Chapter 8:
Therapeutic studies of the leaf extract of
*Eurya japonica* Thunb. and *Ficus auriculata* Lour. for their antidiabetic properties on the streptozotocin induced diabetic albino mice.
CHAPTER 8:

THERAPEUTIC STUDIES OF THE LEAF EXTRACT OF EURYA JAPONICA THUNB. AND FICUS AURICULATA LOUR. FOR THEIR ANTIDIABETIC PROPERTIES ON STREPTOZOTOCIN INDUCED DIABETIC ALBINO MICE.

8.1: Introduction:
Diabetes mellitus (DM) is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level (Debra et al., 1991). This disorder occurs worldwide and its occurrence is increasing at a faster rate in most of the countries (Siddharth, 2001). Diabetes mellitus is the sixth leading cause of death globally (Nash et al., 2001). Conventionally, type I diabetes is managed with exogenous insulin and type 2 with oral hypoglycemic agents (sulphonylureas, biguanides etc), but have side effects associated with their uses (Valiathan, 1998). Thus searching for a new class of compounds is essential to overcome diabetes related problems (Noor et al., 2008).

In traditional practice, medicinal plants are used in many countries to control diabetes mellitus. DM has recently been identified by Indian Council of Medical Research (ICMR) as one of the refractory diseases for which satisfactory treatment is not available in modern allopathic system of medicine and suitable herbal preparations are suggested to be investigated (Verma et al., 2010). Therefore, there is continuous search for alternative drugs (Hansotia and Drucker, 2005). The medicinal plants may provide the useful source of new oral hypoglycemic compounds for the development of pharmaceutical entities or as dietary adjunct to existing therapies (Pepato et al., 2005; Sharma et al., 2006; Tanaka et al., 2006). Furthermore, even the World Health Organization (WHO) approves the use of plant based drugs for the treatment of diabetes mellitus. Investigation on hypoglycemic agents from medicinal plants has become more important. The ethnobotanical information/reports state that about 800 plants may possess antidiabetic potential (Aguilera et al., 2008). Recently the medicinal values of various plant extracts have been studied by many scientists in the field of diabetic research (Noor et al.; 2008; Daisy and Eliza 2007).
In the present study an attempt has been made to investigate the blood sugar lowering activity and antihyperlipidemic activities of the methanol leaf extract of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. in normal and diabetes induced mice to confirm their ethnobotanical use.
8.2 Materials and methods:

8.2.1 Plant Materials:
The powdered leaves of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. plant (100g) were weighed and subjected to soxhlation with methanol for 72 hrs. The excess solvents were distilled off at lower temperature under reduced pressure in the rotary evaporator and concentrated to dryness. The obtained crude extracts were stored in airtight container in refrigerator for further studies.

8.2.2 Experimental animals:
Adult swiss albino mice weighing about 20-30 g each were used in the present investigation. All the experimental mice were given a period of acclimatization for 15 days, before starting the experiment. They were fed with standard food pellet and water *ad libitum*. Animals were housed in a temperature (25°±1°C), humidity controlled room and a 12 hours light- dark cycle. All the experimental procedures are done as per the animal research guidelines of the care and use of laboratory animals and were approved by the Ethical Committee of the Assam University, Silchar.

8.2.3 Acute toxicity study:
Acute oral toxicity test was performed as per OECD-423 guidelines (OECD, 2001). All the animals were randomly distributed into one control group (I) and three treated groups, containing five animals in each group. Group II, III and IV were orally administered 1000 mg/kg, 2000 mg/kg and 3000 mg/kg body weight of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. The control group received distilled water alone. The animals were observed continuously for first 24 hours and then continued for 7 days to observe any signs of behavioral changes, toxicity, mortality and change in body weight etc.

