Summary

Conclusions
The present study was undertaken to systematically investigate the beneficial effects of *C. roseus* in insulin deficient and insulin resistance conditions. Type-1 diabetes is a metabolic disorder due to insulin deficiency whereas insulin resistance is the prominent feature of type-2 diabetes, both types of diabetes are characterized by hyperglycemia. Chronic hyperglycemia by itself and by its associated oxidative stress play an important role in the initiation and progression of diabetic complications. Due to increasing obesity, altered dietary habits and sedentary life style both in western and developing countries. The prevalence of both types of diabetes are growing at an exponential rate.

In the present study STZ induced diabetic rats (i.p. injection at a dose of 55 mg/kg b.wt, in 0.1 ml of 0.05 M citrate buffer, pH 4.5) represent the insulin deficient model and chronic feeding of Wistar rats with fructose rich diet (66 %) served as a model for insulin resistance.

*Catharanthus roseus* Linn (G. Don) (Apocyanaceae) has gained acceptance from the pharmaceutical industries as it is widely used as an infusion in different parts of world to treat diabetes (Alexandrova et al., 2000; Heijden et al., 2004). Although, earlier reports indicate blood glucose lowering activity by alcohol extract of leaves (Chattopadhya, 1999) and dichloromethane-methanol extract of leaves and twigs (Somanath et al., 2001), very little information on the biochemical basis of its antihyperglycemic and antioxidant potential is available and no studies are available on the efficacy of *C. roseus* in preventing insulin resistance. So, the present study was undertaken to examine the beneficial effects of *C. roseus* in STZ diabetic and fructose feed induced insulin resistant rat models.
The experimental animals were divided into six groups of 8 animals each viz., control rats (C-group); control rats treated with *C. roseus* (C + CR-group); STZ diabetic rats (D-group); STZ diabetic rats treated with *C. roseus* (STZ + CR-group); fructose fed rats (F-group); fructose fed rats with *C. roseus* treatment (F + CR-group). Aqueous leaf powder suspension of *C. roseus* was administered orally to C + CR, D + CR and F + CR-groups at a dose of 100 mg/kg b.wt, in ~ 2 ml of distilled water per rat once a day for 60 days using intubation tube.

STZ diabetic rats (D-group) showed a gradual increase in blood glucose level with gradual decrease in plasma insulin and body weights during experimental period. Fructose fed rats (F-group) showed a gradual increase in plasma glucose, insulin and body weight during experimental period. Thus fructose fed rats exhibited insulin resistance from its increased HOMA values during experimental period. There are reports showing increase in energy intake, body weight and adiposity with the consumption of high fructose diets both in humans and animals (Tordoff and Alieva, 1990). The intensity of hyperglycemia is more prominent in STZ diabetic rats compared to fructose fed rats. Oral administration of *C. roseus* leaf powder suspension partially prevented the weight loss in STZ diabetic treated rats (D + CR-group) and completely protected the fructose feed induced weight gain in F + CR-group. The clinical symptoms of diabetes like polyphagia, polydipsia and polyuria observed in the STZ diabetic rats were reversed within 15 days of *C. roseus* administration in D + CR-group.

*C. roseus* administration resulted in gradual decrease in plasma glucose with gradual increase in plasma insulin levels in D + CR-group. By the end of experimental period plasma glucose levels of D + CR-group reached near normal values but the observed
increase in the plasma insulin level still significantly lesser than control rats. *C. roseus* treatment completely prevented the fructose induced hyperglycemia and partially prevented hyperinsulinemia, resulting in a significantly decreased HOMA values in F + CR-group compared to F-group. No toxic effects of *C. roseus* administration was observed in control rats treated with *C. roseus*. Further C + CR rats showed normoglycemia with significantly lower plasma insulin levels indicating the beneficial effects of *C. roseus* in maintaining normoglycemia with lower insulin levels.

