Results

&

Discussions
General observations

During the experimental period of 60 days about 15-20 % mortality was observed in STZ induced diabetic control group, where as no mortality was observed in the remaining groups. No visible side effects and variation in animal behavior (respiratory distress, abnormal locomotion and catalepsy) were observed in C + CR-group representing the non-toxic nature of *C. roseus* leaf powder. D-group rats showed the characteristic signs of diabetes such as polyuria, polydipsia and polyphagia and failure to gain weight. A significantly higher intake of food and water was observed in F-group from 10 days onwards of the experimental period compared to C-group. No significant variation was observed in the intake of food and water among the three groups-C, C + CR and F + CR. Traditional medicines for the treatment of diabetes mellitus are probably based mainly on treatment of its obvious symptoms of pronounced thirst (polydipsia) and polyuria. Administration of *C. roseus* leaf powder suspension resulted in rectification of the clinical signs of diabetes within 10 days of treatment in D + CR-group.

Body weight

Mean body weights of the six experimental groups at 15 day interval are summarized in Table 1 and Fig 7. No significant variation in the initial body weights was observed among C, C + CR, F and F + CR-groups, whereas D and D + CR-groups showed a slight but significantly lesser initial body weight compared to the remaining groups. During experimental period D-group showed a gradual decrease in body weight, while the remaining groups showed a trend of gradual increase in body weight. The data given in Table 1 and Fig 7 & 8 revealed a gradual increase in the body weight of C-rats by 13.3, 27.3,
Table 1. Effect of *C. roseus* treatment on bodyweight in STZ diabetic and fructose fed rats

<table>
<thead>
<tr>
<th>Experimental days</th>
<th>C</th>
<th>C+CR</th>
<th>D</th>
<th>D+CR</th>
<th>F</th>
<th>F+CR</th>
</tr>
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<td>179.37±7.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>168.12±7.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>166.87±6.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>187.37±6.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>184.25±8.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
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<td>201.25±8.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>157.50±7.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>170.00±8.33&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>225.62±7.28&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>176.25±10.60&lt;sup&gt;d&lt;/sup&gt;</td>
<td>248.75±9.10&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>143.12±6.51&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>274.37±4.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>131.25±7.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>200.62±7.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>303.87±8.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>282.87±10.22&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan’s multiple range test).
Fig 7: Body weights of Control (C), Control treated (C + CR), Diabetic (D), Diabetic treated (D + CR), Fructose (F), Fructose treated (F + CR) rats during experimental period.

Fig 8: Per cent change in body weight of six experimental groups during the experimental period.
Results & Discussion

37.0 and 51.4 %, C + CR- rats by 12.2, 25.8, 38.0 and 53.0 %, D + CR-rats by 1.8, 5.6, 11.6 and 20.5 %, F-rats by 18.6, and 32.7, 46.8 and 62.1 % and group-F + CR by 16.7, 30.2, 45.5 and 53.5 % respectively at 15, 30, 45, and 60 days of experimental period when compared to corresponding initial weights. In D-group, body weight decreased gradually during the experimental period from 168.12 ± 7.2 to 131.25 ± 7.9 g. Per cent decrease in body weight of D-group at 15, 30, 45 and 60 days of experimental period was 6.3, 11.5, 14.8, and 22.0 respectively when compared to the initial weight. At the end of experimental period, body weights of F-group were significantly higher (9.2 %) than C-group. Though the D + CR-group showed a gradual increase in body weight at the end of experimental period the body weight of D + CR was significantly lower (27.8 %) than C-group, but significantly (52.8 %) higher than D-group. At the end of experimental period, body weight of F + CR-group did not deviate from C-group. *C. roseus* treatment completely prevented the fructose induced abnormal weight gain and partially prevented the weight loss observed in STZ induced diabetic animals.

A different trend in body weight was observed in STZ diabetic and fructose diet induced insulin resistance rat models during experimental period. STZ diabetic rats as model of Type-I diabetes showed decreased body weight whereas F-group animals as a model of insulin resistance showed excess gain in body weight when compared to the control group.

In spite of polyphagia, decrease in body weight of STZ rats is possible due to defect in glucose metabolism and increased muscle wasting due to excessive break down of tissue proteins. Muscle wasting, negative nitrogen balance and enhanced gluconeogenesis are
characteristic features of uncontrolled diabetes (Swanston et al., 1990; Chatterje and Shinde, 2000).

There are reports indicating increase in energy intake, bodyweight and adiposity with consumption of high fructose diets both in humans (Kanarek and Orthen-Gambill, 1982; Kasim-Karakas et al., 1996) and animals (Tordoff and Allewa, 1990; Astrup et al., 2002; Reddy et al., 2008). One explanation for this observation could be that fructose ingestion did not increase the production of hormones, insulin and leptin that have a key role in the long-term regulation of food intake and energy expenditure (Curry, 1989; Rohner-Jeanrenaud and Jeanrenaud, 1996).

Changes in diet were studied as contributing factors to the development of obesity. The digestion, absorption, and metabolism of fructose differ from those of glucose. Hepatic metabolism of fructose favors de novo lipogenesis, and this may be linked with both hyperlipidemia and increased body fat stores (Herman et al., 1970; Kok et al., 1996). In contrast with glucose, fructose enters cells via a GLUT-5 transporter that does not depend on insulin (Burant et al., 1992). This transporter is present at low concentrations in pancreatic β cells (Sato et al., 1996) and the brain (Payne et al., 1997; Sasaki et al., 2004), which indicates limited entry of fructose into these tissues. Fructose, unlike glucose, does not stimulate insulin secretion from pancreatic β-cells and fructose cannot provide satiety signals because it is transported in less quantity to the brain.

Insulin is involved in the regulation of body adiposity via its actions on the central nervous system (CNS) to inhibit food intake and increase energy expenditure (Woods et al., 1996; Schwartz et al., 2000). Insulin does not enter the brain, but is transported into the CNS
via a saturable receptor-mediated process. Insulin receptors localized in CNS are involved in the control of food intake and energy homeostasis. Insulin administration into the CNS inhibits food intake in animals including non-human primates. Impairment of CNS insulin transport was inversely related to an increase in body weight (Kaiyala et al., 2000). Specifically, knocking out the insulin receptor in neurons results in hyperphagia and obesity in mice (Bruning et al., 2000). Thus, reduced insulin delivery into the CNS or disruption of the insulin-signaling pathways in the CNS may result in weight gain and development of obesity.

Plasma leptin concentrations are strongly correlated with adiposity in rodents (Maffei et al., 1995; Ahren et al., 1997), non-human primates (Havel, 1997) and humans (Weigle et al., 1997). Leptin secretion is dependent on insulin mediated adipocyte glucose transport and metabolism (Mueller et al., 1998). Insulin stimulates leptin gene expression and secretion and has a major role in physiological regulation of leptin production (Havel, 2002). Leptin, the protein product of ob gene secreted from adipose tissue, functions as a circulating signal from body fat stores to the CNS, where it acts to limit adiposity by inhibiting food intake and increasing energy expenditure (Caro et al., 1996; Rohner-Jeanrenaud and Jeanrenaud, 1996).

Thus inability of fructose to stimulate insulin and induction of insulin resistance by high fructose diet may result in decreased insulin production, transportation to CNS and leptin synthesis from adipocytes and secretion further reflected by increased body adiposity and weight.
Increasing body weight together with decreasing physical activity is expected to increase the incidence of several diseases related to lifestyles such as adult type diabetes and vascular atherosclerotic diseases. It has been postulated that increasing consumption of fructose may be a contributory factor in the development of obesity and the accompanying metabolic abnormalities.

*C. roseus* administration for 60 days partially prevented the weight loss in D + CR-group compared to D-group and which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis and may also be due to the improvement in insulin secretion and glycemic control.

*C. roseus* treatment prevented the weight gain in fructose fed rats compared to F-group. Obesity is almost invariably associated with insulin resistance and any reduction of excess body fat contributes to an improvement of insulin sensitivity (Hauner, 1999). Many studies demonstrated that when weight loss occurs, the plasma levels of insulin go down, insulin resistance is decreased, and all of the metabolic syndrome are improved (Groop *et al*., 1992; Dengel *et al*., 1996).

Thus *C. roseus* treatment is found to be beneficial under both STZ diabetic and fructose conditions in regulating the body weights towards the control level.

**Fasting plasma glucose**

The fasting plasma glucose levels of six groups of the animals during experimental period are represented in Table 2 and Fig 9 & 10.
Table 2. Effect of *C. roseus* treatment on fasting plasma glucose levels in STZ diabetic and fructose fed IR rats

<table>
<thead>
<tr>
<th>Experimental days</th>
<th>C</th>
<th>C+ CR</th>
<th>D</th>
<th>D+CR</th>
<th>F</th>
<th>F+ CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>380.87±10.20^b</td>
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<td>73.00±6.66^a</td>
<td>75.01±7.85^a</td>
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<tr>
<td>15</td>
<td>78.91±5.00^a</td>
<td>72.75±7.12^a</td>
<td>388.75±10.45^b</td>
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<td>89.75±5.12^d</td>
<td>76.25±6.92^a</td>
</tr>
<tr>
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<td>75.63±6.07^a</td>
<td>397.06±9.95^b</td>
<td>115.96±9.56^c</td>
<td>99.37±7.35^d</td>
<td>78.25±6.92^a</td>
</tr>
<tr>
<td>45</td>
<td>73.87±6.43^a</td>
<td>72.75±5.57^a</td>
<td>410.37±9.07^b</td>
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<td>110.75±6.34^d</td>
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</tr>
<tr>
<td>60</td>
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<td>73.37±4.74^a</td>
<td>440.25±7.92^b</td>
<td>90.62±9.82^c</td>
<td>118.75±6.47^d</td>
<td>80.45±6.04^a</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan’s multiple range test).
Fig 9: Plasma glucose levels of Control (C), Control treated (C + CR), Diabetic (D), Diabetic treated (D + CR), Fructose (F), Fructose treated (F + CR) rats during experimental period.

Fig 10: Change in body weight of six experimental groups during the experimental period.
There was no difference in plasma glucose levels in C, C + CR, F, and F + CR-groups at the beginning of the experimental period, where as the initial plasma glucose levels of STZ diabetic rats (D and D + CR-group) were ~5.2 folds greater compared to C-group. In D- group, the plasma glucose level increased gradually during the experimental period from 380.8 ± 10.2 to 440.2 ± 7.9 mg/dl. Per cent increase in plasma glucose level of D-group was 15.5 % at the end of experimental period compared to initial level. During the experimental period the plasma glucose level in F-group increased gradually from 73.0 ± 6.6 to 118.7 ± 6.4 mg/dl and the per cent increase was 21.9, 36.1, 51.6 and 62.6 at 15, 30, 45, 60 day intervals compared to initial level. Whereas in C. roseus treated diabetic group (D + CR-group), a significant antihyperglycemic effect was evident from the 15th day onwards and the decrease in the glucose was maximum and reached near normal values by 60 days of treatment and the per cent decrease in serum glucose was 48.7, 70.4, 76.4 and 76.8 at 15, 30, 45 and 60 day intervals compared to initial levels. Groups-C, C+ CR and F + CR remained persistently euglycemic throughout the course of the study. The plasma glucose level of C + CR, D + CR and F + CR-groups during the experimental period clearly indicates that C. roseus does not exhibit hypoglycemic activity, instead, it shows antihyperglycemic effect.

**Plasma Insulin**

The fasting plasma insulin levels of six groups of the animals during experimental period are represented in Table 3 and Fig 11 &12.

Initial plasma insulin levels of D and D + CR-groups were around 4 fold lower than remaining 4 groups. Control-group showed no significant variation in the insulin levels
Table 3. Effect of *C. roseus* treatment on fasting insulin levels in STZ diabetic and fructose fed IR rats

<table>
<thead>
<tr>
<th>Experimental days</th>
<th>Fasting insulin (μU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>0</td>
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</tr>
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</tr>
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<td>45</td>
<td>42.75±3.71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>44.87±3.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
Fig 11: Plasma insulin of Control (C), Control treated (C + CR), Diabetic (D), Diabetic treated (D + CR), Fructose (F), Fructose treated (F + CR) rats during experimental period.

Fig 12: Change in insulin levels of six experimental groups during the experimental period.
during the experimental period. By the end of experimental period, D-group plasma insulin decreased by 17.44 % i.e. from 11.1 ± 0.5 to 9.2 ± 0.5 µU/ml. D + CR-group showed a significant increase in insulin concentration and the per cent of increase was 26.5, 91.2, 90.4 and 103.2 at 15, 30, 45 and 60 days respectively compared to initial level. By the end of experimental period, the insulin level of D + CR-group was significantly higher (115.2 %) than D-group but still significantly lower than C-group. Group-F exhibited a gradual increase in plasma insulin during experimental period showing a significantly higher concentration of insulin at 15, 30, 45 and 60 days with 10.7, 48.6, 77.2 and 139.8 % increase respectively when compared with corresponding values of C-group. The insulin level of F + CR-group at the end of experimental period was significantly lower (46.4 %) than F-group but still significantly higher (28.4 %) than C-group. Control rats treated with C. roseus showed a gradual decrease in the insulin level during experimental period with statistically significant lower insulin level at 45 and 60 days when compared to corresponding data of C-group. Thus C. roseus treatment for 60 days led to given partial protection from STZ diabetic induced depletion in plasma insulin level and also fructose diet induced insulin resistance.

**HOMA**

HOMA approach has been widely used in clinical research to assess insulin sensitivity.

Table 4 and Fig 13 & 14 show HOMA data for insulin resistance. There was no deviation in the initial HOMA values among C, C + CR, F and F + CR-groups, whereas D
Table 4. Effect of *C. roseus* treatment on insulin sensitivity in STZ diabetic and fructose fed IR rats

<table>
<thead>
<tr>
<th>Experimental days</th>
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<th>D</th>
<th>D+CR</th>
<th>F</th>
<th>F+CR</th>
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<td>10.43±0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.13±0.32&lt;sup&gt;c&lt;/sup&gt;</td>
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</tr>
<tr>
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<td>5.35±0.40&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>11.44±0.62&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
Fig 13: HOMA values of control (C), control treated (C + CR), diabetic (D), diabetic treated (D + CR), fructose (F), fructose treated (F + CR) rats during experimental period.

Fig 14: Per cent recovery from diabetic induced and fructose feed induced alterations in plasma insulin and HOMA values by *C. roseus* administrations in D + CR and F + CR-groups.
and D + CR-groups initial HOMA value are significantly higher than C-group. During the experimental period, no change in the HOMA value was observed in C and D-groups, while F-group showed a gradual increased in the HOMA value with 38.2, 107.2, 183.6 and 327.8 % increase at 15, 30, 45 and 60 days compared to initial value. By the end of experimental period F-group showed 327 % increase in HOMA value whereas C. roseus treatment resulted in only 51.3 % increase in HOMA value in F + CR-group. However CR treatment resulted in a gradual decrease in HOMA value in C + CR and D + CR-groups with 12 % and 54 % decrease by the end of the experimental period.

STZ diabetic rats showed severe hyperglycemia and hypoinsulinemia. Fructose feeding for 60 days resulted in hyperglycemia and hyperinsulinemia in F-group animals. STZ is a diabetogenic agent because it selectively destroys the insulin producing β-cells of pancreas by inducing necrosis. It is postulated that selective β-cell toxicity of STZ is related to glucose moiety in its chemical structure (Fig 3). This enables STZ to enter the cells via the GLUT-2 in the plasma membrane of β-cells. Karunanayak et al. (1976) claimed that β-cells of pancreas possesses a high affinity to glucose molecules which was also evident from the accumulation of large portions of STZ in β-cells when administered intraperitoneally which induces degenerative damage directly on β-cells (Karasu and Altan, 1987). Thus STZ diabetic rats showed a marked depletion in plasma insulin levels.

Insulin plays a central role in the regulation of glucose homeostasis. Thus decreased circulating levels of insulin observed in STZ diabetic rats resulted in hyperglycemia. The gradual decrease in the plasma insulin levels during experimental period in STZ diabetic rats may be further due to hyperglycemia induced oxidative damage to β-cell of pancreas.
The antihyperglycemic activity by *C. roseus* administration was evident from the blood glucose levels of D + CR and C + CR-groups. Control rats treated with *C. roseus* showed normoglycemia throughout the experimental period indicating that *C. roseus* does not exhibit hypoglycemic activity. Hypoglycemic plants act through a variety of mechanism such as improving insulin sensitivity, augmenting glucose dependent insulin secretion and stimulating the regeneration of islets of Langerhans in pancreas of STZ induced diabetic rats. The elevation in the plasma insulin levels in the *C. roseus* treated STZ diabetic rats is due to substances present in the plant extract which stimulate insulin secretion or which protect the intact functional β-cells from further deterioration so that they remain active and continue to produce insulin or due to regeneration of STZ destructed β-cell. This is probably due to the fact that pancreas contains stable (quiescent) cells which have the capacity of regeneration (Banerjee *et al.*, 2005; Cano *et al.*, 2008).

In addition, the phytochemicals like flavonoids and alkaloids in the *C. roseus* leaf might have protected the intact functional β-cells from hyperglycemia induced oxidative stress on pancreas. It was also claimed that antioxidants possibly be beneficial in preventing type-1 diabetes by stopping the oxidative damage on pancreas and increases insulin secretion (Eriksson and Kahvakka, 1995; Knekt *et al.*, 1999). This possibility is strengthened by the observation that insulin concentration also increased by antioxidant treatment.

It is well known that high fructose diet leads to IR, hyperinsulinemia, dyslipidemia and hypertension in animal models (Elliott *et al.*, 2002). However, the effect of fructose treatment on plasma glucose levels as reported by different researchers varied from no
change (Sambandam et al., 1997), transient elevation (Lee et al., 1994), to a significant elevation (Rosen et al., 1997). In the present study fructose fed animals showed a significantly enhanced plasma glucose, insulin and IR as reflected by HOMA value from 15 days on words till the end of the experimental period. The consequences of fructose induced insulin resistance include impaired glucose tolerance (Elliott et al., 2002; Reddy et al., 2008). Dietary fructose metabolism leads to the creation of glucose. In addition, the increased concentration of FFA in the liver increases hepatic glucose production (Elliott et al., 2002). Fructose consumption, however, does not directly promote insulin secretion from pancreatic cells, which is necessary for glucose metabolism. Glucose produced as a result of fructose metabolism stimulates insulin release, but the fructose induced insulin resistance prevents the insulin from effectively metabolizing glucose resulting in hyperglycemia (Bezerra et al., 2000; Teff et al., 2004). Insulin resistance also led to compensatory hyperinsulinemia, where the body attempts to balance the reduced effect of insulin by producing and releasing more insulin (Zavaroni et al., 1980; Suga et al., 2000). Insulin resistance, a wide spread feature of atherogenic diseases, predisposes the affected individuals to various diseases including hypertension, dyslipidemia, cardiovascular problems and type-2 diabetes mellitus (Zheng et al., 2005). Lowering endogenous insulin level is a key step to successful therapy directed at IR related diseases (Goldstein, 2002). Fructose fed rats contain normal number of insulin receptors in liver and muscle, but reduced receptor autophosphorylation in the liver after stimulation with insulin which is correlated with insulin resistance in different animal models (Carvalho et al., 1996; Saad et al., 1997)
Unlike fructose fed rats, *C. roseus* administration along with fructose feeding prevented the rise in blood glucose level and maintained normoglycemia. However *C. roseus* treatment partially prevented the hyperinsulinemia observed in fructose fed rats with significant decrease in HOMA values. These results indicate enhanced insulin sensitivity by *C. roseus* administration in F + CR-groups compared to F-group. Insulin sensitizing activity has been reported to reduce plasma insulin levels leading by the same occasion to a reduction of plasma glucose levels and blood pressure (Dai and Mc Neill, 1995). The significant decrease in insulin and HOMA values with normoglycemia observed in control rats treated with *C. roseus* also reflects beneficial effect of this plant in enhancing the insulin sensitivity.

*C. roseus* is thus advantageous in maintaining normoglycemia both under type-1 and insulin resistance conditions.

Hypoglycemic activity has been reported for many plants in STZ and alloxan induced diabetes and fructose feed induced insulin resistance in experimental animals. Myrtle oil from *Myrtus communis* (Sepici et al., 2004), chloroform extract of *Andrographis paniculata* roots (Koteswara Rao, 2006), an aqueous extract of rice bran (*Oryza sativa*) (Hikino et al., 1988), leaf extracts of *Terminalia catappa* (Mansoor et al., 2005), aqueous extract of the bark of *Pterocarpus marsupium* (Dhanabal et al., 2006), seeds of *Trigonella foenum-graecum* (Raju et al., 2001), and aqueous-ethanolic extract of flowers of *Punica granatum* (Jafri et al., 2000) are reported to have hypoglycemic activity in alloxan induced hyperglycemic animals. Aqueous root extract of *Rubia cordifolia* (Baskar et al., 2006), gel extract of *Aloe vera* (Noor et al., 2008), aqueous and butanolic extracts of *Tournefortia*

**Plasma lipids**

Abnormalities of plasma lipid and lipoprotein metabolism are very common in diabetes and insulin resistance and have long been thought to play a role in atherogenesis under these two clinical conditions.

The plasma lipid profile at 0 and 60 days of six experimental groups are represented in Table 5 and Fig 15.

