Validation of anti-diabetic activity of *Naringi crenulata* in *streptozotocin induced diabetic rats*

**INTRODUCTION**

Diabetes mellitus has become a growing problem in the contemporary world (Piyush *et al.*, 2006). This astronomic increase in the prevalence of diabetes has made diabetes a major public health challenge for India and is become important human ailment afflicting many from various walks of life in different countries and once again the whole world being looked upon Ayurvedic the oldest healing system of medicine for the treatment of diabetes (Joseph, 2011). India has today become the diabetic capital of the world with over 20 million diabetes and this number is likely to increase to 57 million by 2025 (Cook and Plotnick, 2008).

The classic well-known symptoms of diabetes are polyuria, polydipsia, polyphagia and loss of body weight (Guthrie and Guthrie, 2003). Diabetes and its complications are among the leading causes for mortality and morbidity worldwide. The global population is in the midst of a diabetes epidemic with people in south East Asia and Western Pacific being mostly at risk. The number of cases for diabetes is predicted to reach 366 million by the end of 2030. Diabetes mellitus is an endocrine disease which is related to the disorders of carbohydrate metabolism brought about by deficiency in insulin production, insulin resistance or both (Raju *et al.*, 2006).

Diabetes mellitus is characterized by hyperglycemia in the postprandial fasting state and its severe form leads to ketosis and protein wasting (Bell, 1991). Blood glucose elevation is a common phenomenon of diabetes mellitus and is primarily due to reduced
glucose intake by the tissues and its production increased via gluconeogenesis and glycogenolysis.

Diabetes mellitus is basically characterized by high levels of blood glucose caused by defective insulin production and action that are often responsible for severe health problems and early death (Leahy, 2005). One of the reasons for injury related to hyperglycemia is the formation of glycated proteins, glucose oxidation and increase free fatty acids (Devi and Falco, 2005). Chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels (Hung et al., 2005). It is a commonest endocrine disorder that affects more than 100 million people worldwide (about 6% of population) and in the next 10 years, it may affect about 5 times more people than it does now (WHO, 1992; ADA, 1997). According to WHO report, India has 19.4 million diabetes patients (King et al., 1998). It is the fourth leading cause of death in the most developed countries and there is substantial evidence that it is epidemic in many developing and newly industrialized nations. Diabetes mellitus is a syndrome resulting from a variable interaction and environmental factors and is characterized by depleted insulin secretion, hyperglycemia and altered metabolism of lipid, carbohydrates and proteins, in addition to damaged β-cells of pancreas and increased risk of complications of vascular diseases (Davis and Granner, 1996).

The concept of polyherbalism is peculiar to Ayurveda although it is difficult to explain in term of modern parameter. Sarandghar Samhita highlights the concept of synergism behind polyherbal formulations. Ayurveda has fundamental aspects for drug formulation. The herbs are selected according to disease and some other herbs are used to prevent side effect arising from chief herb. It is evident that there are many herbal
formulations of varying potency since these preparation act by different mechanism, it is theoretically possible that different combination of these extract will do better job in reducing blood glucose. In the traditional system of plant medicine it is usual to use plant formulation and combined extracts of plants are used as a drug of choice rather than individual ones (Kumar, 2010), to get the benefit of synergism and to find suitable anti-diabetic and antioxidant combination therapy. In the present study, it is aimed to focus the potential of *N. crenulata* extract in lowering the blood glucose level in experimental diabetic animal set up.

**MATERIALS AND METHODS**

**Estimation of blood glucose**

Blood glucose was estimated by the method O-toluidine using the modified reagent of Sasaki *et al.* (1972).

**Reagents**

1. Trichloro acetic acid- 10%

2. O-toluidine reagent- 12.5g of thiourea and 12.0g of boric acid were dissolved in 50.0ml of distilled water by heating over a mild flame. 75.0ml of redistilled O-toluidine and 375 ml of acetic acid were mixed and the total volume was made upto 500 ml with distilled water. The reagent was left in a refrigerator overnight and filtered.

3. Stock standard – 1 mg/ml (100mg of anhydrous D-glucose was dissolved in 100ml of water containing 0.01% benzoic acid)

4. Working standard- 1µg/ml: 10.0ml of stock glucose solution was diluted to 100ml distilled water.
**Procedure**

0.1ml of freshly drawn blood was immediately mixed with 1.9ml of 10% TCA to precipitate the proteins and then centrifuged. 1.0ml of the supernatant was mixed with 4.0ml of O-toluidine reagent and was kept in boiling water bath for 15 minutes. The green color developed was read colorimetrically at 620nm. A set of standard glucose (20-100µg) was treated simultaneously using reagent blank. Glucose concentration was expressed as mg/dl of blood.