The number of mice died within 24 hours and then upto 7days after exposure was observed and noted and their LD$_{50}$ of the extract was calculated using the arithmetic method of Karber as modified by Aliu and Nwude (1982) as shown below:

\[
\text{LD}_{50} = \frac{\text{Least dose that killed all the animals} - \text{Sum of (Dose difference X Mean dose)/ Number of animals.}}}{\text{Number of animals.}}
\]

8.2.4 Antidiabetic activity:

8.2.4a Induction of diabetes:
Streptozotocin (STZ) was freshly dissolved in 0.1 M citrate buffer (pH = 4.5) at the dose of 70 mg/kg body weight and injected intraperitoneally within 15 min of dissolution in a vehicle volume of 0.3 ml whereas normal control group was given
citrate buffer only (0.4 ml). Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of streptozotocin. A rest period of two days was allowed for the blood glucose level to stabilize. During this period the animals used to have free access to both water and food. The hyperglycemic mice (blood glucose level > 200 mg/dl) were separated and divided into different groups comprising of 6 mice in each group for the anti-diabetic study (Hepcy et al., 2012).

8.2.4b Glibenclamide:
Single dose of glibenclamide provokes a brisk release of insulin from the pancreas. It acts on β-cell membrane leading to enhance calcium flux across it, causing degranulation. After chronic administration the insulinemic action of glibenclamide declined, but improvement in glucose tolerance is maintained. Thus it is an oral antidiabetic preparation with an efficient hypoglycemic action (Prasad et al., 2009). Daonil (glibenclamide) manufactured by Aventis Pharma Ltd. Goa, India was collected from the market and preserved at room temperature.

8.2.4c The various groups of mice used in the experiments:
Mice were divided into the following groups.

**Group I:** Consisted of 6 mice which served as normal control and were given only distilled water, daily.

**Group II:** Consisted of 6 STZ induced diabetic mice and served as diabetic control and were given distilled water only.

**Group III:** Consisted of 6 STZ induced diabetic mice and were daily treated orally with methanol extract of *Eurya japonica* Thunb. (EJ) leaves at the dose of 300 mg/kg body weight for 15 days, once a day.

**Group IV:** Consisted of 6 STZ induced diabetic mice and were daily treated orally with methanol extract of *Eurya japonica* Thunb. (EJ) leaves at the dose of 600 mg/kg body weight for 15 days, once a day.

**Group V:** Consisted of 6 STZ induced diabetic mice and were daily treated orally with methanol extract of *Ficus auriculata* Lour. (FA) leaves at the dose of 300 mg/kg body weight for 15 days, once a day.

**Group V:** Consisted of 6 STZ induced diabetic mice and were daily treated orally with methanol extract of *Ficus auriculata* Lour. (FA) leaves at the dose of 600 mg/kg body weight for 15 days, once a day.
Group VII: Consisted of 6 STZ induced diabetic mice and were daily given Glibenclamide at the dose of 10 mg/kg body weight for 15 days, once a day.

After 15 days of herbal treatment the experiment was terminated and experimental observations were made. Body weights were estimated on 0th and 15th day of the treatment. Blood was collected from the tail for glucose estimation just before drug administration on the first day and 1 h after sample administration on days 5, 10, and 15th day. The animals were sacrificed after blood collection under chloroform anesthesia on the 15th day and pancreas, liver and kidney were harvested for histopathological studies. Blood samples collected were centrifuged to separate serum for estimation of serum protein and lipid profile. Total cholesterol, HDL-Cholesterol, LDL-Cholesterol, VLDL-Cholesterol, and triglycerides were analyzed from the serum.

