Hypoglycemic and hypolipidemic activity of aqueous extract of *Ficus racemosa* seeds

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ABSTRACT: Introduction: The incidence of diabetes mellitus is on rise all over the world. Moreover the synthetic drugs are likely to give serious side effects. That is why the expert committee on diabetes at WHO recommends the screening of medicinal plants for the management of diabetes. Objective: The present study was designed to investigate the hypoglycemic and hypolipidemic potential of *F. racemosa* seeds in streptozocin (STZ) induced diabetic mice. Materials and Methods: Swiss albino mice of both sexes, aged 7-8 weeks, average weight of 20-30 gm were used for the experiments. Animals were treated with aqueous extract of *F. racemosa* seeds at a dose of 200 mg/kg body weight. Blood glucose, triglycerides, LDL, HDL and cholesterol was measured at the beginning and end of the experiment. Results: Blood glucose and other studied parameters were elevated in the diabetic mice and were brought about near to the control group (except HDL) by the aqueous extract of *F. racemosa* seeds (200 mg/kg body weight). The decrease in all the parameters (except HDL) were statistically significant (*P* < 0.001). Conclusion: The present study suggests that the aqueous extract of *F. racemosa* seeds can be used for further isolation and identification of active principles with hypoglycemic and hypolipidemic potential.

KEY WORDS: *F. racemosa* seeds, diabetes, streptozocin (STZ), hypoglycemic, hypolipidemic

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by abnormalities in carbohydrate, lipid and lipoprotein metabolism, which not only lead to hyperglycemia but also cause many complications, such as hyperlipidemia, hyperinsulinemia, hypertension and atherosclerosis.¹⁻⁸ The chronic hyperglycemia of diabetes is associated with dysfunction, damage and failure of various organs over the long term.¹⁴ Despite the availability of many antidiabetic medicines in the market, diabetes and its related complications continue to be major medical problems. Plant derivatives with purported hypoglycemic properties are used in folk medicine and traditional healing systems around the world.⁹

Over 400 traditional plant treatments for diabetes have been reported although only a small number of these have received scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs should be further investigated.⁶

*Ficus racemosa* belonging to the family Moraceae is a medium tall tree with quite rich green foliage that provides good shade. It is popularly known as “country fig” in English and “Atti” in Tamil. Different parts of *F. racemosa* are traditionally used as fodder, as food and for ceremonial purposes.¹⁷ All parts of this plant (leaves, fruits, bark, latex and sap of the root) are medicinally important in the traditional system of medicine in India.¹⁸ The leaves, bark and fruits of *F. racemosa* are employed in native medicine to treat several diseases.¹⁸ Several experimental studies have demonstrated the anti-inflammatory, hepatoprotective and hypoglycemic effects of the plant *F. racemosa*.¹⁰⁻¹²

However to the best of our knowledge no work has been reported previously on the hypoglycemic and hypolipidemic
activity of the seeds of *F. racemosa*. The present study was undertaken to investigate the hypoglycemic and hypolipidemic effect of *F. racemosa* seeds.

**MATERIALS AND METHODS**

**Collection and Identification of the Plant**
The fresh seed of *Ficus racemosa* was collected in February 2009 from the area of Purana Palton, Dhaka. The plant was identified by the National Herbarium where a voucher specimen was deposited having the accession number 34479.

**Drying and Pulverization**
The fresh seed was first washed with water to remove adhering dirt and then cut into small pieces and sun dried for 4 days. After complete drying, the entire portion was pulverized into a coarse powder with the help of a grinding machine and was stored in an airtight container at room temperature for further process.

**Extraction of Plant Material**
Ten grams of powdered seeds were mixed with 1000 ml distilled water, boiled for 10 min and then cooled for 15 min. Thereafter, the aqueous extract was filtered using a Millipore filter (Millipore 0.2 mm) to remove particulate matter. The filtrate was then freeze-dried from BCSIR (Bangladesh Council of Scientific and Industrial Research), Dhaka, Bangladesh.

**Drugs, Chemicals and Reagents**
Metformin was purchased from Square Pharmaceuticals Ltd. Dhaka, Bangladesh. All other reagents, assay kits and chemicals used in this work were purchased from Sigma Chemical Co. St Louis, MO, USA.

**Experimental Animals and Their Management**
Swiss-albino mice of both sexes aged 7-8 weeks and with an average weight of 20-30 gm were used for the experiment. The mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental conditions for one week for acclimatization after their purchase and fed ICDDR, B formulated rodent food and water ad libitum. They were housed individually in cages and were kept at constant room temperature (25.0 ± 3.0 °C), humidity 35-60% and 12 hours light/dark cycle. Excreta were removed from the cages every day. The animals were divided into four groups having 6 mice in each group and named as following:

- **Group 1**: Mice treated with 200 mg/kg extract
- **Group 2**: Mice treated Metformin (500 µg/ kg)
- **Group 3**: Diabetic Control
- **Group 4**: Control

**Induction of Diabetes Mellitus and Measurement of Plasma Glucose**
Diabetes was induced by a single intraperitoneal (i.p.) injection of 100 ml of sterile phosphate buffered solution (PBS—pH 7.4) containing streptozotocin (STZ) (65 mg/kg), (Zanosar, Pharmacia & Upjohn, ON, Canada). After 4 days, the hyperglycemia was established.[13] Glucose concentration was measured in a blood sample obtained from tail puncture using glucometer (One Touch Ultra). Only animals that had a blood glucose concentration higher than 10 mM, 4 days after treatment with STZ, were used for the study.[13]

**Blood Sample Collection and Preparation of Plasma**
At the end of 14 days treatment, after 24 h fasting, blood samples were collected from post vena cava of the mice anaesthetizing with Ketamine (500 mg/kg body, intra peritoneal) and transferred into heparinised tubes immediately. Blood was then centrifuged at 4000 g for 10 min using a bench top centrifuge (MSE Minor, England). The supernatant serum samples were collected using dry Pasteur pipette and stored in the refrigerator for further analyses. All analyses were completed within 24 h of sample collection.