**Estimation of haemoglobin**

Haemoglobin in the blood was estimated by the method of Drabkin and Austin (1932). The dilution of blood in an alkaline solution containing potassium cyanide and potassium ferricyanide form the basis of this method. Haemoglobin gets oxidized forming cyanmethaemoglobin whose absorbance was then measured at 540 nm.

**Reagents**

1. Drabkin’s reagen: 200mg of potassium ferricyanide, 50 mg of potassium cyanide and 1.0 g of Na₂Co₃ were dissolved in distilled water and made up to one liter. The reagent had a pale yellow color of pH 9.6 and was stored in brown bottle.

2. Cyanmethaemoglobin standard solution: 16g/dl.

**Procedure**

To 0.02 ml of blood, 5.0 ml of Drabkin’s reagent was added, mixed well and allowed staining for 10 minutes. The solution was read at 540 nm together with the standard solution against a reagent blank. Values were expressed as g/dl for blood.

**Estimation of glycosylated haemoglobin (HbA₁C)**

Glycosylated haemoglobin in the blood was estimated by the method of Sudhakar and Pattabiraman (1981).
Reagents

1. 1 M potassium oxalate in 2 M hydrochloric acid (Oxalate hydrochloric acid).
2. Phenol: 80%
3. Concentrated H2So4
4. TCA: 40%
5. Saline
6. Stock solution: Stock solution was prepared by dissolving 100mg of fructose in 100 ml of distilled water.
7. Working standard: Stock standard was diluted to get a concentration of 100µg/ml

Procedure

0.5 ml of saline washed erythrocytes were lysed with 5.5ml of water, mixed and incubated at 37°C for 15 minutes. The contents were centrifuged and the supernatant was discarded, then 0.5ml of saline water was added, mixed and processed for estimation. To 0.2ml of aliquot, 4ml of oxalate hydrochloric solution was added and mixed. The contents were mixed at 100°C for 4 h, cooled and precipated with 2ml of 40% TCA. The mixture was centrifuged and to 0.5ml of supernatant, 0.5ml of 80% phenol and 3.0ml of concentrated sulphuric acid was added. The colour developed was read at 480nm after 30 minutes. The concentration of glycosylated Hb was expressed as mg/g of haemoglobin.

Estimation of glycogen

Liver glycogen was extracted and estimated by the method of Morales et al., 1973.

Reagents

1. Potassium hydroxide- 30%
2. Absolute alcohol
3. Anthrone reagent - 0.2% of anthrone in Conc. Sulphuric acid was prepared just before use.

4. Ammonium acetate- 1M

5. Stock standard- 1mg/ml: 100mg of anhydrous D-glucose was dissolved in 100ml of water containing 0.01 % benzoic acid.

6. Working solution- 100 µg/ml: `10.0ml stock glucose solution was diluted to 100ml with distilled water.

**Procedure**

The alkali extract of the tissue was prepared by digesting 50 mg of fresh tissue with 3.0 ml of 30% potassium hydroxide solution in boiling water bath for 15 minutes. The tubes were cooled and mixed with 5.0ml of absolute alcohol and a drop of 1M ammonium acetate to precipitate glycogen and left in the freezer over night for complete precipitation. Glycogen was collected by centrifugation at 2000g for 20 minutes. The precipitate was dissolved in water with the aid of heating and again the glycogen was reprecipitated with alcohol and 1M ammonium acetate and centrifuged. The final precipitate was dissolved in saturated ammonium chloride solution by heating in a boiling water bath for 5 minutes. Aliquots of glycogen solution were taken up after suitable dilution and 4.0ml of anthrone reagent was added by cooling the tubes in an ice bath. The tubes were shaken well, covered with marble caps and heated in a boiling water bath for 20 minutes. After cooling, the absorbance was read at 640nm against water blank treated in similar manner. Standard glucose was also treated similarly.

The glycogen content was calculated from the amount of glucose present in the sample and was expressed as mg/g tissue.
Quantitative determination of plasma insulin

The plasma insulin was assayed by ELISA method (Enzyme Linked Immunosorbant Assay) using Boehringer Manheim Kit (Boehringer analyzer ES 300).