8.2.4d Biochemical analysis:

Blood glucose was estimated by the Glucocard 01-mini blood glucose monitoring kit. Serum protein was estimated by Lowry’s method. Total cholesterol (TC), Triglycerides (TG) and HDL were estimated by Crest Biosystem cholesterol kit (CHOD/PAP method), Crest Biosystem Triglycerides kit (GPO/PAP method) and Crest Biosystem HDL Cholesterol kit (PEG precipitation method). For determination of VLDL (Very Low Density Lipoprotein) and LDL (Low Density Lipoprotein) Friedewald’s formula was used (Friedewald et al., 1972). LDL cholesterol was calculated using the formula,

\[
LDL \text{ cholesterol} = \text{Total cholesterol} - \text{HDL} + \left(\frac{TG}{5}\right)
\]

and VLDL cholesterol was calculated using the formula

\[
VLDL \text{ cholesterol} = \left(\frac{TG}{5}\right)
\]

8.2.4e Histopathological study:

Pancreas, liver and kidney were harvested and preserved in 10% neutral formalin fixative solution for histopathological examination. After fixation the tissues were embedded in paraffin, solid sections were cut at 5µm and sections were stained with haematoxylin and eosin (Strate et al., 2005).

8.2.4f Statistical analysis:

The data are expressed as mean ± SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Post hoc multiple comparisons test (Tukey Test). The results were considered statistically significant if the \(^a\) P values were 0.001 or less and \(^b\) P values were 0.05 or less.
8.3 Results and Discussion:

8.3.1 Acute Toxicity:

Experimental screening method is imperative in order to establish the safety and efficacy of traditional and herbal products and also to set up the active components of the herbal products (Mythilypriya et al., 2007). In the acute toxicity test of the methanol leaf extracts of *Eurya japonica* Thunb. and *Ficus auriculata* Lour., there was no mortality or any sign of behavioural changes or toxicity observed at the dose of 1000 mg/kg, 2000 mg/kg and 3000 mg/kg body weight in mice. The calculated LD$_{50}$ was found to be 3000 mg/kg b.w (Table 8.3.1).

There was an increase in the body weight of treated groups (Figure 8.3.1a & b) compared to the control group of mice for the acute toxicity test of the leaf extracts of *Eurya japonica* Thunb. and *Ficus auriculata* Lour.

Table 8.3.1: LD$_{50}$ of the leaf extracts of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. as Calculated by arithmetic method of Karber Groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Mice</th>
<th>Dose of extract (mg/kg)</th>
<th>Number of dead Mice</th>
<th>Dose Difference</th>
<th>Mean death (Md)</th>
<th>Dose difference X Md</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>1000</td>
<td>0</td>
<td>1000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>2000</td>
<td>0</td>
<td>1000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>3000</td>
<td>0</td>
<td>1000</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

LD$_{50}$ = Maximum dose – Sum of (Dose difference X Mean dose)/ Number of animals.

LD$_{50}$ = 3000mg/kg.
Figure 8.3.1a: Changes in body weight of control and treated groups of mice for the acute toxicity test of the leaf extract of *Eurya japonica* Thunb.

Figure 8.3.1b: Changes in body weight of control and treated groups of mice for the acute toxicity test of the leaf extract of *Ficus auriculata* Lour.
8.3.2 Blood glucose level:

