Summary and Conclusion
Diabetes mellitus and its complications constitute a major health problem in modern societies. The number of patients with diabetes mellitus worldwide is increasing and it is estimated that there will be more than 220 million people with this disease by the year 2010 (Zimmet, 1999). In India, there are currently 20 million patients with type II diabetes (non insulin dependent diabetes mellitus, NIDDM) accounting for more than 90% of all patients with diabetes, and this will increase to 57 million in the next 25 years (Sharma, 2001).

The cardiovascular diseases constitute the main cause of morbidity and mortality in diabetes, especially in type II diabetes. Diabetic individuals have 2-4- folds increased risk of clinical atherosclerotic disease (Nathan, 1997).

Specific diabetic risk factors such as glycation of proteins and coagulation abnormalities, and subsequent endothelial dysfunction, may also contribute. In addition, type 2 diabetes is a part of the metabolic syndrome, a cluster of cardiovascular risk factors including dyslipidaemia, hypertension and visceral obesity (Zimmet, 1999).

Blood pressure is also a major risk factor for cardiovascular events, such as myocardial infarction and stroke, as well as for microvascular complications in diabetes. Tight blood pressure control reduces the risk of both micro- and macrovascular complications in patients with hypertension and type 2 diabetes (Stearne, 1998).

There is also considerable evidence that oxidative damage is increased in diabetes, through mechanisms are not clear (West, 2000). Furthermore, there is widespread acceptance of possible role of reactive oxygen species, generated as a result of hyperglycaemia, in causing many of the secondary complications of diabetes such as nephropathy, retinopathy, neuropathy (Giugliano et al., 1996) and cardiomyopathy.
(Rodrigues and Mc Neil, 1992). Alterations in myocardial structure and function occur in the late stages of diabetes, these chronic alterations are believed to result from acute cardiac responses to suddenly increased glucose levels at the early stages of diabetes. Advances in the application of various strategies for targeting the prevention of hyperglycemia-induced oxidative myocardial injury may be fruitful.

In traditional medicine, several Indian medicinal plants or their extracts have been used to treat diabetes (Akhtar and Ali, 1984). Traditional herbal remedies represent new avenues in the search for alternative hypoglycaemic and cardioprotective drugs. Hence, it is the need of the time to study these drugs scientifically with the help of prevailing modern research methodology.

In the scope of a research proposal, addressing the aforementioned problem, it is aimed to study the hemodynamic effects (heart rate and BP) and cardiometabolic changes in experimental diabetes mellitus and to assess the hypoglycaemic (blood glucose and glycosylated hemoglobin), cardioprotective (lactate dehydrogenase and creatine kinase in serum, glutathione in blood, catalase, superoxide dismutase and glutathione in tissue) and antioxidant potential (Thiobarbituric acid reactive substance) of aqueous extract of Clitoria ternatea and Aloe vera gel.

Active oxygen metabolism plays an important role in the normal functioning of the β cells of the pancreas. Free oxygen radicals and oxidative stress appears to be an important factor in the production of secondary complications in diabetes (Thornally et al., 1996). There are many ways by which hyperglycemia may increase the generation of free radicals. The term oxidation describes the capability of glucose to enolize, thereby reducing molecular oxygen and yielding oxidizing molecular oxygen and oxidizing intermediates (Hunt et al, 1988). The reduced oxygen
products formed in the autooxidative reactions are superoxide anion (O$_2^-$), the hydroxyl radical (OH), and hydrogen peroxide (H$_2$O$_2$). All can damage lipids as well as proteins through cross linkage and fragmentation.

The circulating level of malondialdehyde is higher in the plasma of diabetic subjects as compared with those in nondiabetic individuals (Nishigaki et al., 1981). It must be stressed that in vivo measurement of reactive free radical is difficult because of their high reactivity, short half life, and very low concentration (Pryor et al., 1991). Thus, the indirect methods usually used for measuring secondary products of oxidative stress, such as TBARS (thio barbituric acid reactive substance), thought to be the convenient assay of lipid peroxidation in tissue.

