CHAPTER 5

Anti-diabetic activity of Phyllanthus amarus extract
5.1 INTRODUCTION

Diabetes mellitus is the commonest endocrine disorder that affects more than 100 million people worldwide and in the next 10 years it may affect about five times more people than it does now (ADA., 1997). In India the prevalence rate of diabetes is estimated to be 1-5% (Patel et al., 1986, Verma et al., 1986, Rao et al., 1989).

Genetic disposition has been ascribed for type I (early-onset type) of diabetes, which represents only 5% of the total diabetes. The etiology of type II diabetes has not been well documented. Presence of endogenous insulin did not produce any regulation of blood sugar in type II diabetes (Cline et al., 1991). Free radicals and lipid peroxides are generated in vivo under various pathological conditions (Sato et al., 1979). It has been postulated that the etiology of complications of diabetes involves oxidative stress, as a result of hyperglycemia. It has been demonstrated that glucose can undergo oxidation in blood, which is catalyzed by trace metals generating free radicals, hydrogen peroxide and reactive ketoaldehydes (Hunt et al., 1988). Although the rate of glucose auto oxidation is slow, it is relevant to the tissue damage in diabetes. Decreased levels of anti-oxidants are reported in diabetes (Karpen et al., 1984).

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethnobotanical information reports about 800 plants that may possess potential medicinal properties (Alarcon-Aguilara et al., 1998). Many indigenous Indian Medicinal plants have
been found to be successfully used to manage diabetes (Rajasekharan and Tuli., 1976, Bhaskaran Nair and Santhakumari., 1985, Nagarajan et al., 1987, Chattopadhyay et al., 1993, Ponnachan et al., 1993, Subramoniam et al., 1996) and some of them have been tested and the active principle isolated. *Momordica charantia* commonly known as bitter gourd belongs to Cucurbitaceae family. It is a widely used vegetable in India and its fruits, leaves and roots are recommended for treating diabetes mellitus (Warier., 1995). Indians have used *Eugenia gambolana* seeds as anti-diabetic remedy (Chopra et al., 1958). *Mucuna pruritens* has been reported to be useful in diabetes (Dhawan et al., 1980). *Tinospora cordifolia* has been indicated in Ayurvedic treatment as a tonic, vitalizer and anti-diabetic (Nadkarni., 1954, Chopra et al., 1958). There is still a need to look for, as no drugs have been shown to modify the course of diabetic complications. In the present studies we have looked into the effect of *P. amarus* extract in reducing blood sugar in normal and alloxan induced diabetes.

**5.2 METHODS**

**5.2.1 Effect of *P. amarus* extract on blood glucose levels of normal rats**

Male Wistar rats were divided into three groups of six rats each.

- **Group I:** normal
- **Group II:** Treated with 200 mg/ kg b. wt of *P. amarus*
- **Group III:** Treated with 1000 mg/ kg b. wt of *P. amarus*
A single dose of *P. amarus* extract was administered (p.o) and blood samples were collected from the tail vein just prior to and 1, 2, 3, 4, 6h intervals. Serum was separated and sugar levels were estimated by enzymatic GOD/POD method (Trinder., 1969).

5.2.2 Effect of continued administration of *P. amarus* extract on blood glucose levels in normal rats

Male Wistar rats were divided into three groups of six rats each.

Group I: Normal

Group II: Treated with 200mg/kg b. wt of *P. amarus*

Group III: Treated with 1000mg/kg b. wt of *P. amarus*

*P. amarus* extract was administered (p.o) once daily for 15 days. Serum glucose levels of all the animals were measured on 3, 6, 9, 12 and 15th day respectively.

5.2.3 Determination of toxicity of *P. amarus* in normal rats

Total WBC count (Cheesbrough., 1976) of the above animals was estimated using haemocytometer and recorded on various days. The above groups of animals were sacrificed on day 16 and following biochemical parameters were assessed in serum by the method indicated. Glutamate pyruvate transaminase (Bergmeyer and Bernt., 1980), blood urea nitrogen (Marsh et al., 1980), creatinine (Brod and Siorta., 1950). Biochemical changes in liver like glutamate pyruvate transaminase (Bergmeyer and Bernt., 1980), alkaline phosphatase (Kind and king., 1954) and protein (Lowry et al., 1951) were estimated. Detailed methods are given in chapter II. The organs like liver, kidney, spleen and thymus were removed and their weights were recorded.
5.2.4 Determination of the effect of single dose of *P. amarus* extract on alloxan induced diabetes in rats

Diabetes was induced in male Wistar rats by injecting a single intraperitoneal injection of 120 mg/kg b. wt. of alloxan monohydrate (Cooperstien and Walkins., 1981). Serum glucose level was checked by glucose oxidase method (Trinder., 1969) after 72 hr. Animals with serum glucose levels > 250 mg/dl were considered diabetic and were used for the study (Perfumi and Tacconi., 1996). Rats were divided into four groups with 6 rats in each group.

