare worldwide, including cardiovascular disease. In spite of great advances of modern scientific medicine, traditional medicine is still the primary form of treating diseases of majority of people in developing countries including India; Even among those to whom western medicine is available, the number of people using one form or another of complementary alternative medicine is increasing worldwide. Increasing knowledge of metabolic processes and the effect of plants on human physiology has enlarged the range of application of medicinal plants. Therefore the present study is focused on complementary medicine for the treatment of diabetes and protective effect of myocardium using the plant extract of Albizia saman leaves and white Nelumbo nucifera flowers in experimental swiss albino rats.
The present work entitled, "Biochemical changes in patients with diabetic cardiomyopathy during drug therapy and the cardio protective effect of selected medicinal plants on Isoproterenol induced Swiss albino rats" was organised in three phases. Phase I included a detailed analysis of the clinical data of the patients with diabetic cardiomyopathy (with the help of their family history and clinical status). Adverse effect, biochemical and clinical conditions of the study subjects were examined before and after taking the oral medications. Phase II was designed to assess the in vitro antioxidant capacity and phytochemical constituents of the leaves of A.saman and N.nucifera flowers. Phase III included the assessment of in vivo antioxidant, glucose lowering property and cardio protective effect of MeAsL and MeNnF in experimental animals. The antioxidative role was assessed against the induced drugs streptozotocin and isoproterenol.

The persons affected by cardiomyopathy was found to be in the age group of 60-70 years followed by 50-60. The survey also reported the occurrence of diabetic cardiomyopathy to be more in women than men. During the survey medical history of the patient was noted and physical examination was done. Medical history of thirty percent subjects showed the symptoms such as hypertension, iron deficiency, joint pain, obesity, dyspnea, orthopnea, paroxysmal nocturnal dyspnea, abdominal discomfort, leg swelling and low back pain. Twenty percent of female subjects showed risk factors such as iron deficiency anemia, heavy menstrual cycles, mal absorption, fatigue and unusual food cravings (pica due to iron deficiency). Forty percent of the male subjects had the history of recent physical/psychological stress and chest pain symptoms which depends on the nature/cause of electrolyte abnormality.

The study subjects were fifty female and fifty male patients. Clinical and medical status were observed for a treatment period of one year. Patients with diabetic cardiomyopathy were diagnosed at an early stage was selected for the study (above 30 years). The study subjects were compared with normal persons (control) who are not suffering from any other disorder. The level of HbA1c, lipid profile, liver marker enzymes and cardiac markers were measured initially and at an
interval of 3, 6 and 12 months. During the treatment there was a significant improvement in the study subjects and the levels of HbA1c, Triglycerides, total cholesterol and LDL-C decreased significantly, at the same time HDL-C level increased. During the treatment adverse effect was noted in 1-5% of the patients such as abnormal weight gain, headache, swelling of the abdomen, sweating, mental stress and depression, abdominal pain, constipation, facial flushing and GI distress.

Alternative treatments are directed towards the control of the effects of heart disease. An ancient ayurvedic herbal treatment for heart disease, has been discovered to be as effective as allopathic medication. In phase II medicinal plants A.saman leaves and white N.nucifera flowers were selected for the study. Various extracts (aqueous, benzene, chloroform, ethanol, ethyl acetate, methanol and petroleum ether) of A.saman and N.nucifera were analysed for the presence of phytochemicals. The preliminary qualitative phytochemical screening reported the presence of phytochemicals such as tannins, flavonoids, steroid, saponins, cardiac glycosides, alkaloids and phenolic compounds in both the plants. Among all, methanolic extract showed the highest free radical scavenging activity in both the plants.

Bioactive constituents in the selected samples were characterized using HPTLC, HPLC, FT-IR and GC-MS analysis. HPLC analysis of A.saman leaves showed two constituents and N.nucifera flowers showed seven constituents with different retention times. The FT-IR spectrum of A.saman leaves showed 28 peaks and white N.nucifera flowers displayed 26 peaks at the range of 3784.46 cm\(^{-1}\) to 613.38 cm\(^{-1}\) and 3776.75 cm\(^{-1}\) to 424.35 cm\(^{-1}\) respectively.

