Evaluation of Anti-hyperlipidemic activity of *Naringi crenulata* in control and Streptozotocin induced diabetic rats

**INTRODUCTION**

The prime cause of mortality in the Western world involves cardiovascular diseases (CVD) such as myocardial or cerebral infarction. Illustratively, in the Netherlands, CVD account for 34% of all deaths (Statistics Netherlands CBS, 2002). The underlying process leading to these disorders is the development of atherosclerosis which is characterized by endothelial dysfunction, vascular inflammation, and the progressive accumulation of lipids, cholesterol, calcium, and cellular debris in the intima of the vessel wall (Ross, 1999). Hyperlipidemia contributes significantly in the manifestation and development of atherosclerosis and coronary heart diseases (CHD). Atherosclerosis is the most common cause of mortality and morbidity worldwide. Although several factors, such as diet high in saturated fats and cholesterol, age, family history, hypertension and lifestyle play a significant role in causing heart failure, the high levels of cholesterol particularly TC, TG and LDL cholesterol is mainly responsible for the onset of CHDs. 20% reduction of blood cholesterol level can decrease about 31% of CHD incidence, and 33% of its mortality rate (Zamani Marzyieh *et al.*, 2007).

The expression of lipid-related genes under normal conditions is tightly coordinated in order to maintain lipid homeostasis. Unfortunately, in western societies
and in emerging economies a large part of the population suffer from a perturbed lipid homeostasis resulting in unbalanced plasma levels of the different lipoproteins and an increased incidence of coronary events and cardiovascular deaths. Although factors such as high cholesterol consumption and increasing age are important, undesirable lipoprotein profiles are also determined by genetic factors such as dysfunctional genes.

Diabetes mellitus is often linked with abnormal lipid metabolism (Shukla et al., 1995). Higher mortality occurs for type II diabetes mainly due to myocardial infarction. Hence, the aggressive therapy of dyslipidemia is necessary, since the risk of myocardial infarction is the same as in non-diabetic patients with previous myocardial infarction (Haffner et al., 1998). Lipid disorders are common in both insulin dependent and non-insulin dependent diabetes mellitus and are related to the degree of glycemic control. Hyperlipidemia is major risk factor for initiation and progression of cardiovascular disease (The lipid research clinic program, 1981 and national cholesterol education program expert panel, 1996). Accelerated cardiovascular disease is a leading cause of both morbidity and mortality in diabetic patients (Wu and Huan, 2007).

Experimental diabetes in animals is very useful to know the exact changes happening in the physiological and biochemical levels of the diabetic state. Many of this derangement are in the form of significant changes in lipid metabolism and structure (Sochar, 1985). The structural changes are clearly oxidative in nature and are associated with development of vascular disease (Baynes and Thrope, 1999). In diabetes there is inability to store fat and protein along with breakdown of existing fat and protein stores.
Streptozotocin-induced diabetic rats showed significant increases from the levels of cholesterol, phospholipids, and triglycerides and free fatty acids (El-Agouza et al., 2000; Ravi et al., 2005).

The elevation of lipids in plasma leads to the deposition of lipids especially cholesterol on the arterial walls, subcutaneous tissues, tendons and cornea. The important manifestation of hyperlipidemia is due to the accumulation of lipids on arterial walls and resultant pathological changes leading to atherosclerosis (Vasudevan and Sreekumari, 1995). Excess of fatty acids in the serum of diabetic rats are converted into phospholipids and cholesterol in the liver. These two substances along with excess triglycerides formed at the same time in the liver may be discharged into the blood in the form of lipoproteins (Bopanna et al., 1997). Diabetes mellitus is usually associated with abnormal levels of serum lipids and such an increase causes the risk factor for coronary heart diseases (Poonam et al., 2008). NIDDM has also been associated with an increased risk for developing premature atherosclerosis due to increase in triglycerides (TG) and low-density lipoprotein (LDL) levels and decrease in high density lipoprotein levels (HDL) (Bierman, 1992). Hypercholesterolemia and atherosclerosis will increase the probability for myocardial infarction. The risk is still higher with obesity, emotional stress and nicotine (Vasudevan and Sreekumari, 1995).