Reagents

2. Phosphate buffer 40mM/l, pH-7.0.
3. Anti-insulin POD conjugates (POD-peroxidase).
5. Substrate: Phosphate/citrate 100mM/l, H$_2$O$_2$ Ph-4.4/ (as perborate) 3.2 mM/l
6. Chromogen: Di-ammonium 2, 2-azino-bis (3- ethyl benzothiazoline-6-sulphonate).

Procedure

0.1 ml of plasma was injected into the plastic tubes coated with monoclonal anti-insulin antibodies. Phosphate buffer and anti-insulin POD conjugate were added to form anti-insulin antibody-POD conjugate. Substrate-chromogen solution was then added to form indicator reaction. A set of standards were also treated in similar manner. After the development of colour, the absorbance was read at 420nm. The values were expressed as µU/ml of plasma.

Estimation of urea

Urea in the plasma was estimated by using the diagnostic kit based on the method of Fawcett and Scott (1960).

Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. Under alkaline conditions, the ammonia so formed reacts with hypochlorite and sodium salicylate in the presence of sodium nitroprusside to forma
green colored chromophore. The intensity of the colour produced is proportional to the concentration of urea in the sample.

**Reagents**

1. Buffered enzyme: Phosphate buffer, Urease, Sodium nitroprusside and ethylenediamine tetra acetic acid.

2. Colour developing reagent: Buffer, Sodium hypochlorite, Sodium salicylate and Sodium hydroxide.

3. Urea standard: 40mg/dl.

**Procedure**

To 1.0 ml of buffered enzyme, 10µl of sample was added, mixed well and kept at 37°C for 5 minutes. 10 µl of standard and 10µl of distilled water (blank) were also processed simultaneously. To all the tubes, 1.0 ml of colour developing reagent was added and mixed well. Exactly after 5 minutes of incubation at 37 °C, 1.0 of distilled water was added and the colour developed was read at 600 nm. The value was expressed as mg/dl of plasma.

**Estimation of Creatinine**

Creatinine in the plasma was estimated by using the diagnostic kit based on the method of Tietz, (1987) using Jaffe’s (1886) colour reaction. The assay of creatinine has been measured based on the reaction of creatinine with alkaline picrate as described by Jaffe. Most of the contaminants reacting with the Jaffe reagent produce a colour at a lower rate of colour formation are proportional to the concentration of creatinine in the sample.
Reagents

1. Saturated picric acid
2. Sodium hydroxide: 0.75 N
3. Creatinine standard: 2.0 mg/dl

Procedure

0.1ml of sample was added to a reagent mixture containing 0.5ml picric acid solution and 0.5 ml of sodium hydroxide. The tubes were mixed well and incubated for 20 seconds. With the spectrophotometer adjusted to zero absorbance with distilled water, reading was taken at 510 nm at 20 seconds (A₁) and exactly after 45 seconds (A₂). Changes in absorbance (A₂-A₁) were measured for test and standard which was used to determine the creatinine concentration in the test sample. The values were expressed as mg/dl of plasma.

Statistical Analysis

All the data were expressed in Mean ± S.D and analyzed statistically using ANOVA followed by Dunnett’s test and compare with respective control group. A value of P<0.05 was considered statistically significant.

RESULTS

Table.1 shows the study of blood glucose, body weight, plasma insulin and total haemoglobin in normal diabetic and *N.crenulata* extract treated animals in different groups.

BODY WEIGHT CHANGES

In the present study, the initial (0 day) and final body weight (45th day) of each group were observed. In the control group, the initial and final bodyweight was 159.92±12.18 grams and 192.98±8.72 grams, respectively. The average growth recorded
in control group is 36g. The growth was slightly increased in *N.crenulata* extract treated diabetic groups. The initial and final growth of *N.crenulata* treated group was 158±12.12 grams and 199.93±4.65 grams, respectively. At the same time *N.crenulata* acetone extract treated group exhibited average weight gain of 22.01±13.28 grams which is lesser than the methanolic extract treated group (Fig.2). The effect of glibenclamide treated group exhibited the weight gain which is meare equal to that of *N.crenulata* extract treated group.

**PLASMA GLUCOSE**

Plasma glucose level has extremely shot up to a level of 320±15.9 mg/dl in diabetic induced rats, whereas it is normal in the control group of 110.52±0.9 mg/dl. *N.crenulata* extract treated diabetic rats has shown considerable plasma glucose reducing effect as in case of methanolic extract of 600 mg/kg dose the plasma glucose level has reduced to 115.10±6.6 mg/dl and in case of acetone extract treated group 139.8±9.2 mg/dl level of glucose reduction was observed (Fig.3).

