Effect of N-Trisaccharide on carbohydrate metabolism enzymes, lipid profile and pancreas in streptozotocin-nicotinamide induced type 2 diabetic rats

Introduction

Diabetes is always considered as a disturbance in the metabolism of carbohydrate accompanied by alteration in the metabolism of fats and proteins. The changes are due to low insulin/glucagon ratio which controls hepatic glucose output. In type 2 diabetes, fasting blood glucose level is raised in direct proportion to hepatic glucose output (Bogardus et al., 1984), and appears unlikely to be the result of decreased insulin action at the periphery as it has not been shown to correlate closely with insulin-stimulated glucose disposal (Defronzo et al., 1982). The disturbances to glucose homeostasis appear to result from insulin insensitivity, as fasting plasma insulin and C-peptide concentrations are normal in type 2 diabetes.

Hyperglycemic conditions arise due to high rates of glycogenolysis, gluconeogenesis and decreased utilization of glucose by the peripheral tissues due to the decreased peripheral uptake of glucose from blood. Due to lack of insulin, the action of hyperglycemic hormones becomes more prominent because carbohydrates cannot be used as fuel in diabetes, instead fat is used. High glucagon level decreases the hepatic fructose-2,6-bisphosphate level, thereby decreasing the utilization of glucose. The insulin dependent enzymes are less active and the net effect is inhibition of glycolysis and stimulation of gluconeogenesis leading to hyperglycemia.

Glycosylated haemoglobin (HbA1c), a measure of hyperglycemia is a sensitive and reliable marker of impaired glucose metabolism (Dunn et al., 1979). It refers to exposure of haemoglobin to high concentrations of blood glucose and formation of a series of stable minor haemoglobin components slowly and non-enzymatically. The process is multistep condensation of a free primary amine on haemoglobin with the carbonyl of the glucose resulting in the formation of Schiff’s base. This product is
unstable and may undergo an amadori rearrangement to form a stable ketoamine (Gonen and Rubenstein, 1979). The rate of formation of HbA1c is directly proportional to the glucose concentration. Since the human erythrocyte is freely permeable to glucose, the concentration of HbA1c provides glycemic history of the previous 3 months, which is the average erythrocyte life span (Randle, 1995). Hence, HbA1c reflects long term glycemic control and is a more accurate and stable measure than fasting blood glucose level (Goldstein et al., 2003).

Glycogen is the intracellular storable form of glucose and its levels in different tissues directly reflect insulin activity, which regulates its deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Assessment in the levels serves as a marker for studying insulinomimetic activity. Due to deficiency of insulin in diabetic state, glycogen is degraded and gluconeogenesis is increased while glucose utilization is inhibited in skeletal muscle and liver and this alteration is normalized by insulin treatment (Grover et al., 2000).

The entry of glucose into the cell and its phosphorylation for further metabolism through glycolytic pathway is regulated by insulin. The activities of the regulatory enzymes like hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PDH) and gluconeogenic enzymes such as Fructose-1,6-bisphosphatase (FB) and glucose-6-phosphatase (GP) are markedly altered during diabetes (Hers et al., 1987; Adisakwattana et al., 2005). Glucose is phosphorylated to glucose-6-phosphate by HK immediately after entering into the cell. This glucose-6-phosphate may get converted into glycogen or broken down in the HMP shunt pathway. Also, it may enter Embden Meyerhoff pathway or could be hydrolyzed by glucose-6-phosphatase and released from the hepatic cells as free glucose or simply get diverted for the synthesis of glycoproteins. In diabetes, enhanced levels of glucose-6-phosphate formed from fructose-6-phosphate due to the inhibition of phosphofructokinase I decreases HK activity (Bihari et al., 1997). The deficiency of insulin enhances hepatic glucose production either through direct hepatic action (Hue, 1987) or indirectly by enhancing the availability of substrates and energy for gluconeogenesis (Williamson et al., 1969).
Glucose-6-phosphatase catalyzes the terminal step of glucose production and plays a key role in the maintenance of blood glucose homeostasis. The enzyme is present in the membranes of endoplasmic reticulum of liver and kidney and a key determinant in the production of glucose due to its critical position as the final step in both glycogenolytic and gluconeogenic pathways. In diabetic condition, GP increases in the liver, facilitating glucose release into the blood and continues to produce glucose even with severe hyperglycemia by glycogenolysis (Mitra et al., 1996). Fructose-1,6-bisphosphatase serves as a site for the regulation of the process and catalyzes one of the irreversible steps in gluconeogenesis. Increased activities of hepatic GP and FB in diabetic rats have been reported (Baquer et al., 1998).

