ANTIDIABETIC AND HYPOLIPIDEMIC ACTIVITIES OF 
HIBISCUS TILIACEUS (L.) FLOWERS EXTRACT IN 
STREPTOZOTOCIN INDUCED DIABETIC RATS

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Summary

The present study was carried out to evaluate antidiabetic and hypolipidemic activities of Hibiscus tiliaceus methanolic flowers extract in streptozotocin induced diabetic wistar rat by administering graded oral doses (250 and 500 mg/kg body weight) for 21 days. The extract showed significant antidiabetic activity with significant improvement in body weight. Daily oral treatment with the extract for 21 days also resulted in significantly reduction serum cholesterol and triglycerides. HDL-cholesterol level was found to be improved ($p<0.01$) as compared to diabetic control group.

Keywords: Hibiscus tiliaceus; antidiabetic; hypolipidemic; rat; streptozotocin.

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Introduction

Diabetes mellitus is syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting impaired metabolism of glucose and other energy-yielding fuels such as lipids and protein (1). It affects nearly 25% of population and afflicts 150 million people and is set to rise to 300 million by 2025 (2-3). It causes number of complications like retinopathy, neuropathy, and peripheral vascular insufficiencies (4). Synthetic antidiabetic agents can produce serious side effects and they are not suitable for use during pregnancy. In view of the adverse effects associated with the synthetic drugs and as plants are safer, cheaper and much effective, conventional antidiabetic plants can be explored (5). World Health Organization has also recommended the evaluation of traditional plant treatments for diabetes (6). Also, diabetes has been treated orally with several medicinal plants or their extracts based on folklore medicine since ancient times.
Hibiscus tiliaceus L. (Malvaceae), commonly known as “bola” is a mangrove plant growing in tropical Asia and abundant in forests. In folk medicine, the leaves of this plant used to treat fevers, soothe coughs, ulcer, wounds and various skin diseases (7). In Indian system of medicine H. tiliaceus had been used as febrifuge, laxative, resolvent and emollient. An infusion of leave is employed to wash ulcer and wounds. The fruit juice is rubbed on skin to cure weakness (8-10). The flower extract also exhibited significant reducing power and free radical scavenging effect on hydroxyl, superoxide, hydrogen peroxide radicals(7). The plant also showed antinociceptive and anti-inflammatory effects (11). Traditionally, the plant has been used for diabetes (12). Furthermore, Hibiscus rosa sinensis and Hibiscus sabdariffa from the same genus has antidiabetic effect (13-14). The literature survey revealed that there is no experimental evidence of antidiabetic effect of the plant. Therefore, the present work was undertaken to explore the antidiabetic and hypolipidemic potentials of H. tiliaceus methanolic flowers extract (HTMFE) of the plant in streptozotocin induced diabetic wistar rat.

Materials and Methods

Plant material
Hibiscus tiliaceus flowers were collected during month of August from the campus of Kurukshetra University, Kurukshetra, India and were identified by Dr. B.D. Vashishta, Department of Botany, Kurukshetra University, Kurukshetra, India. A voucher specimen of the plant is preserved in the herbarium of the Faculty of Pharmaceutical Sciences, Kurukshetra University (No. IPS/KUK/HT/2009).

Extract preparation
The flowers were dried under shade and powdered to coarse particles. The powdered material was defatted with petroleum ether (60-80°C) in a Soxhlet extraction apparatus and further the same amount plant material extracted with methanol. The extract was dried at 45°C in rotary evaporator to produce a semisolid mass and stored in airtight containers in refrigerator below 10°C.

Chemicals
Streptozotocin (STZ) was purchased from Sigma-Aldrich, India. The STZ solution was prepared by freshly dissolving in citrate buffer (0.01 M, pH 4.5). Total cholesterol, High density lipoprotein (HDL)-cholesterol and triglyceride (TC) standard kits were purchased from Erba diagnostics.
Mannheim Gambh, Germany. All reagents used in study were analytical grade.