No significant variation was observed in the plasma lipid profile i.e. triglycerides (TG), total cholesterol (TC), LDL-C, VLDL-C, HDL-C and atherogenic index of C, C + CR, F and F + CR-groups at 0 days of experimentation whereas STZ diabetic groups i.e. D and D + CR-groups showed significantly increased plasma TG (13.7 and 10.0 %), TC (11.3 and 11.9 %), LDL-C (14.3 and 9.4 %), VLDL-C (49.3 and 63.3 %) and a significantly decreased
Table 5. Effect of *C. roseus* treatment on plasma lipid profile in STZ diabetic and fructose fed IR rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Days</th>
<th>C</th>
<th>C + CR</th>
<th>D</th>
<th>D + CR</th>
<th>F</th>
<th>F + CR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cholesterol</strong></td>
<td>Initial</td>
<td>65.26±3.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.19±2.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.67±3.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.03±2.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.60±3.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.18±4.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>72.89±3.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.21±4.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.51±5.86&lt;sup&gt;c&lt;/sup&gt;</td>
<td>75.60±3.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>118.20±6.19&lt;sup&gt;d&lt;/sup&gt;</td>
<td>76.27±3.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Triglycerides</strong></td>
<td>Initial</td>
<td>69.91±3.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.01±3.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.51±2.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.89±2.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.25±4.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.88±5.79&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>Final</td>
<td>76.28±4.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.78±2.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.37±5.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>77.46±2.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>156.54±8.80&lt;sup&gt;d&lt;/sup&gt;</td>
<td>80.48±8.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>HDL-cholesterol</strong></td>
<td>Initial</td>
<td>33.71±1.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.26±0.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.41±1.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.18±1.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.35±2.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.94±1.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>Final</td>
<td>29.43±1.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.76±0.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36.10±1.45&lt;sup&gt;d&lt;/sup&gt;</td>
<td>32.50±0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.91±1.81&lt;sup&gt;d&lt;/sup&gt;</td>
<td>28.93±1.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>LDL-cholesterol</strong></td>
<td>Initial</td>
<td>13.90±0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.99±0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.90±0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.21±0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.04±0.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.77±0.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>Final</td>
<td>15.25±0.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.75±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.87±1.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.49±0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.30±1.71&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.09±1.65&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>VLDL-cholesterol</strong></td>
<td>Initial</td>
<td>17.65±3.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.93±2.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.36±2.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.83±2.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.20±3.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.47±2.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>Final</td>
<td>28.17±2.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.76±3.82&lt;sup&gt;c&lt;/sup&gt;</td>
<td>38.70±4.27&lt;sup&gt;d&lt;/sup&gt;</td>
<td>27.60±2.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59.97±4.36&lt;sup&gt;e&lt;/sup&gt;</td>
<td>31.05±2.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Atherogenic index</strong></td>
<td>Initial</td>
<td>1.93±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.05±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.39±0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.56±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.10±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.13±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>2.47±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.64±0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.20±0.21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.32±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.55±0.20&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.63±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different from initial values of controls at P<0.05 (Duncan’s multiple range test).
Fig 15: At the end of experimental period percent change in plasma lipids of control treated (C + CR), diabetic (D), diabetic treated (D + CR), fructose (F) and fructose treated (D + CR) rats compared to control rats.
HDL-C (9.8 and 13.4 %) with significantly increased atherogenic index (23.8 and 32.6 %) compared to C-group at 0 days of experimentation. At the end of experimental period of 60 days C-group showed significantly increased plasma TG (9.1 %), TC (11.7 %), LDL-C (9.7 %), VLDL-C (59.6 %) and a significantly decreased HDL-C (12.6 %) when compared to corresponding initial values. These changes in the lipid profile during aging process were further intensified in D and F-groups. *C. roseus* treatment for 60 days prevented the STZ diabetic and fructose diet intensified alterations in the plasma lipid profile during ageing process. After completion of 60 days of experimental period both STZ diabetic (D-group) and insulin resistance (F-group) rats showed significantly enhanced plasma TG (17.2 and 105.2 %), TC (13.2 and 62.1 %), LDL-C (17.4 and 105.2 %) and VLDL-C (37.7 and 112.8 %) with significantly decreased HDL-C (11.3 and 12.0 %) in comparison with C-group. These alterations in lipid profile resulted in increased atherogenic index in both D (29.5 %) and F (84.2 %) groups compared to C-group. Thus the hyperlipidemia (dyslipidemia) observed in F-group is more severe than D-group. *C. roseus* administration for 60 days restored the plasma lipid profile to normal values both in D + CR and F + CR-groups. A significant decrease in plasma TG (9.8 %), TC (14.6 %), LDL-C (9.8 %), VLDL-C (61.8 %) and a significant increase in HDL-C (28.3 %) with a significant decrease in atherogenic index (33.5 %) in C + CR-group was observed when compared to C-group.

The present study revealed the existence of hyperlipidemia both in STZ diabetic and fructose fed insulin resistant rat models. The intensity of dyslipidemia is more prominent in F-group rats than STZ diabetic rats. The lipid abnormalities observed in both rat models (type-1 and IR rats) affected all lipid fractions.
C. roseus administration for 60 days reflected its beneficial effect in rectifying dyslipidemia observed both under STZ diabetic and fructose-fed conditions. Further the significant alteration in the lipid profile of control rats treated with C. roseus (C + CR-group) when compared to C-group indicates hypolipidemic effect of C. roseus. Likewise, Antia and Okokon, (2005) also reported the hypolipidemic activity by C. roseus leaf extract.

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. On the other hand, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (Al-shamaony et al., 1994).

There are numerous studies in which dietary fructose has been shown to induce hyperlipidemia in rodents. Numerous mechanisms have been suggested to explain this phenomenon. These include enhanced hepatic lipogenesis, overproduction of VLDL, and impairment in their peripheral catabolism (Busserolles et al., 2002). As previously discussed, the two monosaccharides-glucose and fructose are metabolized differently. Hellerstein, (1996) showed that there is little de novo lipogenesis from glucose under eucaloric conditions in humans. In contrast, Schwarz et al. (2000) reported 3- to 15-fold increase in fractional de novo lipogenesis from fructose above fasting concentrations in obese and lean subjects and nearly 30 % of circulating triacylglycerol palmitate after fructose ingestion resulted from de novo lipogenesis derived from fructose.
In the past, fructose was considered to be beneficial in the dietary management of diabetes mellitus and insulin resistance because fructose ingestion results in smaller postprandial glycemic and insulin excursions than do glucose and complex carbohydrates (Gliksmann and Bowman, 1993). In contrast with low doses of fructose, when much larger amounts of fructose are consumed, fructose continues to enter the glycolytic pathway distal to phosphofructokinase, and hepatic triacylglycerol production is facilitated. Fructose can provide carbon atoms for both the glycerol and the acyl portions of acylglycerol molecules (Mayes, 1993). Thus, unlike glucose metabolism, in which the uptake of glucose is negatively regulated at the level of phosphofructokinase, high concentration of fructose can serve as a relatively unregulated source of acetyl-CoA. Indeed, studies in human subjects have shown that fructose ingestion results in markedly increased rates of de novo lipogenesis (Schwarz et al., 1993, 1994) whereas de novo lipogenesis does not increase in response to eucaloric glucose ingestion (Hellerstein et al., 1996). Thus, fructose is more lipogenic than is glucose, an effect that might be exacerbated in subjects with existing hyperlipidemia (Jeppesen et al., 1995) or insulin resistance or type-2 diabetes (Abraham et al., 1998).

In the present study dyslipidemia observed in STZ diabetic and fructose fed rats are characterized by elevated plasma TG, TC, LDL-C, VLDL-C with decreased HDL-C. This pattern strongly correlates with cardiovascular risk.

The enhanced plasma LDL-C along with severe hyperglycemia observed in STZ diabetic rats is favorable for increased non-enzymic glycation of LDL. There are reports that LDL gains or increases its atherogenic potential after chemical modification including glycation (Numano et al., 1997). This explains the role of LDL in premature development of
atherosclerosis under diabetic conditions (Retsky et al., 1999). In STZ diabetic rats due to deficiency of insulin, the ineffective utilization of glucose leads to the breakdown of fat and protein. The increased mobilization and utilization of fat for energy requirements results in hyperlipidemia especially an increase in non-esterified fatty acids, triacylglycerols and cholesterol levels of plasma STZ diabetic rats. Elevated plasma TG concentration was reported in both IDDM and NIDDM (Laws et al., 1989; Baynes et al., 1991; Paterson et al., 1991). In IDDM, decreased clearance seems to be the main cause of the high triacylglycerol level (Nikkila et al., 1977; Taskinen and Nikkila, 1979) whereas in NIDDM the triacylglycerolaemia may be due to increased production by the liver (Greenfield et al., 1980; Kissebah et al., 1982; Dunn et al., 1984) and decreased clearance (Nikkila et al., 1977; Taskinen et al., 1982). Triacylglycerol secretion rates are generally higher in NIDDM than in IDDM (Greenfield et al., 1980).

There is an overwhelming evidence that fructose increases plasma TG. Many studies, both of animals and humans, have reported increases in TG after consumption of diets with fructose compared with complex carbohydrates and other sugars. In humans, some groups are more susceptible than others to hypertriglyceridemia that follows fructose consumption. Postmenopausal women, hyperinsulinemic men, and type-2 diabetes are more likely to become hypertriglyceridemic after fructose consumption than normoinsulinemic and non-diabetic men and young women (Reiser, 1985). So, fructose has been discouraged for use in diabetic patients on the basis of its supposed effects on plasma triglycerol and there is concern about a relation between fasting and non-fasting triglycerols and cardiovascular diseases (Bansal et al., 2007; Nordestgaard et al., 2007; Sarwar et al., 2007).
Carbohydrate induced hypertriglycerolemia results from a combination of both TG over production, and inadequate TG clearance (Kazumi et al., 1986; Parks and Hellerstein, 2000). Glucose stimulates both TG production, and TG removal, maintaining homeostasis. Fructose stimulates TG production, but impairs removal, creating the known dyslipidemic profile (Kazumi et al., 1997). Also, high rates of lipolysis in visceral adipose depots can increase availability of non-esterified fatty acids (NEFA) and promote hepatic TG synthesis (Fried and Rao, 2003).

Hypertriglyceridemia may be secondary to increases in the very low-density lipoprotein-triglyceride (VLDL-TG) secretion rate since elevations in plasma triacylglycerol levels have been correlated with rises in this rate (Zavaroni et al., 1982). Previous studies have shown that the VLDL-TG secretion rate in the liver of rats fed a high-sucrose or high-fructose diet was higher than that of controls (Frayn, 1993; Bjorntorp, 1994). Because insulin resistance and reduced insulin binding have been reported in hypertriacylglycerolemic persons (Bieger et al., 1984), this may be one mechanism by which fructose diets promote insulin resistance. Administration of benfluorex and bezafibrate, hypolipidemic agents, reversed the insulin resistance induced by fructose feeding in rats. The improvement was associated with the normalization of triacylglycerol concentrations (Storlien et al., 1993; Matsui et al., 1997). Further insulin resistance may contribute to hypertriglyceridemia by reducing the inhibitory effect of insulin on VLDL-TG secretion.

Although effects of fructose-containing diets on plasma TG are often substantial, the reported effects on plasma cholesterol in response to fructose in the diet are not clear-cut.
The increase in the synthesis of VLDL, which contains 10-20% cholesterol, to transport TG produced in the liver may be a cause of the increase in plasma cholesterol after dietary fructose consumption reported in a few studies. Another mechanism by which fructose could increase plasma cholesterol is by interaction between fructose and copper. Animal (Failla and Seidel, 1988) and human (Reiser et al., 1985) studies report that diets containing fructose aggravate copper deficiency, which results in hypercholesterolemia and may be related to coronary disease (Klevay, 1975). The hypercholesterolemia of copper deficiency is not well understood, but may result from reduced lecithin:cholesterol acyl transferase and lipoprotein lipase activities found in copper deficient animals.

Elevated NEFA concentrations are one of the metabolic consequences of a chronic positive energy balance and increased body adiposity (Ji Equier and Tappy, 1999). The exposure to increased concentrations of NEFA may reduce insulin sensitivity by increasing the intramyocellular lipid content (Virkamaki et al., 2001). Increased portal delivery of NEFA, particularly from visceral adipose tissue, could also lead to impaired carbohydrate metabolism, because elevated portal NEFA concentrations increases hepatic glucose production (Rebrin et al., 1995; Steil 1998). In addition, over time increased NEFA concentrations may have a deleterious effect on cell function (Bergman and Ader, 2000).

In the present study 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.

Various phytochemicals reported in C. roseus, like phenolic compounds, flavonoids and alkaloids (Mustafa and Verpoorte, 2007) may be responsible for its hypolipidemic
activity. In addition in the present study as we have used the leaf powder suspension, other potent compounds such as fibers may be responsible for lipid lowering action.

Reports are available for lipid lowering activity of many plant extracts and phytochemicals. Flavonoids extracted from the fruits of *Solanum melongena* showed significant hypolipidemic action in normal and cholesterol fed rats (Sudheesh *et al*., 1997). Flavonoids from mulberry leaves showed hypoglycemic activity in triton WR-1339 induced hyperlipidemic mice (Chen and Li, 2007). Flavonoid rich extract from *Eugenia jambolana* seeds showed hypoglycemic activity in streptozotocin induced diabetic rats (Sharma *et al*., 2008). Gugullipid, an alkaloid of *Commiphera mukul* (Dalvi *et al*., 1994), dried flowers of *Adenocalymma alliaceum* (Srinivasan and Srinivasan, 1995), *Trigonella graceum* (Sharma *et al*., 1990) possess hypolipidemic activity in hypercholesterolaemic rats and in Type-1 diabetic animals. S-methyl cysteine sulphoxide from *Allium cepa* (Kumari and Mathew, 1995) and polysaccharide from *Lycium barbarum*, showed hypolipidemic activity in high-fat diet induced insulin resistance rats (Rui *et al*., 2005). *Brassica juncea* seeds (Yadav *et al*., 2004) and *Panax ginseng* root (Attele *et al*., 2002) are reported to have hypolipidemic activity in fructose feed induced insulin resistance rats.

Hyperlipidemia observed in both STZ diabetic rats and fructose fed rats may be due to pathogenesis of lipid metabolic disturbances under insulin deficiency or of inadequate insulin efficiency seen in these animals. The possibility that there is a causative link between elevated circulating TG and impaired insulin action warrants further study because of the prevalence of hypertriglycerideremia in diabetes. Also, because of the recent demonstration of significant negative correlation between TG levels and insulin mediated glucose disposal in
a population with normal glucose tolerance but a high risk for development of a noninsulin-
dependent diabetes mellitus. *C. roseus* has given protection against hyperlipidemia observed
under STZ and fructose fed condition. In order to understand the details of hypolipidemic
activity of *C. roseus*, tissue lipid content and key enzymes involved in lipid metabolism in
the liver and adipose tissue are assayed.

Transaminases

Tissue aspartate transaminase (AST) and alanine transaminase (ALT) are important
enzymes that aid in making amino groups available for entry into the urea cycle. Measurement
of their activity provides an indication of amino acid catabolism.

The liver is the major site of nitrogen metabolism in the body. In times of dietary
surplus, the potentially toxic nitrogen of amino acids is eliminated via transaminations,
deamination, and urea formation; the carbon skeletons are generally conserved as
carbohydrate, via gluconeogenesis, or as fatty acid via fatty acid synthesis pathways.
Moreover, AST and ALT levels also act as indicators of liver function.

Animals must metabolize proteins to amino acids, at the expense of muscle tissue,
when blood sugar is low. The preference of liver transaminases for oxaloacetate or alpha-
keto glutarate plays a key role in funneling nitrogen from amino acid metabolism to aspartate
and glutamate for conversion to urea for excretion of nitrogen. Similarly, in muscles the use
of pyruvate for transamination gives alanine, which is carried by the bloodstream to the liver
(the overall reaction being termed "glucose/alanine cycle"). Tissue transaminase activities
are increased in situations associated with enhanced gluconeogenesis. Risk of chronic liver
disease is higher in diabetics. Serum ALT is sensitive predictor of mortality from disease.

The data presented in the Table 6 reveals the hepatic and renal transaminase (ALT
and AST) activities of the six experimental groups. A significant increase in tissue
transaminase activities was observed both in D and F-groups compared to C-group. Increase
in transaminase activities was more pronounced in D-group than F-group. The per cent
increase in hepatic ALT and AST activities of D and F- groups compared to C-group are
70.4 and 60.1 %, and 12.2 and 13.5 % respectively. The per cent increase in renal ALT and
AST activities of D and F-groups compared to C-group are 90.1 and 71.4 %, and 38.1 and
19.0 % respectively. *C. roseus* treatment for 60 days resulted in no significant alterations in
the activities of hepatic and renal transaminases in C + CR-groups compared to C-group.
Whereas except renal ALT activity of D + CR-group, *C. roseus* administration for 60 days
prevented the increased transaminase activities observed both in STZ induced diabetic
condition (D + CR-group) and fructose fed condition (F + CR-group).

The observed elevation of transaminase activities in liver and kidney of STZ-diabetic
and insulin resistance rats is an indication of increased protein degradation and amino acid
catabolism under hyperglycemic conditions, thus providing precursors for gluconeogenesis.
In addition, enhanced non-enzymic glycation of proteins under hyperglycemic conditions
may decrease the half life of proteins, thus contributing to the enhanced protein degradation
(Vlassara and Palace, 2002). More prominent enhancement in transaminase activities of
diabetic rats compared to fructose fed rats indicates more intensified protein degradation and
Table 6. Effect of *C. roseus* treatment on tissue transaminases in STZ diabetic and fructose fed IR rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>C</th>
<th>C+ CR</th>
<th>D</th>
<th>D+CR</th>
<th>F</th>
<th>F+CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (μg of pyruvate formed / min/ mg protein)</td>
<td>Liver</td>
<td>1.22±0.03a</td>
<td>1.20±0.01a</td>
<td>2.08±0.04b</td>
<td>1.28±0.03a</td>
<td>1.38±0.04c</td>
<td>1.26±0.02a</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>0.21±0.01a</td>
<td>0.22±0.01a</td>
<td>0.41±0.02b</td>
<td>0.26±0.02c</td>
<td>0.29±0.02d</td>
<td>0.22±0.01a</td>
</tr>
<tr>
<td>AST (μg of pyruvate formed / min/ mg protein)</td>
<td>Liver</td>
<td>0.81±0.02a</td>
<td>0.82±0.02a</td>
<td>1.30±0.03b</td>
<td>0.86±0.03a</td>
<td>0.92±0.02c</td>
<td>0.78±0.01a</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>0.42±0.01a</td>
<td>0.38±0.03a</td>
<td>0.73±0.02b</td>
<td>0.44±0.01a</td>
<td>0.51±0.01c</td>
<td>0.44±0.01a</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan’s multiple range test).
amino acid catabolism under insulin deficient condition than insulin resistance condition. Higher ALT concentrations were cross-sectionally associated with obesity and whole-body and hepatic insulin resistance and prospectively associated with a decline in hepatic insulin sensitivity and the development of type-2 diabetes (Barbora et al., 2002). According to Barbora et al. (2002) high ALT is a marker of risk for type-2 diabetes and suggest a potential role of the liver in the pathogenesis of type-2 diabetes.

The increased levels of transaminases are active in the absence of insulin because of increased availability of amino acids in diabetics (Felig et al., 1970; Shreepad and Jamee, 1996). This may be responsible for the increased gluconeogenesis and ketogenesis observed in STZ diabetic and fructose fed conditions. Ineffective utilization of glucose in insulin deficiency/insulin insensitivity state leads to enhanced breakdown of proteins thereby providing substrate for gluconeogenesis. Muscle wasting, negative nitrogen balance and accelerated gluconeogenesis are among the hallmarks of uncontrolled diabetes (Buse et al., 1972).

Earlier literature (Maiti et al., 2004; Prakasam et al., 2004) are in accordance with our observations of enhanced transaminases activity under insulin deficient condition (STZ diabetic condition). Enoka et al. (2004) reported decreased concentrations of amino acids in plasma, liver and kidney of Zucker diabetic fatty rats (insulin resistance rats) which is an indication of enhanced catabolism of these amino acids.

Liver, an insulin dependent tissue, plays a pivotal role in glucose and lipid homeostasis and it is severely affected during diabetes. The restoration of transaminase
activities of liver and kidney of D + CR and F + CR-rats to their respective normal levels further strengthens the protective effect of *C. roseus* against diabetes induced alterations.

**CARBOHYDRATE METABOLIC STUDIES**

The concept of over and under utilization of glucose by peripheral tissues during diabetes and insulin resistance plays a pivotal role during the disarray of glucose metabolism leading to elevated systemic glucose. In an attempt to gain an insight into the underlying biochemical mechanism of antihyperglycemic effect of *C. roseus*, the hepatic and muscle glycogen content and the key enzymes of carbohydrate metabolism i.e. glycolysis, HMP shunt, gluconeogenesis, glycogenolysis were assayed in six experimental groups.

**Glucose metabolism**

Liver is the first major tissue to have an opportunity to remove glucose from the portal vein blood. Uptake of glucose in liver cells occurs independent of insulin by means of GLUT-2, a low affinity, high capacity glucose transporter. When blood glucose is high, the liver removes glucose for the glucose-consuming processes of glycogenesis and glycolysis. When blood glucose is low, the liver supplies the blood with glucose by the glucose-producing processes of glycogenolysis and gluconeogenesis. The liver is also the first organ exposed to the blood flowing from the pancreas and therefore "senses" the highest concentrations of hormones (glucagon and insulin) released from this endocrine tissue. These important hormonal regulators of blood glucose levels affect key enzyme-catalyzed steps in the liver.
Muscle and heart cells readily utilize glucose. Insulin stimulates transport of glucose into these cells by way of GLUT-4 (glucose transporter isoform 4). Binding of insulin to its receptor on the plasma membrane initiates a signaling cascade that promotes translocation and fusion of GLUT-4 containing vesicles with the plasma membrane thereby placing GLUT-4 in a position where it can facilitate glucose transport. Glucose taken into muscle and heart cells can be utilized by glycolysis and TCA cycle to provide ATP. Muscle is capable of synthesizing significant quantities of glycogen, an important fuel that the cells of these tissues store for later consumption.

