ANTIHYPERTHYPERGLYCEMIC ACTIVITY OF METHANOLIC EXTRACT OF MADHUCA LONGIFOLIA BARK

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Key words: Madhuca longifolia, hypoglycemia, streptozotocin

SUMMARY

The study was carried out to assess the antihyperglycemic effects of methanolic extract of Madhuca longifolia bark in normal, glucose loaded and streptozotocin induced diabetic rats. All three animal groups were administered the methanolic extract of Madhuca longifolia at a dose of 100 and 200 mg/kg body weight (p.o.) and the standard drug glibenclamide at a dose of 500 µg/kg. Serum glucose level was determined on days 0, 7, 14 and 21 of treatment. The extract exhibited a dose dependent hypoglycemic activity in all three animal models as compared with the standard antidiabetic agent glibenclamide. The hypoglycemia produced by the extract may be due to the increased glucose uptake at the tissue level and/or an increase in pancreatic β-cell function, or due to inhibition of intestinal glucose absorption. The study indicated the methanolic extract of Madhuca longifolia to be a potential antidiabetic agent, lending scientific support for its use in folk medicine.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia, hypertriglyceridemia and hypercholesterolemia, resulting from defects in insulin secretion or action or both (1). Diabetes is crudely grouped into two types: insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). Both types are associated with excessive morbidity and mortality(2).

Madhuca longifolia, synonym M. indica, belonging to the family Sapotaceae, is an important economic tree growing throughout India. Traditionally, Madhuca longifolia bark has been used against diabetes, rheumatism, ulcers, bleeding and tonsillitis (3).

The flowers, seeds and seed oil of madhuka have great medicinal value. Externally, the seed oil massage is very effective to alleviate pain. In skin diseases, the juice of flowers is rubbed for oleation. It is also beneficial as a nasya (nasal drops) in diseases of the head due to pitta, like sinusitis (4).
The aim of the present study was to demonstrate the antihyperglycemic effects of methanol bark extract of Madhuca longifolia in normal and streptozotocin (STZ) induced diabetic rats.

MATERIAL AND METHODS

Plant material

Bark samples were collected from Udupi, Karnataka, India, during January and February 2009 and authenticated by Dr. Gopalkrishna Bhat, a taxonomist from Department of Botany, Poornaprajna College, Udupi. The specimen sample No.14 is kept at the Department, B. Pharmacy College, Rampura, Kakanapur, Godhara.

Preparation of extract

Dried bark samples (7 kg) were ground in a Waring blender and sifted through a wire screen (mesh size 2 mm × 2 mm). The powdered leaves (500 g) were exhaustively extracted with methanol. The extracts were filtered and evaporated to dryness at a temperature below 30 °C. The extract obtained with methanol was 28 g.

Animals

Wistar rats of both sexes (150-200 g) were maintained under standard animal house conditions, fed standard pellet diet (Hindustan Lever Ltd., Bombay) and allowed water ad libitum. Fasted animals were deprived of food for at least 16 h, but were allowed free access to water. The study was approved by the Institutional Animal Ethics Committee of B. Pharmacy College, Rampura, Godhara.

Acute toxicity and selection of doses

The acute toxicity studies were carried out in adult female albino rats weighing 150-200 g, by up and down method as per OECD 425 guidelines (5). Overnight fasted animals received test drug at a dose of 2000 mg/kg body weight orally. Then the animals were observed continuously once in half an hour for the next 4 hours and then after 24 hours for general behavioral, neurologic and autonomic profiles and to find out mortality. The extract was found safe to up to a dose of 2000 mg/kg body weight.

Oral glucose tolerance test

The oral glucose tolerance test was performed in overnight fasted normal animals (6). Rats divided into four groups (n=6) were administered 2% gum acacia solution, methanolic extract 100 mg/kg, 200 mg/kg and glibenclamide (500 µg/kg). Glucose (2 g/kg) was fed 30 min after the administration of methanolic extract. Blood was withdrawn from the retro-orbital sinus at 0, 30, 60, 90 and 120 min of methanolic extract administration. Fasting serum glucose levels were estimated by the Radio Immuno Assay kit (BRAC, Mumbai).

Normoglycemic study

For normoglycemic study, rats were divided into five groups (n=6) and administered 2% gum acacia solution, methanolic extract 100 mg/kg, 200 mg/kg and glibenclamide (500 µg/kg) (7). Blood glucose levels were estimated on days 0, 4, 8 and 12.