Glucose-6-phosphate dehydrogenase is a key enzyme that maintains normal blood sugar level (Mayes, 2000) by catalyzing the oxidation of glucose-6-phosphate in presence of NADP⁺. This produces NADPH in the initial step of HMP shunt pathway (Gupta et al., 1996) which is required for lipogenesis (Fitch and Chaikoff, 1960). The rate of lipogenesis is correlated with the activity of this enzyme. Therefore, changes in the activity may affect the utilization of ingested carbohydrates or the process of lipogenesis. The activity of this enzyme is reduced in diabetic state (Gomathy et al., 1990) and the reason could be decreased HK activity (Gandhi and Raychowdhury, 1982).

**Lipid profile in diabetes**

Diabetes is associated with profound alterations in lipoprotein profile, triglycerides and plasma lipid along with an increased risk of coronary heart disease (Fontbonne et al., 1989; Motta et al., 2013). Lowering lipid levels through diet or drug results in decrease risk of vascular disease (Grundy et al., 1999). In diabetic subjects, abnormal high concentration of lipids (such as VLDL particles, small dense LDLs, triglyceride content of LDLs and HDLs) is found due to absence or less concentration of insulin, which is required for the inhibition of hormone sensitive lipase (Rhoads et al., 1976). Whereas, enhanced lipolysis is mediated by glucagon and other hormones. The diabetic state characterized by marked hyperlipidemia is a consequence of uninhibited actions of lipolytic hormones on the fat depots (Goodman and Gilman, 1985).
**Role of insulin**

Insulin inhibits hormone-sensitive lipase and reduces free fatty acids levels by inhibiting VLDL production thereby promotes the storage of triglycerides in the adipocytes. On the other hand, insulin is a potent activator of lipoprotein lipase (LPL), promoting the catabolism of triglyceride-rich lipoproteins (Mazzone et al., 1984). Insulin also acts on HDL metabolism by activating hepatic lipase (Ruotolo et al., 1994). Due to deficiency of insulin, increased number of VLDL (Howard and Howard, 1994) and, IDL particles (Steiner et al., 1998) and plasma triglycerides level is frequently increased in type 2 diabetes, which is augmented to the increased lipid pool in hepatocytes by increased flux of free fatty acids into the liver (Shimomura et al., 2000).

Type 2 diabetic patients have reduced catabolism of IDL and VLDL particles which promotes diabetic hyperglyceridaemia. This has been shown by *in vivo* kinetic studies using stable isotopes (Duvillard et al., 2000) and also using radio isotopes (Taskinen et al., 2003). This defect reflects mainly the reduced activity of lipoprotein lipase that mediates degradation of triglycerides within IDL and VLDL particles. The decrease in HDL-cholesterol is due to increased pool of triglyceride-rich lipoproteins (mainly VLDL) that activates cholesteryl ester transfer protein (CETP), which transfers triglycerides to HDL from VLDL. Triglycerides enriched with HDL become good substrate for hepatic lipase, leading to increased catabolism of HDL particles.

In this chapter, an attempt has been made to elucidate the possible mechanism of antihyperglycemic action of the active principle (N-Trisaccharide) of *C. Prophetarum* fruits by studying its effects on pancreatic β-cell function, glycemic control, carbohydrate metabolism enzymes and lipid metabolism in STZ-NA induced diabetic rats on long term treatment.

**Materials and Methods**

A total of 36 rats were divided into six groups each consisting of 6 rats.