Group I: Normal

Group II: Diabetic untreated animals

Group III: Diabetic rats treated with + 200mg/ kg b. wt of *P. amarus*

Group IV: Diabetic rats treated with + 1000mg/ kg b. wt of *P. amarus*

*P. amarus* extract was given orally on 3rd day after alloxan treatment. Fasting blood samples were collected from the tail vein on 3rd day after alloxan treatment prior to the oral administration of the drug and at 1, 2, 3, 4, and 6h intervals. Serum was separated and glucose levels were estimated.

5.2.5 Determination of the effect of the continued administration of *P. amarus* extract on blood glucose in alloxan induced diabetes in rats

Male Wistar rats were made diabetic by a single i.p injection of 120mg/ kg body weight of alloxan monohydrate. Three days later blood samples were drawn and glucose levels were determined to confirm the
development of diabetes (> 250 mg/dl). The diabetic rats were divided into four groups, each containing six animals.

**Group I: Normal untreated animals**

**Group II: Diabetic untreated animals**

**Group III: Diabetic rats treated with 200mg/ kg b. wt of P. amarus**

**Group IV: Diabetic rats treated with 1000mg/ kg b. wt of P. amarus**

P. amarus extract was given orally on 3rd day after alloxan treatment and continued for 15 days.

Blood was collected at random and serum glucose levels were measured on 6, 9, 12, 15 and 18th day after alloxan treatment. Total WBC count was measured using haemocytometer and recorded on various days after bleeding from the tail vein.

**5.2.6 Determination of the effect of the continued administration of P. amarus extract in ameliorating toxicity induced by alloxan**

The above rats were sacrificed on day 19, blood was collected and serum was separated and Glutamate pyruvate transaminase (Bergmeyer and Bernt., 1980), Blood urea nitrogen (Marsh et al., 1980), Creatinine (Brod and Siorta., 1950) were estimated. The organs like liver, kidney, spleen and thymus were removed and their weights recorded. Biochemical changes in liver like Glutamate pyruvate transaminase (Bergmeyer and Bernt., 1980), alkaline phosphatase (Kind and King., 1954) and protein (Lowry et al., 1951) were estimated.
5.3. RESULT

5.3.1 Effect of *P. amarus* extract on blood glucose levels of normal rats

As shown in Table 5.1 administration of *P. amarus* was found to reduce the serum glucose level in normal rats. The maximum reduction in blood sugar was noticed by first hour after the administration of the extract i.e., 34.5% at dose level of 200mg/ kg b. wt and 47.4% at dose level of 1000mg/ kg b. wt. Values came back to normal levels by 6th h.

5.3.2 Effect of continued administration of *P. amarus* extract on blood glucose levels in normal rats

The rats treated with 200 and 1000 mg/kg b. wt for 15 days appeared healthy and active for the entire observation period. None of the rats died. The treatment with different doses of *P. amarus* extract in normal rats showed continued reduction in blood sugar (Fig 5.1). *P. amarus* extract at dose of 200mg/ kg b. wt showed 35.5% decrease in the blood glucose level on 15th day. Treatment with 100mg/ kg b.wt of *P. amarus* resulted into a 51.9% of fall of blood glucose on 15th day.

5.3.3 Toxicity of *P. amarus* in normal rats

Normal rats treated with 200 and 1000mg/ kg b.wt of *P. amarus* extract did not produce any weight loss (Fig 5.2). There was no significant alteration in the liver GPT, ALP, and BUN and serum GPT and creatinine levels (Table 5.2). This indicated that in animals treated with *P. amarus* extract, *P. amarus* did not produce any toxic effect in the liver and kidney. The organ weights of *P. amarus* treated animals were found unaltered at 200 and 1000mg/ kg b. wt. Effect of
P. amarus extract on total white blood cells count (WBC) in normal animals is shown in Fig 5.3. Administration of P. amarus extract did not produce any significant change on WBC.