Obesity contributes to the development of insulin resistance which may underlie a number of the manifestations and cardiovascular complications of diabetes and the metabolic syndrome (Reaven, 1988). Abdominal obesity involves in insulin resistance, a
metabolic abnormality linked to the development of type 2 diabetes mellitus and cardiovascular disease (CVD). Insulin resistance is associated with increased cardiovascular risk and early intervention to treat insulin resistance is an important preventive health strategy (Daniel, 2007). The pathophysiologic mechanisms known to increase CVD risk in individuals with insulin resistance include formation of advanced glycation end products, hypertension, proinflammatory and prothrombotic states and dyslipidemia (i.e., low levels of high density lipoprotein (HDL), increased levels of triglycerides, low density lipoprotein (LDL), apolipoprotein B, and inflammation). The increased flux of free fatty acids from adipose tissue to the liver promotes dyslipidemia. Insulin resistance and impaired glucose tolerance are associated with increased CVD risk. Individuals with coexisting metabolic syndrome and diabetes have the highest prevalence rates of CVD. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death.

Generally, lipids are useful as energy reserves (triglycerides), insulating material to maintain the temperature, constituents of membrane structure (phospholipids and cholesterol), source for fat soluble vitamins (A, D, E and K), cellular metabolic regulators (steroid hormones and prostaglandins) and many other functions in the body. Hypercholesterolemia, hypertriglyceridaemia, platelets hyper aggregativeness and lipoprotein abnormalities are very common in diabetic patients (Jain et al., 2000). The above said problems ultimately lead to atherosclerosis and coronary heart disease (Tada et al., 2005). Hyperlipidemia in diabetic patients consists of elevated triglycerides, LDL
and low HDL cholesterol. Diabetes has a two to four times increased risk for CAD than
general population (Hogan et al., 2003). Saturated fatty acids are non-essential fatty acids
and are harmful if ingested excessively in food. They favour excess weight, insulin
resistance (Folsom et al., 1996), increased LDL-cholesterol and are atherogenic.

The abnormality in lipid level causes many unwanted diseases and current
medications are not appropriate for diabetic condition i.e., prolonged medication leads to
other related diseases. The current allopathic medications for diabetes mellitus only
possess glucose lowering effect. The drug having glucose lowering activity along with
lipid lowering potential is preferable for diabetes mellitus treatment. Most of the herbal
medicines are having potential glucose lowering activity and lipid lowering effect. In the
present study, the anti-hyperlipidemic activity of *N. crenulata* extract was evaluated in the
streptozotocin induced diabetic rats.

**MATERIALS AND METHODS**

**Extraction of lipids**

The method of Folch *et al.* (1957) was adopted for lipid extraction. A known
volume of tissue homogenate was homogenized with three or four times of cold methanol
extracts were filtered in Whatman No. 1 filter paper and the residue on the filter paper
was scrapped off and then it was homogenized in chloroform with twice the volume of
methanol. The residue was again homogenized in chloroform methanol 2:1 v/v mixture
and the extract was filtered. The lower chloroform layer containing lipids was evaporated
to dryness and finally suspended in a known volume of chloroform-methanol mixture
(2:1 v/v). To the lipid extract, 1 ml of 0.1 M potassium chloride was added, shaken well and centrifuged. The chloroform layer obtained was mixed with chloroform: methanol: potassium mixture (10:10:1 v/v). The washed layer was made up to known volume with and aliquots that were used for analysis of lipids.

**Total cholesterol**

Total cholesterol was estimated by the method of Parekh and Jung (1970).

**Reagents**

1. Ferric chloride-uranyl acetate:

   10 ml of water and 3 ml of concentrated ammonia was added to 500 mg of ferric chloride. The precipitate obtained was washed several times with distilled water, dissolved in glacial acetic acid and made up to 1 litre with acetic acid. 100 mg of uranyl acetate was then added and the contents were shaken well and left over night in a brown bottle.

2. Ferrous sulphate and sulphuric acid reagent:

   100 mg of anhydrous ferrous sulphate in 100 ml of glacial acetic acid was made upto 1 litre with concentrated sulphuric acid.