- **Group I**: normal untreated rats
- **Group II**: normal rats treated with 50 mg N-Trisaccharide/kg.b.w/day
- **Group III**: diabetic untreated rats
Chapter IV

Group IV: diabetic rats treated with 25 mg N-Trisaccharide/kg.b.w/day
Group V: diabetic rats treated with 50 mg N-Trisaccharide/kg.b.w/day
Group VI: diabetic rats treated with 25 mg glibenclamide/kg.b.w/day

N-Trisaccharide/glibenclamide was administered by gastric intubation with a force feeding needle into the animals of the respective groups every day morning for 28 days. Rats of all the six groups were sacrificed on 29th day after an overnight fasting under anesthesia. Blood, pancreas, liver and kidney were collected and immediately stored at -20°C till further use. Prior to the treatment and sacrifice, body weight of all the animals were recorded. Estimation of blood glucose, plasma insulin, HbA1c, activities of enzymes of carbohydrate metabolism and serum lipid profiles were carried out using specific methods as described under materials and methods section in chapter II. The data were analyzed statistically using one way ANOVA.

Results

Anti-diabetic activity of active compound

Fasting blood glucose levels of untreated diabetic rats were significantly $p \leq 0.05$ higher than those of normal control rats (Table 4.1). Significant decrease in blood glucose levels were observed in diabetic rats ($325 \pm 46.47$ to $99.5 \pm 10.4$ mg/dL) treated with N-Trisaccharide at 50 mg/kg.b.w. The treatment showed significant $p \leq 0.05$ antihyperglycemic activity (69.3%) by bringing down the blood glucose level to near normal on day 14 and maintained at the same level thereafter till the end of experimental period (28 days) in diabetic rats. At the dose level of 25 mg/kg.b.w, N-Trisaccharide treated rats showed 47.7% fall in blood glucose level. No hypoglycemic effect was observed in normal treated rats. Treatment of diabetic rats with standard antidiabetic drug glibenclamide at 25 mg/kg.b.w resulted in 62.3% fall in blood glucose level on day 14.

Effect on biochemical parameters

Effect on plasma insulin level and other variables

There was a significant $p \leq 0.05$ decrease in plasma insulin levels of diabetic untreated group compared to those in normal rats (Fig. 4.1). The insulin levels were
further decreased in untreated diabetic rats at the end of 28 days. In diabetic rats, after treatment with N-Trisaccharide, the insulin level was significantly $p \leq 0.05$ increased to $16.93 \pm 0.33$ IU/mL from an initial value of $8.8 \pm 0.71$ IU/mL at the end of the experimental period. An increase from $8.7 \pm 0.75$ to $15.01 \pm 0.40$ IU/mL was observed in diabetic rats treated with N-Trisaccharide at 25 mg/kg.b.w. In the normal treated rats with N-Trisaccharide, there was a slight increase from an initial level of $16 \pm 0.62$ to $19.28 \pm 0.56$ IU/mL. 

Fig. 4.2 and 4.3 shows the level of HbA1c (%) and hepatic glycogen levels respectively. The change in body weight of the normal and experimental diabetic rats is shown in Fig. 4.4. Higher levels of HbA1c and lower levels of hepatic glycogen were observed in diabetic rats as compared to normal controls. Treatment with N-Trisaccharide decreased HbA1c and increased hepatic glycogen levels to near normal. Diabetic rats showed marked reduction in body weight when compared to normal control rats. Treatment with N-Trisaccharide significantly increased the body weight of diabetic rats but not to near normal control rats.

**Effect on glucose metabolism and hepatic glycogen enzymes**

The effect of oral administration of N-Trisaccharide on carbohydrate metabolic enzymes in liver and kidney of normal and diabetic control rats is illustrated in Table 4.2 and 4.3 respectively. Diabetic rats showed decreased activity of hexokinase and increased activities of glucose-6-phosphatase, fructose-1,6-bisphosphatase and glucose-6-phosphate dehydrogenase. Treatment with N-Trisaccharide (25 and 50 mg/kg.b.w) restored these enzyme levels to near normal levels.

The effect of N-Trisaccharide on glycogen synthase and glycogen phosphorylase enzymes in the liver of normal and diabetic rats is shown in Table 4.4. Diabetic rats showed increased activity of glycogen phosphorylase and decreased activity of glycogen synthase. The enzyme levels were significantly ($p \leq 0.05$) reverted to near normal levels after treatment with N-Trisaccharide.