The observed elevation of transaminase activities in liver and kidney of D-group and F-group rats is an indication of increased protein degradation and amino acid catabolism in these metabolic conditions, thus providing precursors for gluconeogenesis. Increase in transaminase activities was more pronounced in D-group than F-group. *C. roseus* administration for 60 days prevented the increased transaminase activities observed both in D + CR-group and F + CR-group.

Both STZ and fructose fed rats showed dyslipidemia (increased levels of plasma triglycerides, TC, VLDL-C and LDL-C and decreased HDL-C level). The intensity of hyperlipidemia was more in insulin resistance rats (F-group) than insulin deficient rats (D-group). The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase. The fructose is more lipogenic than glucose. High levels of plasma triacylglycerols is a well-established consequence of dietary fructose intake (Kelley *et al.*, 2004). Enhanced hepatic lipogenesis, overproduction of VLDL, and impairment in their peripheral catabolism (Busserolles *et al.*, 2002) are responsible for the
observed dyslipidemia in fructose fed rats. *C. roseus* administration improved the lipid profile by lowering TC, TG, LDL-C and VLDL-C and increasing HDL-C resulting in decreased atherogenic index in both STZ diabetic and fructose fed rats. Increase in plasma insulin levels in D + CR-group and increasing insulin sensitivity in F + CR-group may be the key factor for correction of dyslipidemia in these rats.

The tissue lipids (total cholesterol, triglycerides, phospholipids and free fatty acids) of liver and heart are markedly elevated in both type-1 diabetic and IR rats compared to C-group. Tissue lipid accumulation was more pronounced in F-group than D-group. It was well known that the process of lipid accumulation interferes with utilization of glucose through principles of Randle cycle. The lipogenic character of fructose was evident with increased activity of FAS and malic enzyme in liver and decreased activity of lipoprotein lipase in adipose tissue of fructose fed animals. In contrast to F-group, D-group showed a significantly decreased activity of hepatic fatty acid synthase and malic enzyme compared to C-group. Thus lipogenesis may not be the reason for increased tissue lipid content in D-group rats. The enhanced tissue lipid content was brought down to normal value by *C. roseus* treatment both in D + CR and F + CR-groups. *C. roseus* also rectified the alterations seen in enzyme activities of lipid metabolism in D + CR and F + CR-groups.

The decreased glycogen content (liver and muscle), increased hepatic glycogen phosphorylase activity and glucose-6-phosphatase, decreased activities of glycolytic enzymes (hexokinase, phosphofructokinase and pyruvate kinase in liver and skeletal muscle); hepatic glucose-6-phosphate dehydrogenase activity and increased activities of
gluconeogenic enzymes (fructose, 1-6-bisphosphatase and glucose-6-phosphatase in liver and kidney) were observed in D-group animals.

Similar to STZ diabetic rats fructose fed rats also showed enhanced operation of gluconeogenesis in liver and kidney as evident by enhanced activities of gluconeogenic enzymes (fructose, 1-6-bisphosphatase and glucose-6-phosphatase) and decreased activities of those key glycolytic enzymes (HK, PFK in liver and muscle) seen before the entry of fructose metabolites into glycolytic pathway. Unlike D-group of animals enhanced PK enzyme activity was observed in liver and muscle of F-group animals. In contrast to STZ diabetic animals fructose fed rats (F-group) showed enhanced glycogen stores of liver and muscle with decreased hepatic glycogen phosphorylase activity and enhanced operation of HMP shunt (G6PDH). The increased activity of G6PDH in liver provides large amounts of NADPH needed for lipid synthesis and it may be an adaptatery mechanism to combat the oxidative stress as NADPH is need for regenerating GSH from GSSG.

No change in hepatic fructokinase activity was observed in C+ CR, D and D + CR-groups compared to C-group. However, F-group showed a significantly increased hepatic fructokinase activity compared to C-group. *C. roseus* treatment did not correct the enhanced fructokinase activity by fructose feeding in F + CR-group. The increased activity of liver fructokinase in fructose fed rats (F and F + CR-group) was due to fructose overload on liver.