3. Cholesterol standard: 2mg/ml chloroform.

**Procedure**

A known volume of the serum/lipid extract was added to 10 ml of ferric acetate-uranyl acetate reagent. The mixture was kept for 5 minutes and centrifuged at 3000 rpm for 5 minutes and 2 ml of ferrous sulphate-sulphuric acid reagent was added to 3.0 ml
aliquot of the supernatant. After 20 minutes, the color was read at 540 nm along with a series of standard cholesterol solutions 925-100 μg and a blank containing the reagent processed in a similar manner. Values were expressed as mg/dl for serum and mg/g for liver.

**Phospholipids**

Phospholipids were estimated by the method of Zilversmith and Davis (1950). The organic phospholipids phosphorus is converted to inorganic phosphorus which reacts with ammonium molybdate to form phosphomolybdic acid. This is on reduction with aminonapthanol sulfonic acid forms a stable blue color.

**Reagents**

1. Concentrated sulphuric acid
2. Concentrated nitric acid.
3. Ammonium molybdate: 2.5% in 5N H₂SO₄.
4. 1-amino 2-naphthol 4-sulphuric acid (ANSA): 500 mg ANSA was dissolved in a mixture of 195 ml 15% sodium bisulphate and 5 ml of 20% sodium sulphite solution. This solution was stored in a brown bottle.
5. Standard phosphorus solution: 35.1 mg of potassium dihydrogen phosphate was dissolved in water. To this 1 ml of 10 N sulphuric acid was added and made upto 100 ml with distilled water. 10 ml of this solution was diluted to 100 ml, to prepare a working standard containing 8μg phosphorus/ml.
Procedure

0.1 ml of serum/tissue homogenate was mixed with 1.9 ml of distilled water and 1.5 ml of 10% TCA. The precipitated proteins were sedimented by centrifugation. The supernatant was discarded. 1 ml of concentrated nitric acid and 1 ml of concentrated sulphuric acid was added to the residue and digested on a sand bath till the solution become colorless. In liver homogenate, a known amount of the lipid extract was digested with 1.0 ml of 70% perchloric acid over a sand bath with until the sample become colorless and clear. After cooling, the volume was made upto 5 ml with water. Standard solution of phosphorus in the range of 8-32 µg and a blank with double distilled water were also prepared. Then 1 ml of ammonium molybdate solution was added, followed by 0.4 ml of ANSA reagent. The absorbance was read at 680 nm after 5 minutes. Phospholipids level was expressed as mg/dl for serum and mg/g for liver and kidney after multiplying by the factor 25 (Scheig factor).

Triglycerides

Triglycerides were estimated by the method of Foster and Dunn (1973). The triglycerides were extracted by isopropanol which upon saponification with potassium hydroxide, yields glycerol and soap. The glycerol liberated is treated with metaperiodate which release formaldehyde, formic acid and iodide. The formaldehyde released is reacts with acetylacetone and ammonia forming yellow colored compound which is read in a spectronic 20 at 45 nm.
Reagents

1. Isopropanol

2. Activated alumina (neutral)

3. Saponification agent: 5.0 g of potassium hydroxide was dissolved in 60 ml distilled water and 40 ml isopropanol was added to it.

4. Acetyl acetone reagent: 0.75 ml of acetyl was dissolved in 60 ml distilled water and 40 ml isopropanol was added to it.

5. Sodium metaperiodate reagent: 77 g of anhydrous ammonium acetate was dissolved in about 700 ml of distilled water 60 ml glacial acetic acid was added to it followed by 650 mg sodium metaperiodate. The mixture was dissolved and diluted to one litre with distilled water.

6. Standard solution of triolein: 1.0 g of triolein was dissolved in 100 ml isopropanol. 1.0 ml of stock standard was diluted to 100 ml to prepare working standard containing 100 µg of triolein/ml.

Procedure

To an aliquot of serum/lipid extract, evaporated to dryness 0.1 ml methonal was followed by 4.0 ml isopropanol, 4.0 g of alumina was added to all the tubes and shaken well for 15 minutes, centrifuged and then 2 ml of the supernatant fluid was transferred to appropriately labeled tubes. The tubes were placed in a water bath at 65 °C about 15 minutes for saponification after adding 0.6 ml of the saponification regent followed by 0.5 ml acetyl acetone reagent. After mixing, the tubes were kept in a water bath at 65 °C
for an hour. The contents were cooled and the absorbance was read in a spectronic 20 at 420 nm. A series of standards of concentration 8 to 40 µg triolein were treated similarly along with a blank containing only the reagent. All the tubes were cooled and read in a spectronic 20 at 405 nm. The triglyceride content was expressed as mg/dl for serum and mg/g for liver and kidney.