Determination of total protein, total cholesterol, creatinine and blood urea nitrogen

Total proteins were determined by the method described by Lowry et al. (8). Creatinine and blood urea nitrogen were determined by the kit supplied by Merck Specialties, Ltd. Total cholesterol was determined by the kit supplied by Beacon Diagnostics, Kabilpore, India.

Induction of experimental diabetes

Diabetes was induced by administering intraperitoneal injection of a freshly prepared STZ solution (60 mg/kg of body weight) in 0.1M cold citrate buffer to the overnight fasted rats. Because of the STZ instability in aqueous media, the solution is made using cold citrate buffer (pH 4.5) immediately before administration. Animals with blood glucose values above 250 mg/dL on day 3 of STZ injection were considered as diabetic rats. The treatment was started after day 5 of diabetes induction and was considered as day 1 of treatment.
Experimental design

The animals were divided into five groups of six animals, as follows: group I, normal healthy control; group II, diabetic control (STZ 60 mg/kg i.p.); group III, diabetic + methanolic extract (100 mg/kg body weight, orally); group IV, diabetic + methanolic extract (200 mg/kg body weight, orally); and group VI, diabetic + glibenclamide (500 µg/kg body weight, orally).

Blood sampling

At the end of day 12, blood samples were collected from the inner canthus of the eye under light ether anesthesia using capillary tubes (Micro Hemocrit Capillaries, Mucaps). Blood was collected into fresh vials containing anticoagulant antiserum and separated in a centrifuge at 2000 rpm for 2 min. Serum insulin levels were estimated by the Radio Immuno Assay kit supplied by the Board of Radiation and Isotope Research, Bhaba Atomic Research Centre (BRAC), Mumbai, India.

Statistical analysis

Data were statistically evaluated by use of one-way ANOVA, followed by post hoc Scheffe’s test using 7.5 version of the SPSS computer software. The values were considered significant at \( P < 0.05 \).

RESULTS

Acute toxicity studies

Acute toxicity studies revealed the non-toxic nature of methanolic extract at the two dose levels tested. There were no morphological changes like distress, hair loss, restlessness, convulsions, laxative effect, coma, weight loss, etc. At the end of the treatment period, there was no lethality or toxic reaction at any of the doses selected.

Glucose tolerance test

In all groups except for glibenclamide, at 30 min of initiating glucose tolerance test, blood glucose concentration was higher than at zero time but decreased significantly from 30 min to 120 min (Table 1). Methanolic extracts were enhancing glucose utilization, thus the blood glucose level was significantly decreased in glucose loaded rats.

Normoglycemic study

In normoglycemic rats, the doses of 100 and 200 mg/kg reduced hyperglycemia on days 4, 8 and 12 of treatment (Table 2). A significant hypoglycemic activity was found on day 12 with 100 and 200 mg/kg doses.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (vehicle)</td>
<td>89.2±2.9</td>
<td>108.3±1.1</td>
<td>103.7±2.1</td>
<td>100.2*±1.4</td>
<td>96.3*±1.6</td>
</tr>
<tr>
<td>2</td>
<td>Methanolic extract (100 mg/kg)</td>
<td>80.4±1.1</td>
<td>92.7±2.1</td>
<td>86.7±3.1</td>
<td>83.6±1.8</td>
<td>79.8±1.3</td>
</tr>
<tr>
<td>3</td>
<td>Methanolic extract (200 mg/kg)</td>
<td>89.3±1.7</td>
<td>97.4±2.2</td>
<td>90.5±2.5</td>
<td>84.2*±1.2</td>
<td>75.4*±1.3</td>
</tr>
<tr>
<td>4</td>
<td>Glibenclamide (500 µg/kg)</td>
<td>83.4±1.5</td>
<td>79.5±1.1</td>
<td>80.1±2.7</td>
<td>75.1*±2.1</td>
<td>73.6±2.9</td>
</tr>
</tbody>
</table>

Table 1. Effect of methanolic extract on serum glucose level (mg/dL) on glucose tolerance test in glucose loaded rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Day 0</th>
<th>Day 4</th>
<th>Day 8</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (Vehicle)</td>
<td>78.4±3.3</td>
<td>76.1±36</td>
<td>79.4±2.2</td>
<td>76.3±2.4</td>
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<tr>
<td>2</td>
<td>Methanolic extract (100 mg/kg)</td>
<td>82.1±2.6</td>
<td>80.3±3.3</td>
<td>79.8*±3.7</td>
<td>77.5*±1.3</td>
</tr>
<tr>
<td>3</td>
<td>Methanolic extract (200 mg/kg)</td>
<td>74.4±2.3</td>
<td>70.6±2.4</td>
<td>67.3±3.6</td>
<td>61.8*±1.3</td>
</tr>
<tr>
<td>4</td>
<td>Glibenclamide (500 µg/kg)</td>
<td>80.1±2.3</td>
<td>73.2±3.9</td>
<td>66.7*±3.2</td>
<td>59.6*±1.7</td>
</tr>
</tbody>
</table>