A significant reduction in blood glucose level after administration of methanol extract of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. (300 mg/kg b.w. & 600 mg/kg b.w.) in the STZ induced diabetic mice on 5th, 10th and 15th days treatment were observed. In STZ induced diabetic control group, the blood glucose level increased from 255 to 362mg/dl during the experimental observation period. The blood glucose level in STZ induced diabetic mice treated with glibenclamide and *Eurya japonica* Thunb. and *Ficus auriculata* Lour. (300 mg/kg b.w, 600 mg/kg b.w) decreased from 249.5 mg/dl, 276.75 mg/dl, 266.0 mg/dl, 279.0 mg/dl, 275.5 mg/dl to 97.75 mg/dl, 120.25 mg/dl, 104.25 mg/dl, 140.25 mg/dl, 136.75 mg/dl respectively. Significant decrease (p<0.001) in blood glucose level were observed in the standard and extract treatment when compared to diabetic control. The methanol leaf extract of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. showed a dose dependent reduction in blood glucose level and this hypoglycemic effect were comparable with that of standard oral hypoglycemic agent, glibenclamide. The hypoglycemic effect of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. at 600 mg/kg b.w showed 60.80% fall and 59.36% fall in the blood glucose level on the 15th day as compared to 0th day which were found to be closely comparable with that of glibenclamide treatment, at 10mg/kg b.w (60.82%) (Table 8.3.2). There was an increase in body weight of the mice in the standard and extract treated groups of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. as compared to STZ induced diabetic control mice.
Table 8.3.2: Effects of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. methanolic leaf extracts on the body weight and blood glucose level in the normal and STZ induced diabetic mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial body weight (g) (0\textsuperscript{th} day)</th>
<th>Final body weight (g) (15\textsuperscript{th} day)</th>
<th>Blood glucose concentration (mg/dl)</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0\textsuperscript{th} day</td>
<td>5\textsuperscript{th} day</td>
</tr>
<tr>
<td>Group I</td>
<td>Normal</td>
<td>20.55±0.08</td>
<td>23.81±1.0</td>
<td>83±5.1</td>
<td>75.75±8.7</td>
</tr>
<tr>
<td>Group II</td>
<td>Diabetic control</td>
<td>26.57±0.8</td>
<td>24.07±2.0</td>
<td>255±10.8</td>
<td>306.75±12.7</td>
</tr>
<tr>
<td>Group III</td>
<td>Glibenclamide standard</td>
<td>22.85±0.2</td>
<td>24.14±1.0</td>
<td>249.5±4.1</td>
<td>210.5±13.6</td>
</tr>
<tr>
<td>Group IV</td>
<td>Methanolic extract (EJ) 300mg/kg body weight</td>
<td>25.72±1.5</td>
<td>23.90±0.7</td>
<td>276.75±6.0</td>
<td>153.25±11.7</td>
</tr>
<tr>
<td>Group V</td>
<td>Methanolic extract (EJ) 600mg/kg body weight</td>
<td>24.63±1.9</td>
<td>24.43±1.6</td>
<td>266±18.1</td>
<td>175.5±11.9</td>
</tr>
<tr>
<td>Group VI</td>
<td>Methanolic extract (FA) 300mg/kg body weight</td>
<td>28.97±0.4</td>
<td>26.84±0.3</td>
<td>279±5.0</td>
<td>250.25±11.1</td>
</tr>
<tr>
<td>Group VII</td>
<td>Methanolic extract (FA) 600mg/kg body weight</td>
<td>27.42±1.8</td>
<td>26.89±1.5</td>
<td>275.5±7.2</td>
<td>217.75±3.1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM n= 6, \textsuperscript{a} P values<0.001 and \textsuperscript{b} P values <0.05 when compared to diabetic control. % Reduction in blood glucose level as compared to 0\textsuperscript{th} day.
8.3.3 Biochemical parameters:

In the STZ-induced diabetic mice, the rise in blood glucose was accompanied by an increase in the serum cholesterol, TG, LDL, VLDL and the decrease in serum protein and HDL level, whereas the treatment with glibenclamide standard and methanol leaf extract of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. reduced cholesterol, TG, LDL, VLDL and increased serum protein with improved HDL were observed in the diabetic induced mice (Table 8.3.3).

Table 8.3.3: Antihyperlipidemic effects of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. methanolic extracts in STZ-induced diabetic mice.