**Fructose metabolism**

The consequence of the digestion of sucrose and other fructose containing foods such as honey, fruits, and some vegetables, is absorption and transport of fructose by the intestinal epithelium into the hepatic portal vein. Therefore, all fructose absorbed flows through the liver initially. Uptake of fructose in liver and skeletal muscle is through insulin independent facilitative glucose transporters i.e. GLUT-2 in liver and GLUT-5 in muscle. Because of the presence of an active hepatic enzyme system for metabolizing fructose, fructose readily passes into the liver. In humans it was shown that the liver metabolized at least 50% of the fructose injected intravenously (Mendeloff and Weichselbaum, 1953). As a consequence of the high rate of extraction of fructose by the liver, correspondingly low concentrations of fructose are found in the systemic blood vessels after meals containing fructose or sucrose are consumed (Crossley and Macdonald, 1970). Some 20% of fructose administered intravenously is taken up by the kidney (Bj Orkman and Felig, 1982) and considerably smaller fraction would be available for adipose tissue (Froesch and Ginsberg,
1962) and skeletal muscle (Bergstrom and Hultman, 1967). Recent studies revealed that GLUT-5 is specifically localized in the plasma membrane in human skeletal muscle, and fractionation studies using human adipocytes or skeletal muscle demonstrated that it is not further recruited to the plasma membrane in response to insulin stimulation (Shepherd, 1992). Previous study showed that when fructose is infused into exercising subjects to maintain a concentration of 5.5 mmol/L, which is above physiological concentrations and above the glucose concentration, there is considerably more fructose oxidation by exercising and resting muscles (Ahlborg and Bj Orkman, 1990).

The metabolism of fructose appears to be different in different tissues depending on the tissue distribution of the various enzymes involved in fructose metabolism (Herman and Zakim, 1968).

Fructose is rapidly phosphorylated by ATP in the liver to form fructose-1-phosphate. Fructose-1-phosphate is split by liver aldolase (aldolase B) into glyceraldehyde and dihydroxyacetone phosphate, a member of the glycolysis sequence of intermediates. Glyceraldehyde so formed is phosphorylated by triokinase to form glyceraldehydes-3-phosphate, another intermediate of the glycolytic pathway. Thus, the pathways of glucose and fructose metabolism in the liver converge at the triose phosphate stage of metabolism and from this point on their metabolism are qualitatively similar (Mayes, 1993). The two trioses formed by the cleavage of fructose-1-phosphate can each follow three paths

1) Dihydroxyacetone phosphate can be isomerized to glyceraldehyde-3-phosphate and continue through the glycolytic pathway to ultimately yield pyruvate, which is converted to either lactic acid under anaerobic conditions or enters the citric acid cycle as acetyl-CoA
under aerobic conditions. The acetyl-CoA can then either produce energy via the respiratory chain or be used as the substrate for fatty acid synthesis. 2) Dihydroxyacetone phosphate may be reduced to glycerol-3-phosphate and provide the glycerol backbone of synthesized triacylglycerols, phospholipids, and other lipids. 3) Dihydroxyacetone phosphate may also be condensed with glyceraldehydes-3-phosphate by aldolase to form fructose-I, 6-diphosphate, and ultimately glucose or glycogen (Hallfrisch, 1990)

In muscle and adipose tissue hexokinase coverts fructose to fructose-6-phosphate (F-6-P) which enters in to the glycolysis (Strickland and Ellis, 1975). The fructokinase content of muscle and adipose tissue seems to be quite low if present at all. Muscle contains an NADPH-dependent glycerol dehydrogenase which is absent in liver (Herman and Zakim, 1968).

Liver and skeletal muscle glycogen

Glycogen, the principal storage form of carbohydrate in animals, is present mainly in liver and muscle. Muscle and liver glycogen stores serve completely different roles. Liver glycogen functions as a glucose reserve for the maintenance of blood glucose concentrations. The function of muscle glycogen is to act as readily available source of hexose units to glycolysis within the muscle itself. The concentration of tissue glycogen also depends upon the rate of its formation (glyogenesis) and degradation (glycogenolysis).

The glycogen content of liver and skeletal muscle of six experimental groups are presented in Table 7 and Fig 16. When compared to C-group STZ diabetic rats (D-group), insulin resistance rats (F-group) showed differential trend regarding the changes in glycogen
Table 7. Effect of *C. roseus* treatment on glycogen and liver glycogen phosphorylase activity in STZ diabetic and fructose fed IR rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>C</th>
<th>C + CR</th>
<th>D</th>
<th>D + CR</th>
<th>F</th>
<th>F + CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen (mg glucose/g tissue)</td>
<td>Muscle</td>
<td>2.36 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.48 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.70 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.43 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.80 ± 0.13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.51 ± 0.06&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>29.24 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.89 ± 10.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.42 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.25 ± 0.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36.53 ± 1.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>31.60 ± 0.79&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glycogen phosphorylase (μmol of Pi formed/min/mg protein)</td>
<td>Liver</td>
<td>0.131 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.137 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.288 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.149 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.092 ± 0.008&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.118 ± 0.007&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan’s multiple range test).
Fig 16: Per cent change in liver glycogen phosphorylase activity, liver glycogen and muscle glycogen levels of diabetic (D), diabetic rats treated with *C. roseus* (D + CR), fructose fed (F) and fructose fed rats treated with *C. roseus* compared to control rats.
content of liver and muscle. Group-D showed significantly decreased glycogen content in liver (60.9 %) and muscle (70.3 %), whereas F-group showed significantly enhanced glycogen content in liver (24.7 %) and muscle (18.6 %) when compared to C-group. *C. roseus* treated control rats (C + CR-group) showed no significant variation in the glycogen content of liver and muscle when compared to C-group. The significant increase observed in the glycogen content of liver (94.8 %) and muscle (104.2 %) by *C. roseus* administration in D + CR-group when compared to D-group did not restore to normal values. However, *C. roseus* administration for 60 days to fructose fed rats (F + CR-group) resulted in a significant decrease in glycogen content of liver (13.5 %) and muscle (10.7 %) when compared to F-group. In the present study hepatic and muscle glycogen content was reduced significantly in STZ diabetic rats along with hypoinsulinemia.

Insulin plays a direct role in the control of liver glycogen metabolism by the regulation of the glycogenesis process. It controls this process by regulating the conversion of a glucose-6-phosphate-dependent form into a glucose-6-phosphate-independent form of glycogen synthase (Tan and Nuttall, 1976; Khandelwal *et al.*, 1977). Basal amounts of insulin inhibit glycogenolysis by about 60 %. Hence selective insulin deficiency in STZ diabetic rats may increase the glycogen breakdown and decrease the glycogen synthesis.

It is rationale that the glycogen levels in these tissues of STZ diabetic rats (skeletal muscle) decrease as they depend on insulin for influx of glucose (Bishop, 1970; Whitton and Herns, 1975; Golden *et al.*, 1979). Muscle is the principal site of insulin stimulated glucose disposal *in vivo*. Previous studies indicate that in muscle glucose transport across the plasma membrane was the rate limiting step for the glucose metabolism for normal
Results and Discussion

Subjects and in diabetics (Yki-Jarvinen et al., 1990; Butler et al., 1990; Rothman et al., 1992). Glycogen levels in various tissues especially in skeletal muscle is direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase (Ortmeyer et al., 1997).

Two months of treatment with C. roseus partially prevented the depletion of glycogen in the liver and muscle of STZ diabetic rats. This may be due to increased circulatory insulin concentrations observed in the D + CR-group compared to D-group. Thus C. roseus treatment resulted in protection/regeneration of pancreatic β-cells which is further reflected by partial correction in the altered glycogen metabolism observed in D-rats.

Studies of Belfiore et al. (1986) in STZ induced diabetic rats indicated a decrease in the glycogen content in insulin dependent tissues like liver and muscle and the reverse was reported in insulin independent tissues (kidneys). The decreased glycogen content in liver and skeletal muscle of diabetic rats (Hornbrook, 1970; Migliorni, 1971; Whitton and Hems, 1975) was normalized by insulin treatment (Welihinda and Karunanayake, 1986; Grover et al., 2000).

In the present study unlike insulin deficient rats (D-rats) fructose fed rats showed enhanced liver and muscle glycogen content when compared to control rats. C. roseus treatment for 60 days partially prevented the fructose feeding induced enhancement in the glycogen content of liver and muscle observed in F + CR-group.

A high flux of fructose to the liver, the main organ capable of metabolizing this simple carbohydrate, disturbs normal hepatic carbohydrate metabolism leading to two major
consequences—perturbations in glucose metabolism and glucose uptake pathways and a significantly enhanced rate of de novo lipogenesis and TG synthesis (Basciano et al., 2005).

Once absorbed, dietary fructose is mainly taken up by the liver where it is metabolized via bypassing glucokinase and phosphofructokinase, regulatory steps in glycolysis. Fructose is phosphorylated to fructose-1-phosphate by fructokinase which is then converted to DHAP and glyceraldehydes-3-phosphate. Increased concentrations of DHAP and glyceraldehydes-3-phosphate in the liver drive the gluconeogenic pathway towards glucose-6-phosphate, glucose-1-phosphate and glycogen formation (Mayes, 1993). It appears that fructose is a better substrate for glycogen synthesis than glucose and that glycogen replenishment takes precedence over TG formation. Once liver glycogen is replenished, the intermediates of fructose metabolism are primarily directed towards TG synthesis (Parniak, 1998).

Studies of Forster, (1974) demonstrated greater deposition of glycogen in animals infused with fructose than those infused with glucose. Glycogen synthesis is impaired in diabetes either because of glycogen synthase inhibition or impaired hepatic glucose uptake and glucose phosphorylation. Because fructose phosphorylation does not require hexokinase it may overcome the glycogen synthesis in diabetes. Studies of Cuidad et al. (1988) demonstrated enhanced glycogen synthesis in isolated hepatocytes both from diabetic and normal rats when incubated in solutions containing fructose compared to glucose, due to stimulation of glycogen synthesis enzymes.
Our results of enhanced glycogen content in fructose fed rats are in accordance with earlier studies (Youn et al., 1987; Murakami et al., 1997). However a few contradictory observations of decrease hepatic glycogen content in fructose fed rats also appeared in literature (Rajasekar and Anuradha, 2007).

When compared with glucose, fructose is most predominantly utilized by skeletal muscle. Fructose appears to be more readily incorporated into glycogen. After an intravenous infusion, fructose has been demonstrated to disappear from the blood stream more rapidly than glucose and leads to a greater production of lactate (Zierath et al., 1995). In man, skeletal muscle glycogen content has been reported to increase in response to fructose infusion (Nillson and Hultman, 1974). The observed increase in muscle glycogen has been reported to occur despite alterations in circulatory levels of glucose, and offers indirect evidence that a portion of circulating fructose is converted directly into muscle glycogen. Despite these findings, no change in diaphragm muscle glycogen content (Vrana et al., 1978) and decreased soleus muscle glycogen content (Thorburn et al., 1989) are observed.

**Glycogen phosphorylase**

In order to understand the cause for the observed alterations in the hepatic glycogen content of diabetic and fructose fed rats and C. roseus treated diabetic and fructose fed rats, glycogen phosphorylase a key regulatory enzyme of glycogen degradation was assayed.

The control of synthesis and degradation of glycogen in the liver is central to the regulation of blood glucose level. Glycogen metabolism is profoundly affected by several
hormones. Insulin induces the synthesis of glycogen. Glucagon and epinephrine in contrast trigger the breakdown of glycogen.

Glycogen synthesis and breakdown is controlled by a complex series of reactions involving covalent modification by protein phosphorylation and dephosphorylation. Briefly, regulation centers around two rate-controlling enzymes—glycogen synthase and glycogen phosphorylase. The active form of glycogen synthase (synthase a) is the dephosphoenzyme, whereas the inactive synthase b is phosphorylated. On the other hand, active glycogen phosphorylase a is the phosphoenzyme whereas the inactive b form is dephosphorylated. Protein kinases carry out phosphorylations and protein phosphatases carry out dephosphorylations of these enzymes. Both processes are controlled by hormonal and allosteric modifiers (Ercan et al., 1996; Klinov and Kurganov, 2001).

α-D-glucose is a weak inhibitor of glycogen phosphorylase b and acts as a physiological regulator of hepatic glycogen metabolism. Glucose binds to phosphorylase at the catalytic site and results in a conformational change that stabilizes the inactive T state of the enzyme promoting the action of protein phosphatase 1 and stimulating glycogen synthase. It has been suggested that, in the liver, glucose analogues with greater affinity for glycogen phosphorylase may be a more effective regulatory agent (Kimberly et al., 1994).

The hepatic glycogen phosphorylase activity of six experimental groups are presented in Table 7 and Fig 16. STZ diabetic rats (D-group) showed a significantly enhanced hepatic glycogen phosphorylase activity (119.4 %) compared to C-group, whereas
insulin resistance rats (F-group) showed significant decrease (29.7 %) in the activity of glycogen phosphorylase when compared to C-group. The significant decline in the activity of hepatic glycogen phosphorylase (48.3 %) observed in D + CR-group compared to D-group by *C. roseus* administration for 60 days did not restore to normal values. *C. roseus* treatment along with fructose feed (F + CR-group) reduced the fructose feed induced decline in the glycogen phosphorylase activity, but it is still significantly lower than C-group. Control rats treated with *C. roseus* (C + CR-group) showed no change in the hepatic glycogen phosphorylase activity when compared to C-group.

Inhibitors of glycogen phosphorylase have been proposed as a therapeutic strategy for improving glycaemic control in Type 2 diabetes mellitus and various studies have shown the efficacy of such compounds in lowering blood glucose or inhibiting liver glycogenolysis *in vivo* (Mc Cormack *et al.*, 2001; Treadway *et al.*, 2001; Oikonomakos, 2002).

In untreated diabetics the level of insulin is too low and that of the glucagon is too high relative to the needs of the patient (Kuhl and Holst, 1976). The high glucagon-insulin ratio in diabetes also promotes glycogen breakdown. Glucose-6-phosphatase, one of the key enzymes in the homeostatic regulation of blood glucose levels, catalyses the terminal step in both gluconeogenesis and glycogenolysis.

Increased activity of hepatic glycogen phosphorylase and G6Pase (Table 10 and Fig 19) observed in STZ diabetic rats may be one contributing factor for decreased hepatic glycogen content. The elevated hepatic glycogen content and decreased plasma glucose levels of *C. roseus* treated STZ-diabetic rats compared to STZ diabetic control rats can be
explained by diminished activity of glycogenolytic enzyme namely glycogen phosphorylase. Decline in the activity of hepatic glycogen phosphorylase of C. roseus treated diabetic rats may be due to observed increased plasma insulin levels.

A survey of the literature reveals a disparity in results on whether fructose promotes liver glycogen deposition, with the balance of studies in vivo in the fed condition indicating that fructose is a better promoter of glycogenesis than is glucose (Youn et al., 1987). The net deposition of glycogen results from both activation of glycogen synthase (Whitton and Hems, 1975) and inhibition of glycogen phosphorylase (Thurston et al., 1974). This appears to be brought about by several mechanisms. Phosphorylase a is inhibited by fructose-1-phosphate (Vanden Berghe et al., 1973) which accumulates after administration of fructose. Also, glucose-6-phosphate increases in concentration and activates glycogen synthase and inhibits phosphorylase (Kaufmann and Froesch, 1973).

In the present study, the enhanced hepatic glycogen stores in fructose fed rats can be explained by the observed decrease in the activity of glycogen phosphorylase. This can be explained by enhanced production of fructose-1-phosphate by increased activity of fructokinase observed in these animals (Table 9).

Previous investigations using $^{31}$P magnetic-resonance spectroscopy have confirmed the accumulation of fructose-1-phosphate in the human liver after intravenous administration of fructose (Segebarth et al., 1991). Although most of these effects were obtained by using unphysiological concentrations of fructose, experiments with isolated hepatocytes using graded concentrations of fructose demonstrated that significant elevations
of fructose-1-phosphate occurred at concentrations of 0.5 and 1.0 mmol fructosc/L which are available in the portal vein in vivo after a fructose meal (Vanden Berghe, 1986).

Further, inhibition of glycogen phosphorylase and activation of glycogen synthase by increased concentration of glucose-6-phosphate may also be possible because of enhanced operation of gluconeogenesis (Table 10 and Fig 19) in fructose fed animals. Thus conversion of fructose to liver glycogen is increase because of enzyme adaptation.

Partial correction of enhanced glycogen content by fructose feed in Ci roseus treated fructose fed rats can be explained by the observed significant enhancement in glycogen phosphorylase activity of F + CR-group compared to F-group.

**Glycolytic enzymes**

Glycolysis is an almost universal central pathway of glucose catabolism with the largest flux of carbon in most cells. The glycolytic breakdown of glucose is the main source of metabolic energy with the caveat that regulation of flux through glycolysis is dependent on the tissue under consideration and nutritional and hormonal state of the tissue. Activities of Hexokinase (HK), Phosphofructokinase (PFK) and Pyruvate kinase (PK) have shown to be very sensitive indicators or markers of the glycolytic pathway (Murphy and Anderson, 1974). Measuring these enzyme activities represents a method to assess the peripheral utilization of glucose.

The data on activities of key glycolytic enzymes i.e., HK, PFK and PK of liver and muscle of six experimental groups are presented in the Table 8 and Fig 17 & 18.
Table 8. Effect of *C. roseus* treatment on glycolytic enzyme activities of liver and muscle in STZ diabetic and fructose fed IR rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>C</th>
<th>C + CR</th>
<th>D</th>
<th>D + CR</th>
<th>F</th>
<th>F + CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase (μmol of G6P formed/mi/mg protein)</td>
<td>Liver</td>
<td>3.93±0.08a</td>
<td>4.05±0.03a</td>
<td>2.23±0.04b</td>
<td>3.80±0.12a</td>
<td>2.84±0.08c</td>
<td>3.00±0.08c</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>4.39±0.07a</td>
<td>4.52±0.08a</td>
<td>2.60±0.05b</td>
<td>4.53±0.07a</td>
<td>2.84±0.06c</td>
<td>4.68±0.27a</td>
</tr>
<tr>
<td>Phosphofructokinase (μmol of F16Bis phosphate formed/mi/mg protein)</td>
<td>Liver</td>
<td>3.35±0.05a</td>
<td>3.59±0.05b</td>
<td>2.10±0.05c</td>
<td>3.27±0.04a</td>
<td>2.50±0.04d</td>
<td>2.62±0.08d</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>4.17±0.07a</td>
<td>4.30±0.07a</td>
<td>1.75±0.02b</td>
<td>4.15±0.07a</td>
<td>2.61±0.07c</td>
<td>4.07±0.10a</td>
</tr>
<tr>
<td>Pyruvate kinase (μmol of NADH oxidized/min/mg protein)</td>
<td>Liver</td>
<td>2.57±0.04a</td>
<td>2.76±0.06b</td>
<td>1.74±0.02c</td>
<td>2.69±0.06a</td>
<td>3.15±0.05d</td>
<td>2.64±0.06a</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>2.87±0.03a</td>
<td>2.92±0.05a</td>
<td>1.79±0.04b</td>
<td>2.78±0.06a</td>
<td>3.08±0.04c</td>
<td>2.80±0.03a</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan’s multiple range test).
Fig 17: Per cent change in the activities of hepatic and skeletal muscle glycolytic enzymes and hepatic G6PDH in STZ diabetic (D) and fructose fed (F) rats compared to control (C) group.

Fig 18: Per cent recovery in the activities of hepatic and skeletal muscle glycolytic enzymes and hepatic G6PDH in STZ diabetic and fructose fed rats.
STZ induced diabetic rats showed significantly decreased activities of glycolytic enzymes both in the liver and muscle compared to control rats. The per cent decrease in HK, PFK and PK activities in liver and muscle of D-group are 43.2, 37.3 and 32.2 %, and 40.7, 58.1 and 37.6 % respectively. Similar to STZ induced diabetic rats fructose fed rats (F-group) also showed significantly decreased HK and PFK activities in liver (27.7 and 25.3 %) and muscle (35.3, 37.4 %) compared to control rats. But the decrease in these enzyme activities of F-group was significantly lower than D-group. Unlike HK and PFK activity, PK activity was significantly enhanced both in the liver (22.5 %) and muscle (7.3 %) of F-group compared to C-group. *C. roseus* treated control rats (C + CR) showed no alteration in the activities of hepatic HK and muscular HK, PFK and PK compared to C-group, whereas, significantly enhanced hepatic PFK (7.1 %) and PK (7.3 %) activities were found in C + CR-group compared to C-group. *C. roseus* treated diabetic rats i.e. D + CR-group showed no deviation in the activities of HK, PFK and PK both in the liver and muscle compared to C-group. Thus *C. roseus* treatment in D + CR-group prevented the diabetic induced alterations in glycolytic enzyme activities. *C. roseus* treated fructose fed rats (F + CR-group) showed significantly enhanced muscle HK (64.7 %) and PFK (55.9 %) activities and significantly decreased hepatic and muscular PK activity (16.1 and 9.0 %) compared to F-group.