Increased levels of serum lipids like TG, TC, HDL-C, LDL-C and VLDL-C of the experimental groups of animals are shown in Fig. 4.5. Diabetic rats had elevated levels of
serum total cholesterol, TG, LDL-C, and VLDL-C and decreased level of HDL-C. Treatment with N-Trisaccharide of diabetic group showed significant reversal of TG (75%), TC (91%), HDL-C (109%), LDL-C (87%) and VLDL-C (60%) compared to untreated diabetic group.

**Histologic changes in the pancreas**

Pancreatic tissue of normal control (Fig. 4.6.A) and normal treated with N-Trisaccharide at 50 mg/kg.b.w (Fig. 4.6.B) showed the exocrine portion consisting of normal acini (a), endocrine portion with the Islets of Langerhans (IL) containing alpha cells (α) at the periphery of islets, beta cells (β) in the core and delta cells (δ) of a relatively larger size. The normal architecture of the islets in the diabetic rats were shrunken (Fig. 4.6.C) with less number of β-cells. The islets showed vacuolation (v) and irregular hyperchromatic nuclei (hcn). In animals treated with 25 and 50 mg/kg b.w of N-Trisaccharide (Fig. 4.6.D and 4.6.E), the restoration of the normal cellular population and size of islets were evident especially in the central β-cell region.
Table 4.1: Effect of N-Trisaccharide treatment on the fasting blood glucose levels in normal and experimental diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose level (mg/dL) at weekly interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Normal</td>
<td>87 ± 11.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal + N-Trisaccharide (50 mg/kg)</td>
<td>84 ± 11.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic</td>
<td>303 ± 38.0</td>
</tr>
<tr>
<td>Diabetic + N-Trisaccharide (25 mg/kg)</td>
<td>306 ± 30.68&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + N-Trisaccharide (50 mg/kg)</td>
<td>325 ± 46.47&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (25 mg/kg)</td>
<td>313 ± 28.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n = 6)

Values in the same column not sharing common superscripts differ significantly at p ≤ 0.05.
Table 4.2: Effect of N-Trisaccharide treatment on enzyme activities of carbohydrate metabolism in liver of different group of experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hexokinase 1*</th>
<th>Glucose-6-Phosphatase 2</th>
<th>Fructose-1,6-bisphosphatase 3</th>
<th>Glucose-6-Phosphate dehydrogenase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.16 ± 0.017^a</td>
<td>0.052 ± 0.010^b</td>
<td>0.044 ± 0.011^a</td>
<td>0.324 ± 0.054^a</td>
</tr>
<tr>
<td>Normal + N-Trisaccharide (50 mg/kg)</td>
<td>0.18 ± 0.015^b,c</td>
<td>0.056 ± 0.011^b</td>
<td>0.046 ± 0.010^b</td>
<td>0.44 ± 0.04^b,c</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.08 ± 0.011</td>
<td>0.148 ± 0.011</td>
<td>0.129 ± 0.010</td>
<td>0.2 ± 0.025</td>
</tr>
<tr>
<td>Diabetic + N-Trisaccharide (25 mg/kg)</td>
<td>0.13 ± 0.013^b,c</td>
<td>0.085 ± 0.012^b,c</td>
<td>0.053 ± 0.010^b</td>
<td>0.27 ± 0.04^b,c</td>
</tr>
<tr>
<td>Diabetic + N-Trisaccharide (50 mg/kg)</td>
<td>0.14 ± 0.010^b,c</td>
<td>0.075 ± 0.011^b,c</td>
<td>0.059 ± 0.010^b,c</td>
<td>0.31 ± 0.03^b</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (25 mg/kg)</td>
<td>0.139 ± 0.012^b,c</td>
<td>0.082 ± 0.010^b,c</td>
<td>0.081 ± 0.012^b,c</td>
<td>0.29 ± 0.014^b</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD (n = 6)
Values in the same column not sharing common superscripts differ significantly at p ≤ 0.05.