<table>
<thead>
<tr>
<th>Treatment (mg/kg body weight)</th>
<th>Serum protein (g/ml)</th>
<th>Serum cholesterol (mg/dl)</th>
<th>Serum triglycerides (mg/dl)</th>
<th>Serum HDL (mg/dl)</th>
<th>Serum LDL (mg/dl)</th>
<th>Serum VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>6.27±0.42^a</td>
<td>102.88±3.45</td>
<td>75.33±2.90^a</td>
<td>31.62±2.03^a</td>
<td>58.55±1.66^a</td>
<td>15±0.57^b</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>3.98±0.32</td>
<td>281.17±53.04</td>
<td>168.20±30.34</td>
<td>16.49±2.17</td>
<td>185.35±34.67</td>
<td>33.63±6.06</td>
</tr>
<tr>
<td>Glibenclamide standard</td>
<td>5.27±0.25^b</td>
<td>126.09±2.80</td>
<td>93.42±4.89^b</td>
<td>62.66±1.06</td>
<td>82.11±3.24^a</td>
<td>18.68±0.98^b</td>
</tr>
<tr>
<td>Methanolic extract (FA) 300mg/kg body weight</td>
<td>4.8±0.26</td>
<td>167.07±11.77</td>
<td>106.99±12.42^b</td>
<td>25.78±3.34</td>
<td>102.60±16.66^b</td>
<td>21.39±2.47^b</td>
</tr>
<tr>
<td>Methanolic extract (FA) 600mg/kg body weight</td>
<td>4.96±0.15</td>
<td>168.58±5.12</td>
<td>117.48±6.69</td>
<td>30.06±1.93^b</td>
<td>110.92±8.13^b</td>
<td>23.49±1.33</td>
</tr>
<tr>
<td>Methanolic extract (EJ) 300mg/kg body weight</td>
<td>5.08±0.17</td>
<td>141.91±12.04</td>
<td>105.29±3.64^b</td>
<td>34.94±3.62^b</td>
<td>91.41±4.38^b</td>
<td>21.05±0.73^b</td>
</tr>
<tr>
<td>Methanolic extract (EJ) 600mg/kg body weight</td>
<td>5.71±0.61^b</td>
<td>155.32±10.13</td>
<td>110±2.07</td>
<td>43.48±3.30^a</td>
<td>88.52±4.57^b</td>
<td>22±0.42^b</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n= 6, ^a^ P values<0.001 and ^b^ P values <0.05 when compared to diabetic control.
8.3.4 Histopathological studies:

Light photomicrographs of pancreas (Figure 8.3.4i-xiv) in the normal mice, showed normal histological structure of β-cells at the central zone in the islet of Langerhans in the endocrine portion and the normal histological structure of the acini in the exocrine portion were recorded. In the diabetic untreated mice, atrophy and degeneration were observed mostly in the β-cells of the central zone at the islet of Langerhans in the endocrine portion. Necrosis of the pancreatic tissues of the acini in the exocrine portion was also recorded. In the treatment of diabetic mice by glibenclamide and methanol leaf extracts (300 mg/kg b.w. & 600mg/kg b.w.) of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. exhibited restoration of normal cellular architecture of the affected β-cells of the central zone at the islet of Langerhans in the endocrine portion and pancreatic tissues of the acini in the exocrine portion were recorded. Results are comparable to that of the standard glibenclamide treatment.

Light photomicrographs of liver (Figure 8.3.4xv-xxviii) in a normal control mice showed a normal appearance of lobules which are roughly hexagonal or pentagonal in shape, with portal triads at the vertices and a central vein (CV) in the middle. Within each lobule, hepatocytes (H) are arranged into hepatic cords running radiantly from the central vein and are separated by adjacent blood sinusoids (S) containing Kupffer cells. Liver of diabetic mice revealed dilated sinusoids, degenerative changes in the hepatocytes and some necrotic regions. Cells all over the hepatic lobules were observed to have many vacuoles giving them foamy appearance and some of them showed pyknotic nuclei. This was probably a result of increased quantity of fat within the cells due to impaired metabolism of fatty acids. Liver of diabetic mice treated with glibenclamide and plant extracts (300 mg/kg b.w. & 600mg/kg b.w.) showed regenerative effect of hepatocytes and decreased number of vacuolized cells and degree of vacuolisation. This observation revealed that the consumption of leaf extracts of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. could reverse most of the histopathological and biochemical changes in the liver of the diabetic groups comparable to the glibenclamide standard. These findings suggest that treatment by *Eurya japonica* Thunb. and *Ficus auriculata* Lour. methanol leaf extract may be able to improve the impairment of fatty acid metabolism in diabetes.
8.3.4 Photomicrograph of the Histopathological studies of pancreas:

Figure 8.3.4:

i) Section of the Pancreas showing normal appearance of islet of langerhans (IL) in the pancreas of the normal control group.

ii) Section of the Pancreas showing marked degeneration of islet of langerhans (IL) in the pancreas of the diabetic control group.

iii) Section of the Pancreas showing marked regeneration of islet of langerhans (IL) in the pancreas of the group treated with glibenclamide.

iv) & v) vi) & vii) Pancreas section showing marked regeneration of islet of langerhans (IL) in the pancreas of the group treated with methanol leaf extract of *Eurya japonica* Thunb. & *Ficus auriculata* Lour. (300mg/kg b.w & 600mg/kg b.w.) respectively
viii) Section of the Pancreas showing normal appearance of the pancreatic acini
(PAn in the pancreas of the normal control group.
ix) Section of the Pancreas showing marked degeneration of the pancreatic acini
(PA) in the pancreas of the diabetic control group.
x) Section of the Pancreas showing marked regeneration of the normal cellular
architecture of pancreatic acini (PA) in the pancreas of the group treated with
glibenclamide.
xii) & xii) Pancreas section showing marked regeneration of the cellular
architecture of the pancreatic islets (PA) in the pancreas of the group treated with
methanol leaf extract of *Eurya japonica* Thunb. & *Ficus auriculata* Lour. (300mg/kg
b.w & 600mg/kg b.w.) respectively.
8.3.4 Photomicrograph of the Histopathological studies of liver:

Figure 8.3.4:

xv) Section of the Liver showing normal appearance of lobules with portal traits at the vertices, central vein (CV) in the middle and normal sinusoid (NS) in the liver of normal control group.

xvi) Liver section showing dialated sinusoid (DS), necrosis in the diabetic control group.

xvii) Liver section showing regenerative effects of hepatocytes (H) and sinusoid (S) in glibenclamide treated group.

xviii) & xix); xx) & xxi) Liver section showing regenerative effects of hepatocytes (H) and sinusoid (S) in treatment with methanol leaf extract of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. (300 mg/kg b.w & 600 mg/kg b.w.) respectively
Figure 8.3.4:

xxii) Section of the Liver showing normal appearance of hepatocytes (H) in the liver of normal control group.

xxiii) Liver section showing degenerative hepatocytes (DH), vacuolisation (V) in the diabetic control group.

xxiv) Liver section showing regenerative effects of hepatocytes and sinusoid in glibenclamide treated group.

xxv) & xxvi); xxvii) & xxviii) Liver section showing regenerative effects of hepatocytes and sinusoid in treatment with methanol leaf extract of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. (300mg/kg b.w & 600mg/kg b.w.) respectively.
Light photomicrographs (Figure 8.3.4xxix-xxxv) in the kidney of the normal control mice showed normal glomerulus surrounded by the Bowman’s capsule, proximal and distal convoluted tubules without any inflammatory changes. However, Streptozocin treatment causes slight glomerular proliferation. Histopathological study of the STZ induced kidney of the diabetic mice exhibited distorted and slightly expanded glomeruli with slightly thickening glomerular basement membranes (GBMS) and mild necrosis were also noticed in some sections of the convoluted tubules. The group that was treated with methanol extract of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. (300 mg/kg b.w. & 600 mg/kg b.w.) respectively for 15 days showed features of healing comparable to that of glibenclamide treated mice that is normalization of glomerulus, absence of inflammatory cells, improvement in basement membrane and capillaries.
8.3.4 Photomicrograph of the Histopathological studies of kidney:

Figure 8.3.4: xxix) Section of the normal kidney of the normal control mice revealed normal glomerulus surrounded by the Bowman’s capsule, proximal and distal convoluted tubules without any inflammatory changes.