In the present study the significantly decreased activities of these glycolytic enzymes observed both in the liver and skeletal muscle of STZ diabetic rats indicated decreased operation of glycolysis under diabetic condition both in the insulin dependent tissue (skeletal muscle) and insulin independent tissue (liver). Decreased HK activity also
indicates decreased availability of glucose -6-phosphate for HMP shunt operation under diabetic condition. Similar trend of decreased glycolytic enzyme activities was reported in various tissues of diabetic animals (Williams, 1993; Grover et al., 2000; Rathi et al., 2002). Enhanced glucose flux through polyol pathway is a well established observation in diabetic state. Thus the decreased trend of glycolysis in the diabetic animals can be explained by the competition for availability of NAD which is channeled into the sorbitol pathway instead of glycolysis in diabetes (Williamson et al., 1993).

The data presented in the Table 8 and Fig 18 clearly indicate the beneficial effects of C. roseus in normalizing the decreased glycolytic enzyme activities both in liver and muscle and in rectifying the decreased utilization of glucose for energy production under diabetic condition. Studies of Weber and Convery, (1966) indicated that insulin administration restored the decreased glycolytic enzyme activities of diabetic animals. Insulin upregulates the transcription of glucokinase, phosphofructokinase, and pyruvate kinase genes (Howard, 1995). Improvement in plasma insulin levels by C. roseus treatment in D + CR-group might be responsible for increased activity of glycolytic enzymes by enhancing their gene expression by insulin.

The decreased activity of HK and PFK, the key glycolytic enzymes observed in liver and skeletal muscle of fructose fed rats indicates decreased rates of glucose oxidation in these tissues. The decreased utilization of glucose, as found in the whole organism when placed on a high-sucrose or fructose diet, was also reported in studies of individual tissues (Bender and Thadini, 1970; Tuovinen and Bender, 1975). Similarly decreased activity of
HK and PFK in liver and muscle of fructose fed rats as in the present study were also reported (Rewana et al., 1993; Kannappa et al., 2006).

The development of insulin resistance in fructose fed rats as also reported in the present study is well documented in the literature. Defects in post-receptor events in insulin signaling (Bezerra et al., 2000) and in enzymes involved in glucose metabolism (Blakely et al., 1981) were reported. The hyperglycemia prevailing in fructose fed conditions may lead to increased uptake of glucose into hepatocytes through GLUT-2, an insulin independent glucose transporter. In addition decreased operation of glycolysis is also responsible for the enhancement in the cellular glucose concentration in the liver. High intracellular glucose exerts toxic effects on structure and function of organs and induces insulin resistance, a phenomenon referred to as glucose toxicity was observed in skeletal muscle of diabetic rats (Kahn et al., 1991)

In the span of glycolytic reactions from glyceraldehyde-3-phosphate to pyruvate and lactate, the rate-controlling step is catalyzed by pyruvate kinase.

In contrast to HK and PFK, PK activity significantly increased in liver of fructose fed rats. This can be explained by the hepatic fructose metabolism. Rapid uptake of fructose by liver and its entry in to the glycolysis at the triose phosphate level after bypassing the PFK regulatory steps takes place. As a result of the loading of the initial pathways of fructose metabolism there is a tendency for intermediates of glycolysis to increase in concentration resulting in an increased flux through the pathway (Sahebjami and Scalettar, 1971) as evidenced by enhanced PK activity in fructose fed rats. Pyruvate kinase is normally under feed-forward control because of allostERIC activation by fructose-1, 6-bisphosphate.
Although this metabolite may double in concentration when fructose is added to hepatocytes (Van den Berghe, 1986), of more significance are the large increases in fructose-1-phosphate concentrations which extend a similar but more enhanced activation of pyruvate kinase (Eggleston and Woods, 1970).

Impaired insulin sensitivity with compensatory hyperinsulin and hyperglycemia are common pathogenic factors observed in the fructose fed rats. Under these conditions, skeletal muscle represents the main site of insulin resistance as skeletal muscle is one of the major sites for glucose consumption.

Even though low concentrations of fructokinase are reported in muscle and adipose tissue, tissue hexokinase converts fructose to fructose-6-phosphate which enters the glycolysis (Katzen et al., 1965; Herman and Zakim, 1968; Strickland and Ellis, 1975) because of the decreased HK activity observed in fructose fed rats resulting in less production of fructose-6-phosphate the substrate of PFK. Thus fructose fed rats also showed decreased PFK activity indicating decreased entry of fructose into glycolysis. The observed decreased activity of the first two key enzymes of glycolysis also indicates decreased oxidation of glucose in the muscle of fructose fed rats. Muscle tissue shows decreased activity to metabolize glucose and increased ability to oxidize fatty acids after animals have been fed high fructose diets (Vrana et al., 1978).

*C. roseus* administration to fructose fed rats prevented the fructose feed induced decrease in HK and PFK activity in muscle and increase in PK activity in muscle and liver. Even though improvement was observed in hepatic glycolytic enzyme activity in F + CR-group compared to F-group, the plant treatment does not restore the liver PK and PFK
enzymes to normal level. The current results of glycolytic enzyme activities represent the beneficial effects of *C. roseus* treatment in rectifying the impairment in the insulin mediated glucose transport observed in the muscle tissue of fructose fed rats whereas insulin resistance is partially rectified at hepatic level.

**Fructokinase**

Fructose is rapidly phosphorylated by ATP in liver to form fructose-1-phosphate, catalyzed by the first enzyme of the fructose pathway—fructokinase. This enzyme is virtually specific for fructose because it is a ketohexokinase, and fructose is the only ketohexose of physiological significance in the diet. Fructokinase is virtually confined to the liver (Cori *et al.*, 1951). Although fructokinase is negligible in tissues other than liver, some activity is present in kidney and intestine of several species including humans (Heinz *et al.*, 1975; Crouzoulon, 1979).

The results presented in Table 9 represent the activity of fructokinase in the liver of six experimental groups. No significant variation in the activity of hepatic fructokinase activity was observed among C, C+ CR, D and D + CR-groups. However, significantly increased hepatic fructokinase activity was observed both in F-group (33.1 %) and F + CR-group (26.2 %) compared to C-group. *C. roseus* treatment resulted a slight but not significant decrease in hepatic fructokinase activity in F + CR-group compared to F-group.

Glucose is a general substrate for all body tissues while fructose has to be processed in the liver first and its intake represents a carbohydrate load targeted on the liver. A further aspect favoring the hepatic fructose metabolism is the high activity of fructokinase relative
Table 9. Fructokinase activity of six experimental groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>C+CR</th>
<th>D</th>
<th>D+ CR</th>
<th>F</th>
<th>F+CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructokinase (µmol NADH oxidized/mim/mg protein)</td>
<td>1.09 ± 0.03^a</td>
<td>1.04 ± 0.02^a</td>
<td>0.99 ± 0.07^a</td>
<td>1.17 ± 0.05^a</td>
<td>1.45 ± 0.03^b</td>
<td>1.38 ± 0.04^b</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
to that of glucose phosphorylating enzymes. This is a surprising mammalian characteristic feature since glucose is the major hexose from exogenous and endogenous sources available for energy production. Yet the maximal capacity of fructokinase exceeds that of hexokinase and glucokinase combined by a factor of 3 to 5 in different species including humans (Heinz et al., 1968). The high capacity of this enzyme ensures the channeling of the majority of fructose into hepatic metabolism.

More recently it was demonstrated that prolonged feeding is necessary for meaningful induction of enzyme, for example fructokinase activity rises gradually up to 2 fold during 4 weeks on a 50 % sucrose diet (Fukuda et al., 1983). Fructokinase induction is preventable by actinomycin both in liver and intestines confirming the transcriptable mode of substrate regulation of its synthesis (Grand et al., 1974). Pathological conditions also affect fructokinase activity. STZ diabetes in rats causes a decrease while insulin treatment restores the activity. Sucrose feeding of diabetic rats has a fructokinase inducing effect even in the absence of insulin (Shafrir and Overvi, 1984). The substantial inducibility of fructokinase shows that the mammalian liver is capable of adaptation to fluctuations in dietary fructose content by expanding its capacity for fructose utilization. In contrast to earlier report, in the present study fructokinase activity of STZ-diabetic rats is not deviated from control rats.

Gluconeogenesis

Glucose is produced through gluconeogenesis and glycogenolysis. Both phenomena are inhibited by insulin and enhanced by a deficiency of insulin action (Friedmann et al.,
1967). Increased hepatic glucose production is a major component of diabetes and insulin resistance induced hyperglycemia.

In order to assess the role of gluconeogenesis in STZ diabetic and fructose diet induced hyperglycemia and to study the antihyperglycemic efficacy of *C. roseus*, the key gluconeogenic enzymes i.e, Fructose-1, 6-bisphosphatase (F1,6BPase) and Glucose-6-phosphatase (G6Pase) are assessed in the liver and kidney of all experimental groups and data are presented in Table 10 and Fig 19 & 20.

Gluconeogenesis and glycolysis are reciprocally regulated so that one pathway is relatively inactive while the other is highly active. Gluconeogenesis mostly takes place in liver and to some extent in kidney.

Significantly increased activities of tissue F1,6BPase and G6Pase activities were observed both in D and F-groups compared to C-group. However, the enhancement in these enzyme activities is more prominent in D-group than F-group. The per cent increase in hepatic F1,6BPase and G6Pase activities of D-group are 76.9 and 133.3 % and in F- group are 43.5 and 59.5 % respectively compared to C-group. The per cent increase in kidney F1,6BPase and G6Pase activities of D and F-groups compared to C-group are 63.6 and 42.9 %, and 45.5 and 23.2 % respectively. *C. roseus* treatment for 60 days resulted in no significant alterations in the activities of hepatic and kidney F1,6BPase and G6Pase in C + CR-group compared to C-group. *C. roseus* treated diabetic (D + CR-group) and fructose fed (F + CR-group) rats showed a significant decrease in these enzyme activities both in the liver and kidney when compared to D and F-groups respectively and this decrease further
Table 10. Effect of *C. roseus* treatment on activity of gluconeogenic enzymes in liver and kidney of STZ diabetic and fructose fed IR rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>C</th>
<th>C + CR</th>
<th>D</th>
<th>D + CR</th>
<th>F</th>
<th>F + CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose-1, 6-bisphosphatase</td>
<td>Liver</td>
<td>0.39±0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.40±0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.69±0.013&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.42±0.010&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.56±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.40±0.007&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>0.11±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12±0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18±0.011&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.12±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16±0.006&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.12±0.005&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>Liver</td>
<td>20.58±0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.37±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.02±2.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.00±0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.83±1.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.51±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>17.45±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.32±0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.92±0.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.25±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.49±0.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.06±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
Fig 19: Percent change in hepatic and renal gluconeogenic enzyme activities of STZ diabetic (D) and fructose fed insulin resistance (F) rats compared to control group (C-group).

Fig 20: Per cent recovery from STZ and fructose induced alterations in gluconeogenic enzyme activities in D + CR and F + CR-group by *C. roseus* treatment.
resulted in bringing these enzyme activities to normal values. Thus C. roseus treatment in D + CR and F + CR-groups prevented the STZ diabetic and fructose induced enhancement in the activities of these enzyme.

Insulin regulates hepatic glucose production and gene expression of several proteins at transcription level including regulatory enzymes of gluconeogenesis (phosphoenolpyruvate carboxykinase and G6Pase) (Brien et al., 1995; Argaud et al., 1996; Gabbay et al., 1996). In the presence of insulin resistance insulin may also fail to regulate the transcription of these genes. In adult rat liver, phosphoenolpyruvate carboxykinase (PEPCK) gene transcription is upregulated by glucagon, glucocorticoids, and epinephrine and downregulated by insulin which exerts a dominant effect (Lucas and Granner, 1992). Activities of liver microsomal G6Pase have been reported to be up-regulated in both fasting and diabetic states (Ashmore et al., 1954; Segal and Washko, 1959), and administration of insulin to diabetic rats resulted in a reduction in the enzyme activity (Nordlie and Arion, 1965). Recent studies suggested that adipose hormones may be involved in the control of glucose metabolism in liver. Indeed the suppression of hepatic glucose production by insulin is enhanced by leptin and adiponectin (Fruhbeck and Salvador, 2000; Berg et al., 2001; Combs et al., 2001) but impaired by resistin (Rajala and Obici, 2003).

The glycolysis and gluconeogenic pathways make use of many common intermediates and enzymes but are controlled by several non-equilibrium enzymes unique to each pathway (Murray et al., 1993). Two pivotal reactions, for which the activities are reciprocal and coordinated, dominate these pathways. These are catalyzed by phosphofructokinase in glycolysis and F1,6BPase in gluconeogenesis. Their activities are
both induced by hormones and also by allosteric modifiers often acting on the key regulatory molecule-fructose-2,6-bisphosphate-which activates phosphofructokinase and inhibits fructose-1,6-bisphosphatase (Hers and Schaftingen, 1982).

The glucagon-insulin antagonism that is implicated in the short term regulation of hepatic gluconeogenesis is also manifested in the regulation of PFK activity. The pair F1,6BPase-PFK is one of the sites of action of glucagon in the regulation of hepatic gluconeogenesis. These facts support a relevant role of PFK in the control of gluconeogenic pathway as it has been shown for PK. The coordinate hormonal control of these two major regulatory glycolytic enzymes could explain, at least in part, the increase of hepatic gluconeogenesis by glucagon, for both substrates enter at the pyruvate and the triosephosphate levels (Donkin et al., 1997).

Increased activity of F1,6BPase and G6Pase was observed in STZ diabetic rats indicating enhanced operation of gluconeogenesis under the insulin deficient conditions. Enhanced gluconeogenesis under diabetic condition may also be due to increased availability of gluconeogeneic substrates by enhanced catabolism of proteins and lipids.

The redox state of the liver cell is highly reduced in diabetes together with the changes in the energy state. ATP levels are lowered in the cytosol, the mitochondrial ATP; however not much is affected and could be the energy source for the higher levels of gluconeogenesis in diabetes both in liver and kidney.
Results & Discussion

C. roseus treatment to STZ diabetic rats restored the enhanced gluconeogenic enzyme activities to the normal level which may be due to increased insulin levels observed in these animals.

The liver can convert fructose into glucose but this occurs by a pathway not involving sorbitol. The pathway probably involves the formation of fructose-1-phosphate followed by cleavage to D-glyceraldehyde and DHAP. D-glyceraldehyde is converted to glyceraldehyde-3-P which reacts with DHAP to form fructose-1, 6-bisphosphosphate and successively fructose-6-Phosphate, glucose-6-Phosphate, and finally glucose (Ballard, 1965)

Increased activity of F1,6BPase and G6Pase in F-group animals indicates enhanced operation of gluconeogenesis in liver and kidney under insulin resistance condition. This may be attributed to the enhanced availability of gluconeogenic precursors.

Fructose is a highly lipogenic nutrient because its metabolism produces the precursors for lipogenesis and TG synthesis i.e., acetyl-CoA and glycerol (Mayes, 1993). Fructose infusions in humans resulted in dangerous increases in blood lactic acid, especially in patients with preexisting acidotic conditions such as anoxia, diabetes, postoperative stress, or uremia (Hallfrisch, 1990). Many studies revealed that more lactate is formed from fructose than from glucose (Sahebjami and Scalettar, 1971). The increased lactate production occurs because fructokinase activity is increased, the rate limiting step of glycolysis (PFK) is bypassed and PK activity is stimulated by activity of fructose-1-phosphate. Thus, fructose metabolism favours the formation of gluconeogenic substrates like glycerol, acetyl-CoA, pyruvate and lactate. In addition, enhanced protein degradation
reflected by increased tissue transaminases in fructose fed rats also provides gluconeogenic amino acid pool.

Functional activity of many enzymes is regulated intracellularly by the availability of their substrates and by the presence of various low-molecular-weight activators and inhibitors. Adaptive changes in total assayable activity is a common property of hepatic enzymes (Wimhurst and Manchester, 1973). Thus the enhanced operation of gluconeogenesis in fructose fed rats can be explained by availability of gluconeogenic substrates.

Increased visceral adiposity is a risk factor for insulin resistance (Coon et al., 1992; Carey et al., 1996). Although post-absorptive hyperinsulinemia has been shown to maintain glucose production within normal limits, its suppression by insulin is significantly impaired in individuals with visceral obesity (Shaughnessy et al., 1995). The ability of insulin to inhibit glucose production is markedly impaired in an obese rodent model of aging (Barzilai and Rossetti, 1996). The link between visceral fat and peripheral and hepatic insulin resistance can be speculated to the endocrine function of adipose tissue (Hotamisligil et al., 1996). Hence enhanced accumulation of visceral fat in the F-group animals might also contribute to the insulin resistance, thus resulting in enhanced gluconeogenesis in these animals.

The present work emphasizes the suggestion made by Buschiazzo et al. (1970) that substrates as well as hormones may be potent initiators of adaptation. *C. roseus* administration for 60 days along with fructose feed prevented the enhanced gluconeogenesis
by fructose diet. Activities of enzymes of gluconeogenesis and insulin sensitivity are reciprocally related. Activities of gluconeogenic enzymes are inhibited by enhancing insulin sensitivity (Barzilai et al., 1999).

Barzilai et al. (1999) reported that suppression of PEPCK is associated with enhanced hepatic insulin sensitivity after removal of visceral fat in rats. They also demonstrated that lowering visceral fat by caloric restriction or leptin administration dramatically improved hepatic insulin sensitivity independent of whole body fat mass (Barzilai et al., 1997; 1998).

Adiponectin has been implicated as a factor that suppresses hepatic glucose production and plays a major role in the regulation of insulin action (Havel, 2002). Adiponectin expression is reduced in insulin resistance state (Hu et al., 1996), increased with caloric restriction and thiazolidinedione treatment (Combs et al., 2002), and correlates with insulin sensitivity determined by euglycemic clamps (Hotta et al., 2001). Administration of this hormone increases insulin sensitivity, inhibits hepatic glucose production, and reduces glycemia in mice (Berg et al., 2001).

*C. roseus* administration for 60 days with fructose feed prevented the fructose feed induced enhancement in the activity of gluconeogenic enzymes both in the liver and kidney. Thus *C. roseus* treatment restored the enzyme activities to normal level. This can be explained by the enhanced insulin sensitivity by *C. roseus* treatment in F + CR-group which can be attributed to its hypolipidemic activity reflected by preventing lipid accumulation in liver and muscle as well as maintaining normal plasma lipid profile thus preventing fructose
induced abnormalities in metabolism reflected by decreased visceral fat in F + CR-group and hence correcting the endocrine functions of the adipose tissue.

**Glucose-6-phosphate dehydrogenase (HMP shunt pathway)**

Glucose-6-phosphate dehydrogenase is the key regulating enzyme of pentose phosphate pathway and controls the flow of carbon through this pathway. Specifically, the enzyme catalyzes the first reaction in the pathway leading to the production of pentose phosphates and reducing power in the form of NADPH for reductive biosynthesis and maintenance of the redox state of the cell. Alterations in G6PDH activity can significantly alter oxidative stress induced cell death (Beutler, 1992; Vulliamy et al., 1992). It plays an important role in cell death by regulating intracellular redox levels. Glucose-6-phosphate dehydrogenase gene can be considered to be a constitutively expressed "housekeeping gene" in many tissues. There are several other tissues (liver, adipose, lung and proliferating cells) wherein modulation of cellular G6PDH activity represents an important component of the integrated response to external stimuli (hormones, growth factors, nutrients, and oxidative stress) (Rudack et al., 1971; Berdanier and Shubeck, 1979).

Hepatic G6PDH activity has long been known to respond to several hormonal and dietary manipulations (Kletzien et al., 1994). In addition G6PDH has long been considered as a member of the family of lipogenic enzymes which also includes fatty acid synthase, acetyl-CoA carboxylase, and malic enzyme. This group of enzymes are induced in liver during episodes of dietary carbohydrate excess and is responsible for fatty acid synthesis, a
Table 11. Effect of *C. roseus* treatment on hepatic glucose-6-phosphate dehydrogenase enzyme activity in STZ diabetic and fructose fed IR rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>C + CR</th>
<th>D</th>
<th>D + CR</th>
<th>F</th>
<th>F+CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-6-phosphate dehydrogenase (µmol of NADP reduced/min/mg protein)</td>
<td>2.43±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.60±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.11±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.06±0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.22±0.04&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.10±0.06&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan’s multiple range test).
process generally regulated by insulin (Rudack et al., 1971; Katsurada et al., 1989). The elevated activity seems to be dependent upon the presence of insulin, glucocorticoids, and carbohydrate in the diet (Berdanier and Shubeck 1979; Miksicek and Towle, 1982) whereas insulin is generally considered to be the predominant signal responsible for up regulation of expression of the lipogenic enzymes including G6PDH. Other studies have shown that the glucocorticoids play an important role (Bouillon and Berdanier, 1980; Berdanier, 1989).

The data on activity of hepatic G6PDH of six experimental groups is furnished in the Table 11 Fig 17 & 18. An opposite trend in the alteration of G6PDH activity was observed in STZ diabetic (D-group) and insulin resistance rats (F-group) compared to C-group. Hepatic G6PDH activity decreased significantly (54.3 %) in D-group and enhanced significantly (32.5 %) in F-group compared to C-group. *C. roseus* treatment resulted in a significant enhancement in the activity of G6PDH in the liver of C + CR and D + CR-groups (7.0 and 85.5 %) compared to C and D-groups respectively. However, the improved activity of G6PDH observed in D + CR-group did not reach normal value whereas *C. roseus* treatment did not caus significant alterations in the activity of hepatic G6PDH in F + CR-group compared to F-group.