*(1) µ moles of glucose phosphorylated/h/mg protein, (2) µ moles of Pi liberated/h/mg protein, (3) µ moles of Pi liberated/h/mg protein, (4) U/mg protein.
<table>
<thead>
<tr>
<th>Groups</th>
<th>Hexokinase (^{1*})</th>
<th>Glucose-6-Phosphatase(^{2})</th>
<th>Fructose-1,6-bisphosphatase(^{3})</th>
<th>Glucose-6-Phosphate dehydrogenase(^{4})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.138 ± 0.014(^{a})</td>
<td>0.048 ± 0.014(^{a})</td>
<td>0.042 ± 0.012(^{a})</td>
<td>0.3 ± 0.09(^{a})</td>
</tr>
<tr>
<td>Normal + N-Trisaccharide (50 mg/kg)</td>
<td>0.169 ± 0.02(^{b,c})</td>
<td>0.043 ± 0.011(^{b})</td>
<td>0.036 ± 0.012(^{b})</td>
<td>0.428 ± 0.053(^{b,c})</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.049 ± 0.012</td>
<td>0.118 ± 0.02</td>
<td>0.126 ± 0.012</td>
<td>0.14 ± 0.011</td>
</tr>
<tr>
<td>Diabetic + N-Trisaccharide (25 mg/kg)</td>
<td>0.119 ± 0.014(^{b,c})</td>
<td>0.046 ± 0.011(^{b})</td>
<td>0.054 ± 0.009(^{b})</td>
<td>0.259 ± 0.037(^{b})</td>
</tr>
<tr>
<td>Diabetic + N-Trisaccharide (50 mg/kg)</td>
<td>0.13 ± 0.012(^{b})</td>
<td>0.052 ± 0.010(^{b})</td>
<td>0.049 ± 0.011(^{b})</td>
<td>0.314 ± 0.029(^{b})</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (25 mg/kg)</td>
<td>0.13 ± 0.012(^{b})</td>
<td>0.043 ± 0.009(^{b})</td>
<td>0.048 ± 0.011(^{b})</td>
<td>0.282 ± 0.02(^{b})</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD (\(n = 6\))

Values in the same column not sharing common superscripts differ significantly at \(p \leq 0.05\).

\(^{1*}\)(1) \(\mu\) moles of glucose phosphorylated/h/mg protein, (2) \(\mu\) moles of Pi liberated/h/mg protein, (3) \(\mu\) moles of Pi liberated/h/mg protein, (4) U/mg protein.
Table 4.4: Effect of N-Trisaccharide treatment on enzyme activities of Glycogen synthase and Glycogen phosphorylase in liver of different group of experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glycogen synthase(^{1})</th>
<th>Glycogen phosphorylase(^{2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>786.66 ± 29.09(^{a})</td>
<td>598 ± 27.36(^{a})</td>
</tr>
<tr>
<td>Normal + N-Trisaccharide (50 mg/kg)</td>
<td>758.16 ± 28.67(^{b,c})</td>
<td>551 ± 31.01(^{b,c})</td>
</tr>
<tr>
<td>Diabetic</td>
<td>478.33 ± 24.34</td>
<td>737.5 ± 22.16</td>
</tr>
<tr>
<td>Diabetic + N-Trisaccharide (25 mg/kg)</td>
<td>634.16 ± 20.74(^{b,c})</td>
<td>631 ± 23.6(^{b,c})</td>
</tr>
<tr>
<td>Diabetic + N-Trisaccharide (50 mg/kg)</td>
<td>661.83 ± 29.57(^{b,c})</td>
<td>532.66 ± 22.99(^{b,c})</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (25 mg/kg)</td>
<td>636.66 ± 25.36(^{b,c})</td>
<td>606 ± 13.97(^{b})</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD (\(n = 6\)).
Values in the same column not sharing common superscripts differ significantly at \(p \leq 0.05\).

\(^{1}\) µ moles UDP formed/h/mg protein, \(^{2}\) µ moles of Pi liberated/h/mg protein
Fig. 4.1: Effect of N-Trisaccharide treatment on plasma insulin levels of normal and experimental diabetic animals. Values are mean ± SD (n = 6). For group I and III, BT refers to initial plasma insulin level on day 0 and AT refers to insulin level after 28 days of experimental period. BT and AT denote before treatment and after treatment respectively for groups II, IV, V and VI. #p < 0.01 different from control diabetic group. *p ≤ 0.05 different from group I
**Fig. 4.2:** Effect of N-Trisaccharide treatment on glycosylated haemoglobin (HbA1c). Values are mean ± SD (n = 6). *p ≤ 0.01* different from control diabetic group

**Fig. 4.3:** Effect of N-Trisaccharide treatment on Hepatic glycogen levels. Values are mean ± SD (n = 6). *p ≤ 0.01* different from control diabetic group
**Fig. 4.4:** Effect of N-Trisaccharide treatment on change in body weight (g) of normal and experimental diabetic rats. Values are mean ± SD (n = 6). *p ≤ 0.05, #p ≤ 0.01 different from control diabetic group.