In general, antioxidant enzyme such as SOD, catalase and glutathione peroxidase have been reported to be decreased, increased or unaltered in nonvascular beds of diabetic animals, with wide variations from one tissue to another. In vascular beds (Pieper et al., 1995) and cardiac tissue (Wohieb et al., 1987) of diabetic animals, there is a selective increase in catalase, but not in SOD or glutathione peroxidase, which has been interpreted as evidence that diabetic blood vessels are chronically exposed to peroxidative stress due to elevated production of H$_2$O$_2$ in vivo. Meticulous glycemic control with islet transplantation after 8 weeks of untreated streptozotocin (STZ) induced diabetes restores vascular catalase concentrations to normal (Glugliano et al., 1996).

Since STZ treatment do not completely destroy pancreas, still few β cells remain functional and therefore rats injected with STZ do not require insulin to survive, STZ- treated rats are considered to be an extremely useful model of Non Insulin Dependent Diabetes Mellitus (NIDDM) in humans. The present proposal was designed to study the effect of the aqueous extract of *Clitoria ternatea* and 30% *Aloe vera* gel on diabetic
hyperglycaemia using the murine model of streptozotocin induced-diabetes. Cardiomyopathy was induced by lisoproterenol in normal and diabetic rats. The cellular events at the target organs will be assessed on the basis of biochemical studies from blood sample and tissue homogenate, cardiotoxicity will be assessed by light morphometry of heart sample after sacrificing the animal on 21st day of study and the hemodynamic parameters monitored and recorded throughout the duration of the experiment.

Diabetes mellitus is a syndrome initially characterized by a loss of glucose homeostasis, similarly streptozotocin action in β cells is accompanied by characteristic alteration in blood insulin and glucose concentration. Hyperglycemia, defining established diabetes, can induce oxidative stress by free radical generation and over production of superoxide anion. Hyperglycemia can simply inactivate existing enzymes by glycating their protein, also leads to DNA cleavage (Wiernsperger, 2003).

Glycosylated hemoglobin is an easily measured biochemical marker that strongly correlates with the level of ambient glycemia during last 1 to 3 months. In our study we also observed tight regulation of blood glucose level and glycosylated hemoglobin level after treatment of C. ternatea and Aloe vera gel and results are good with dose of 100 mg/kg of C. ternatea and 200 mg/kg of Aloe vera gel. The antidiabetic effect of both the drugs may be due to increased release of insulin from existing β cells of pancreas similar to that observed after gliclazide administration.

The cytotoxicity of a xenobiotic can evaluated using the serum activities of marker enzyme. One such enzyme is serum lactate dehydrogenase (LDH) which, though distributed throughout the body, posses isoenzymes recognized as marker for liver and muscle lesion
(Aldrich, 2003). We have observed the antihyperglycemic activity of Aloe vera gel and C. ternatea. They also reduce the level of LDH in serum of diabetic rats indicating protection of liver and muscles from damage caused by OFR produced by glycation of protein and fat.

Diabetes mellitus is associated with oxidative reactions. Much of the evidence concerning the role of oxidation in the induction of diabetes mellitus comes from the studies of alloxan and streptozotocin, which produce experimental diabetes in animal (Wolf et al., 1993). The drug appears to selectively destroy the islets of langerhans by oxidant production. It has been proved that cytotoxicity of STZ is due to the function of three factors: efficient uptake, alkylation of cellular DNA, and generation of reactive oxygen species along with reduction in antioxidant system.

The circulating level of malondialdehyde is higher in the plasma of diabetic subjects as compared with those in nondiabetic individuals. It must be stressed that in vivo measurement of reactive free radicals is difficult because of their high reactivity, short half-lives and very low concentration (Pryor et al., 1991). Thus, the indirect methods usually used for measuring secondary products of oxidative stress, such as TBARS (thio barbituric acid reactive substance), thought to be the convenient assay of lipid peroxidation in tissue. Oxygen free radicals (OFR) are suggested to increase lipid peroxidation (Lukic, 1998; Spinas, 1999). The increase in TBARS could be due to decrease in antioxidant enzymes and also due to decrease in the non-enzymatic antioxidant such as glutathione (Behrens and Madere, 1991). The accumulation of TBARS during the progression of diabetes may play a role in secondary complications associated with diabetes.