Lowered hepatic G6PDH activity was observed in STZ-diabetic rats. Our results are in consistence with earlier reports (Siddiqui and Rahman, 1980; Ugochukwu and Babady, 2003; Bugdayci et al., 2006). This decrease may be considered as an adoptive phenomenon by which the supply of NADPH for aldose reductase is cut down.
Reduced activity of this enzyme in diabetic state shows that glucose does not enter into pentose phosphate pathway to a greater extent. G6PDH activity and NADPH/NADP ratio vary inversely in relation to blood glucose concentration. Studies of Margarita et al. (2006) indicated that decrease in hepatic G6PDH activity was dependent on the severity of hyperglycemia. The decreased activity of G6PDH also indicates that the HMP shunt activity is unable to meet the requirement of cellular NADPH for the enzymes that continuously maintain Hb and GSH in their reduced states. The decreased activity of this enzyme further indicates the accumulation of glucose-6-Phosphate which is a potent glycosylating agent that causes GSH depletion and thereby boosts glycation (Jain, 1998) and it may also promote the final step of gluconeogenesis (G6Pase). C. roseus administration induced moderate reversal of this effect in D + CR- rats. Insulin is reported to stimulate oxidation of glucose by increasing the activation of G6PDH in dose dependent manner (Stumpo and Kletzien, 1984; Asavari et al., 1998). Thus increased circulatory insulin level observed in C. roseus treated STZ diabetic rats may cause increased activity of G6PDH in these rats.

In fact the role of G6PDH in the cell response to oxidative stress has been well established. Thus the decreased activity of this enzyme in diabetic rats gives an indication of decreased potential to combat oxidative stress. G6PDH deficient cells had decreased growth rates and are highly susceptible to oxidative stress compared with cells expressing endogenous levels of G6PDH (Wang et al., 1999). Improved activity of G6PDH by C. roseus administration in D + CR-rats indicates effective operation of pentose phosphate pathway and increased potential to overcome oxidative stress. Similar results of enhanced G6PDH activity were reported in experimental diabetic animals treated with some anti
diabetic plants like Momordica charantia (Shibib et al., 1993), Trigonella foenum graceum (Raju et al., 2001), Gymnema sylvestre (Shanmugasundaram et al., 1983) and Gongronema latifolium (Ugochukwu and Babady, 2003).

A significantly enhanced activity of hepatic G6PDH observed in fructose fed rats is in accordance with earlier reports (Moser and Berdamier, 1974; Fiebig et al., 1998). The liver is an organ in which glucose regulated gene expression has already been investigated. When glucose flux increase in the hepatocytes, flux through the pentose phosphate pathway also increases. In addition, pressure overload induces G6PDH, the enzyme catalyzing the flux generating step in oxidative pentose phosphate pathway (Zimmer, 1996). The induction of G6PDH in different tissue by a variety of non-hormonal agents suggested that the G6PDH gene may be a sentinel for oxidant stress and may be capable of responding rapidly to the need for NADPH for maintenance of the cellular redox state.

Dietary carbohydrate also influenced the level of expression. It was suggested that a metabolite of the sugars is involved in upregulation of hepatic G6PDH expression (Fukuda et al., 1992). However, since both glucose and sucrose can elicit insulin secretion, it has been difficult to sort out secondary effects of the carbohydrates versus primary effects on gene expression in animals. Other studies demonstrated that fatty acids in the diet can repress induction of G6PDH activity, mRNA abundance (Tomlinson et al., 1988; Katsurada et al., 1989), and G6PDH gene transcription (Iritani, 1992) following fasting/refeeding. Several studies have explored the possible role of carbohydrate on G6PDH expression, since earlier studies in animals and hepatocytes in culture suggested that nutrient influenced G6PDH activity (Kelley and Kletzien, 1984; Salati et al., 1988). However, the reports
demonstrate a lack of consensus; some groups indicate that glucose does not influence expression while others suggest that it does. A study by Fukuda et al. (1992) showed that glucose can enhance the insulin and glucocorticoid-induced expression of all the lipogenic enzymes in addition to G6PDH, although the influence of the sugar in the absence of the hormones was not addressed. Thus, one is not sure if the sugar effect is directly linked to enhancement of gene expression or indirectly linked through an enhancement of hormonal signaling. Fukuda et al. (1992) also showed that fructose could replace glucose and that glycerol was also effective suggesting that a metabolite or glycolytic intermediate is mediating the effect of carbohydrate on G6PDH expression. Thus in the present study the hyperglycemia along with hyperinsulinemia in fructose fed rats might have mediated the G6PDH expression. In addition fructose as well as its metabolite-glycerol and fructose induced oxidative stress has given additive effect in enhancing the G6PDH expression.

Basing on our observations in carbohydrate metabolism the antihyperglycemic effect of this plant appears to be at least in part, due to extra pancreatic activity, including increased glucose utilization by liver and muscle (glycolysis), enhanced glucose oxidation through shunt pathway via activation of G6PDH and decreased glucose production by depression of key gluconeogenic and glycogenolytic enzymes.

Weber et al. (1971) after an analysis of the observations made in diabetes and in insulin therapy concluded that the impact of insulin in the liver is to increase glycolysis and decrease gluconeogenesis, "i.e. predominance of glycolysis over gluconeogenesis". Further, insulin integrates hepatic carbohydrate metabolism by increasing the biosynthesis of enzymes of glycolysis, glycogenesis, pentose oxidative pathway and lipogenesis while
inhibiting gluconeogenesis. This is well evident from the study of key enzymes of carbohydrate metabolism of liver, kidney and muscle of diabetic animals under *C. roseus* administration. This indicates that the above changes may be due to the regeneration of damaged pancreatic β-cells leading to elevated levels of circulating insulin in STZ diabetic and improved insulin sensitivity in fructose feed induced insulin resistance condition by *C. roseus* treatment.

**Intestinal disaccharidases**

The intestinal digestive and absorptive processes of carbohydrates are mediated by the disaccharidases and hexose transporters that are localized in the brush-border membranes of intestinal absorptive cells (Semenza, 1986; Thorens, 1996).

Diabetes mellitus is a state of nutrient starvation that frequently results in severe metabolic imbalances and pathological changes in many tissues. In the small intestine this severe disease causes significant changes in the morphology and function of the mucosa (Miyamoto *et al.*, 1991; Zoubi *et al.*, 1995). Diabetes stimulates the functional activity of the intestinal brush border membrane with enhancement of both hydrolytic enzyme activity (Schedl *et al.*, 1983; Mc Anuff-Harding *et al.*, 2006) and membrane transport system (Hopfer, 1975).

Chronic diabetes enhances glucose transport by non-specific increases in intestinal mass (Olsen and Korsme, 1975; Ferraris *et al.*, 1993). Release of glucose and transport across the intestinal brush border membrane down to the blood stream has attracted much attention recently as potential targets to control postprandial hyperglycemia. Since both postprandial hyperglycemia and hyperinsulinemia were improved by treatment with a
disaccharidase inhibitor in patients with NIDDM, the possibility that this drug might improve insulin resistance was suggested (Friedman et al., 1991; Shinozaki et al., 1996). Therefore, a disaccharidase inhibitor could be recommended to prescribe to obese and insulin resistance patients even without metabolic derangement in glucose homeostasis, i.e. diabetes mellitus.

So the present study was extended to understand the efficacy of *C. roseus* administration in regulating the intestinal disaccharidases activity under both insulin deficient and insulin resistance conditions.

The data presented in the Table 12 and Fig & 21 reveals the activities of intestinal disaccharidases (maltase, sucrase and lactase) of the six experimental groups. Both STZ diabetic group (D-group) and insulin resistance rats (F-group) showed significantly enhanced activities of maltase (56.0 and 12.6 %), sucrase (15.2 and 19.3 %) and lactase (26.1 and 15.6 %) respectively when compared to C-group. Thus STZ diabetic rats showed significantly higher maltase and lactase activities than F-group rats whereas no significant variation was observed regarding intestinal sucrase activity between D and F-groups. *C. roseus* administration for 60 days caused no significant alteration in the activities of intestinal disaccharidases in C + CR-group from C-group. However it caused a significant decreases in the activities of intestinal disaccharidases in D + CR-group and F + CR-group compared to D and F-groups respectively. Thus *C. roseus* treatment resulted in the restoration of enhanced intestinal disaccharidases observed in SZT diabetic condition and fructose fed conditions to normal values.
Table 12. Effect of *C. roseus* treatment on intestine disaccharidases

<table>
<thead>
<tr>
<th>Disaccharidase (nmol of disaccharide hydrolysed/min/mg protein)</th>
<th>C</th>
<th>C + CR</th>
<th>D</th>
<th>D + CR</th>
<th>F</th>
<th>F + CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltase</td>
<td>3.96±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.80±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.18±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.82±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.46±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.85±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sucrase</td>
<td>3.73±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.63±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.20±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.70±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.45±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.64±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactase</td>
<td>2.75±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.65±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.47±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.84±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.18±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.67±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
Fig 21: Plasma glucose and intestinal disaccharidases (maltase, sucrase and lactase) activity of Control (C), Control treated (C + CR), Diabetic (D), Diabetic treated (D + CR), Fructose (F), Fructose treated (F + CR) rats.
The increased activities of intestinal disaccharidases (maltase, sucrase and lactase) observed in present study are in agreement with earlier reports in STZ diabetic induced insulin deficient rats (Goda et al., 1983; Suresh and Sivakami, 1998) and fructose feed induced insulin resistance rats (Tominaga et al., 1997; Kishi et al., 1999).

Insulin deficiency stimulates the functional activity of brush border membrane of the intestinal absorptive cell. For example, experimental diabetes has been reported to increase the enzymatic activity of many brush border hydrolases including the disaccharidases (Caspary et al., 1972) and stimulates a number of transport systems which are believed to reside in this membrane (Caspary, 1973; Hopfer, 1975). In diabetic patients and animals, in particular in rats with STZ induced IDDM, abnormal increases in the activities of sucrase and isomaltase are observed in the small intestine (Olsen and Korsto, 1997; Hoffmann and Chang, 1992). Furthermore, it has been reported that the postprandial metabolic profile is improved by the administration of an inhibitor of the sucrase and isomaltase in the experimental IDDM rats (Matsuo et al., 1992) suggesting that the abnormal increases in these enzyme activities exert a harmful influence on the metabolic imbalance under diabetic conditions (Porteous et al., 1979). Diabetes mellitus in man has also been associated with increased intestinal disaccharidase activity (Cerda et al., 1972; Tandon et al., 1975) and enhanced glucose absorption (Vinnik et al., 1965). Although negative studies in man have also been reported (Genel et al., 1971; Arvanitakis and Olsen, 1974), perhaps because of differing severity of the disease and effects of treatment. According to Olsen and Korsto, (1977) enhanced sucrase-isomaltase enzyme activity of STZ diabetes was the consequence
of an increase in concentration of sucrase-isomaltase protein due to decreased rate of its degradation.

Hormone secretion by the gut and the pattern of response after feeding may be abnormal in diabetes and might be regulatory for disaccaridases. The intestinal effects are prevented or markedly diminished by insulin therapy (Olsen and Rosenberg, 1970; Caspary et al., 1972) suggesting that they are in fact the result of insulin deficiency or insulin resistance. In addition, change in the pattern of food consumption i.e., polyphagia of diabetes might also stimulate the intestinal disaccharidases in STZ diabetic rats.

The traditional view of the interaction of nutritional substances and intestinal enzymes is that the enzymes act to digest and metabolize the foodstuffs that one eats. More recent knowledge has demonstrated that intestinal enzyme activity will vary according to the diet; i.e., the concept of adaptation to the diet ingested. This should not be surprising since adaptation to the environment is a fundamental principle of many biological systems.

Previous studies demonstrated the adaptable nature of intestinal disaccharidase activity in rats (Deren et al., 1967). Specific dietary sugars can alter enzyme activity in the small intestine of man in a specific fashion. Sucrose and fructose are able to regulate sucrase and maltase activity (Rosensweig and Herman, 1968). Yasutake et al. (1995) reported that dietary sucrose enhanced both sucrase-isomaltase mRNA and sodium/D-glucose transporter-1 (SGLT-1) mRNA levels in the rat jejunum within 12 h. Similarly, Miyamoto et al. (1993) demonstrated that the mRNA levels of jejunal hexose transporters (SGLT-1, GLUT-5, and GLUT-2) were elevated by feeding fructose diet to rats for 5 days.
The α-glucosidase inhibitors are a new class of antihyperglycemic drugs that have a unique effect on the glycemic profile. Their major action is to lower postprandial plasma glucose levels by inhibiting the breakdown of complex carbohydrates within the intestine resulting in delayed absorption of glucose with the small bowel and a consequent reduction is postprandial glucose levels. The α-glucosidase inhibitors not only delay carbohydrate absorption but they also alter the gastrointestinal hormonal axis. These drugs decrease postprandial secretion of gastric inhibitory polypeptide (potentiates insulin secretion under hyperglycemic condition) and increases postprandial levels of glucagon-like peptide which may play a role in the regulation of insulin secretion (Sunil and Sadekar, 1999). Therefore, α-glucosidase inhibitors such as acarbose, voglibose and miglitol are widely used either alone or in combination with insulin secretagogues in patient with Type-2 diabetes (Johnston et al., 1998; Saito et al., 1998; Standl et al., 1999). Acarbose is the first commercially available α-glucosidase inhibitor and inhibits both amylase and membrane bound α-glucosidase with approximately equal affinity. It has a potent inhibitory effect on sucrase but weak effect on the maltase and no effect on β-glucosidase such as lactase. Thus lactose is digested normally (Chait and Brunzell, 1996). Acarbose does not interfere with the sodium dependent glucose transporter thereby not affecting absorption of glucose. Friedman et al. (1991) reported that the decreased levels of GLUT-4 of fatty Zucker rats were normalized by the treatment with a disaccharidase inhibitor, acarbose. Since GLUT 4 level is the rate-limiting step of insulin stimulated glucose disposal, normalization of GLUT-4 level might considerably improve in vivo insulin resistance of fatty Zucker rats.

Voglibose, an N-substituted derivative of valiolamine isolated from the fermentation broth of Streptomyces hygroscopicus, is a potent and structurally novel inhibitor of the
intestinal disaccharidases. It had a potent inhibitory effect on maltase but with a short inhibitory duration (Kameda et al., 1984; Goto et al., 1995; Taira et al., 2000; Koyama et al., 2000). Shinozaki et al. (1996) found an improvement of in vivo sensitivity to insulin in non-diabetic hyperinsulinemic patients by administration of a disaccharidase inhibitor A0-128 (voglibose). It is hardly thought that a disaccharidase inhibitor by itself corrects the abnormality of post-receptor pathway of both the peripheral tissue and liver in insulin resistant diabetic rats. Tominaga et al. (1997) also reported that insulin resistance of Wistar fatty rats was improved by a disaccharidase inhibitor. Even though acarbose and voglibose are included in the drug list of diabetes management of either Type-1 or Type-2, they show some common side effects. The most common adverse effects of these drugs are gastrointestinal disturbance induced by fermentation of unabsorbed carbohydrate in the bowel, and increments of gastrointestinal motility and hepatotoxicity (Nakamura, 1993; Goke et al., 1995; Uribe et al., 1998; Nagai et al., 2000). Unfortunately, the disturbance of digestive system also exists in diabetics (Quigley, 1999). Luo et al. (2001) reported combined effect of these drugs with gymnemic acid (GA), a mixture of triterpene glucuronides, found in the leaves of the Indian plant Gymnema sylvestre which augmented their intestinal disaccharidases inhibitory effects with diminished adverse effects (Yoshioka, 1986; Imoto et al., 1991).

In this category, majority of recent studies report the potential of antidiabetic medicinal plants on inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase and manipulation of intestinal glucose transporters. A wealth of literature has emerged now showing the potential effect of phytochemicals in inhibiting α-amylase (Kim, 2000) and α-glucosidase (Watanabe et al., 1997). Watanabe et al. (1997) also reported the
potential effect of phytochemicals in inhibiting α-glucosidase activity resulting in lowering of in vivo postprandial hyperglycemia.

In the present study the enhanced or elevated activities of intestinal disaccharidases of insulin deficient diabetic and insulin resistance rats were prevented by the C. roseus treatment. Therefore the improvement of glycemic control by the delayed absorption of glucose with a disaccharidase inhibitor is likely responsible for the slight improvement of insulin resistance in fructose fed rats Thus, antidiabetic property of C. roseus may also contribute due to its intestinal disaccharidase inhibitory activity.

LIPID METABOLIC STUDIES
Tissue lipids

The data presented in the Table 13 and Fig 22 reveal the hepatic and heart tissue total cholesterol, triglycerides, phospholipids and FFA of the six experimental groups. The data from six experimental groups revealed that liver tissue contains higher proportions of lipid fractions than heart. All fractions of tissue lipids are significantly enhanced in both D and F-group compared to C-group. When compared to C-group, the per cent increase in hepatic and cardiac TC, TG, phospholipids and FFA are 12.8, 9.13, 5.7 and 15.2 %, and 21.6, 17.9, 16.5 and 32.8 % respectively in D-group and 21.6, 17.8, 51.7 and 32.2 %, and 41.4, 21.6, 63.9 and 53.7 % respectively in F-group. Thus insulin resistance rat model showed higher lipid accumulation in both liver and heart tissues compared to STZ diabetic rats. C. roseus administration for 60 days restored the tissue lipid fractions to normal values both in STZ diabetic rats (D + CR) and insulin resistance rats (F + CR). However C + CR-
Table 13. Effect of *C. roseus* treatment on tissue lipids in STZ diabetic and fructose fed IR rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>C</th>
<th>C + CR</th>
<th>D</th>
<th>D + CR</th>
<th>F</th>
<th>F + CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC mg/100g tissue</td>
<td>Liver</td>
<td>149.67±2.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>137.41±3.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>168.80±2.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>149.16±2.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>181.92±3.55&lt;sup&gt;d&lt;/sup&gt;</td>
<td>152.37±2.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>62.53±1.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.34±1.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.17±1.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>61.52±1.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.41±3.73&lt;sup&gt;d&lt;/sup&gt;</td>
<td>63.65±1.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TG mg/100g tissue</td>
<td>Liver</td>
<td>156.61±2.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>145.82±2.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>170.97±2.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>149.78±2.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>184.48±4.55&lt;sup&gt;d&lt;/sup&gt;</td>
<td>160.14±2.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>67.74±0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.85±0.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.77±0.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>68.52±1.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.47±2.86&lt;sup&gt;d&lt;/sup&gt;</td>
<td>69.82±1.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PLP μg/100g tissue</td>
<td>Liver</td>
<td>887.6±13.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>847.7±12.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>938.5±17.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>869.4±16.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1034.2±14.28&lt;sup&gt;d&lt;/sup&gt;</td>
<td>892.4±9.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>447.5±5.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>412.3±4.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>634.4±5.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>425.6±6.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>733.1±6.10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>430.5±5.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FFA mg/100g tissue</td>
<td>Liver</td>
<td>210.24±3.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>191.49±4.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>242.35±3.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>205.66±3.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>279.80±5.32&lt;sup&gt;d&lt;/sup&gt;</td>
<td>212.38±3.89&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>122.50±2.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.71±2.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>159.81±2.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>126.50±3.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>188.44±2.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>128.11±2.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan’s multiple range test).
Fig 22: Liver fatty acid synthase activity and liver tissue lipids (total cholesterol and free fatty acids).
group showed a slight but a significantly decrease in tissue lipid fractions when compared to C-group.

Liver shows a marked increase in the lipid concentration during diabetes (UmeshYadav et al., 2005) and fructose fed condition (Agheli, 1998) resulting into fatty liver. In order to understand the contribution of lipogenic enzyme activities for the fatty liver in STZ diabetic and fructose fed rats and their alterations by C. roseus administration, the following hepatic lipogenic enzymes i.e. FAS, malic enzyme and G6PDH were assayed in the liver of six experimental groups.

**Fatty acid synthase (FAS), Malic enzyme and Lipoprotein lipase (LPL)**

Fatty acid synthase plays a central role in *de novo* lipogenesis in animals by catalyzing all the reactions in conversion of acetyl-CoA and malonyl-CoA to palmitate (Wakil et al., 1983). Malic enzyme and G6PDH play a key role in generation of NADPH for lipogenesis. The activities of hepatic malic and G6PDH enzymes correlate closely with the rate of lipid biosynthesis (Revilla *et al*., 1987). Lipoprotein lipase is an enzyme responsible for the hydrolysis of triacylglycerols from plasma lipoproteins, mainly chylomicrons and very low-density lipoproteins and its activity is influenced by nutritional and hormonal status and by environmental conditions (Kraemer *et al*., 1998; Merkel *et al*., 2002). Adipose tissue LPL, is the enzyme that initiates the entry of lipoprotein packaged fatty acids into adipose tissue for storage (Appel and Fried, 1992).

The data on activity of hepatic G6PDH of six experimental groups are furnished in the Table 11 and Fig 17 & 18. The data on activity of hepatic malic enzyme and FAS of six
Table 14. Effect of *C. roseus* treatment on lipid metabolic enzymes in STZ diabetic and fructose fed IR rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>C</th>
<th>C+CR</th>
<th>D</th>
<th>D +CR</th>
<th>F</th>
<th>F+CR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Malic enzyme</strong> (µmol of NADP reduced /min/mg protein)</td>
<td>Liver</td>
<td>5.88 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.13 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.80 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.68 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.08 ± 0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.92 ± 0.21&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Fatty acid synthase</strong> (µmol of NADPH utilized /min/mg protein)</td>
<td>Liver</td>
<td>0.47 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.42 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.50 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Lipoprotein lipase</strong> (µmol of PNP/ min/mg protein)</td>
<td>Adipose</td>
<td>8.33 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.25 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>8.04 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.35 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.41 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan’s multiple range test).
experimental groups are furnished in the Table 14 and Fig 22. An opposite trend in the alteration of malic enzyme and FAS activities were observed in STZ diabetic (D-group) and insulin resistance rats (F-group) compared to C-group. Group-D showed a significantly decreased hepatic malic enzyme (18.3 %) and FAS (21.2 %) activities, whereas, F-group showed a significantly enhanced activity of hepatic malic enzyme (37.4 %) and FAS (25.3 %) when compared to C-group. The significant enhancement in the hepatic malic enzyme and FAS activities of D + CR-group compared to D-group resulted in the restoration of these enzyme activities to normal levels. *C. roseus* administration to fructose fed rats (F + CR-group) resulted in a significant decrease in hepatic malic enzyme (14.3 %) and FAS (15.2 %) activities when compared to F-group. These alterations in F + CR-group by *C. roseus* administration restored the hepatic FAS activity to normal level, whereas activity of malic enzyme of F + CR group is still significantly higher than C-group. However these enzyme activities of C + CR-group did not deviate from C-group.