Characterization of bioactive component in A.saman showed the presence of N, HC, OH and COOH group which indicates the presence of flavonoids, alkaloids, phenols and glycosides in the extracts, N.nucifera contains COOH group, C=O group, HC group and acetate which may be due to the presence of flavonoids, phenols and cardiac glycosides. HPTLC chromatogram studies confirmed the
presence of phenols, flavonoids, cardiac glycosides in A.saman leaves and white N.nucifera flowers. Thus phase II confirmed the presence of antioxidants and bioactive components in the selected plants.

In Phase III, animal experiment was carried out to find out the glucose lowering property and cardio protective effect of methanolic extract of A. saman leaves (MeAsL) and white N. nucifera flowers (MeNnF).

In this study the rats were divided into nine groups. Each group consisted of five animals. Group I served as non-diabetic control, Group II, III, IV, V, VI and VII animals were induced with Streptozotocin (diabetes induced group). The rats having fasting blood glucose (FBG) values of 250 mg/dl or above were considered for the study. Group III rats were treated with standard drug metacord (200 mg/kg) and metformin (50 mg/kg) intraperitoneally for a period of 30 days. Group IV and Group V rats were pre-treated with MeAsL (100 mg/kg and 200 mg/kg respectively) for a period of 30 days. Group VI and Group VII rats were pre-treated with MeNnF (100 mg/kg and 200 mg/kg respectively). Group VIII and Group IX rats (plant extract alone group) were treated with MeAsL and MeNnF (200 mg/kg) for a period of 30 days. Group II, III, IV, V, VI and VII were induced with isoproterenol (20 mg/100g subcutaneously twice at an interval of 24 hours) at the end of the treatment period (on 29th and 30th days).

Changes in body weight were noted initially and after induction of diabetes and cardiomyopathy. There was a significant difference in body weight between control (Group I) and ISO-control group (Group II). When compared to A.saman treated group (IV and V) better result was observed in treatment with MeNnF (group VI and VII) since it showed a significant increase in body weight.

Glucose lowering property was examined in different experimental groups. Changes in blood glucose level was measured before and after the treatment. In non-diabetic control group (group I) there was no significant change in fasting blood glucose (FBG) level. In group II (diabetic control group) there was a significant
increase in FBG and it maintained hyperglycemia till the end of the experiment. When compared with standard drug treated group (group III) plant extract treated groups (group IV, V, VI and VII) showed a significant decrease in glucose level. The enhanced fasting blood glucose level in diabetic rats were significantly reduced with the treatment of *N. nucifera* (group VI and VII). Thus, *N. nucifera* had the highest ability to reduce blood glucose when compared to *A. saman* (group IV and V).

At the end of the experiment after dissection wet heart weight was examined. There was a significant increase in heart weight in group II showing abnormal heart which indicates cardiac hypertrophy. Groups IV and V (MeAsL treatment group) showed reduction in heart weight when compared to standard drug treated group (group III). MeNnF treatment to ISO groups (group VI) significantly reduced the heart weight. Thus a higher dose of MeNnF (group VII) significantly reduced the cardiac hypertrophy caused by isoproterenol.

Activity of serum cardiac marker enzymes were analysed in the heart homogenate of different experimental groups. In isoproterenol induced control rats (Group II), a significant increase in the activities of serum CK-MB, AST and ALT were observed as compared to normal control rats (Group I). A similar trend was observed in the level of cardiac troponin-T in serum. Rats pre-treated with MeAsL and MeNnF (Group IV, V, VI and VII) showed significant decrease in the levels of these cardiac markers during cardiomyopathy compared to isoproterenol treated rats (group II). Rats pre-treated with MeNnF 100 mg/Kg body weight (Group VII) showed minimum increase in the activities of these cardiac marker enzymes indicating better cardioprotection among the doses of MeAsL. Decrease in the activity of these enzymes was observed in isoproterenol induced rats compared to normal rats, whereas significant increase in the activity was seen in rats pre-treated with *N. nucifera* compared to isoproterenol induced rats (group II). Enzyme activities in the heart of group VII rats were similar to that of normal control rats.