**PLASMA INSULIN**

Plasma insulin of normal control was relatively higher as compared to that of diabetic control rats. Plasma insulin level in normal control and diabetic control were observed as 15.09±1.11 µU/ml and 7.49±0.3 µU/ml. The plasma insulin level was increased in diabetic treated with *N.crenulata* extract 13.16±1.02µU/ml and glibenclamide 13.1±1.51 µU/ml. There was a promising increase in the level of plasma insulin in normal rats when treated with *N.crenulata* extract respectively more than that of standard glibenclamide (Fig. 4).
TOTAL HEMOGLOBIN

Fig. 5. shows the level of total haemoglobin which was totally decreasing in the diabetic rats 7.69±0.9 g/dl than control group 13.49±1.51 g/dl, and there was a surprising increase in total haemoglobin of diabetic rats when treated with *N.crenulata* formulation 12.49±1.23 g/dl which is highly significant to all other treated group.

GLYCOXYLATED HEMOGLOBIN (HbA1C)

Glycosylated haemoglobin (HbA1C) level in diabetic was 8.04±0.57 HbA1c % which seems very high as compared to that of normal control which was 42.04±2.09 HbA1c % and it was found that *N.crenulata* extract has vigorously lowered the level of glycosylated haemoglobin and was found 5.04±0.5 HbA1c % (Table. 2 and Fig. 6).

LIVER GLYCOGEN

Fig. 7 shows the level of liver glycogen in diabetic rats which is relatively less 23.14 ± 2.14 mg /g compared to that of control animals which was observed 40.1 ± 4.14 mg /g. As a contrary, *N.crenulata* extract treated group exhibited efficient level of glycogen in diabetic rats up to a promising level of 33.24± 3.43 mg /g which was near to standard glibenclamide treated 35.54± 4.12 mg /g.

UREA LEVEL

Urea level was totally found more in diabetic group 30.24±3.13 mg/dl and the same was found recovering in *N.crenulata* extract treated 26.20±2.52 mg/dl (Fig.8) near to standard glibenclamide treated group.

CREATININE LEVEL

Similarly, creatinine level was found more in case of diabetic induced 1.28±0.18 mg/dl which is totally reverse to that of normal control level of 0.56±1.04 mg/dl. *N.crenulata* extract was found significant enough in lowering the level of creatinine up to
level of 0.77±0.07 mg/dl and 0.84±0.08 significantly (Fig.9). The evaluation of creatinine was found decreased when treated with *N.crenulata* extract.

From all the above experimental diabetic analysis the results are proved to be evident that there was a progressive fall of blood sugar level and significant increase in body weight and insulin level after the *N.crenulata* leaf extract treated group. These results were on par with the standard drug used.
Table 1. The effect of *Naringi crenulata* on Blood glucose, Plasma insulin, Total haemoglobin and changes in body weight of normal and experimental animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Net weight gain (g)</th>
<th>Plasma glucose (mg/dl)</th>
<th>Plasma Insulin (µU/ml)</th>
<th>Total Hb (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>159.92±12.18</td>
<td>192.98±8.72</td>
<td>35.8±8.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.52±8.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.09±1.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic</td>
<td>156.26±14.15</td>
<td>151.99±12.17</td>
<td>-4.27±12.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>320.5±15.9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.49±0.3&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>N. crenulata</em> (M)/300</td>
<td>160.1±13.28</td>
<td>180.66±11.43</td>
<td>26.66±11.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>205.5±11.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.95±0.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>N. crenulata</em> (M)/600</td>
<td>158.99±12.12</td>
<td>199.93±4.65</td>
<td>32.66±4.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>115.10±6.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.16±1.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>N. crenulata</em> (A)/300</td>
<td>154.99±12.17</td>
<td>177±13.28</td>
<td>22.01±13.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>184.79±10.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.34±1.31&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>N. crenulata</em> (A)/600</td>
<td>157.63±6.87</td>
<td>180±10.44</td>
<td>22.37±10.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>139.8±9.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.51±0.94&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>154.21±11.06</td>
<td>187.16±11.43</td>
<td>27.16±11.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>121.18±7.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.1±1.51&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD (n=6 rats)
Values that are not sharing a common superscript letter in the same column differ significantly at p<0.05
Fig. 2. Effect of *N. crenulata* extract on the body weight of diabetic induced rats.