The activities of LPL of experimental groups are represented in Table 14. The abdominal adipose tissue was not visible in the D-group animals. Whereas huge quantity of adipose tissue in the abdominal region was seen in F-group animals. A significantly decreased activity (11.7 %) of LPL of adipose tissue was observed in fructose fed rats (F-group) when compared to C-group. *C. roseus* administration for 60 days showed no significant variation in this enzyme activity in C + CR and D + CR-groups. However F + CR-group showed a significantly enhanced adipose LPL activity when compared to F and C-groups.
Liver plays an important role in glucose and lipid metabolism and gets severely affected metabolically during diabetes due to lack of insulin. Liver is involved in lipid metabolism through uptake, oxidation and metabolic conversation of plasma lipoproteins. Thus under both insulin deficient condition (STZ diabetic rats) and fructose fed insulin resistance condition, fatty liver results due to accumulation of lipids. Both STZ and fructose fed rats showed enhanced lipid content by significantly increased TG, TC, phospholipids and FFA in liver and heart tissue compared to control rats.

Hepatic lipid accumulation may be a result of one or several of the following factors: increased delivery of adipose tissue or dietary fatty acids to the liver, increased de novo synthesis of fatty acids in the liver, decreased rate of hepatic fatty acid oxidation, or decreased rate in the exit of fatty acid from the liver in the form of TG. Delivery of fatty acids to the liver appears to be the most potent mechanism for hepatic lipid accumulation (Nielsen et al., 2004; Donnelly et al., 2005).

During diabetes liver shows decrease in weight due to elevated catabolic process such as glycogenolysis, lipolysis and proteolysis, which is the outcome of lack of insulin and/or cellular glucose in liver cells. During diabetes lipogenesis is decreased, while lipolysis is increased in the hepatic tissue, which is the outcome of underutilization of glucose resulting in increased lipolysis and stimulation in the activities of gluconeogenic enzymes (West, 1982). However the increase in the hepatic lipid levels in STZ diabetic rats may be due to increased uptake from the portal system as shown earlier (Gupta et al., 1999) and not due to de novo synthesis. The present study also reflects decreased activity of most
Results of lipogenic enzymes i.e. FAS, G6PDHase and malic enzyme in liver of STZ diabetic rats which supports this view (Table 11 and 14).

A regulatory protein, called sterol regulatory element binding protein (SREBP), binds to sterol responsive elements found on multiple genes, and activates a cascade of enzymes involved in lipid biosynthesis pathway such as HMG-CoA reductase (Brown and Goldstein, 1997) and fatty acid synthase (Bennett et al., 1995). Insulin controls hepatic SREBP expression. Expression of SREBP is enhanced by insulin in all three major insulin target tissues, liver, fat and skeletal muscle (Kim et al., 1998; Foretz et al., 1999; Sewter et al., 2002). Also Shimomura et al. (1999) demonstrated that transcription factor SREBP-1 was down regulated in the liver of STZ diabetic rats due to the depletion of insulin. Sun et al. (2002) showed that insulin treatment corrected the decreased SREBP-1 expression in the liver of diabetic rats. Thus in the present study establishment of near normal values of blood glucose, with significantly increased concentration of plasma insulin in C. roseus administered STZ diabetic rats may be responsible for normalization of lipogenesis and tissue lipids preventing further degradation of stored lipids.

The human liver possesses a large capacity to metabolize fructose to lipids because of its ability to shunt metabolism toward serum TG production. Triglyceride stores supply an energy 'sink', providing an almost unlimited TG production capacity (Herman et al., 1970). As discussed earlier, the effects of fructose in promoting TG synthesis are independent of insulinemia. Hirsch, (1995) argued that carbohydrate overload results in elevated TG because the large amounts of sugar that need to be absorbed so rapidly from the intestine lead to the involvement of other metabolic pathways, such as the hexose
monophosphate shunt that favor the synthesis of FFA. If FFA are not removed from tissues, as occurs in fructose fed insulin resistant models, there is an increased energy and FFA flux that leads to the increased secretion of TG. Again, the liver takes up dietary fructose rapidly where the unregulated fructose metabolism generates glycerol and acyl portions of acyl-glycerol molecule, the substrate for TG synthesis. Increase in the acyl-coA corboxylase and diacyl glycerol acyl transporter activities has been reported in liver of a similar model system, fructose fed hamster (Casaschi et al., 2005). The activity of SREBP in liver is reported to be enhanced in insulin resistant fructose fed mice (Miyazaki et al., 2004), and this explains the increased levels of cholesterol and fatty acids during fructose feeding by inducing the enzymes of lipogenesis.

Peripheral insulin resistance in fat and muscle leads to increased delivery of free fatty acids to the liver increasing TG synthesis. Fatty liver develops when hepatic TG synthesis exceeds hepatic synthesis and export of VLDL-TG (Anna and Hannele, 2008). The prevalence of non-alcoholic fatty liver disease (NAFLD) is becoming increasingly recognized in different countries reporting high prevalence in obese individuals. NAFLD may progress to non-alcoholic steatohepatosis, steatohepatitis, fibrosis or cirrhosis, representing increasing liver damage (Neuschwander-Tetri and Caldwell, 2003)

The liver, once fatty, is insulin resistance and overproduces both glucose and VLDL leading to hyperglycemia, hypertriglyceridemia, and a low HDL-C concentration (Wasastjerna et al., 1972). In humans, exogenous insulin therapy (70 IU/d for 7 months) decreases liver fat significantly suggesting that hyperinsulinemia may be a consequence rather than cause of liver fat (Juurinen et al., 2007). Insulin normally inhibits the production
of VLDL from the liver. Once fatty, this action of insulin is impaired whereas VLDL clearance remains unchanged. The overproduction of VLDL results in hypertriglyceridemia and a lowering of HDL-C (Nikkila and Taskinen, 1978).

Thus in the present study the enhanced tissue lipids in fructose fed rats can be explained by enhanced hepatic lipogenesis as reflected by the increased activities of FAS, malic enzyme and G6PDH in the liver along with decreased activity of LPL of adipose tissue under fructose fed condition.

The protective role of C. roseus against fructose feed induced lipid accumulation in tissues is also evident from preventing the enhanced lipogenesis observed under fructose fed conditions by keeping the lipogenic enzymes of liver FAS, malic enzyme and G6PDH and LPL activity of adipose tissue to the normal values. Thus C. roseus treatment had given protection against fructose feed induced insulin resistance in target tissues by preventing lipid accumulation.

**OXIDATIVE STRESS**

The oxidative stress and resultant tissue damage are hallmark of chronic diseases and cell death, and diabetes is not an exception. During diabetes persistence hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose autooxidation and protein glycation (Jiang et al., 1990; Baynes and Thorpe, 1999; George and Mary, 2004). In addition, hyperinsulinemia in insulin resistance condition and enhanced FFA observed in diabetes and insulin resistance condition are also sources of free radicals (Henry et al., 1996; Strother et al., 2001; Itani et al., 2002; Evans et al., 2003). Diabetes mellitus and insulin resistance are postulated to be a state of
increased free radical activity. Oxidative stress is currently suggested as the mechanism underlying diabetes and diabetic complications. Normalizing ROS generation not only reversed these changes, but also prevented the long-term complications of diabetes (Nishikawa et al., 2000).

The direct measurement of free radicals, particularly in vivo is extremely difficult. Usually, the products of radical damage in the cell-viz., lipids, proteins and DNA are considered good markers of oxidative stress (Piconi et al., 2003). In order to understand the extent of oxidative stress and to assess the protective effect of C. roseus administration in insulin deficient and insulin resistance conditions, oxidative markers like lipid peroxidation (LPO), protein oxidation and antioxidant status were assessed in tissues of six experimental groups. Lipid peroxidation has probably been the most extensively investigated process induced by free radicals. Lipid peroxides are derived from the oxidation of poly unsaturated fatty acids of membranes and are capable of further LPO by a free radical chain reaction (Das et al., 2002). All bio macromolecules are faced with oxidative stress including proteins. Protein oxidation is defined as the covalent modification of a protein induced either directly by reactive ROS or indirectly by reactions with secondary byproducts of oxidative stress (Sermin et al., 2007).

Lipid peroxidation (LPO) and Protein oxidation

The extent of LPO and protein oxidation in liver, pancreas and heart of six experimental groups are summarized in the Table 15 and Fig 23 & 24. The extent of LPO in all experimental groups indicates that the intensity of LPO is highest in liver followed by
Table 15. Tissue lipid peroxidation, protein oxidation and reduced glutathione levels in six experimental groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>C</th>
<th>C + CR</th>
<th>D</th>
<th>D + CR</th>
<th>F</th>
<th>F+CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxidation</td>
<td>Liver</td>
<td>20.33±0.36 a</td>
<td>17.81±0.31 b</td>
<td>29.94±0.40 c</td>
<td>20.85±0.24 a</td>
<td>24.08±0.63 d</td>
<td>19.21±0.53 a</td>
</tr>
<tr>
<td>(nmol of MDA formed/ min/mg protein)</td>
<td>Pancreas</td>
<td>7.41±0.13 a</td>
<td>6.366±0.22 a</td>
<td>11.27±0.37 b</td>
<td>8.43±0.18 c</td>
<td>8.79±0.22 d</td>
<td>7.67±0.37 a</td>
</tr>
<tr>
<td>Heart</td>
<td>Heart</td>
<td>11.59±0.23 a</td>
<td>10.64±0.37 b</td>
<td>15.31±0.21 c</td>
<td>12.52±0.17 a</td>
<td>14.48±0.23 d</td>
<td>12.04±0.47 a</td>
</tr>
<tr>
<td>Protein oxidation</td>
<td>Liver</td>
<td>7.51±0.49 a</td>
<td>6.51±0.54 b</td>
<td>9.58±0.86 c</td>
<td>7.60±0.43 a</td>
<td>8.67±0.56 d</td>
<td>7.72±0.47 a</td>
</tr>
<tr>
<td>(µmol of protein carbonyls/mg protein)</td>
<td>Pancreas</td>
<td>3.11±0.05 a</td>
<td>3.02±0.10 a</td>
<td>4.04±0.11 b</td>
<td>3.30±0.52 a</td>
<td>3.71±0.06 c</td>
<td>3.23±0.06 a</td>
</tr>
<tr>
<td>Heart</td>
<td>Heart</td>
<td>4.48±0.13 a</td>
<td>4.29±0.10 a</td>
<td>5.26±0.09 b</td>
<td>4.61±0.07 a</td>
<td>5.12±0.14 c</td>
<td>4.31±0.13 a</td>
</tr>
<tr>
<td>GSH</td>
<td>Liver</td>
<td>5.55±0.20 a</td>
<td>6.33±0.20 b</td>
<td>3.92±0.32 c</td>
<td>5.11±0.16 d</td>
<td>4.78±0.25 c</td>
<td>5.58±0.18 a</td>
</tr>
<tr>
<td>(µg/ mg protein)</td>
<td>Pancreas</td>
<td>2.07±0.21 a</td>
<td>2.50±0.18 b</td>
<td>1.36±0.13 c</td>
<td>2.20±0.14 a</td>
<td>1.89±0.13 a</td>
<td>2.40±0.23 d</td>
</tr>
<tr>
<td>Heart</td>
<td>Heart</td>
<td>3.72±0.31 a</td>
<td>4.16±0.034 b</td>
<td>2.12±0.30 c</td>
<td>3.62±0.31 a</td>
<td>3.22±0.22 d</td>
<td>3.59±0.15 a</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan’s multiple range test).
Fig 23: Per cent change in the LPO, protein oxidation and GSH of liver, pancreas and heart of D and F-groups compared to control group.

Fig 24: Per cent recovery from STZ diabetic and fructose feed induced alterations in LPO, protein oxidation and GSH levels in D + CR and F + CR-groups.
heart and pancreas (L > H > P). Both STZ diabetic (D-group) and insulin resistance rats (F-group) showed significantly enhanced LPO in liver (47.3 and 22.0 %), pancreas (52.1 and 18.6 %) and heart (32.1 and 24.9 %) when compared to corresponding values of C-group. Thus under STZ diabetic condition the per cent increase in LPO is more in pancreas than liver and heart. Whereas under insulin resistance condition (F-group) per cent increase in LPO is more in heart than liver and pancreas under insulin resistance condition (F-group). Except pancreas LPO of D + CR-group C. roseus treatment for 60 days prevented the increased tissue LPO observed in STZ induced diabetic condition and fructose fed conditions. The significantly decreased pancreas LPO (25.2 %) of D + CR-group compared to D-group did not reach the normal value. The beneficiary effect of C. roseus treatment is also reflected in significantly decreased LPO observed in liver (12.5 %) and heart (8.2 %) of C + CR-group compared to C-group.

Similar to the LPO, the protein oxidation levels in all experimental groups are in the following order i.e. L > H > P. Statistically significant increased levels of protein oxidation were observed in the liver (27.5 %), pancreas (29.8 %) and heart (10.1 %) of STZ diabetic rats (D-group) compared to C-group whereas F-group showed enhanced protein oxidation in the liver (15.4) pancreas (19.2 %) and heart (7.1 %) when compared to C-group. The altered protein oxidation levels are reinstated to normal values by C. roseus supplementation both in D + CR and F + CR-groups. However C. roseus treated control group i.e. C + CR showed no deviation in the protein oxidation in pancreas and heart tissues with a significantly decreased protein oxidation levels in the liver compared to C-group.
From the results obtained, it is evident that increased oxidative stress as measured by the index of LPO increased both in STZ diabetic and fructose fed rats and it could cause initial β-cell damage in STZ diabetic (insulin deficient) (Robertson et al., 1992; Krauss et al., 2003) or impaired insulin function in insulin resistance condition (Evans et al., 2005). Further, the oxidative stress as indicated by severity of LPO and protein oxidation is comparatively more in insulin deficient condition than insulin resistance condition. In the present study, among the tissues in which oxidative stress studies conducted (liver, pancreas and heart), pancreas and heart tissues have shown more prominent oxidative stress under insulin deficient and insulin resistance conditions respectively.

Increased LPO impairs membrane functions by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors (Chen et al., 2002). Malondialdehyde (MDA) is a breakdown product of peroxidation of long chain fatty acids which accumulates when LPO increases (Ceconi et al., 1992). MDA levels in liver may also be used to investigate the oxidative damage of proteins and lipoproteins which is a possible pathogenic mechanism for liver injury (Kojic et al., 1998). Oxidative stress as measured by indices of LPO, increased in both Type-1, and Type-2 diabetes mellitus and even in diabetic patients without complications (Nishikawa et al., 2000; Ceriello et al., 2001; Mohora et al., 2006; Stephens et al., 2006). Several studies have also shown increased concentrations of ROS and oxidative stress in STZ diabetic rats (Wilson et al., 1984; Thomas and Ramwell, 1989; Sakurai and Ogiso, 1994; Kroncke et al., 1995) as well as fructose fed rats (Srividhya and Anuradha 2002; Girard et al., 2005; Sandrine et al., 2005).
Mechanisms that contribute to increased oxidative stress in diabetes may include not only the persistence hyperglycemia, increased non-enzymatic glycosylation (glycation) and autooxidative glycosylation but also metabolic stress resulting from changes in energy metabolism, alterations in sorbitol pathway activity, changes in the level of inflammatory mediators, the status of antioxidant defense systems, localized tissue damage resulting from hypoxia and ischemic reperfusion injury. Damage caused by free radicals is possibly involved in β-cell destruction and in the pathogenesis of diabetes mellitus (Oberley, 1988). Alterations of metabolic processes in diabetes also influence enzymatic defenses, and these changes may be associated with late complications of diabetes. In addition, hypoinsulinemia observed in diabetes (Type-1) increases the activity of the enzyme, fatty acyl-CoA oxidase, which initiates β-oxidation of fatty acids resulting in LPO (Oberley, 1988). Low levels of lipoxygenase peroxides stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases it may initiate uncontrolled LPO leading to cellular infiltration and islet cell damage in Type-1 diabetes (Metz, 1984).

Insulin resistance is also associated with increased LPO and free radical formation and increased formation of TBARS (thiobarbituric acid reactive substances) is associated with insulin perturbations. Previous studies strongly suggest that oxidative stress occurs in rats fed a high fructose diet (Srividhya and Anuradha, 2002). Detrimental effects of fructose are enhanced when antioxidant defenses are decreased or when free radical production is increased (Rayssiguier et al., 1981, 1993). High fructose diet has prooxidant effects (Busserolles et al., 2002). Both enhanced oxidative damage to cellular constituents and
Results & Discussion

 diminished antioxidative capacity have been reported in fructose fed-rats (Faure et al., 1997; Busserolles et al., 2002).

 Elevated plasma insulin levels reflect an insulin resistance state such that inducing higher plasma glucose levels results in increased ROS production (Paolisso and Giuliano, 1996). Earlier studies showed direct correlation between ROS levels and fasting insulin concentration in patients with type-2 diabetes (Paolisso et al., 1994). In fructose fed rats, free radical production can be enhanced during hyperinsulinemia and hyperglycemia by mechanisms such as autoxidation of glucose, enhanced glycation, and altered polyol pathway (Paolisso and Giuliano, 1996). FFA could directly increase reactive oxygen species via peroxidation reactions and via mitochondrial production (Bakker et al., 2000). A study by Pennathur et al. (2005) showed that rats with diet-induced hyperlipidemia without hyperglycemia fail to exhibit increased protein and lipid oxidation products in the retina. Whereas Randall et al. (2002) with linear-regression analysis demonstrated a significant positive correlation between plasma glucose concentration and levels of plasma TBARS, but neither plasma cholesterol nor plasma triglyceride levels were correlated with plasma TBARS. However, Sies et al. (2005) observed that hyperglycemia and/or hyperlipidemia can give rise to nutritional oxidative stress under postprandial conditions. Thus the presence of elevated lipid alone can cause oxidation of proteins and lipids that can be enhanced in the association with hyperglycemia.

 High fructose diets may have a hypertriglyceridemic and prooxidant effect, and fructose fed rats have shown less protection from lipid peroxidation. Moreover, the susceptibility of tissues to oxidative stress may depend on alterations in lipid composition.
Enhanced lipid accumulation observed in the tissues of fructose fed rats may also contribute to increase LPO in these animals. Further heightened catabolism of fructose would result in energy depletion in cells making them more susceptible to peroxidation (Fields et al., 1992). Besides hyperglycemia, hypertriglyceridemia and hyperinsulinemia along with lipid over load in non-adipose tissues by fructose feeding can be related to increased lipid peroxide levels found in these rats. Similar trend as reported in Zucker diabetic fatty rats, a model of type-2 diabetes (Atkinson et al., 2003). Kelley et al. (2004) hypothesized that prooxidant stress response pathways might mediate hepatic increases in VLDL secretion and delayed clearance upon fructose feeding. Fructose is a highly lipogenic nutrient. The present study also revealed enhanced levels of cholesterol, TG and FFA in blood and liver of fructose fed rats.

Oxidation of biomolecules always impairs its original physiological function, which means that it is not useful any more and should be removed. Otherwise, the cell gets older or even dies (apoptosis) (Tsu Chung et al., 2000). It has been established that protein oxidation is associated with aging, oxidative stress and a number of diseases.

IDDM subjects have enhanced protein oxidation levels with decreased plasma levels of total protein, albumin, globulin and their ratio (Rama Krishna and Jailkhani, 2008). Similarly enhanced protein oxidation was reported in experimentally induced diabetic animals (Rajasekar and Anuradha, 2007).

Oxidation of a protein molecule always induces inactivation of the molecule and introduces a tag to that molecule. These modified protein molecules are prone to degradation in vivo by the proteasome system (Berlett and Stadtman, 1997). Coupling of
protein modification and degradation of chemically modified proteins is one of the normal protein turnover pathways \textit{in vivo}. Many of these oxidative products have newly formed carbonyl groups (Tsu-Chung \textit{et al.}, 2000).

Ceriello \textit{et al.} (1991) reported that protein modification through increased free radical generation could reduce insulin activity. Sandrine \textit{et al.} (2005) showed that insulin resistance induced by high fructose diet in rats was associated with oxidative stress. Thus enhanced protein oxidation under fructose fed conditions may be one of the factors for development of insulin resistance. Oxidative stress could also participate in the progression of insulin resistance since the incubation of adipocytes in the presence of $\text{H}_2\text{O}_2$ decreases the sensitivity of cells to insulin and glucose transport (Hansen \textit{et al.}, 1999). Kocic \textit{et al.} (2007) reported that positive correlation was found between the malondialdehyde (MDA) level and index of insulin sensitivity (Fasting Insulin Resistance Index).