**HDL**

The HDL cholesterol was estimated by the heparin-manganese chloride precipitation method of Gidez and Webb (1950).

**Reagents**

1. Heparin
2. Manganese chloride
3. Heparin-manganese chloride: 3.1167 g manganese chloride and 1.0 ml heparin made up to 8.0 ml with distilled water.

**Procedure**

To 1.0 ml of serum, 0.18 ml of heparin manganese chloride reagent was added and mixed. After standing in an ice bath for 30 minutes, the contents were centrifuged at 2500 g for 30 minutes to get the HDL fraction. An aliquot of the HDL supernatant was used for cholesterol estimation by the method of Parekh and Jung (1970). HDL cholesterol is expressed as mg/dl serum.

**LDL**

LDL was calculated by using the formula of Friedewald *et al.*, 1972.
LDL was calculated by using the formula,

\[
LDL = \text{Total Cholesterol} - (\text{HDL} + \text{VLDL}).
\]

**Atherogenic Index (AI) and Coronary Risk Index (CRI)**

AI and CRI were calculated by using the formula of Abbott *et al.*, (1988) and Alladi and Shanmugasundaram (1989), respectively.

\[
\text{Atherogenic Index (AI)} = \frac{\text{LDL} - \text{Cholesterol}}{\text{HDL} - \text{Cholesterol}}
\]

\[
\text{Coronary Risk Index (CRI)} = \frac{\text{Total Cholesterol}}{\text{HDL} - \text{Cholesterol}}
\]

**RESULTS**

Table 3. shows the serum cholesterol, triglycerides, HDL, LDL and phospholipids levels of control and streptozotocin induced diabetic rats.

**TOTAL CHOLESTEROL IN SERUM**

There was a significant elevation in total cholesterol level in serum observed in diabetic induced rats 130.39±7.73 mg/dl when comparing with control rats which was found 79.10±5.25 mg/dl. It was found reducing level in serum tissues treated with
N.crenulata extract of 600mg/kg 88.19±5.15 mg/dl (Table.3) which is highly significant when compared to the rats treated with glibenclamide 80.49±5.55 mg/dl. (Fig.10).

TRIGLYCERIDES IN SERUM

The levels of triglycerides were found very high in serum of diabetic induced 155.29±8.18 mg/dl (Table.3) as compared to that of normal control 70.09±4.14 mg/dl. It was found reducing incase of diabetic induced treated with N.crenulata extract 125.49±6.26 mg/dl which is slightly high as compared to that of glibenclamide treated rats 115.09±6.16 mg/dl (Fig. 11).

HIGH DENSITY LIPOPROTEIN IN SERUM

Vigorous decreases in HDL were found in diabetic induced 30.04±1.51 mg/dl when compared to that of normal control rat’s 39.39±2.72 mg/dl. This significant decrease in diabetic rats were found recovering when treated with N.crenulata extract 35.39±1.31 mg/dl which were found very near to that of glibenclamide treated rats 38.10±1.21 mg/dl (Fig. 12).

LOW DENSITY LIPOPROTEIN and PHOSPHOLIPIDS

Similarly, LDL were significantly increased in diabetic induced treated with streptozotocin 68.10±5.25 mg/dl and reduction was found in diabetic induced rats when treated with N.crenulata extract 42.20±3.23 mg/dl slightly higher than that of glibenclamide treated 35.50±2.82 mg/dl (Fig. 13). Phospholipids significantly increases on streptozotcin diabetic induced rats 159.26±7.09 mg/dl and there was a lowering in diabetic rats treated with N.crenulata extract 135.29±6.26 mg/dl (Fig. 14)
Table 4 shows the Liver cholesterol, triglycerides and phospholipids levels of control and streptozotocin induced diabetic rats.