**Determination of Lipid Profile**
Triglycerides, total cholesterol and HDL concentration were evaluated according to the instruction of manufacturer of assay kits (purchased from Sigma Chemical Co, St Louis, MO, USA). According to Friedewald's formula,[14] VLDL and LDL were calculated as: VLDL cholesterol = TG/5 and LDL cholesterol = TC – (VLDL+HDL cholesterol).

**STATISTICAL ANALYSIS**
The value of glucose (mmol/l) and lipid profile parameters (mg/dl) were expressed as mean ± SEM (standard error of mean) and analyzed for ANOVA and post hoc Dunnet's t-test. SPSS (Statistical Package for Social Science) for WINDOWS (Ver. 18) was applied for the analysis of data. Differences between groups were considered significant at $P < 0.05$, 0.001 levels.

**RESULTS**
The hypoglycemic effect of *Ficus racemosa* seeds is shown in Table 1. It was found that the seed extract of *F. racemosa* reduced blood glucose level in streptozocin-induced diabetic mice and produced substantial hypoglycemic effects. In the first week after induction of diabetes, the blood glucose level was 15.98 ± 0.67 mmol/l and it was 6.57 ± 0.82 mmol/l in the third week after treatment and it was near to the control group 6.0 ± 1.03 mmol/l. Incase of lipid profile, the plant extract decreased the level of cholesterol, triglycerides and LDL. (Table 2). The decrease in cholesterol,
Table 1: Effect of F. racemosa seeds extract on fasting blood glucose

<table>
<thead>
<tr>
<th>Groups</th>
<th>1st week Glucose (mmol/l)</th>
<th>3rd week Glucose (mmol/l)</th>
<th>Decrease/increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (200 mg/kg)</td>
<td>15.98 ± 0.67</td>
<td>6.57 ± 0.82(**)</td>
<td>−55.680</td>
</tr>
<tr>
<td>Group 2 Metformin (500 µg/ kg)</td>
<td>10.68 ± 0.11</td>
<td>5.85 ± 0.18(*** timeframe)</td>
<td>−51.546</td>
</tr>
<tr>
<td>Group 3 (Diabetic Control)</td>
<td>11.52 ± 0.42</td>
<td>15.40.73 ± 0.67(*** timeframe)</td>
<td>24.479</td>
</tr>
<tr>
<td>Group 4 (Control)</td>
<td>6.0 ± 1.03</td>
<td>6.8 ± 1.90</td>
<td>1.493</td>
</tr>
</tbody>
</table>

All the values (mmol/l) are expressed as mean ± SEM (standard error of mean). In each group 6 mice were taken. Level of significance was taken as *p < 0.05, **p < 0.01, ***p < 0.001, NS = Not Significant.

Table 2: Effect of F. racemosa seeds extract on lipid profile parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=6)</th>
<th>Diabetic control (n=6)</th>
<th>Standard control (metformin 500 µg/kg)</th>
<th>Extract, 200 mg/kg (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>54 ± 2.6</td>
<td>102 ± 6.6</td>
<td>66.13 ± 6.7</td>
<td>61.042 ± 10.20 (*** timeframe)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>120.7 ± 10.4</td>
<td>259.7 ± 19.4</td>
<td>218.01 ± 16.67</td>
<td>162.96 ± 22.91 (*** timeframe)</td>
</tr>
<tr>
<td>LDL</td>
<td>180.3 ± 22.8</td>
<td>304.3 ± 22.8</td>
<td>255.328 ± 17.77</td>
<td>212.53 ± 4.22 (*** timeframe)</td>
</tr>
<tr>
<td>HDL</td>
<td>9.6 ± 1.7</td>
<td>14.6 ± 1.7</td>
<td>18.13 ± 0.64</td>
<td>15.54 ± 0.095 NS</td>
</tr>
</tbody>
</table>

All the values (mg/dl) are expressed as mean ± SEM (standard error of mean). In each group 6 mice were taken. Level of significance was taken as *p < 0.05, **p < 0.01, ***p < 0.001, NS = Not Significant.

The mechanism involved may be due to binding to the insulin receptors to act as insulin secretagogue, like biguanides. Other probable mechanisms by which the extracts of F. racemosa lowered blood glucose levels in diabetic mice may be by increasing glycogenesis, inhibiting gluconeogenesis in the liver, or inhibiting the absorption of glucose from the intestine or these might have improved insulin resistance. Further experiments are needed to determine the actual mechanism of action of the active constituents of the relative plant fractions.

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease. This abnormally high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots; thus, hypercholesterolemia and hypertriglyceridemia are known to occur in STZ induced diabetic mice. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyzes triglycerides. However, lipoprotein lipase is not activated in conditions of insulin deficiency, thus resulting in hypertriglyceridemia. The observed hypocholesterolemic and hypotriglyceridemic effects of F. racemosa seeds therefore may be due to the activation of the enzyme, lipoprotein lipase. So the next step should be to isolate the compounds that are responsible for the observed antidiabetic activity and to elucidate the exact mechanism of action.

REFERENCES