5.3.4 Effect of P. amarus extract on alloxan induced diabetes in rats

The blood glucose levels of diabetic untreated rats were significantly higher than the blood glucose levels of normal untreated rats. The single administration of the extract 200mg/ kg b. wt on 3rd day after alloxan administration produced insignificant reduction in serum glucose level (6.07%) at 4h, and at 1000mg/ kg b.wt there was 18.7% reduction (Table 5.3)

5.3.5 Effect of continued administration of P. amarus extract in alloxan induced diabetes in rats

Continued administration of the extract (200mg/ kg b.wt) showed significant reduction from 6th day onwards and produced 75.95% reduction in the glucose level on 18th day (Table 5.4). At 1000mg/ kg b. wt, there was 81.2% reduction in blood glucose level on 18th day after alloxan treatment. In animals treated with 1000mg/ kg b.wt blood sugar was almost similar to normal rats on 18th day of alloxan treatment (Fig 5.4). In the case of untreated control, percent of reduction was only 44.4% on 18th day.

5.3.6 P. amarus extract in amelioration of toxicity-induced alloxan

A decreasing trend in the body weight was noted in alloxan induced diabetic rats (Fig 5.5). In P. amarus extract treated rats, the body weight remains almost same. The treatment therefore, not only
arrests loss of weight due to diabetic state but also results in weight gain. This may be due to the amelioration of alloxan toxicity and probably due to its role in initiating an improvement in the overall metabolic status.

Serum GPT, blood urea nitrogen and creatinine were elevated in alloxan induced diabetes indicating that alloxan administration produced hepatic and renal damage. When treated with the extract 200 and 1000mg/ kg b.wt, there was significant reduction in the elevated levels of SGPT, blood urea nitrogen and creatinine. Liver GPT was found to be elevated from 528.1 to 869.1 U/ mg proteins in alloxan induced diabetes in rats and ALP was found to be elevated from 33.94 to 56.9 KA/ dl, 15 days after alloxan administration (Table 5.5). Animals treated with 200mg/ kg b.wt of P. amarus extract along with alloxan had lowered liver GPT levels by 23.5%. Administration of P. amarus extract, 1000mg/ kg b.wt significantly reduced liver GPT levels by 31.4%. Similarly elevated ALP levels in liver during alloxan induced diabetes were found to be significantly lowered by 200mg/ kg b. wt of P. amarus extract to 15.1%. 1000mg/ kg b.wt of P. amarus showed 25.3% decrease in elevated ALP levels in liver.

Total WBC was considerably reduced from 10366 to 6066 on 3rd day after alloxan injection and was gradually increased to 7800 on 18th day (Fig 5.6). Administration of P. amarus (1000mg/ kg b.wt) prevented alloxan induced cellular damage as seen from the increased number of total WBC which was 9800 in the case of 200mg/ kg b. wt and 10533 in the case of 1000mg/ kg b. wt on 18th day.
5.4 DISCUSSION

The results of the present study indicated that *P. amarus* extract was found to reduce the glucose level in normal animals and in those made diabetic with alloxan. Alloxan has been shown to induce free radical production, which causes tissue injury (Halliwell and Gutteridge., 1985) which could be responsible for increased blood sugar seen in the animals. The pancreas is especially susceptible to the action of alloxan induced free radical damage. However, it is found that action is not specific to pancreas as other organs such as liver, kidney and haemopoietic system are also affected by alloxan administration as seen from the elevation of marker enzymes and reduction of hematological parameters. This was reversed by the continued administration of *P. amarus* extract.

Present study indicated that in alloxan induced diabetes elevation of blood sugar was reversed by simultaneous administration of *P. amarus*. Decrease in blood sugar was seen within hours and upon continued administration the blood sugar value was found to be almost normal. Results shown in this study indicate that *P. amarus* extract reduces blood sugar in alloxan induced diabetic rats. It was reported earlier that *P. amarus* extract could act as a free radical scavenger *in vitro* (Joy and Kuttan., 1995). The present finding indicates that administration of *P. amarus* can reduce the level of blood sugar as well as ameliorate the destruction of WBC and confirms the possibility that the major function of the extract is on the protection of vital tissues.
including pancreas, there by reducing the causation of diabetes in these animals.

Since *P. amarus* is a known hepatoprotective agent the improvement of liver function and subsequent increase in uptake of blood glucose and its utilization may be another mechanism of action of the extract.

Other possible mechanism includes the stimulation of β-cells and subsequent release of insulin and activation of insulin receptors. Estimation of insulin level and insulin receptor may give more insight into the mechanism of the anti-diabetic activity shown by the extract. *P. amarus* extract contains several polyphenols such as ellagic acid (Ishimaru et al., 1992), Flavonoids (Agarwal and Tiwari., 1991) and lignans (Satyanarayana et al., 1980), which are potent anti-oxidants and anti-carcinogens. The active ingredient in the extract, which reduces the blood sugar, is not known at present.
Table 5.1 Effect of *P. amarus* extract on blood sugar levels in normal animals (single dose short term study)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood sugar in mg/dl (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>Normal</td>
<td>75.6±2.8</td>
</tr>
<tr>
<td>II</td>
<td><em>P. amarus</em> (200mg)</td>
<td>78.4±15.4</td>
</tr>
<tr>
<td>III</td>
<td><em>P. amarus</em> (1000mg)</td>
<td>73.2±18.3</td>
</tr>
</tbody>
</table>