xxx) Histopathological study of the STZ induced kidney of the diabetic mice exhibited distorted and slightly expanded glomeruli with slightly thickening glomerular basement membranes (GBMS) and mild necrosis were also noticed in some sections of convoluted tubules.

xxxi) Glibenclamide treated mice showing normalization of glomerulus, absence of inflammatory cells, improvement in basement membrane and capillaries.

xxxii) & xxxiii); xxxiv) & xxxv) The group that was treated with methanol extract of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. (300 mg/kg b.w. & 600 mg/kg b.w.) respectively for 15 days showed features of healing comparable to that of glibenclamide treated mice that is normalization of glomerulus, absence of inflammatory cells, improvement in basement membrane and capillaries.
The calculated value obtained from the LD$_{50}$ determination and lack of mortality when orally administered may be an indication that the methanol extract of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. treatment is of low toxicity. All the treated animals were alive, active and healthy during the entire period of observation. It is therefore concluded that the methanol leaf extract of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. was found to be non toxic during the oral acute toxicity studies in mice.

From the above result, we can confirm that the methanol leaf extract of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. at doses of 300 and 600 mg/kg possesses significant anti-hyperglycemic activity on the period of experimentation (15-day) treatment in Albino mice. In the STZ-induced diabetic mice, the rise in blood glucose is accompanied by an increase in the serum cholesterol, TG, LDL, VLDL and the decrease in HDL, whereas the treatment with standard and methanol leaf extract of of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. reduced cholesterol, TG, LDL, VLDL and improved HDL in diabetic mice.

Streptozotocin induced diabetes is characterized by severe weight loss (Ravi *et al.*, 2004). Hence, the weight gain after administration of the extract in diabetic mice is simply due to the ability of the extract to reduce hyperglycemia. There was an increase in body weight of the mice in the standard and sample-treated groups, when compared to diabetic control. On the basis of the current investigation it was noted that the methanol leaf extract of of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. acted in a similar fashion to glibenclamide (Standard drug) and it can be suggested that these results provide pharmacological evidence for its folklore claim as an anti-diabetic agent.

Diabetic individuals were observed to have increased plasma lipids, which are responsible for several cardiovascular disorders (Alarcon *et al.*, 2002). The higher lipid levels seen in the diabetic mice was due to increased mobilization of free fatty acids from the peripheral depots and also due to the lipolysis caused by hormones (Eisoud *et al.*, 2007; Nikkhila and Kekki, 1973). The methanol leaf extract leads to regeneration of the b-cells of the pancreas and potentiation of insulin secretion from surviving b-cells; the increase in insulin secretion and the consequent decrease in blood glucose level may lead to inhibition of lipid peroxidation and control of lipolytic hormones. It is well known that LDL plays an important role in arteriosclerosis and that hypercholesterolemia is associated with a defect relating to
the lack of LDL receptors (Yadav et al., 2008). The decrease of cholesterol and LDL levels achieved by administration of methanol extract, demonstrates a possible protection against hypercholesterolemia and the harm this condition brings about.

A number of plants have been used traditionally to treat diabetes and some of them have been proven to have hypoglycemic effects. Previous Phytochemical investigations on the leaf extracts of *Eurya japonica* Thunb. revealed the antioxidant properties of the plants and the presence of phenolics, flavonoids, alkaloids and terpinoids. *Ficus auriculata* Lour. also revealed the presence of antioxidant activities and the presence of phenolics, flavonoids, alkaloids, and terpinoids. Flavonoids, sterols/triterpenoids, alkaloids, and phenolics are known to be bioactive and having antidiabetic principles (Oliver-Bever, 1986; Ivorra et al., 1989; Atta-Ur-Rhemann and Khurshid Zaman, 1989; Kameswara et al., 1997). Flavonoids are known to regenerate the damaged pancreatic β-cells in the chemically-induced diabetic animals (Chakravarthy et al., 1980). Phenolic compounds are also known to help in the regulation of plasma glucose concentration (Manickam et al., 1997), and therefore it is suggested that the extracts exhibited antihyperglycemic effects through increased insulin secretion due to the presence of phenolic compounds. In the present study the leaf extract of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. have shown a potent antioxidant activity and the leaf extracts were found to have phenolic and flavonoid content in them. The antidiabetic effect of the leaf extracts may be due to the presence of more than one antihyperglycemic principle and their synergistic properties. In this study, the antihyperglycemic activity caused by glibenclamide in chemically induced diabetic mice is an indication of the presence of some β cells, as glibenclamide is known to stimulate insulin secretion from β cells (Kameswara et al., 2003). This study suggests that the plant extracts may act through the mechanisms of glibenclamide by reversing the abnormalities in the pancreatic islets. Based upon the results it can be hypothesized that the leaf extracts in the present study probably act by releasing insulin from the pancreatic β-cells by stimulating the insulin secretion.