**Fig. 4.5:** Effect of N-Trisaccharide treatment on serum TG, TC, HDL-C, LDL-C and VLDL-C in normal and experimental diabetic animals. Values are mean ± SD (n = 6).
**Fig. 4.6:** Photomicrographs of rat pancreas showing normal (A), normal treated with N-Trisaccharide (B) and diabetic (C) rats. 25 and 50 mg/kg.b.w of N-Trisaccharide (D) and (E) respectively. The effect of glibenclamide (F) at 25 mg/kg.b.w. Microscope magnification (40x)
Discussion

The use of medicinal plants is increasing due to the development of many successful drugs and chemotherapeutic agents with their application as traditional rural herbal remedies (Tiwari and Madhusudhana, 2000). STZ-NA induced diabetes mellitus is a type 2 diabetic model; this could be due to partial destruction of β-cells of the islets of Langerhans in the pancreas. Type 2 diabetes is the consequence of number of defects, including impaired insulin secretion with resistance of peripheral tissues to the glucose-utilizing effect of insulin and augmented hepatic glucose production (Shulman, 2000).

The efficacy of N-Trissacharide, the active principle from *C. prophetarum* is comparable to standard antidiabetic drug glibenclamide and is mediated by improving the glycemic control mechanisms and increasing insulin secretion from remnant pancreatic β-cells. N-Trissacharide showed improvement in reduction of blood glucose level at 50 mg/kg.b.w against the baseline level of 300-330 mg/dL and did not cause hypoglycemia or any mortality, proving its safety or no toxic effects. Whereas, glibenclamide causes hypoglycemia and its treatment could be contraindicated for those with G6PDH deficiency, as it may cause acute haemolysis. The potency of N-Trissacharide is assured and could be safer to use over-time when compared to glibenclamide and may be attractive alternative to synthetic drugs or reinforcements to currently used treatments.

In the present investigation, treatment for 28 days with N-Trisaccharide showed significant antihyperglycemic activity. Maximum reduction in glucose level was observed in groups receiving 50 mg/kg.b.w of active compound, as it is evident by the significant increase in the level of insulin in diabetic rats. The possible mechanism for exhibiting antihyperglycemic action in diabetic rats could be due to increased pancreatic secretion of insulin from the existing β-cells. Similar stimulatory effect on insulin release has been reported by the use of a number of plants possessing antihyperglycemic activity by several workers (Nmila *et al*., 2000; Sharma *et al*., 2006; Gupta *et al*., 2009). STZ-NA induced diabetes is characterized by severe loss in body weight (Pari and Srinivasan, 2010). Due to absolute or relative deficiency of insulin and decreased production of ATP, protein synthesis decreases in all tissues (Murray *et al*., 2003). This insulin deficiency
causes hyperglycemia and when blood glucose level exceeds the renal threshold, glucose gets excreted in urine. Water accompanies glucose due to osmotic effect and to compensate for this loss of water, thirst center is activated and more water is consumed. The loss and ineffective utilization of glucose lead to breakdown of fat and protein. Structural proteins contribute to body weight, the loss or degradation of which causes reduction in body weight (Ramesh and Pugalendi, 2006). The excessive catabolism of protein to provide amino acids for gluconeogenesis during insulin deficiency results in muscle wasting and weight loss in diabetic untreated condition. In this study, diabetic rats showed marked reduction in their body weight. The protective effect of N-Trisaccharide in preventing body weight loss could be due to its ability to increase insulin level thereby improving glycemic control.

HbA1c levels are monitored as a reliable index of glycemic control in diabetes. Glycosylation of haemoglobin increases in diabetes mellitus and the amount of increase is directly proportional to fasting blood glucose level (Babu et al., 2007). In this study, diabetic rats showed high levels of HbA1c. Administration of N-Trisaccharide to diabetic rats prevented the increase in glycosylated haemoglobin significantly and this could be due to decrease in glucose levels. Recently, Anand et al. (2012) reported that oral administration of aqueous extract of Biophytum sensitivum (Linn.) reduced HbA1c level in STZ-NA induced diabetic rats.