In animal models, there is a growing recognition that cardiac dysfunction can occur early following induction of diabetes (Wold \textit{et al.}, 2006; Wichit \textit{et al.}, 2007). In parallel, insulin resistance was also associated with an early development of cardiac hypertrophy (Scognamiglio \textit{et al.}, 2004; Thirunavukkarasu \textit{et al.}, 2004). Earlier studies also indicated that cardiac hypertrophy was induced by chronic fructose feeding. Recently the role of oxidative stress in the development of atherosclerosis in the insulin resistance syndrome has been evoked. Major components of insulin resistance syndrome (IRS) (insulin resistance, hypertension, dyslipidemia) generate oxidative stress in response to an overproduction of superoxide anion by the activation of NADPH oxidase (Lee, 2001). Normalizing ROS
generation not only reversed these changes, but also prevented the long-term complications of diabetes (Nishikawa et al., 2000).

Control of diabetes induced oxidative stress could theoretically be used to reduced the severity of diabetic complications. The antioxidant like vitamin E, vitamin C (Cinar et al., 2001; Kedziora-kornatowska et al., 2003), α-lipoic acid (Kocak et al., 2000; Thirunavukkarasu et al., 2004) and L-carnitine (Rajasekar and Anuradha, 2007) received much attention for their potential role in controlling oxidative stress in STZ diabetic or fructose fed insulin resistance conditions. In addition to the many antioxidants examined a number of commonly used drugs have shown promising antioxidant activity in addition to their primary pharmacological activity. These drugs include thiazolidinediones, metformin and HMG-CoA reductase inhibitors (statins), and inhibitors of the rennin-angiotensin system. Thiazolidinediones have been shown in many animal studies to have antioxidant effect. In one study, pioglitazone-treated rats had reduced urinary excretion of isoprostane, a marker of oxidative stress (Dobrian et al., 2001). In a trial with type-2 diabetic rats, Bagi et al. (2004) demonstrated that treatment with rosiglitazone reduced NAD (P) H-derived ROS and increased the activity of catalase. Another study using type-2 diabetic rats found that treatment with troglitazone lowered hydroperoxides and decreased SOD activity (Fukui et al., 2000). A study using troglitazone and pioglitazone in type-2 diabetic rats found that both agents reduced TBARS levels and increased the aortic vasorelaxation response (Lida et al., 2003).

Clinical trials with conventional antioxidants in diabetic patients are limited. For major cardiovascular outcomes, vitamin E failed to provide any benefit. Recently, it has
been postulated that antioxidant potency of vitamins such as C and V is limited because these antioxidants work as scavengers of existing excess reactive species in a stoichiometric approach to oxidative stress associated clinical problems (Cuzzocrea et al., 2001).

Based on the new developments in our understanding of the pathophysiology of oxidative stress, it is clear that strategies to block the formation of reactive radicals will provide a targeted and causal approach to provide conclusive evidence whether antioxidants should be part of the cardiovascular treatment plan in diabetes.

*C. roseus* supplementation for 60 days to STZ diabetic and fructose fed insulin resistance rats alleviated the lipid accumulation in the skeletal muscle, heart and liver tissues. This may depend upon its TG lowering and insulin sensitivity effects. Further, its antihyperglycemic effect could bring a favorable metabolic environment avoiding the prooxidant conditions with reduced oxidative stress in *C. roseus* treated STZ and fructose fed rats. This protection against oxidative stress by *C. roseus* is further reflected by controlled LPO and protein oxidation in D + CR and F + CR-groups.

**Polyol pathway**

Polyol pathway also known as sorbitol pathway basically involves the conversion of glucose to fructose via sorbitol. Aldose reductase (AR) reduces glucose to sorbitol with the aid of co-factor NADPH and the second enzyme sorbitol dehydrogenase (SDH) with its co-factor NAD⁺, converts sorbitol to fructose.

Under normal conditions the bulk of glucose is metabolized through the glycolytic pathway and the pentose shunt. When hyperglycemia occurs, glucose disposal through pentose phosphate pathway tend to increase and glucose is converted in to sorbitol, via the
polyol pathway. Under normal glycemic conditions, approximately 3% of the glucose metabolized is routed via the polyol pathway. However, under hyperglycemic conditions this pathway accounts for more than 30% of the glucose utilized (Stephen et al., 2003). The polyol pathway enzyme AR catalyzes the reduction of aldose sugars and other saturated and unsaturated aldehydes (Williamson et al., 1993; Busik et al., 1997). This enzyme constitutes the first and the rate limiting step of the polyol pathway. This pathway has been suggested to play an important role in the development of vascular and neurological complications in diabetes (Greene et al., 1987; Kinoshita and Nishimura, 1988). Inhibition of aldose reductase has been shown to ameliorate vascular and other complications in diabetes (Williamson et al., 1993; Greene et al., 1993).

In the pathogenesis of diabetic complications, the polyol pathway is important and it is the one of the major source of diabetes induced oxidative stress. There are three potential mechanisms for the polyol pathway to contribute to oxidative stress. (1). AR activity depletes its co-factor NADPH, which is also required for GR to regenerate GSH. Under hyperglycemic condition, glucose channeled into the polyol pathway, causing a substantial depletion of NADPH and consequently a significant decrease in the GSH level. Thus, during hyperglycemia, AR activity diminishes the cellular antioxidant capacity (Cheng and Gonzalez, 1986; Bravi et al., 1997). (2). Oxidation of sorbitol to fructose by SDH causes oxidative stress because its co-factor NAD+ is converted to NADH in the process, and NADH is the substrate for NADH oxidase to generate ROS (More et al., 2000). Oxidation of sorbitol by NAD+ increases the cytosolic NADH: NAD+ ratio, which tends to inhibit glyceraldehydes phosphate dehydrogenase activity. This can lead to increased levels of
triose phosphates, methylglyoxal, and diacylglycerol. This chain of events is also associated with consumption of NAD$^+$ by activated poly (ADP-ribose) polymerase. This in turn was associated with intracellular AGE formation and activation of PKC, the hexosamine pathway and NF-κB (Du et al., 2003). (3). The polyol pathway converts glucose to fructose. Because fructose and its metabolites fructose-3-phosphate and 3-deoxyglucose are more potent nonenzymatic glycation agents than glucose, the flux of glucose through the polyol pathway would increase AGE formation. AGE, as well as binding of AGE to their receptors, are known to cause oxidative stress (McPherson et al., 1988).

The activities of AR and SDH in liver, pancreas and heart tissues of six experimental groups are given in the Table 16 and Fig 25 & 26.

Aldose reductase enzyme activity was found to be highest in liver, then pancreas followed by heart. A significant increase in the activity of AR was observed in liver (40.0%), pancreas (16.2%) and heart (22.9%) of D-rats compared to C-rats. Whereas a slight but not significant increase in the AR activity was observed in F-group compared to C-group. A significant increase in SDH activities of liver, pancreas and heart tissues were observed both in D and F-groups compared to C-group. Percent increase in the activity of SDH in liver, pancreas and heart are 35.1, 18.6 and 12.4% respectively in D-group and 11.7, 7.6 and 8.2% respectively in F-group compared to C-group. Thus the increase in SDH activity is more prominent in D-group than F-group.

It is well established that glucose flux through AR is increased in diabetes since aldose reductase has a high Km for glucose (Qing et al., 2008). It would mean that increase flux via polyol pathway proportionately. The degree of hyperglycemia is significantly greater
Table 16. Effect of *C. roseus* treatment on activities of aldose reductase and sorbitol dehydrogenase enzyme activities in STZ diabetic and fructose fed IR rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>C</th>
<th>C + CR</th>
<th>D</th>
<th>D + CR</th>
<th>F</th>
<th>F + CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldose reductase ( \mu \text{mol of NADPH oxidized/min/mg protein} )</td>
<td>Liver</td>
<td>0.87±0.03(^a)</td>
<td>0.86±0.04(^a)</td>
<td>1.22±0.03(^b)</td>
<td>0.83±0.04(^a)</td>
<td>0.94±0.04(^a)</td>
<td>0.85±0.02(^a)</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>0.68±0.02(^a)</td>
<td>0.67±0.02(^a)</td>
<td>0.79±0.02(^b)</td>
<td>0.69±0.01(^a)</td>
<td>0.75±0.04(^a)</td>
<td>0.69±0.02(^a)</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>0.48±0.02(^a)</td>
<td>0.47±0.01(^a)</td>
<td>0.59±0.01(^b)</td>
<td>0.49±0.02(^a)</td>
<td>0.55±0.04(^a)</td>
<td>0.50±0.02(^a)</td>
</tr>
<tr>
<td>Sorbitol dehydrogenase ( \mu \text{mol of NADH oxidized/min/mg protein} )</td>
<td>Liver</td>
<td>4.93±0.08(^a)</td>
<td>5.00±0.03(^a)</td>
<td>6.66±0.11(^b)</td>
<td>5.23±0.10(^c)</td>
<td>5.51±0.06(^d)</td>
<td>5.20±0.08(^c)</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>2.09±0.03(^a)</td>
<td>2.06±0.03(^a)</td>
<td>2.48±0.06(^b)</td>
<td>2.11±0.04(^a)</td>
<td>2.25±0.03(^c)</td>
<td>2.07±0.02(^a)</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>2.17±0.03(^a)</td>
<td>2.16±0.03(^a)</td>
<td>2.44±0.04(^b)</td>
<td>2.14±0.03(^a)</td>
<td>2.35±0.03(^c)</td>
<td>2.20±0.01(^a)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan’s multiple range test).
Fig 25: Per cent change in the activities of polyol pathway enzymes (AR and SDH) of liver, pancreas and heart of D and F-groups compared to control rats.

Fig 26: Per cent recovery from STZ diabetic and fructose feed induced alterations in activities of AR, and SDH in C. roseus administered D + CR and F + CR-groups.
in STZ diabetic rats than fructose fed rats. The greater glucose availability time (duration) is in part a key reason for the increased flux through aldose reductase in STZ diabetic rats compared to fructose fed rats. Polyl pathway dependent alterations in the cytosolic redox state could potentially account for increased free radical generation, AGE accumulation, protein kinase C activation and hexosamine formation by causing decreased NADPH and GSH.

Reduction of NADH/NAD⁺ by the oxidation of glucose-derived sorbitol to fructose would favor lactate production (Hwang et al., 2004) and divert glycolytic intermediates to the synthesis of phospholipid precursors such as α-glycerophosphate, phosphatidic acid, diacylglycerol, while at the same time interfering with β-oxidation of long-chain fatty acids giving a metabolic profile similar to that of ischemia (Willioamson et al., 1993).

_C. roseus_ treatment resulted in a significant decrease in tissue AR activity in D + CR-group compared to D-group, thus resulting in normalizing the tissue AR activity of D + CR-group whereas F + CR and C + CR-groups showed no significant change in tissue AR activities from C-group. _C. roseus_ treatment resulted in a significant decrease in tissue SDH activity in D + CR and F + CR-groups compared to D and F-groups respectively. Thus _C. roseus_ treatment restored the SDH activity of pancreas and heart tissues to normal values in D + CR and F + CR-groups. However, the decreased activity of hepatic SDH by _C. roseus_ treatment in D + CR and F + CR-groups did not reach the normal values. Control rats treated with _C. roseus_ (C + CR-group) showed no significant change in tissue SDH activity when compared to C-group.
Thus restoration of enhanced polyol pathway enzymes to normal levels by *C. roseus* supplementation in diabetic and fructose fed animals (D + CR and F + CR) avoided the deleterious alterations due to enhanced operation of polyol pathway towards oxidative stress.

Excessive activation of the polyol pathway increases intracellular and extracellular sorbitol concentrations. The sorbitol cannot cross cell membranes, and when it accumulates, it produces osmotic stresses on cells by drawing water. Osmotic stress, from the accumulation of sorbitol, is more important factor for the development of diabetic cataract (Alan and Stephen, 1999). Earlier studies demonstrated that increased cellular polyol pathway enzymes and increased ischemic injury with duration of diabetes. Further inhibition of AR and SDH resulted in reduced ischemic injury and improved functional recovery (Tracey *et al.*, 2000; Hwang *et al.*, 2002).

Beneficial effects of AR2 inhibitors (ARIs) that block conversion of glucose to sorbitol in animal and human diabetes strongly implicate the sorbitol pathway as a significant factor in the pathogenesis of the long-term complications of diabetes mellitus, particularly diabetic polyneuropathy (Greene *et al.*, 1989). Hotta and Kakuta, (1981) demonstrated that AR activity of the polyol pathway is not affected directly by insulin. It is likely that the specific activities of several enzymes regulated by insulin are altered and that glucose utilization is diverted to metabolic pathways other than the polyol pathway.

Reports are available on polyol pathway inhibiting activity of many plant extracts and phytochemicals. Aqueous extracts of *Ocimum sanctum, Withania somnifera, Curcuma longa, Azadirachta indica* inhibit AR activity (Halder *et al.*, 2003). Flavonoid treatment
inhibited the polyol pathway in experimental diabetes (Vertommen et al., 1994). Turmeric and curcumin supplementation also reduced the oxidative stress encountered by the diabetic rats. This was demonstrated by the lower levels of TBARS which may have been due to the decreased influx of glucose into the polyol pathway (Suryanarayana et al., 2005).

Results from our study revealed another beneficial property of *C. roseus* in decreasing the polyol pathway towards preventing the oxidative stress induced diabetic complications.

**ANTIOXIDANT SYSTEM**

Exposure to free radicals from a variety of sources has led organism to develop a series of defense mechanisms against free radical-induced oxidative stress involving: (1) preventative mechanism, (2) repair mechanism, (3) physiological defenses and (4) antioxidant defenses (Cadenas, 1997). Antioxidants are defined as compounds that can donate at least one hydrogen atom to a free radical resulting in the termination of radical chain reactions. An alternative type of antioxidant is defined by its ability to prevent the initiation of a free radical chain reaction rather than to terminate them. The cell possesses some innate mechanism by which it tries to combat oxidative insult by increasing its reserves of antioxidants. The cellular free radical scavengers and antioxidant enzymes normally protect the cell from toxic effect of ROS which include enzymatic and non-enzymatic antioxidants. Enzymatic antioxidant defenses include glutathione (GSH) independent SOD and CAT and GSH dependent GPx, GR and GST and non-enzymatic antioxidants are represented by ascorbic acid (vitamin C), α-tocopherol (vitamin E), GSH, carotenoids, flavonoids, and other antioxidants. Oxidative stress occurs when there is
imbalance between free radical production reactions and scavenging capacity of antioxidative defense mechanism of the organisms. Enzymatic antioxidant systems SOD, CAT, GR, GPx and GST function by direct or sequential removal of ROS thereby terminating their activities (Sies, 1999).

Consideration of antioxidants in clinical treatment as adjuvant therapy in type-2 diabetes is warranted because of many reports of elevated markers of oxidative stress in patients with this disease, which is characterized by imperfect management of glycemia, consequent chronic hyperglycemia, and relentless deterioration of β-cell function (Ceriello and Motz, 2004).

\[
\begin{align*}
2O_2^- + 2H^+ & \overset{SOD}{\longrightarrow} H_2O_2 + O_2 \\
2 H_2O_2 & \overset{CAT}{\longrightarrow} 2 H_2O + O_2 \\
2GSH + H_2O_2 & \overset{GPX}{\longrightarrow} GSSG + 2H_2O \\
GSSG + NADPH + H^+ & \overset{GR}{\longrightarrow} 2GSH + NADP^+ \\
X + GSH & \overset{GST}{\longrightarrow} X-S-G
\end{align*}
\]

**Glutathione (GSH)**

GSH, comprising a major portion of cellular non-protein thiols, plays a central role in diverse groups of cell metabolic functions; the transport, cellular protection and detoxification of the exogenous as well as endogenous toxins. Thus GSH is an important constituent of cell and considered to be a vital component and redox buffer in the cell. It provides protection to cell non-enzymatically by acting as a free radical scavenger, a nucleophilic shield and as a reductant (Masella *et al.*, 2005).
During ROS over production, intracellular GSH is oxidized to disulfide form GSSG, which is then reconverted to GSH by GR. The GSH/GSSG ratio defines the so-called GSH redox state which plays an important role in cellular activation, gene expression, mRNA stability, protein folding, metabolic regulation, and cell protection against oxidative damage (Cappel and Gilbert, 1988; Tran et al., 2004). The relationship between oxidative stress and diabetic complications as well as between oxidative stress and insulin action is a research area that is extensively investigated (Baynes, 1991; Paolisso et al., 1992, 1993; Giuliano et al., 1996). In particular Mattia et al. (1998) found that GSH infusion in patients with type-2 diabetes mellitus increased intracellular GSH/GSSG ratio and insulin sensitivity (Mattia et al., 1998). Nevertheless, the role exerted by insulin on intracellular oxidative stress is still unclear. Depleted plasma GSH and tissue GSH was well documented in type-1 diabetic animal models (Venkateswaran and Pari, 2003; Pari and Latha, 2004) and NIDDM patients (Seghrouchni et al., 2002). It has recently been shown that patients with type-2 diabetes mellitus have lower GSH levels and higher levels of TBARS (Zancon and Sola-Penna, 2005).

Thus GSH is an important antioxidant that functions directly in elimination of toxic peroxides and aldehydes and indirectly in maintaining vitamin C and vitamin E and SH dependent enzymes in their reduced and functional forms. Resistance of many cells against oxidative stress associated with high intracellular levels of GSH (Meister, 1991; Estrela et al., 1995). Hence, the measurement of cellular GSH provides the information about GSH associated scavenging system against free radicals induced LPO in the metabolic disease conditions and aging.
The reduced glutathione of liver, pancreas and heart of six experimental groups are summarized in the Table 15 and Fig 23 & 24.

A significant decrease in the tissue GSH content was observed in liver (29.2 %) pancreas (34.4 %) and heart (42.9 %) of D-group compared to C-group. Similarly F-group also showed a significant decreased GSH content in the liver (13.7 %) and heart (13.3 %) with no alterations in the pancreas in comparison with C-group. Thus STZ diabetic animals showed significantly lower levels of tissue GSH compared to F-group. *C. roseus* treated control rats (C + CR-group) showed significantly enhanced tissue GSH content compared to C-group. *C. roseus* treated diabetic (D + CR) and insulin resistance rats (F + CR) exhibited significantly enhanced tissue GSH content compared to D and F-groups respectively. The significantly enhanced GSH content of pancreas and heart of D + CR and liver and heart of F + CR-groups reached normal values whereas GSH content of pancreas of F + CR-group increased beyond the normal values. The significant increase in hepatic GSH content (30.2 %) observed in D + CR-group compared to D-group was not restored to normal values.

The significant decrease in GSH content of diabetic rats represents increased utilization of GSH due to oxidative stress (Anuradha and Selvam, 1993). More prominent decrease in tissue GSH content of STZ diabetic rats compared to fructose fed rats indicates more intensified oxidative stress in STZ induced insulin deficient condition than fructose feed induced insulin resistance condition. Earlier studies also revealed the decreased concentration of GSH in STZ diabetic rats (Pari and Latha 2004; Ozsoy-Sacan *et al.*, 2006) and fructose fed insulin resistance rats (Girard *et al.*, 2005; Rajasekar and Anuradha, 2007)
suggesting that the decreased GSH concentration plays a role in development of diabetic complications.

The significantly decreased GSH content of STZ diabetic and fructose fed rats compared to control rats may be due to increased utilization of GSH or decreased synthesis because of enhanced oxidative stress. It has proposed that pathways of glucose metabolism are involved in the control of myocardial GSH (Mak et al., 1996; Bersin and Stacpoole, 1997; Squires et al., 2003). In the case of diabetes, the well-documented decreases in myocardial insulin signaling and glucose utilization (Rodrigues et al., 1995; Huang et al., 2003) are likely factors contributing to alterations in GSH status. Accordingly, insulin replacement therapy in Type-1 diabetic models maintains normal cardiac GSH levels (Yadav et al., 1997; Xu et al., 2002). Recent studies of Shumin et al. (2007) also provide functional evidence for a link between glucose metabolism and cell GSH. Thus significant decrease in GSH content of liver, pancreas and heart of diabetic rats reflected the enhanced oxidative stress in the form of increased LPO and protein oxidation.

Significant increase in the GSH content in liver, pancreas and heart of C. roseus treated STZ diabetic and fructose fed rats indicates that C. roseus treatment activated the compensatory mechanism against the oxidative stress and cell death. Generally, antioxidant treatment can exert beneficial effects in diabetes, with preservation of \textit{in vivo} \(\beta\)-cell function. Antioxidant treatment suppresses apoptosis in \(\beta\)-cells without changing the rate of \(\beta\)-cell proliferation supporting the hypothesis that in chronic hyperglycemia, apoptosis induced by oxidative stress causes reduction in \(\beta\)-cell mass (Kaneto et al., 1999; Wiemsperger, 2003).
The observed significant elevation of GSH content of the tissues of D + CR and F + CR-rats compared D and F-rats indicates that *C. roseus* might have either increased the biosynthesis of GSH or lowered the utilization of GSH due to decreased oxidative stress or both. Particularly in D +CR-group, *C. roseus* protection against STZ diabetic induced depletion of GSH is also evident from improved insulin secretion of these animals compared to D-group thus indicating the suppression of β-cell apoptosis induced under oxidative stress.

The antioxidant treatment also preserved the amounts of insulin content and insulin mRNA making the extent of insulin degranulation less evident. Furthermore, expression of pancreatic and duodenal homeobox factor-1, a β-cell-specific transcription factor, was more clearly visible in the nuclei of islet cells after the antioxidant treatment (Kaneto *et al*., 1999). Cristina *et al.* (2006) also reported a direct correlation between GSH/GSSG ratio and plasma insulin levels in diabetic patients indicating that the increase in GSH/GSSG ratio is directly due to the hormone effect.