Effect of MeAsL and MeNnF on serum total cholesterol and lipid profile in different experimental groups were examined. Subcutaneous administration of ISO
(group II) caused a significant rise in the levels of cholesterol, triglycerides, LDL-C and decreased level of HDL-C in serum of rats compared with control (group I). In group VII, there was a significant reduction in the levels of cholesterol, triglycerides and LDL-C. Group VIII and Group IX (plant extracted treated group) maintained the cholesterol level at near normal.

Antioxidant activities were found out in the heart homogenate of different experimental groups. Antioxidant activity was decreased in isoproterenol induced control rats (group II) compared to normal control rats (group I) and an increase in activity was seen in pre-treated groups (IV, V, VI and VII). Among all the experimental groups *N. nucifera* showed highest antioxidant activity. Lipid peroxidase activity was also examined in different experimental groups. Intraperitoneal administration of Isoproterenol caused a significant elevation in the level of lipid peroxidation in the heart tissue of group II rats as compared to that of group I normal rats. The Intraperitoneal administration of MeNnF showed considerable reduction in the levels of lipid peroxidation in heart tissue of group VII animals as compared to group III (pre-treated with standard drug) rats, which indicates the ability of *N. nucifera* to protect the heart tissue from isoproterenol induced myocardial injury.

The protective role of *A. saman* leaves and white *N. nucifera* flowers against isoproterenol-induced myocardial infarction was further confirmed by histopathological examination.

Histopathological studies of the heart section was carried out to find out the cardioprotective effect of *A. saman* and *N. nucifera*. In normal rats the myocardium membrane showed adequate cellularity and normal morphology. Myocytes were healthy and there was no evidence of myocyte necrosis, vascular proliferation, macrophage activity and muscle hypertrophy. Group VIII and IX showed that the myocardium was similar to that of group I with adequate cellularity and normal morphology. Less vascular proliferation and necrosis was observed in group III and group V. No evidence of necrosis, vascular proliferation, macrophage activity and muscle hypertrophy observed in group VII. The morphological changes in group II
strongly suggested isoproterenol-induced myocardial injury. Large areas of coagulative necrosis were seen with neutrophilic infiltrate, diffused interstitial edema and pale myocytes with fading nuclei and decreased striations.

In ISO groups treated with MeNnF (group VII), many areas of myocyte debris were disintegrated with the presence of macrophages, suggesting that the myocytes were removed by macrophage activity. Macrophage activity was prominent in areas of injury and it increased with increasing doses of MeNnF. This was also true for areas of vascular proliferation which were both present in numerous areas throughout the myocardium and increased with increasing doses of MeNnF.

Histopathological study also confirmed the myocardial damage with ISO treatment. There were necrosis in the myocardium and vascular proliferation, increased macrophage activity, scar formation and myocardial hypertrophy in group II.

Histopathological and biochemical findings of this study indicates the antioxidant properties of white N.nucifera and its protective effect on myocardium against isoproterenol-induced oxidative stress. The most important protective mechanism offered is through its ability to decrease lipid hydro peroxides and to increase superoxide dismutase and glutathione level. Thus white N.nucifera flowers has been shown to possess cardioprotective effect against isoproterenol-induced cardiomyopathy in rats. These findings suggest that MeNnF at a dosage of 200 mg/kg is a good choice for controlling blood glucose level and it also protected the heart muscle from oxidative damage. Thus it could be concluded that white N.nucifera flower could be used as alternative for diabetic treatment in patients to reduce the risk of cardiovascular disease.
Recommendations

✓ Plant-based diet could be combined with cholesterol lowering therapies.

✓ Screening strategies could be formulated with selected cardiac markers as attempted in the present study.

✓ Future studies could be carried out to find out the specific component responsible for the cardioprotective effect.

✓ Variation in gene expression for the development of plants with enhanced medicinal value through technological interventions could be studied.