Streptozotocin (STZ) is well known for its selective pancreatic islet β-cells cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with the cellular metabolic oxidative mechanisms (Papaccio et al., 2000). This well established model is characterized by insulin deficiency associated with insulin resistance (Bar et al., 1976). Consequently, there is reduced secretion of insulin leading to clinical conditions such as hyperglycemia, polyphagia, polydipsia,
polyuria and weight loss observed in the STZ treated animals (Murali et al., 2002; Alese et al., 2013). The β-cells were also observed to be degenerated with varying degree of transversing tiny fibrosepta and distortion in the tissue architecture. Also, a significant decrease in blood glucose and evidence of ameliorative effects on the β-cells was observed in the group of diabetic animals treated with leaf extracts and glibenclamide (Alese et al., 2013). Normalisation of β-cells in the histopathological studies of the islet of Langerhans in pancreas of diabetes mice fed with leaf extracts may explain both hypoglycemic and hypolipidemic actions of the plant extracts under study.

It has been well established that diabetes mellitus alters the normal metabolism of tissues like liver, kidney and heart (Leite et al., 2008). Buko (Buko et al., 1996) reported that the diabetic liver is characterized by hydropic dystrophy and lymphocytic infiltrations. These damages may be due to the oxygen free radicals (OFR) exerting their cytotoxic effect by peroxidation of membrane phospholipids leading to a change in permeability and loss of membrane integrity. Decreased endothelium-dependent relaxation in diabetes is linked to the release of OFRs. Hyperglycemia causes increased production of OFRs, during diabetes, from glucose oxidation and protein glycosylation (Tesfamariam, 1994). The level of total protein in serum decreased in diabetic groups compared to those of normal control, while it increased in the diabetic mice treated with of Eurya japonica Thunb. and Ficus auriculata Lour. compared to those of diabetic mice.

The results indicate a primary and a secondary effect of the diabetic state on the kidney of the mice (Ragavan and Krishnakumari, 2006). The primary effect, the diabetes factor was associated with hyperglycemia and was responsible for dilatation of proximal and distal tubules in the cortex. The secondary effect, named as the individual response factor, was associated with inflammatory processes (Leegwates and Kuper, 1984). Diuresis is a common feature associated with diabetes which may be the reason for structural changes observed with glomerulus (Das et al., 1996). The recovery of renal function expected with the treatment of of Eurya japonica Thunb. and Ficus auriculata Lour. can be explained by the regenerative capability of the renal tubules.

Admittedly, the research study was carried out for a shorter duration and this might be insufficient for significant vascular changes in the kidney of the diabetic mice. The treated diabetic mice however showed healing features, which is comparable to that of
a normal kidney. Further studies are needed to identify the chemical constituents of the methanol extract of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. that may be responsible for the hypoglycemic and hypolipidemic activity.

8.4 Conclusion:

In the light of the results of the present investigation, it can be concluded that the antidiabetic effect of *Eurya japonica* Thunb. and *Ficus auriculata* Lour. is promising. Further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in the researches on diabetes.