During diabetes there is increased production of OFRs through glucose autoxidation and protein glycation (Hunt et al., 1990; Wolff and...
SUMMARY AND CONCLUSION

Dean, 1987). The oxidative degradation of this oxidant could participate in the formation of lipid peroxidation products. In our study we found an increased level of TBARS in diabetic rats heart, liver and pancreas and the treatment with C. ternatea and 30% Aloe vera gel for 20 days in diabetic rats produced a significant reduction of TBARS levels which is also consistent with the others reports (Yang and Cherian, 1994). Non protein thiol like glutathione (GSH) is one of the important primary defenses that counteract the oxidative stress. We observed a recovery in pancreatic GSH level on 20 days oral treatment with both the test drug. The observed decrease in GSH in diabetic pancreas may be due to utilization of non protein thiols by increased oxygen free radicals produced in hyperglycemic condition associated with diabetes mellitus.

CAT and SOD activity is undoubtedly important to the regulation of oxidative status in diabetes. However, there is variation as to the status of this enzyme in the diabetic state. Some studies have reported decreased SOD activity (Kedziora et al., 2003; Obrosova et al., 2000), while others have shown increase (Rauscher et al., 2001). In our study administration of STZ decreases the activity of SOD in liver heart and pancreas, same finding are reported by others also (Kedziora, 2003). The observed decrease in SOD activity could result from inactivation of SOD by H$_2$O$_2$ or by glycation of the enzyme, which have been reported to occur in diabetes (Sozmen et al., 2001; Soon and Tan, 2002). Likes SOD, variation in the activity of CAT is reported in diabetic rat organs. Some studies has reported decrease in CAT levels in all the vital organ including pancreas while some have reported sharp rise in CAT activity in heart only. (Otsyula et al., 2003), some observed no change in activity (Godin et al., 1988).

Bukan et al. (2004) reported decreased activity of SOD and increased activity of CAT in diabetic rat heart, in contrast to these Babu et al. (2006) have reported a significant rise in SOD, CAT and GPx activity.
in diabetic heart. Kono and Fridovich (1982) reported that an increase in the SOD activity may protect CAT against enzyme inactivation by superoxide radicals. In our study we observed a sharp rise in heart and liver CAT levels in diabetic rats and reduction in pancreatic tissue. This imbalance in CAT activity is normalized by C. ternatea and Aloe vera gel.

Along with hyperglycemia and oxidative stress, hypertension is generally believed to be more prevalent among diabetics. It has been considered that diminished endothelium dependent relaxation due to reduced synthesis or utilization of nitric oxide NO is a common feature in experiment a diabetes. In this study we observed a significant rise in mean blood pressure in STZ induced diabetic rats. Ozcelikay et al. (2000) also found a raised blood pressure in normotensive rats on administration of STZ. Pretreatment with Clitoria ternatea and Aloe vera gel in diabetic rats maintains the blood pressure and did not affect the heart rate in diabetic rats.

Aqueous extract of Clitoria ternatea and Aloe vera gel have been observed to normalize the elevated blood glucose level, mean blood pressure, CAT and SOD levels in liver, heart and pancreatic tissue of diabetic rats.
CONCLUSION

Aqueous extract of *C. ternatea* stems and 30% *Aloe vera* gel showed a good antioxidant and antihyperglycemic activity in animal models in both the doses. Reported chemical constituents of *C. ternatea* are glucoside, palmitic, stearic, oleic acids. It also contains blue anthocynin, apajitin, cliotorin. Further studies are to be carried out for isolation of active principle of *C. ternatea* responsible for its antidiabetic and antioxidant activity. Similarly, *Aloe vera* gel is also reported to contain vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids which possesses antioxidant, anti-inflammatory, hypoglycemic activity, further studies are required for fixation of dose and studies with isolated compound that is responsible for all above pharmacological events.

Studies are also needed to find out the mechanism by which *Clitoria ternatea* and *Aloe vera* gel is maintaining the blood glucose levels.
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