**TOTAL CHOLESTEROL IN LIVER**

Total cholesterol level in liver of diabetic induced rats 8.14±0.1 mg/g when comparing with control rats which was found 3.88±0.14 mg/g. It was found reducing in diabetic rats treated with *N. crenulata* extract 4.12±0.1 mg/g and also in lesser in diabetic rats treated with glibenclamide 4.08±0.8 mg/g (Fig. 15).

**TRIGLYCERIDES IN LIVER**

The level of triglycerides in diabetic induced 18.41±0.4 mg/g as compared to that of normal control 9.01±1.4 mg/g. It was found reducing incase of diabetic induced treated with *N. crenulata* extract 9.1±0.4 mg/g which is slightly high as compared to that of glibenclamide treated rats 9.22±0.7 mg/g (Fig. 16).

**PHOSPHOLIPIDS IN LIVER**

Phospholipids level in diabetic induced rats is 25.47±1.6 mg/g whereas in normal liver it is 11.41±0.10 mg/g. *N. crenulata* methanolic extract treated group showed 12.74±2.1 mg/g and glibenclamide treated group exhibited 11.06±0.6 mg/g (Fig. 17).

Table 5 shows the atherogenic index and coronary index of serum and total cholesterol triglycerides of liver tissues.
ARTHEROGENIC INDEX

Artherogenic index in control rats were ranging 0.62±0.04 and it was found quite high in diabetic induced 2.10±0.22 and the same was found reducing in diabetic rates treated with *N. crenulata* extract 1.44±0.16 (Fig. 18).

CORONARY RISK INDEX

Coronary risk indexes in diabetic induced were quite higher 4.25±0.4 and also there was a contrary change in group treated with *N. crenulata* extract 2.89±0.28 and 3.41±0.3 respectively (Fig.19).
Table 3. Total Cholesterol (serum), Triglycerides (serum), HDL (serum), LDL (serum) and Phospholipids (serum) of normal and experimental animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>Phospholipids (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>79.10±5.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.09±4.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.39±2.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.49±3.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>115.24±7.57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic</td>
<td>130.39±7.73&lt;sup&gt;d&lt;/sup&gt;</td>
<td>155.29±8.18&lt;sup&gt;e&lt;/sup&gt;</td>
<td>30.04±1.51&lt;sup&gt;d&lt;/sup&gt;</td>
<td>68.10±5.25&lt;sup&gt;e&lt;/sup&gt;</td>
<td>159.26±7.09&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>N. crenulata</em> (M)/300</td>
<td>97.99±6.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>138.89±7.37&lt;sup&gt;d&lt;/sup&gt;</td>
<td>33.5±1.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58.10±5.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>145.10±7.27&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td><em>N. crenulata</em> (M)/600</td>
<td>88.19±5.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>125.49±6.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.39±1.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>42.20±3.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>135.29±6.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>N. crenulata</em> (A)/300</td>
<td>105.32±2.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>140.26±7.37&lt;sup&gt;d&lt;/sup&gt;</td>
<td>32.9±0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60.12±2.21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>149.30±2.67&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>N. crenulata</em> (A)/600</td>
<td>94.36±2.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>131.45±2.37&lt;sup&gt;d&lt;/sup&gt;</td>
<td>33.9±3.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56.24±1.68&lt;sup&gt;d&lt;/sup&gt;</td>
<td>141.22±1.24&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>80.49±5.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>115.09±6.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.10±1.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.50±2.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>130.09±6.16&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. 10. Effect of *N. crenulata* extract on Total serum cholesterol level of diabetic induced rats

Fig. 11. Effect of *N. crenulata* extract on Total serum triglycerides level of diabetic induced rats
Fig. 12. Effect of *N. crenulata* extract on Total serum HDL level of diabetic induced rats

Fig. 13. Effect of *N. crenulata* extract on Total serum LDL level of diabetic induced rats
Fig. 14. Effect of *N. crenulata* extract on Total serum phospholipids of diabetic induced rats