Glycogen level in tissues such as liver and skeletal muscle corresponds to the insulin activity as it causes glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. By insulin treatment, recovery of glycogen levels in tissues (liver and muscle) occur due to increased influx of glucose into the liver (Vats et al., 2004). Treatment with N-Trisaccharide for 28 days, in the present study significantly increased the hepatic glycogen level in STZ-NA induced diabetic rats, thereby indicating the presence of insulin, which could be due to secretagogue activity of N-Trisaccharide.

In general, hyperglycemia results from increased hepatic glucose production plus decreased hepatic glycogen synthesis and glycolysis, which are the major symptoms in type-2 diabetes and this seems to be the consequence of low hexokinase and high
glucose-6-phosphatase activities in diabetic state (Guignot and Mithieux, 1999). One of the key enzymes in the catabolism of glucose is hexokinase; an insulin dependent enzyme which plays an important role by phosphorylating glucose to glucose-6-phosphate in the cell system (Laakso et al., 1995). In the present study, due to insulin deficiency, the activities of both hepatic and kidney hexokinase were decreased in diabetic rats as compared to normal rats. Similar results were reported by Bopanna et al. (1997) and Suneetha (2011). Administration of N-Trisaccharide to diabetic rats enhanced the hexokinase activity in liver and kidney, which could be due to increased insulin secretion and thereby greater uptake of glucose from blood by liver cells and increased glycolysis for energy production leading to decreased blood glucose levels.

Under normal conditions, insulin suppresses glucose-6-phosphatase and fructose-1,6-bisphosphatase, the important regulatory enzymes of gluconeogenic enzymes. Glucose-6-phosphatase, the key enzyme in the homeostatic regulation of blood glucose level, catalyzes the terminal step in gluconeogenesis and glycogenolysis (Berg et al., 2001). Fructose-1,6-bisphosphatase, catalyzes one of the irreversible steps in gluconeogenesis and serves as a site for the regulation of the process (Tillmann et al., 2002). The activities of these gluconeogenic enzymes increase in the liver of diabetic rats (Baquer et al., 1998), which could be due to insulin deficiency. Diabetic rats treated with N-Trisaccharide and glibenclamide exhibited lower activity levels of these two enzymes, which could be due to higher secretion of insulin.

Glucose-6-phosphate dehydrogenase (G6PDH) is the enzyme, which maintains intracellular glucose-6-phosphate at optimum levels by diverting it into pentose phosphate pathway, thus maintaining normal blood sugar level. Insulin is reported to increase the activity of G6PDH in a dose dependent manner (Weber and Convery, 1966). The activity of G6PDH significantly gets decreased in diabetic rats as reported by Shibib et al. (1993). Treatment of diabetic rats with N-Trisaccharide restored the activity of G6PDH to near normal, which could be due to increased level of insulin.

Liver plays an important role in buffering postprandial blood sugar level and is involved in glycogen storage. In diabetic condition, the normal capacity of liver to
synthesize glycogen gets impaired (Sirag, 2009). In STZ-NA induced diabetic rats, the activation of glycogen synthase from synthase phosphatase is defective (Kirana and Srinivasan, 2008). In the present study, the activity of glycogen phosphorylase increased and that of glycogen synthase decreased in diabetic rats. In N-Trisaccharide treated diabetic rats, the liver glycogen came to near normal level and this could be due to increased secretion of insulin that enhanced glycogenesis.

The increased activity of hepatic and kidney gluconeogenic enzymes (GP and FB) in diabetic rats was reported by several workers (Pushparaj et al., 2007; Zhang et al., 2004). Increased activities of gluconeogenic enzymes were shown to be decreased by the treatment of *Syzygium cumini* (Prince et al., 1997), an acidic polysaccharide from *Tremella fuciformis* (Kiho et al., 1996); *Coccinia indica* leaves and *Momordica charantia* (Shibib et al., 1993); *Trigonella foenum-graecum* (Gupta et al., 1999); neem seed kernel (Bopanna et al., 1997); S-methyl cysteine sulfoxide from *Allium cepa* L. (Kumari et al., 1995) and *Berberis aristata* root extract containing berberine (Singh and Kakkar, 2009) in experimental diabetic animals and the present results with N-Trisaccharide are similar to the above reports.