Previous studies suggest that GSH may also be important in blood pressure and glucose homeostasis, consistent with the involvement of free radicals in both essential hypertension and diabetes mellitus (Beutler and Gelbart, 1985; Sagar *et al*., 1992; Paolisso *et al*., 1992, 1993 and 1994; Moran *et al*., 1993; Faure *et al*., 1997). Changes in the GSH/GSSG ratio affect the β-cell response to glucose and improve insulin action (Ceriello *et al*., 1991). Paolisso *et al.* (1992) reported that intravenous infusion of GSH in Type-2 diabetic patients improved insulin secretion and glucose tolerance during oral glucose tolerance tests. Maintenance of ample concentrations of antioxidants seems to be necessary
for efficient insulin action. Efficient expression of insulin receptor gene requires certain transcription factors that are activated by GSH (Araki et al., 1991). Vitamin E also has a beneficial effect on insulin action as its supplementation could restore the GSH concentration in fructose fed rats and improve the physical state of plasma membrane and insulin action in NIDDM patients (Paolisso et al., 1993). Thus prevention of GSH depletion seen in fructose fed rats by C. roseus supplementation may also responsible for the enhanced insulin sensitivity observed in F + CR-group.

**Glutathione reductase (GR), Glutathione peroxidase (GPx) and Glutathione-S-transferase (GST)**

Besides transport and *de novo* synthesis, level of GSH is also regulated by GSH redox cycle composed of GPx, GR and G6PDH. GR catalyses the reduction of GSSG to GSH in the presence of NADPH which is generated by a reduction of NADP⁺ via the HMP shunt pathway. Under normal conditions the balance of the equation is far in the direction of maintaining cellular glutathione in its reduced state. The significance of these enzymes lies in their ability to keep high levels of GSH.

Peroxides produced in a cell can be detoxified by the action of GPx and CAT. GPx has a complementary catalytic activity with catalase. The Km value for H₂O₂ of GPx (0.25 mmol/l) is lower than that of CAT (25 mmol/l) providing a preferential pathway for the degradation of low concentration of H₂O₂ present in intact cell (Wohaieb and Godin, 1987). GPx catalyses the reduction of peroxides with GSH to form GSSG and the reduction product of H₂O₂. This enzyme is specific for its hydrogen donor GSH, and non-specific for the hydroperoxides ranging from H₂O₂ to organic peroxides (Freeman and Crapo, 1982) thus
offering a major defense role in cells against peroxidative damage of complex biochemical compounds such as lipids and nucleic acids (Meister and Anderson, 1983; Bandyopadhyay et al., 1999).

GST is a multifunctional protein found in many tissues showing a broad specificity for organic hydroperoxides but not for H$_2$O$_2$ (Bruce et al., 1982). Glutathione-S-transferase catalyses conjugation between GSH with a very wide range of secondary substrates and a large number of xenobiotics with electrophilic center. Thus it plays an important role in detoxification of xenobiotic compounds thereby protecting the cells from peroxidative damage (Deneke and Fanburge, 1989).

The activities of glutathione dependent antioxidant enzymes (GR, GPX and GST) of liver, pancreas and heart of six experimental groups are summarized in the Table 17 Fig 27 & 28.

All these enzyme activities are found to be highest in liver, the main organ involved in detoxification of process, followed by heart and pancreas. Thus pancreas exhibits the lowest activity of antioxidant enzymes compared to other tissues indicating its susceptibility to oxidative stress. β-cells appear to be vulnerable to oxidative stress as they contain relatively low levels of GPx and other protective enzymes compared to other cells (Grankvist et al., 1981; Sigfrid et al., 2004). Moreover, studies on pancreatic islet and β-cell lines revealed the cells to be incapable of increasing their antioxidant enzyme expression in response to cellular stress induced by exposure to glucose (Tiedge et al., 1997; Sigfrid et al., 2004).
Table 17. Effect of *C. roseus* treatment on glutathione dependent enzyme activities in STZ diabetic and fructose fed IR rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>C</th>
<th>C+CR</th>
<th>D</th>
<th>D+ CR</th>
<th>F</th>
<th>F+CR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glutathione reductase</strong> (µmol of NADPH oxidized/min/mg protein)</td>
<td>Liver</td>
<td>34.04±0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.38±0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.02±0.58&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33.67±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.87±0.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.33±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>22.19±0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.10±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.71±0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.79±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.36±0.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.06±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>26.20±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.70±0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.76±0.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.11±0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.42±1.65&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.62±0.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Glutathione peroxidase</strong> (µg of GSH consumed/min mg protein)</td>
<td>Liver</td>
<td>9.45±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.76±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.83±0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.82±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.09±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.00±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>4.75±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.95±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.81±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.6±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.06±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.08±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>6.02±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.91±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.94±0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.75±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.19±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.89±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Glutathione-S-transferase</strong> (mmol of CDNB-GSH conjugate formed/min/mg protein)</td>
<td>Liver</td>
<td>0.232±.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.220±.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.159±.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.226±.006&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.199±.005&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.228±.006&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>0.037±.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.039±.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.026±.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.036±.0005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.032±.001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.036±.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>0.046±.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.045±.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.030±.0007&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.044±.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.033±.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.045±.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan’s multiple range test).
Fig 27: Per cent change in the activities of GR, GPx, and GST of liver, pancreas and heart of D and F-groups compared to control rats.

Fig 28: Per cent recovery from STZ diabetic and fructose feed induced alterations in activities of GR, GPx, and GST in C. roseus administered D + CR and F + CR-groups.
Both D-group and F-group animals showed a significantly decreased activity of these enzymes in all these tissues compared to C-group. When compared to control rats, the percent decrease in the activity of GR in liver, pancreas and heart are 14.7, 15.6 and 20.7 % in STZ diabetic rats and 9.3, 8.2 and 10.6 % in fructose fed rats respectively. Compared to control rats, the activities of GPx and GST decreased in liver (17.1 and 31.4 %), pancreas (19.7 and 29.7 %) and heart (17.9 and 34.7 %) of STZ diabetic rats. Like STZ diabetic rats fructose fed rats also showed decreased activities of GR and GST in liver (14.3 and 14.2 %), pancreas (14.5 and 13.5 %) and heart (13.7 and 28.2 %) respectively compared to control rats. Heart tissue of STZ diabetic rats showed significantly lower activity of GPX and GR when compared to F-group while GST activity of liver and pancreas of D-group is lower when compared to corresponding values of F-group. There is no significant variation in the activities of GPX and GR of liver and pancreas and GST of heart between D and F-groups. Significantly enhanced activities of these enzymes were observed in *C. roseus* treated STZ diabetic (D + CR) and fructose fed rats (F + CR) compared to D and F-groups respectively. Thus *C. roseus* treatment for 60 days normalized the STZ diabetic and fructose feed induced alterations in the activities of tissue GR, GPX and GST activities. Further *C. roseus* treated control group i.e., C + CR-group showed no deviation in the activities of these enzymes from C-group.

The depletion in the activities of these enzymes in both STZ diabetic and fructose fed condition may result in deleterious oxidative stress due to accumulation of toxic products. Earlier studies also support decreased activities of these GSH dependent enzymes in STZ diabetic rats (Anuradha and Selvam, 1993; Venkateswarun and Pari, 2002) and
However, in the published literature the response of antioxidant enzymes to diabetics has been unclear. Diabetes has been reported to be associated with either increased (Ndahinana et al., 1996), decreased (Uzel et al., 1987) or unchanged (Faure et al., 1995) activities of antioxidant enzymes in various tissues.

The observed decrease in GR activity in STZ diabetic and fructose fed rats may be due to the decreased availability of NADPH by decreased G6PDH activity and/or enhanced operation of polyol pathway (Peterson et al., 1990) as well as due to the inactivation of the enzyme by non-enzymatic glycation (Arai et al., 1987). Fructose has a stronger reducing capacity than glucose and the glycation reaction is easily induced by fructose (Mc Pherson et al., 1988). The decreased activity of GR in D and F- rats may lead to decreased regeneration of GSH from GSSG. Maintenance of ample concentrations of antioxidants seems to be necessary for efficient insulin action, for example, expression of insulin receptor gene requires certain transcription factors whose activity is modulated by GSH.

Decreased activity of GPx and GST in D and F-rats could be directly explained by the low content of GSH found in these rats since GSH is a substrate and cofactor of GPx and GST (Domingues et al., 1998). GSH, the most important antioxidant metabolite, plays an important role in maintaining good levels of GPx activity. Flohe, (1971) reported that the kinetics of GPx are in the first order in respect to GSH. Thus the decreased levels of GSH in D and F- rats may be one of the factors for decreased activity of GPx. GPx is a relatively stable enzyme, but it may be inactivated under conditions of severe oxidative stress (Condell and Tappel, 1983). The low activity of GPx causes accumulation of H$_2$O$_2$ in
diabetic rats. This finding could also explain the progressive decrease in SOD in later stages of the diabetes. The depletion in the activities of GST and GPx may result in the involvement of deleterious oxidative changes due to accumulation of toxic products. Thus the decreased activities of these GSH related antioxidant enzymes of D and F-rats may be responsible for elevated LPO observed in these group of rats.

*C. roseus* supplementation prevented the depletion in tissue GR activity in D + CR and F + CR-group by maintaining the normal levels of this enzyme in these animals. Enhanced GR activity in D + CR and F + CR-groups compared to D and F-groups respectively reveals the protective effect of *C. roseus* against oxidative damage by keeping normal GSH levels in tissues in STZ diabetic and fructose fed conditions which is further reflected by enhanced activities of GPx and GST in *C. roseus* treated insulin deficient and resistance animal models.

**Superoxide dismutase (SOD) and Catalase (CAT)**

Superoxide dismutase and CAT, GSH independent antioxidant enzymes are widely distributed in all animal cells. Superoxide dismutase, a Cu/Zn containing enzyme, is a major defense for aerobic cells in combating the toxic effect of superoxide radicals (Mc Cord *et al.*, 1976). Catalase a hemoprotein, reduces H$_2$O$_2$ produced by dismutation reaction and prevents generation of hydroxyl radical.

SOD has been touted as one of the most important enzymes in the enzymatic antioxidant defense system. It provides the primary catalytic cellular defense that protects cells and tissues against potentially destructive reactions of O$_2^{-}$ and their derivatives. Recent
studies demonstrated that a hyperglycemia-induced process of overproduction of $O_2^\cdot$ by the mitochondrial electron-transport chain appears to be the initial and key event in the activation of all other pathways involved in the pathogenesis of diabetes-related complications (Brownlee, 2001). These include increased polyol pathway flux, increased formation of AGEs, activation of protein kinase C and nuclear factor $\kappa B$. An increased expression of inducible nitric oxide synthase (iNOS), accompanied by increased generation of NO, and an overactivity of the reduced form of NADP-which, in turn, overgenerates $O_2^\cdot$ (Garcia Soriano et al., 2001; Guzik et al., 2002). So maintenance of intracellular level of SOD enzyme is necessary to prevent free radical toxicity. Mc Cord and Fridovich, (1969) proved that this enzyme is required to sustain life in aerobic condition. Oxygen free radical ($O_2^\cdot$) has known to inactivate CAT which is involved in the detoxification of $H_2O_2$. Wohaieb and Godin, (1987) suggested that the reactive oxygen free radicals could inactivate and reduce the hepatic SOD and CAT activities.

CAT catalyses the reduction of hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Chance et al., 1952). $H_2O_2$ is considered a key metabolite because of its relative stability, its diffusion and its involvement in cell signaling cascade (Fridovich, 1995; Khan and Wilson, 1995; Pantopoulso et al., 1997; Sujuki et al., 1997). Since, the first description of GPx in 1957, an intense debate was created on whether CAT or GPx was the primary enzyme in the removal of $H_2O_2$ (Scott et al., 1991). CAT decomposes $H_2O_2$ without generation of free radicals by minimizing one electron transfer (Sebastain et al., 1997). Studies of Corrochr et al. (1986) demonstrated that the administration of CAT resulted in protection against $H_2O_2$ mediated LPO.
The activities of SOD and CAT of liver, pancreas and heart of six experimental groups are summarized in the Table 18 and Fig 29 & 30.

Like GSH dependent antioxidant enzymes, activities of SOD and CAT are also found to be highest in liver, followed by heart and pancreas. Both D-group and F-group animals showed a significantly decreased activities of CAT and SOD in three tissues compared to C-group. Between D and F-group, the SOD activity of pancreas is significantly lowered in STZ diabetic rats compared to F-group. Similarly D-group rats showed significantly lower activity of CAT in liver, pancreas and heart tissues when compared to F-group whereas no significant variation was observed in the activities of SOD of liver and heart between D and F-groups. The per cent decrease in the SOD activity in liver, pancreas and heart tissues are 29.0, 46.3 and 28.2 % respectively in D-group and 19.1, 31.3 and 26.0 % respectively in F-group compared to C-group. Respective per cent decrease in the CAT activity of D-group and F-group in liver, pancreas and heart are 24.12, 39.5 and 35.5 %, and 19.1, 29.8 and 24.0 % compared to C-group. The per cent alterations in SOD and CAT activities are prominent in pancreatic tissue compared to liver and heart both in STZ diabetic rat and fructose fed rats. Administration of C. roseus for 60 days resulted in a significant enhancement in the activities of these two enzymes both in D + CR and F + CR-groups compared to D and F-groups respectively. This significant enhancement in the activities of SOD and CAT of F + CR-group compared to F-group resulted in restoration of these enzyme activities to normal values. Similarly C. roseus treatment to D + CR group also normalized SOD activity in liver, heart and pancreatic tissues and CAT activity in
Table 18. Effect of C. roseus treatment on glutathione independent enzyme activities in STZ diabetic and fructose fed IR rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>C</th>
<th>C + CR</th>
<th>D</th>
<th>D + CR</th>
<th>F</th>
<th>F + CR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Superoxide dismutase</strong></td>
<td>Liver</td>
<td>45.30±1.31a</td>
<td>50.85±1.69b</td>
<td>32.16±1.89c</td>
<td>44.13±1.33a</td>
<td>36.66±1.45d</td>
<td>46.72±2.18a</td>
</tr>
<tr>
<td>(Units /mg protein)</td>
<td>Pancreas</td>
<td>26.67±1.18a</td>
<td>31.48±1.13b</td>
<td>14.31±1.14c</td>
<td>25.93±0.98a</td>
<td>18.32±1.71d</td>
<td>29.20±1.31a</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>29.28±1.31a</td>
<td>31.08±1.46a</td>
<td>21.00±1.32b</td>
<td>27.52±1.30a</td>
<td>21.66±1.36b</td>
<td>27.67±1.32a</td>
</tr>
<tr>
<td><strong>Catalase</strong></td>
<td>Liver</td>
<td>99.84±4.40a</td>
<td>111.55±4.22b</td>
<td>75.75±3.77c</td>
<td>91.69±3.20d</td>
<td>80.81±2.92e</td>
<td>100.95±3.77a</td>
</tr>
<tr>
<td>(μmol of H₂O₂ consumed/ min/mg protein)</td>
<td>Pancreas</td>
<td>14.56±0.53a</td>
<td>16.82±0.41b</td>
<td>8.84±0.30c</td>
<td>15.46±0.28a</td>
<td>10.22±0.44d</td>
<td>15.05±0.43a</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>25.75±0.92a</td>
<td>26.10±0.71a</td>
<td>16.65±0.44b</td>
<td>22.77±0.88c</td>
<td>19.56±0.47d</td>
<td>24.45±0.77a</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
Fig 29: Per cent change in the activities of SOD and CAT of liver, pancreas and heart of D and F-groups compared to control rats.

Fig 30: Per cent recovery from STZ diabetic and fructose feed induced alterations in activities of SOD and CAT in *C. roseus* administered D + CR and F + CR-groups.
pancreas. However, CAT activities in hepatic and cardiac tissue of D + CR-group are still significantly lower than C-group. Further C. roseus administration to control group i.e., C + CR-group showed significantly increased activities of SOD and CAT in hepatic and pancreas tissues with no alterations in these enzyme activities in cardiac tissues.

Earlier studies also indicated the decreased activities of these SOD and CAT in STZ diabetic rats (Ugochukwu and Babady, 2002; Pari and Latha 2004; Sathishsekar and Subramanian, 2005) and fructose fed insulin resistance rats (Anuradha and Selvam, 1993; Venkateswarun and Pari, 2002; Joyeux-Faure et al., 2006). Copper and zinc depletion may results in reduced activity of Cu/Zn dependent enzymes like SOD. Earlier studies revealed that decreased Cu and Zn (Busserolles et al., 2002; Joyeux-Faure et al., 2006) in fructose fed animals may contribute to the observed decrease in SOD activity in F-group animals.

The results on the SOD and CAT activity clearly show that C. roseus treatment resulted in enhanced scavenging activity which could exert a beneficial action against pathologic alterations caused by the presence of O$_2^-$ and H$_2$O$_2$. Restoration of SOD activity in D + CR and F + CR-groups reveals an efficient defense against the first line of oxidative stress i.e., O$_2^-$ radicals which are known to inactivate CAT. Benhamou et al. (1998) demonstrated that overexpression of CAT in human islets had given protection against oxidative stress induced by xanthine oxidase-hypoxanthine. Xu et al. (1999) demonstrated that overexpression of CAT in mouse islet cells had given protection against H$_2$O$_2$ induced oxidative stress resulted in normal insulin secretion and reduced the diabetogenic effect of STZ in vivo.
Therefore, as reported, the levels of all biomarkers of oxidative stress are modified in patients with diabetes and diabetic animal models which suggests that diabetes is associated with an over generation of free radicals. Importantly, further support is provided by a study showing that hyperglycemia-induced insulin resistance can be prevented by antioxidant treatment. Several trials have shown that improving glycemic control does not necessarily improve accompanying oxidative stress (Seghrouchni et al., 2002). One might infer from such observations that specific therapy directed towards oxidative stress is obvious. Moreover, the large evidence for oxidative stress in insulin resistance and diabetes has logically prompted the use of antioxidants like a self-evident treatment (Marfella et al., 1995; Ting et al., 1996). There are some clinical studies addressing potential treatment aiming at reduction of oxidative stress, and they have mainly focused on treatment or prevention of cardiovascular disease (Cuzzocrea et al., 2001; Marchioli et al., 2001). Heart tissues of both STZ diabetic and fructose fed rats showed higher per cent decrease in oxidative enzymes when compared to liver and pancreas. Which explains vulnerability of cardiac tissues to oxidative stress because these antioxidant enzymes play a key role in the cell protection against the deleterious effects of the ROS. Increased ROS production and changes in the activities of various antioxidant defenses are associated with alterations in gene expressions in different tissues suggesting ROS as biological subcellular messengers in gene replication and signal transduction path ways (Allen and Tresini, 2000). It has been shown that fructose induced oxidative stress might modulate transcription factors that are sensitive to change in the redox state of the cell (Ramon et al., 2001). This observation indicates that the tissue specific protection of C. roseus that eventually lead to control of LPO in these tissues. The results from the present study indicate that STZ diabetic and
fructose fed insulin resistance rats have increased oxidative stress and a compromised antioxidant defense system in the liver, pancreas and heart. This increase in oxidative stress could be reverted to normal values by *C. roseus* administration. Further, *C. roseus* exerts a protective effect against LPO in liver, pancreas and heart by scavenging ROS and elevating the activities of antioxidant enzymes both in insulin deficient and insulin resistance conditions.

Various natural products have long been used in traditional medical systems for treating diabetes (Shapiro and Gong, 2002). Most of them contain a wide range of antioxidants with a potent scavenging activity for ROS. A similar type of protection against oxidative stress by enhancing the antioxidant enzymes was also observed in diabetic rats treated with other plants such as *Salacia oblonga* (Krishnakumar et al., 1999), *Tinospora cordifolia* (Stanely et al., 1999), *Anoectochilus formosanus* (Shih et al., 2002), *Morinda officinalis* (Soon and Tan, 2002), *Morus indica* (Andallu and Varadacharyulu, 2003), *Ficus carica* (Perez et al., 2003), *Piper betle* (Santhakumari et al., 2003), *Coccinia indica* (Venkateswaran and Pari, 2003), *Gymnema montanum* (Ananthan et al., 2004) and *Eugenia jambolana* (Ravi et al., 2004).

Many phytochemicals are reported to enhance antioxidant enzymes by inducing gene expression of these enzymes. Induction of the hepatic GSH antioxidant system by chemopreventive agents was reported in several studies (Velumurugan et al., 2001; Mahn et al., 2005; Yeh and Yen, 2006). Several phytochemicals were reported to act against the deleterious effects of oxidative stress such as anthraquinones in aloe vegetables (Malterud
et al., 1993), total saponins from *Pinax ginseng* (Yukozawa et al., 1996), polyphenols (Tiwari, 2001) and flavonoids from *Sideritis raeseri* (Gabrieli et al., 2005).

Some of the phytochemicals (alkaloids and flavonoids) (Mustafa and Verpoorte, 2007) present in the *C. roseus* either alone or in combination might also exert similar effects in the present study. However, further studies are needed to prove the assumptions on the exact mechanism of action. Therefore, it may be assumed that these products or isolated natural compounds could play a very important role in adjuvant therapy and for diabetes mellitus.

The major challenge in diabetes research is to define not only the cause-effect relationship between various risk factors and complications, but also to comprehend the effects of pharmaceutical agents that are beneficial in the management of diabetic complications. It appears that apart from acting on carbohydrate metabolic targets compounds present in medicinal plants 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.

The results from the present study suggest that *C. roseus* has antioxidant potential against oxidative stress along with antidiabetic and hypolipidemic potential. These results may lend further support to mount evidence to show that *C. roseus* contains compounds which, if taken in sufficient quantities, could conceivably be beneficial in attenuation and prevention of diabetes and its associated complications.