Investigation of Hypoglycemic Effect of Ceiba Pentandra Root Bark Extract in Normal and Alloxan Induced Diabetic Albino Rats

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Abstract

The present work was designed to investigate the hypoglycemic effect of Ceiba pentandra root bark extract in normal and alloxan induced diabetic rats. To investigate the effect of Ceiba pentandra 40 albino rats were included in this study divided into four groups of ten rats in each group. Group A and B rats were normal while diabetes was induced in group C and D rats by intraperitoneal injection of alloxan dissolved in 1mL of distilled water at a dose of 125mg/kg body weight. The Ceiba pentandra root bark extract was given orally at a dose of 150mg/kg of body weight. Data was taken after seven days of administration of alloxan. After the induction of diabetes the group B and D rats were treated with Ceiba pentandra root bark extract at a dose of 150mg/kg of body weight. Group A and C rats were not treated with Ceiba pentandra root bark extract. The blood samples from all the rats were collected from coccygial vein on 1st day, then on 21st and 42nd day respectively. All the samples were analyzed for blood glucose level by using enzymatic kits. The blood glucose level of treated groups of rats showed significant reduction after 7 weeks of treatment with Ceiba pentandra root bark extract. By statistical analysis of results it was found that Ceiba pentandra root bark extract has hypoglycemic effect in normal and alloxan induced diabetic rats.

Keywords: Albino rats, alloxan, Ceiba pentandra, hypoglycemic effect

Introduction

Diabetes mellitus is a chronic disease marked by elevated blood glucose (Hyperglycemia) and urinary glucose excretion. Diabetes mellitus is a metabolic disorder characterized by high concentration of glucose in the blood. Glucose plays a vital role in the regulation of the β-cell insulin secretion\textsuperscript{11}. Insulin (Latin insula, "island") is a polypeptide hormone primarily playing a pivotal role in the regulation of carbohydrate metabolism. There are two states where insulin disturbance can be pathologic i.e. diabetes mellitus type I and diabetes mellitus type II. Both lead to hyperglycaemia, which largely causes the acute signs of diabetes; excessive urine production, resulting compensatory thirst and increased fluid intake, blurred vision, unexplained weight loss, lethargy and changes in energy metabolism. Other examples of impaired glucose tolerance are insulinoma or reactive hyperglycemia\textsuperscript{9}.

Recently, it is suggested that formation of free radicals also involved in the pathogenesis of diabetes and the development of diabetic complications because a prolonged exposure to hyperglycemia increases the generation of free radicals and reduces capacities of the antioxidant defense system\textsuperscript{26}. High levels of reactive oxygen have been found to play a role in the pathogenesis of several diseases including NIDDM\textsuperscript{10}. Approximately 140 million people worldwide suffer from diabetes (Anonymous 1999). The disease becomes a real problem of public health in developing countries, where its prevalence is increasing steadily\textsuperscript{8}. Diabetic patients, both type I and II exhibit abnormal antioxidant status, auto-oxidation of glucose and excess glycosylated proteins\textsuperscript{15, 17}. Oxidative stress in diabetes leads to tissue damage, with lipid peroxidation, inactivation of proteins, and protein glycation as intermediate mechanisms\textsuperscript{24} for complications including retinopathy, nephropathy and coronary heart disease\textsuperscript{13, 15}. The underlying causes of diabetic complications have been attributed to hyperglycaemia which results in oxidative stress, alterations in enzyme activities, protein glycosylation and several structural changes\textsuperscript{3}.

The individuals with diabetes have a 25-fold increased risk of blindness, a 20-fold increased risk of renal failure, 20-fold increased risk of amputation as a result of gangrene and a 2 to 6 fold increased risk of coronary heart disease and ischemic brain damage\textsuperscript{33}. Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed\textsuperscript{5}. This has led to an increasing demand for natural products with anti-diabetic activity and fewer side effects. Many herbs and plant products have been shown to have hypoglycemic action. Many traditional plant
treatments for diabetes mellitus are used throughout the world. Management of diabetes without any side effect is still a challenge to the medical system. Medicinal plants have become so important in this present generation in the treatment of numerous types of diseases. In many countries it is traditional to use medicinal plants to control diabetes. The hypoglycemic effect of several plant extracts and herbal formulations has been confirmed, which are being used as anti-diabetic remedies and their therapeutic abilities investigated more intensely. These medicinal plants are the sources of important drugs of the modern world. Some of these include guanine from digitoxin from Digitalis leaf, atropine from Belladonna root and leaf, hyoscyamine from Hyoscyamus and Datura leaves and roots, conine from Conium leaf, and vineristine and vinblastine from Catharanthus root.

*Ceiba pentandra*, commonly called the silk-cotton tree belongs to the Bombacaceae family. Various morphological parts of this plant have been reported to be useful as effective remedies against diabetes, hypertension, headache, dizziness, constipation, mental trouble, fever, peptic ulcer, rheumatism and leprosy. It is also used as diuretic.