Fig. 3. Effect of *N. crenulata* extract on the plasma glucose level of diabetic induced rats.
Fig. 4. Effect of *N. crenulata* extract on the plasma insulin level of diabetic induced rats

Fig. 5. Effect of *N. crenulata* extract on the Total Haemaglobin level of diabetic induced rats
Table 2. Glycosylated Hb, Liver glycogen and Urine sugar and Creatinine of normal and experimental animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glycosylated Hb (HbA1C %)</th>
<th>Liver glycogen (mg /g)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.14±1.35\textsuperscript{a}</td>
<td>42.04±2.04\textsuperscript{a}</td>
<td>21.44±1.17\textsuperscript{a}</td>
<td>0.56±1.04\textsuperscript{a}</td>
</tr>
<tr>
<td>Diabetic</td>
<td>8.04±0.57\textsuperscript{e}</td>
<td>23.14±2.14\textsuperscript{d}</td>
<td>30.24±3.13\textsuperscript{d}</td>
<td>1.28±0.18\textsuperscript{d}</td>
</tr>
<tr>
<td><em>N. crenulata</em> (M)/300</td>
<td>5.79±0.5\textsuperscript{c}</td>
<td>34.94±4.14\textsuperscript{c}</td>
<td>24.14±2.27\textsuperscript{c}</td>
<td>0.77±0.07\textsuperscript{b}</td>
</tr>
<tr>
<td><em>N. crenulata</em> (M)/600</td>
<td>5.04±0.4\textsuperscript{b}</td>
<td>33.24±3.43\textsuperscript{c}</td>
<td>26.20±2.52\textsuperscript{c}</td>
<td>0.84±0.08\textsuperscript{c}</td>
</tr>
<tr>
<td><em>N. crenulata</em> (A)/300</td>
<td>7.23±0.42\textsuperscript{d}</td>
<td>30.24±2.14\textsuperscript{b}</td>
<td>27.25±2.82\textsuperscript{c}</td>
<td>0.77±0.07\textsuperscript{b}</td>
</tr>
<tr>
<td><em>N. crenulata</em> (A)/600</td>
<td>7.04±0.58\textsuperscript{d}</td>
<td>33.24±3.43\textsuperscript{c}</td>
<td>30.24±3.13\textsuperscript{d}</td>
<td>0.89±0.08\textsuperscript{c}</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>4.55±0.42\textsuperscript{d}</td>
<td>35.54±4.12\textsuperscript{c}</td>
<td>21.19±2.12\textsuperscript{b}</td>
<td>0.71±0.06\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD (n=6 rats)
Values that are not sharing a common superscript letter in the same column differ significantly at p<0.05
Fig. 6. Effect of *N. crenulata* extract on the glycosylated haemoglobin (HbA1c) of diabetic induced rats

Fig. 7. Effect of *N. crenulata* extract on the Liver glycogen of diabetic induced rats
Fig. 8. Effect of *N.crenulata* extract on the Urea of diabetic induced rats

Fig. 9. Effect of *N.crenulata* extract on the Creatinine of diabetic induced rats
DISCUSSION

The treatment of diabetes with medicines of plant origin that proved much safer than synthetic drugs is an integral part of many cultures throughout the world and has gained importance in recent years. India has a rich history of using various potent herbs and herbal components for treating various diseases including diabetes (Yeh et al., 2003). Several phytomolecules including flavonoids, alkaloids, glycosides, saponins, glycolipids, dietary fibers, polysaccharides, peptidoglycans, carbohydrates, amino acids and others obtained from various plant sources have been reported as potent hypoglycemic agent (Khaled et al., 2008; Baldi et al., 2010).

In the present study Herbal drug and phytoconstituents resembles safely and efficacy, they produces no side effect when compare to synthetic drugs. In the present study, untreated diabetic rats showed severe body weight loss. This characteristic weight loss in diabetic rats could be due to degradation and catabolism of fats and proteins (Nurlan and Garlick, 1979). Thus, increased catabolic reactions leads to muscle wasting which may be the major cause for weight loss in diabetic rats (Rajkumar et al., 1991). However, extract treated groups showed a sign of recovery in the body weight which suggest the protective effect of the extract by preventing it from muscle wastage and other macromolecular degradations.