Disturbances in glucose metabolism, altered lipid levels and oxidative stress are important risk factors of diabetes. In diabetic patients, insulin deficiency causes a variety of derangements in metabolic and regulatory processes, which leads to accumulation of lipids like TG and TC (Goldberg, 1981). Hyperglycemia is accompanied with dyslipidemia (Bierman et al., 1996), a condition which is characterized by increase in TG, TC, LDL, VLDL and fall in HDL representing risk factor for coronary heart diseases. This abnormal high level of serum lipids causes lipolytic hormones their uninhibited actions on fat depots. Under normal conditions, lipoprotein lipase hydrolyses triglycerides, thereby maintaining normal lipid profile. However, this enzyme is not activated in diabetic state due to insulin deficiency resulting in hypertriglyceridemia (Pushparaj et al., 2007) and due to metabolic abnormalities it also leads to hypercholesterolemia (Murali et al., 2002).
In the present study, higher levels of cholesterol were evident in the serum of diabetic rats. Administration of N-Trisaccharide to diabetic rats decreased the levels of cholesterol. In normal conditions, circulating LDL-C gets cleared by reuptake in the liver via specific receptors (Lusis, 2000). Defect in LDL-C receptor in diabetic rat leads to increased LDL concentration in the serum either through failure in its production or function. HDL-C is protective by neutralizing the atherogenic effect of oxidized LDL-C, inhibiting the oxidation of LDL-C and reversing cholesterol transport. A greater increase of VLDL-C and LDL-C may also cause more decrease in HDL-C as there is a reciprocal relationship between the concentration of HDL-C and VLDL-C. Decreased HDL-C may also be due to diminished lecithin cholesteryl acyl transferase activity. Diabetic rats treated with N-Trisaccharide showed a significant elevation in HDL-C and reduction in LDL-C and VLDL-C. Thus, N-Trisaccharide could alleviate the risk of cardiovascular diseases.

Hypertriglyceridemia is a common factor associated vascular complications in diabetic patients (Kudchodkar et al., 1988). Deficiency of lipoprotein lipase (LPL) activity may contribute significantly to the elevation of triglycerides in diabetes (Braun and Severson, 1992). Lopez-Virella et al. (1983) reported that insulin treatment lowers triglyceride levels by returning LPL activity to normal. Thus, decreased triglyceride level following N-Trisaccharide treatment could be due to the increased insulin secretion and increased LPL activity. In the present study, N-Trisaccharide significantly reversed the serum lipid level toward normal by increasing the level of HDL cholesterol. High levels of TC and particularly LDL-C are the predictors of atherosclerosis (Temme et al., 2002) and treatment with N-Trisaccharide markedly reduced both serum TG and LDL levels. Thus the present findings demonstrate that N-Trisaccharide has antihyperlipidemic effect, which could be due to its stimulatory effect on insulin secretion from the remnant β-cells in the diabetic rats.

β-Cell population of the islets of Langerhans in pancreas reflects the production and secretion of insulin. In the present study, destruction of β-cells was observed in STZ-NA induced diabetic rats. Control rats showed normal cellular population and size of β-cells. Restoration of β-cells in the islets of Langerhans was observed in the diabetic rats.
treated with N-Trisaccharide. This correlates the increased level of insulin in diabetic treated group compared to diabetic untreated group. The possible mechanism of action by which the novel N-Trisaccharide exhibits its antidiabetic action may be by stimulation of insulin secretion in injured β-cells.

Administration of N-Trisaccharide significantly increased plasma insulin level in diabetic rats, which could be due to stimulatory effect on the remnant β-cells thereby increasing insulin secretion. This may be as a consequence of significant reduction in the level of gluconeogenic enzymes or due to increased utilization of glucose by the activities of hexokinase, glucose-6-phosphate dehydrogenase and glycogen synthase that caused the decrease in the concentration of glucose in blood.

All these results are going to prove conclusively the antidiabetic effect of N-Trisaccharide on STZ-NA induced type 2 diabetic rats.