**Materials and Methods**

**Ceiba Pentandra (Silk Cotton Tree)**

*Ceiba pentandra* roots were purchased from the local market. The bark from roots was removed, cleaned, sun and oven dried (60°C) and ground to make powder and stored in air tight bags to keep it moisture free till the time of use.

**Extraction of Plant Material:**

One kg roots bark powder of *Ceiba pentandra* was macerated in 1:1 (v/v) mixture of methanol for two days with occasional stirring at room temperature. The filtrate thus obtained was concentrated using a Rota vapor at 80°C. The roots bark extract was given at a dose of 150mg/kg of body weight of each rat dissolved in 1mL of water. The dose was given by dropper.

**Alloxan:** Alloxan induced hyperglycemia has been described as a useful experimental model to evaluate the activity of hypoglycemic agents. Diabetes was induced by a single intra-peritoneal injection of alloxan prepared in 0.1mol/L citrate buffers at a dose of 100 mg/Kg body weight. Diabetes was confirmed in the alloxan treated rats by measuring the fasting blood glucose concentration 8-10 days post-injection.

**Enzymatic Kit:** Commercial kits of the company Human, Germany were used to determine serum glucose levels in albino rats by spectrophotometer.

**Animals:** Total 40 albino rats (*Rattus norvegicus*) of either sex weighing between 175-250 g were used in this study. These animals were housed in steel cages under controlled laboratory conditions. They were maintained on standard pellet diet.

**Experimental Design and Induction of Diabetes**

The animals were divided into four groups (table I) and each group consisted of ten rats. Diabetes was induced by a single intra peritoneal injection of alloxan prepared in 0.1mol/L citrate buffer at a dose of 125 mg/Kg body weight. The control rats were only injected with freshly dissolved 0.1mol/L citrate buffer (pH 4.5). The rats with blood glucose level above 150mg/dl were considered as diabetic and were used in the further experiments. Diabetes was confirmed in the alloxan treated rats by measuring the fasting blood glucose concentration 8–10 days post-injection.

**Blood Collection and Analysis:** Blood was taken from the coccygial vein of overnight fasted albino rats. Collected blood poured into centrifuged glass tubes, and then centrifuged at 3500×g for ten minutes and serum was separated and stored in a deep freezer for further biochemical measurements. The specific enzymatic kits were used to assess glucose levels of rats using spectrophotometer.

**Estimation of Serum Glucose:** Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as an indicator.

**Reaction Principle**

\[ \text{Glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{GOD}} \text{Gluconic acid} + \text{H}_2\text{O}_2 \]
2H₂O₂ + 4-aminophenazone + Phenol → Quinoneimine + 4H₂O

**Procedure:** Total 42 test tubes were taken. 40 tubes were labeled as 1,2,3,………40 for each sample of rat’s serum from each group i.e., normal + normal feed, normal + *Ceiba pentandra* root bark extract, diabetic + normal feed and diabetic + *Ceiba pentandra* root bark extract. The remaining two tubes were labeled as blank and standard. 2ml reagent was taken in all the tubes by pipette. 20µl of distilled water was added to the test tube labeled as blank, 20µl of standard solution from the kit was added to the test tube labeled as standard and 20µl of each serum sample was taken in tubes labeled as 1,2,3 ………40. Then the contents of all tubes were mixed and incubated at 37°C for 10 minutes. Then absorbance of standard (Abs Std) and sample (AbsS) were measured at 546nm against the blank (AbsRB).

**Calculations:** The concentration of glucose in serum was calculated by the following formula as prescribed by Mackey and Macky \(^\text{16}^\).

\[
\text{Glucose concentration (mg/dl)} = \left( \frac{\text{A test}}{\text{A standard}} \right) \times 100
\]

**Statistical analysis:** Descriptive statistics mean and standard deviation were calculated for all the variables of each group. ANOVA was applied for statistical analysis using statistical software Minitab V. 1.5 and p value at<0.05 had been considered significance level \(^\text{29}^\).

**Results**

The present research work was designed to investigate the hypoglycemic effect of methanol root bark extract of *Ceiba pentandra* in normal and alloxan induced diabetic rats.

Total 40 albino rats were used which were divided into four groups as A, B, C and D. Diabetes was induced in group C and D rats by intra-peritoneal injection of alloxan dissolved in 1mL of distilled water at a dose of 125mg/kg of body weight. The *Ceiba pentandra* root bark extract was given orally at a dose of 150mg/kg of body weight. Data was taken after seven days of administration of alloxan. After the induction of diabetes the group B and D were treated with *Ceiba pentandra* root bark extract at a dose of 150mg/kg of body weight. Group A and C were not treated with *Ceiba pentandra* root bark extract. Blood samples were taken from the coccygial vein of each rat for the estimation of glucose level at different time periods i.e. 1\(^{\text{st}}\), 21\(^{\text{st}}\) and 42\(^{\text{nd}}\) days after the treatment. The blood glucose levels of control group as shown in figure 1 have almost similar blood glucose levels throughout the 42\(^{\text{nd}}\) day of study. The average blood glucose levels of group A were recorded as 108.2mg/dl at 1\(^{\text{st}}\) day of experiment. The blood glucose level changed to 107.9mg/dl and 108.9mg/dl at 21\(^{\text{st}}\) and 42\(^{\text{nd}}\) day of the experiment respectively while they were not treated with *Ceiba pentandra* root bark extract. The overall reduction in the blood glucose levels was observed which was non-significant (P>0.05).