The present study confirms the antihyperglycemic and antihyperlipidemic effects of N. crenulata extracts in streptozotocin induced diabetic rats. Administration of N. crenulata extracts to diabetic rats reduced the blood glucose levels to near normal and the optimum activity of the extract was found at the dose of 600 mg/kg bw. Although, the exact mechanism of action of the extract is unknown, the reduction in blood glucose level could
be due to increased pancreatic insulin secretion from existing β-cell of the pancreas (Ghosh and Suryawanshi, 2001). This anti-hyperglycemic activity of N.crenulata was associated with increase in plasma insulin levels. The extents of changes in insulin levels with plant phenolic extract are insufficient to account for the obvious improvement in the glucose profile (Sachdewa and Khemani, 1999).

Amount of STZ it damages a large number of beta-cells and causes decrease in endogenous insulin secretion. The reduction in the serum insulin levels in the STZ treated rats might be attributed to the reduced secretion of the hormone which might be due to the damage of the beta cells of endocrine pancreas. The STZ selectively destroys the pancreatic cells and induce hyperglycemia (Gilman et al., 1990, Kurup and Bhonde., 2000, Jarvenin, 1995). Similar studies were recorded earlier in the STZ treated rats, the levels of serum insulin significantly reduced (Yoon and Ray, 1985). Diabetes affects both glucose and lipid metabolism (Sperling and Saunders, 2000).

The etiology of the complications of diabetes involves oxidative stress perhaps as a result of hypoglycemia, because glucose itself and hyperglycemia-related increased protein glycosylation are important sources of free radicals. In physiological condition, antioxidant enzyme protects the cells against harmful free radicals. A number of plant derived products have been possessed hypoglycemic, hyperlipidemic as well as antioxidant properties (Vasu et al., 1975).

Kidney maintains optimum chemical composition of body fluid by acidification of urine and removal of metabolic wastes such as urea, uric acid, creatinine and ions. During renal diseases, the concentration of these metabolites increases in blood (Jaspreet et al., 2000). In the present study it was observed that, administration of polyherbal formulation at
300 mg/kg doses reduced elevated levels of urea and creatinine, which was comparable to the effect observed with glibenclamide. This indicates the prevention of any significant kidney change, which may be possible in diabetic animals.

The liver is an important organ that plays a vital role in glycolysis and gluconeogenesis pathways. Glucose-6-phosphatase is the key enzyme in homeostatic regulation of blood glucose level (Massillon et al., 1996). Hence in the present study administration of extract of interest to the STZ diabetic rats enormously increase the liver glycogen to its normal level of existence hence in the diabetic rats the glucokinase activity was decreased in the liver of diabetic rats, which may be due to deficiency of insulin. Decrease in the enzymatic activity of hexokinase and increase in the level of glycogen phosphorylase observed in the present study are responsible for the depletion of liver glycogen. In addition fructose1, 6-bisphosphatase catalyzes on the irreversible step in gluconeogenesis and serves as a site in the regulation of the process (Gupta et al., 1999). Glucose is transported out of the liver to increase in blood glucose concentration. Normally insulin inhibits the hepatic glucose production by glucose-6-phosphatase and fructose-1, 6-bisphosphatase activity. Administration of *N.crenulata* extract decreased both enzymes activities in diabetic rats in a dose dependent manner thereby decreasing gluconeogenesis.

**SUMMARY AND CONCLUSION**

The treatment of diabetes with medicines of plant origin that proved much safer than synthetic drugs is an integral part of many cultures throughout the world and has gained importance in recent years. The current investigation focuses on anti-hyperglycemic property of a methanolic and acetone extracts of *N.crenulata* on streptozotocin induced
diabetic rats. The diabetes induced animals were fed with leaf extracts at the dosage of 300mg/kg and 600 mg/kg body wt. Methanolic *N.crenulata* extract administrated animals revealed a significant (P<0.01) increment of serum insulin levels, higher reduction in hyperglycemia when compared to the acetone extract treated and diabetic control rats (P<0.01). The levels of blood glucose, Glycosylated Hb, urea and Creatinine were found to be significantly increased in diabetic rats when compared to control groups. Administration of extract in the treated groups showed altered changes in the above mentioned parameters and found that the 600 mg/kg of methanolic *N.crenulata* extract showed best results when compared to other acetone extracts. Oral administration of 600 mg/kg of methanolic *N.crenulata* extract for 45 days resulted in increase of insulin and liver glycogen compared to the diabetic control. Thus from the above findings, it is clear evident that, methanolic extract of *N.crenulata* exhibited well worsed anti-hyperglycemic activities