Table 2. Effect of methanolic extracts on serum glucose level (mg/dL) in normal fasted animals

Values are expressed as mean ± SEM; n=6 in duplicate for each treatment; *statistically significant difference from the corresponding zero time value; \( P<0.05 \).
After oral administration of 100 and 200 mg/kg of the methanolic extract of *Madhuca longifolia*, a significant reduction was observed in the blood glucose level of STZ induced diabetic rats. A dose dependent effect was seen with doses of 100 and 200 mg/kg of methanolic extract throughout the study period (Table 3). Blood sugar level was also determined before and after glibenclamide treatment. Glibenclamide, a known hypoglycemic agent, reduced blood sugar level.

### Biochemical assay

The protein amount in diabetic control group was reduced as compared to normal control. The lowered levels of protein after treatment with methanolic extracts increased near to the control one. Total cholesterol level, which was increased in diabetic rats, was also reduced after treatment with methanolic extracts. Creatinine and blood urea nitrogen values were significantly increased in STZ induced diabetic rats. Oral administration of methanolic extracts for three weeks significantly lowered creatinine and blood urea nitrogen in STZ induced diabetic rats.
DISCUSSION

A wide range of synthetic oral antidiabetic drugs such as sulfonylureas and biguanides have been used for 50 years now in the treatment of diabetes. However, they have not been of much benefit in controlling the complications of the disease. Streptozotocin is a broad spectrum antibiotic obtained from Streptomyces achromogenes. STZ causes massive reduction in insulin release via destruction of β cells of the islets of Langerhans and thereby induces hyperglycemia (9).

In the present study, the antihyperglycemic activity of methanolic bark extract of Madhuca longifolia was assessed in normal and STZ induced diabetic rats. Oral administration of a single dose of methanolic bark extract of Madhuca longifolia caused a significant decrease in serum glucose level in normal rats. A dose of 200 mg/kg of methanolic extract produced maximum glucose lowering effect, whereas 100 mg/kg of ethanolic extract showed a significant hypoglycemic effect throughout the study period. In the oral glucose tolerance test, the Madhuca longifolia bark extract showed significant reduction of serum glucose levels and these effects were dose dependent. The extract of Madhuca longifolia bark displayed a significant hypoglycemic effect in normal rats. The main mechanism by which the extracts bring the hypoglycemic effects most probably involves stimulation of peripheral glucose consumption. Furthermore, the glycemia profile observed in the glibenclamide group indicates that the extract of Madhuca longifolia acts on the liver or on peripheral glucose consumption (10).

The glibenclamide effects on glucose can be attributed to the enhanced activity of the β cells of the pancreas, resulting in secretion of a large amount of insulin. These results have indicated that some drugs may also be effective in NIDDM. The significant hypoglycemic effects of Madhuca longifolia bark in diabetic rats indicate that this effect can be mediated by stimulation of glucose utilization by peripheral tissues.

Insulin deficiency or insulin resistance is associated with hypercholesterolemia (11). Administration of methanolic extract of Madhuca longifolia bark to diabetic rats lowered the total cholesterol level.

Renal disease is one of the most common and severe complications of diabetes. Insulin is a physiological factor, which plays an important role in the maintenance of protein balance (12). In addition, significant elevations in the urea and creatinine levels indicate an impaired renal function in diabetes. Methanolic extracts of Madhuca longifolia bark improve renal function that is generally impaired in diabetic rats.

The results of the present study clearly indicated the methanolic extract of Madhuca longifolia bark to have a hypoglycemic effect on STZ induced diabetic rats. The extract was highly effective in managing the complications associated with diabetes mellitus, such as hypercholesterolemia and impaired renal function. Therefore, the Madhuca longifolia bark extract showed a therapeutic action against the development and progression of diabetic complications mentioned above. Further studies are in progress to isolate the active principle(s) and elucidate the exact mechanism of action of Madhuca longifolia bark.

REFERENCES