A significant decrease in blood glucose level was observed in non-diabetic rats receive root bark extract of *Ceiba pentandra* (150mg/kg) as shown in and figure 2. The average blood glucose levels of group B rats were recorded as 112.0mg/dl at 1\(^{\text{st}}\) day of the experiment before the treatment with *Ceiba pentandra* root bark extract. The blood glucose levels after the treatment with root bark extract of *Ceiba pentandra* decreased to 98.2mg/dl and 82.2mg/dl at 21\(^{\text{st}}\) and 42\(^{\text{nd}}\) day of the experiment respectively. The overall decrease in glucose was observed which was significant (P<0.05).

In diabetic rats a gradual increase in blood glucose level was observed as shown in figure 3. The average blood glucose levels observed in group C rats were recorded as 163.9 mg/dl at 1\(^{\text{st}}\) day of experiment. The blood glucose level changed to 168.5mg/dl and 171.3mg/dl at 21\(^{\text{st}}\) and 42\(^{\text{nd}}\) day of the experiment respectively while they were not treated with *Ceiba pentandra* root bark extract. The overall increase in blood glucose levels was observed for this group which was significant (P<0.05).

Diabetic rats receive root bark extract of *Ceiba pentandra* (150mg/kg) showed a significant decrease in blood glucose levels as presented in figure 4. The average blood glucose levels of group D rats were recorded as 172.1mg/dl at 1\(^{\text{st}}\) day of experiment before the treatment with *Ceiba pentandra* root bark extract. The blood glucose levels for this group were changed to 154.9mg/dl and 132.2mg/dl at 21\(^{\text{st}}\) and 42\(^{\text{nd}}\) day of the experiment respectively after the treatment with *Ceiba pentandra* root bark extract. The overall decrease in blood glucose levels was found which was significant (P<0.05).

**Discussion**
Considering the importance of natural products, the present study was designed to investigate the hypoglycemic effect of methanol root bark extract of *Ceiba pentandra* in normal and alloxan induced diabetic rats. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and alterations in carbohydrate, fat and protein metabolism, associated with absolute or relative deficiencies in insulin secretion or insulin action. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is a growing interest in herbal remedies, due to the side effects associated with these therapeutic agents\(^3\). Several such herbs have shown anti-diabetic activity when assessed using presently available experimental techniques\(^2,6,12\).

A wide array of plant derived active principles representing numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of NIDDM\(^18\). Control of diabetes by spices and other natural products is becoming popular and more economical for use in developing countries. Spices come from the dried aromatic plants or trees and may be the bark, fruit, bud or the berry of trees/plants\(^25\). Many herbal medicines have been recommended for the treatment of diabetes\(^4\).

This study revealed that diabetic and normal rats had significant effect of methanol root bark extract of *Ceiba pentandra* on their blood glucose levels after 42 days of treatment. Glucose levels of diabetic and non diabetic rats of Group B and Group D decreased having P < 0.000. The results are highly correlated with the work of Olusola *et al*\(^23\) who reported that the aqueous stem bark extract of *Ceiba pentandra* exhibited effective hypoglycemic action. Similarly reduction in blood glucose level has also been reported by Djomeni *et al*\(^7\) which showed the hypoglycemic properties of *Ceiba pentandra* root bark extract.

![Figure 1 Evaluation of glucose level in control group of rats](image-url)
Figure 2 Hypoglycemic effect of root bark extract of *Cebia pentandra* in rats

Figure 3 Evaluation of glucose level in alloxan induced diabetic rats
Figure 4 Hypoglycemic effect of root bark extract of *Cebia pentandra* in alloxan induced diabetic rats

![Graph showing glucose level over time](image)

Table 1: Animal Grouping and Treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A</strong>: Normal (Control)</td>
<td>Normal Feed</td>
</tr>
<tr>
<td><strong>Group B</strong>: Normal</td>
<td>Normal Feed + <em>Cebia pentandra</em> Root bark extract</td>
</tr>
<tr>
<td><strong>Group C</strong>: Diabetic (Control)</td>
<td>Normal Feed</td>
</tr>
<tr>
<td><strong>Group D</strong>: Diabetic</td>
<td>Normal Feed + <em>Cebia pentandra</em> Root bark extract</td>
</tr>
</tbody>
</table>

The present research work reveals that the oral administration of root bark extract of *Cebia pentandra* showed hypoglycemic effects in alloxan induced diabetic rats, therefore it was concluded that root bark extract of *Cebia pentandra* is helpful to lower glucose level in treatment of hyperglycemia.

References