Table 4. Total Cholesterol (Liver), Triglycerides (Liver), Phospholipids (Liver) in normal and experimental animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Cholesterol (mg/g)</th>
<th>Triglycerides (mg/g)</th>
<th>Phospholipids (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.88±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.01±1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.41±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic</td>
<td>8.14±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.41±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.47±1.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<td><em>N. crenulata</em> (M)/300</td>
<td>4.89±7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.26±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.69±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td><em>N. crenulata</em> (M)/600</td>
<td>4.12±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.1±0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.74±2.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><em>N. crenulata</em> (A)/300</td>
<td>7.10±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>24.78±2.8&lt;sup&gt;b&lt;/sup&gt;</td>
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<td><em>N. crenulata</em> (A)/600</td>
<td>6.38±1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.87±1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.74±0.8&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Glibenclamide</td>
<td>4.08±0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.22±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.06±0.6&lt;sup&gt;b&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. 15. Effect of *N. crenulata* extract on Liver cholesterol level of diabetic induced rats

Fig. 16. Effect of *N. crenulata* extract on Liver triglycerides level of diabetic induced rats
Fig. 17. Effect of *N. crenulata* extract on Liver phospholipid level of diabetic induced rats

Table 5. Atherogenic index (serum), Coronary Risk index (serum) of normal and experimental animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Artherogenic index</th>
<th>Coronary Risk index</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.62±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.01±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic</td>
<td>2.10±0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.25±0.4&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>N. crenulata</em> M/300</td>
<td>1.50±0.13&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.41±0.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>N. crenulata</em> M/600</td>
<td>1.44±0.16&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.89±0.28&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>N. crenulata</em> A/300</td>
<td>1.80±0.13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.98±1.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>N. crenulata</em> A/600</td>
<td>1.72±0.24&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.41±2.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>1.25±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.85±0.3&lt;sup&gt;b&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. 18. Effect of *N.crenulata* extract on Total serum Artherogenic index of diabetic induced rats

Fig. 19. Effect of *N.crenulata* extract on Total serum coronary risk index of diabetic induced rats
DISCUSSION

Diabetes is associated with a high risk of cardiovascular disease (CVD). The management of diabetic dyslipidemia, a well-recognized and modifiable risk factor, is a key element in the multifactorial approach to prevent CVD in individuals with type 2 diabetes. Type 2 diabetes is associated with a marked increased risk of cardiovascular disease (CVD). Individuals with diabetes have an absolute risk of major coronary events similar to that of nondiabetic individuals with established coronary heart disease (CHD) (Haffner et al., 1998).

The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase. On the other hand glucagon catecholamines and other hormones enhance lipolysis. Diabetes induced hyperlipidemia led to access mobilization of fat from the adipose due to under utilization of glucose (Krishna kumar et al., 2000). In the present study, anti-hyperlipidemic activities of *N.crenulata* extract have been evaluated in control and streptozotocin induced diabetic rats. Cholesterol is a sterol useful in cell membrane integrity and precursor for steroid hormones. TCL is increased in diabetic group when compared with control rats. Diabetes mellitus leads with impaired carbohydrate metabolism and increased lipolysis causing accumulation of acetyl co A. Increased availability of acetyl co-A leads to synthesis of cholesterol which causes hyperlipidemia. The increased total cholesterol level was restored in the diabetic rats treated with *N.crenulata* extract and glibenclamide. Insulin deficiency results with hypercholesterolemia.
due to metabolic abnormalities (Muralie et al., 2002). The level of serum lipids has been increased by lipolysis due to insulin deficiency in diabetic rats (Shirwaikar et al., 2004). Usually, insulin increases the lipogenesis and decrease lipolysis and ketogenesis. But in diabetic condition, insulin deficiency reverses the above said role in lipid metabolism.

Triglycerides are neutral fats, major energy reserve for the body stored at adipose tissue diabetic condition increase the lipolysis and produce more free fatty acids. Normally, insulin activates the enzyme lipoprotein lipase which hydrolyses triglycerides. But in diabetic state, lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia (Pushparaj et al., 2007). Phospholipids are rich in PUFAs which are very susceptible for super oxide anion and hydroxyl radicals (Ahmed et al., 2001). Significant elevation of serum, liver and kidney phospholipid levels were observed in the present investigation. The elevation of phospholipid level in diabetic group was well controlled by the N.crenulata extract treatment. The maintenance of phospholipid level in N.crenulata extract treated diabetic rats may be due to the good glycemic control, decreased triglyceride level and decreased total cholesterol. The same result was reported by the previous investigator (Jain et al., 2000).