* P< 0.05; **P< 0.01; ***P< 0.001 (Compared to same group of initial value)
Table 5.2 Effect of *P. amarus* extract on biochemical parameters of liver and serum in normal rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver</th>
<th>Blood</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GPT (U/ mg Protein)</td>
<td>ALP (KA/dl)</td>
<td>BUN (mg/dl)</td>
</tr>
<tr>
<td>Normal</td>
<td>528.1 ± 6.3</td>
<td>33.9 ± 2.2</td>
<td>18 ± 2.1</td>
</tr>
<tr>
<td><em>P. amarus</em> (200mg)</td>
<td>522.6 ± 37.9</td>
<td>35.7 ± 4.7</td>
<td>21 ± 2.2</td>
</tr>
<tr>
<td><em>P. amarus</em> (1000mg)</td>
<td>518.3 ± 48.5</td>
<td>36.9 ± 1.7</td>
<td>22 ± 9.1</td>
</tr>
</tbody>
</table>
Table 5.3 Effect of *P. amarus* extract on blood sugar levels in alloxan induced diabetic rats (single dose short term study)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood sugar in mg/dl (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>Normal</td>
<td>75.6±2.8</td>
</tr>
<tr>
<td>II</td>
<td>Control (alloxan)</td>
<td>479.2±16.5</td>
</tr>
<tr>
<td>III</td>
<td><em>P. amarus</em> (200mg)</td>
<td>488.9±27.4</td>
</tr>
<tr>
<td>IV</td>
<td><em>P. amarus</em> (1000mg)</td>
<td>501.4±20.2</td>
</tr>
</tbody>
</table>

**P< 0.01; ***P< 0.001 (Compared to same group of initial value)
Table 5.4 Effect of *P. amarus* extract on blood sugar level in alloxan induced diabetic rats (multi dose long term study)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood sugar in mg/dl (DAYS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Dose/kg b.wt)</td>
<td>3</td>
</tr>
<tr>
<td>I</td>
<td>Normal</td>
<td>75.6±2.9</td>
</tr>
<tr>
<td>II</td>
<td>Control (alloxan)</td>
<td>479.2±16.5</td>
</tr>
<tr>
<td>III</td>
<td><em>P. amarus</em> (200mg)</td>
<td>488.9±27.4</td>
</tr>
<tr>
<td>IV</td>
<td><em>P. amarus</em> (1000mg)</td>
<td>501.4±20.2</td>
</tr>
</tbody>
</table>

***P< 0.001 (Compared to same group of initial value)
Table 5.5 Effect of *P. amarus* extract on biochemical parameters of liver and serum in alloxan diabetic rats

<table>
<thead>
<tr>
<th>Treatment (Dose/ kg b. wt)</th>
<th>Liver</th>
<th>Blood</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GPT (U/ mg Protein)</td>
<td>ALP (KA/dl)</td>
<td>BUN (mg/dl)</td>
</tr>
<tr>
<td>Normal</td>
<td>528.1± 66.3</td>
<td>33.9± 2.2</td>
<td>18± 2.1</td>
</tr>
<tr>
<td>Control (alloxan)</td>
<td>869.1± 84.0</td>
<td>56.9± 6.4</td>
<td>66.0± 3.5</td>
</tr>
<tr>
<td><em>P. amarus</em> (200mg)</td>
<td>665.0± 80.8**</td>
<td>48.3± 5.2*</td>
<td>38.0± 2.7***</td>
</tr>
<tr>
<td><em>P. amarus</em> (1000mg)</td>
<td>596.0± 56.0***</td>
<td>42.5± 13.8*</td>
<td>27.0± 3.8***</td>
</tr>
</tbody>
</table>

*P< 0.05; **P< 0.01; ***P< 0.001

Values are determined 19th day after alloxan administration
Fig 5.1 Effect of *P. amarus* extract on blood sugar level in normal animals.
Fig 5.2 Effect of *P. amarus* extract on body weight in normal rats
Fig 5.3 Effect of *P. amarus* extract on total leucocyte counts in normal rats
Fig 5.4 Effect of *P. amarus* extract on blood sugar level in alloxan induced diabetic rats
Fig 5.5 Effect of *P. amarus* extract on body weight in rats treated with alloxan
Fig 5.6 Effect of P. amarus extract on total leucocytes counts in rats treated with alloxan