The alterations observed in the activities of enzymes of carbohydrate metabolism in D-group and F-group rats were significantly restored to near normal values by *C. roseus* treatment in D + CR and F + CR-group.
Thus the enhanced peripheral utilization of glucose by enhanced activities of glycolytic enzymes and decreased production of glucose by decreasing the gluconeogenic and glycogenolytic enzyme activities of D + CR and F+ CR-groups compared to D and F-groups may be responsible for the observed antihyperglycemic activity of \textit{C. roseus}. This indicates that the above changes may be a sequel to elevated levels of circulating insulin in D + CR-group and improved insulin sensitivity in F + CR-group compared to D and F-groups respectively.

Enhanced activities of intestinal disaccharidases (maltase, sucrase and lactase) were observed both in D-group and F-group rats compared to C-group, indicating the increased rate of digestion of disaccharides in these two metabolic conditions (insulin deficiency and insulin resistance). Therefore, they may play an important role in aggravating postprandial hyperglycemia. \textit{C. roseus} treatment significantly decreased the activities of intestinal disaccharides in D + CR and F + CR-groups which indicates protective effect of \textit{C. roseus} treatment by delaying the absorption of disaccharides from intestine under hyperglycemic conditions.

Chronic hyperglycemia causes oxidative stress in tissues prone to complications in patients with diabetes (Greene et al., 1992; Rosen et al., 2001). Hyperglycemia, advanced glycation end products, autooxidation of glucose, polyol pathway and intracellular accumulation of lipids and metabolic alterations all lead to the increased formation of oxygen-derived reactive oxygen species, which cause damage either directly affecting a specific molecule or indirectly by forming numerous toxic derivatives. Defective
antioxidant protection is certainly present in diabetes (Maxwell et al., 1997) and may well contribute to enhanced lipid peroxidation.

The enhancement of tissue LPO and protein oxidation (liver, pancreas and heart) in diabetic and insulin resistance rats indicates the existence of oxidative stress in these conditions. In addition defective antioxidant status was evident in both groups from the decreased GSH content and significantly lowered activities of GSH dependent (GR, GST, GPx) and independent enzymes (SOD and CAT). On the whole the severity of oxidative stress is more prominent in D-rats than F-rats. These abnormalities were prevented with the treatment of C. roseus in D + CR and F + CR-groups. C + CR-group showed higher values of GSH content and increased activities of antioxidant enzymes with decreased lipid peroxidation and protein oxidation levels compared to C-group represents improved antioxidant status by C. roseus treatment.

Enhanced operation of polyol pathway of STZ diabetic rats was evident by the observed elevated activities of aldose reductase and sorbitol dehydrogenase in liver, pancreas and heart compared to C-group. Whereas F-group showed significantly increased activity of sorbitol dehydrogenase without any alterations in aldose reductase activity compared to C-group. C.roseus treatment rectified the alterations observed in polyol pathway enzymes both in D + CR and F + CR-groups.

Thus C. roseus administration was found to be beneficial in insulin deficient rats by increasing the plasma insulin levels, which may be due to regeneration of the damaged β-cells of islets of pancreas and insulin resistance condition by increasing the insulin sensitivity as reflected by controlling the hyperinsulinemia observed in insulin resistance condition.
It appears that apart from acting on carbohydrate metabolic targets compounds present in medicinal plants either alone or in combination, possess a variety of beneficial activities and have the potential to impart therapeutic effect holistically in complicated disorders like diabetes and its complications.

Multiple defects in the pathophysiology of diabetes are mostly imprecisely understood, and therefore warrant not isolating a single drug target to the reversal of all or majority of aspects of the disease. Therefore, the unidirectional therapeutic approach in the management of diabetes does not appear to be the way to address this problem. The beneficial multiple activities like antihyperglycemic activity by manipulating carbohydrate metabolism by various mechanisms, hypolipidemic activity and restoration of enhanced intestinal disaccharidases of diabetic animals and antioxidant potential of C. roseus offer an exciting opportunity to develop this into a novel therapeutic approach for multifactorial pathogenicity of both types of diabetes.