Since lipid abnormalities’ accompanying with premature atherosclerosis is the major accuse of cardiovascular diseases in diabetic patients, therefore ideal treatment for diabetes, in addition to glycemic control, should have a favorable effect on lipid profile. Cardiovascular diseases are listed as the cause of death in 65% people suffering from diabetes (Kesari, 2007; Kumar et al., 2010; Osadebe et al., 2010; Kumar et al., 2011; Patil
et al., 2011). Diabetes is often associated with dyslipidemia, insulin resistance, and hypertension. Together, these metabolic perturbations greatly increase the risk of developing cardiovascular disease and diabetes. In the present investigation *N.crenulata* extract treated groups for 30 days showed renounced down regulating effect of cholesterol level which includes LDC and Triglycerides to considerable range. It showed positive effect in increasing HDL level with beneficiary effect in diabetic rats. Even the glibenclamide drug has not pronounced the consecutive effect in lowering cholesterol index when compared to polyherbal treated groups. Previous experiments on fish oil supplementation diabetic animals exerted a hypolipidemic effect (lower plasma TG, TC, and NEFA); however, in obese mice, fish oil feeding improved insulin sensitivity (Saraswathi et al., 2009).

LDL transports cholesterol from liver to other peripheral tissues. Increased fatty acid level in the blood stream of diabetic rats reduced by streptozotocin was converted to phospholipids and cholesterol by the liver. Cholesterol and phospholipids along with excess triglycerides are formed as lipoproteins in the liver may be discharged in to blood (Taskinen, 2003).

HDL is commonly called as good cholesterol due to its role in prevention of atherosclerosis by transporting the cholesterol from peripheral tissues to liver for excretion. The decrease in HDL level was observed in present study in diabetic rats which will increase the chance of atherosclerosis and was restored in *N.crenulata* extract administered animals. Reduction in HDL cholesterol level has been well documented in diabetes mellitus (Jonas, 1991). The raised total cholesterol and LDL concentrations are having negative
correlation with HDL (Mayne, 1996). Increase in HDL cholesterol level is associated with a decrease in coronary risk (Santosh et al., 2007).

Atherogenic index (AI) and coronary risk index (CRI) also are very high in streptozotocin induced diabetic rats. Lipoproteins have the major role in the occurrence of premature atherosclerosis in diabetic patients (Balamurgan et al., 2009). Increased glycation of apolipoproteins contributes to the increased atherogenecity of LDL cholesterol. Glycoxidised LDL binds more readily to matrix protein that infiltrates in to the vascular wall (Heart protection study collaborative group, 2002). High levels of TC and TG are major risk factors for atherosclerosis and coronary risk (Chait and Brunzell, 1996). Significant lowering of total cholesterol and rise in HDL cholesterol is very desirable state for prevention of atherosclerosis and ischaemic condition (Luc and Fruchart, 1991) which is attained in the present study after the treatment of diabetic rats with the combined formulation of *N.crenulata* extract up to a great extent.

**SUMMARY AND CONCLUSION**

Diabetes is an increasing risk factor in the present days which affect millions of people’s worldwide. Treatment and medication of this disease with cheap rate of drugs is essential nowadays. From the present findings, it is well documented the *N.crenulata* extract is more appropriate and have the potential to counteract the hyperlipidemic condition in diabetic animals which is being considered as a silent killer to the humanity. Important biomarkers of hyperlipidemic etiology like triglycerides, and total cholesterol were tentatively reduced after the treatment of *N.crenulata* extract in the diabetic rats.
In the mean time phospholipids level in kidney, Liver and pancreas tentatively reduced after the drug treated groups rather than control groups. Potentially hazardous VLDL cholesterol in the serum is drastically reduced to characteristic range which is good hallmark of anti-hyperlipidimic effect of the plant extract.

Methanolic extract of *N.crenulata* seem to have immense anti hyperlipidimic effect in joint venture with anti diabetic effect. *N.crenulata* was used to treat diabetes and lipidosis since time immemorial in traditional medicine of ayurveda and siddha. It is clear evident that the ethno medicine mode of practice of *N.crenulata* extract as drug formulation is advisable form to treat hyperlipidimic and diabetic in experimental model. Sill more clinical mode of experiment in higher animal model and individual compound isolation is necessary to progress towards drug discovery related orientation.