Animals
Wistar rat of either sex, weighing about 150-250 g were used in the study. Animals were maintained under standard environmental conditions i.e. ambient temperature of 22 ± 2 °C and at 45–55% relative humidity for 12 h, each of dark and light cycle and fed with a standard pellet rats diet obtained from Ashirwad Industries, Chandigarh, India and water was supplied ad libitum. All the studies were conducted in accordance with the Animal Ethical Committee of the University.

Induction of diabetes
After one week of acclimatization, the rats were subjected to a 12-h fast. Rats were made diabetic by a single dose of STZ 60 mg/kg body weight i.p. The blood glucose level was checked before and 72 h after streptozotocin injection to confirm the development of diabetes. The diabetic animals were stabilized for five days and the next day (day 0) experiment was started. Only those animals which showed blood glucose levels >250 mg/dl were separated and used for the study.

Experimental design
All the diabetic animals were randomly divided into five groups with six animals each and treated once a day for 21 days as follows:
Group I (Normal healthy control): given only vehicle (Tween 80, 1% v/v).
Group II served as diabetic control: received only vehicle
Group III diabetic rats: received HTMFE 250 mg/kg.b.w.
Group IV diabetic rats: received HTMFE 500 mg/kg.b.w.
Group V diabetic rats: received (Glibenclamide 10 mg/kg.b.w.)

Blood glucose was measured with elegance glucometer (CT-X10, Convergent Technologies, Germany) at weekly intervals i.e. 0, 7, 14 and 21 day after daily administration of extract orally.

Lipid profile
On day 21, blood was collected by retro-orbital puncture under mild ether anesthesia from rats. Total cholesterol and triglyceride were determined by the method of Rifai et al. (15). HDL-cholesterol was also evaluated in normal and streptozotocin induces diabetic rats by autoanalyser (Erba Chem 7, Mannheim, Germany) using Erba diagnostic kits by methods of Burstein et al. (16).
Statistical analysis
All the data were expressed as mean ± S.E.M. Statistical analysis was carried using Student's t-test to analyze the significance between the groups. A value of $p < 0.05$ was considered to be significant.

Results

Effect on blood glucose level
In the study, the antidiabetic potential of HTMFE was measured in STZ induced rats and it was found that daily administration of the extract for three weeks led to a dose dependent fall in blood glucose levels. There was a significant increase in blood glucose level ($p<0.01$) in diabetic rats when compared with normal controls and it was significantly ($p<0.01$) reduced by 21 days treatment with methanolic extract of *Hibiscus tiliaceus*. At the end of experiment (21st day) blood glucose (FBG) level was $158.53 ± 2.1$ and $135.51 ± 2.4$ mg/dl in the doses 250 and 500 mg/kg of HTMFE respectively. The antidiabetic effect of HTMFE on the blood glucose levels in diabetic rats is shown in Table 1.

Table 1. Effect of *H. tiliaceus* on the blood glucose levels in diabetic rat

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial day</td>
</tr>
<tr>
<td>I</td>
<td>115.27 ± 4.5</td>
</tr>
<tr>
<td>II</td>
<td>258.41 ± 2.3</td>
</tr>
<tr>
<td>III</td>
<td>278.32 ± 2.2</td>
</tr>
<tr>
<td>IV</td>
<td>290.31 ± 2.1</td>
</tr>
<tr>
<td>V</td>
<td>274.27 ± 3.5</td>
</tr>
</tbody>
</table>

Data represent means ± S.E.M. *$p<0.05$, **$p<0.01$, When groups III and IV compared with diabetic control i.e. group II

Effect on body weight
There was a significant decrease ($p<0.01$) in the body weight of the diabetic controls (group II) compared with the normal controls (group I). During the weekly of observation of the flower extract-treated diabetic rats at doses of 250 mg/kg and 500 mg/kg, there were significant ($p < 0.05$) weight gains on day 21 relative to day 0 as shown in table 2.
Table 2. Effect of HTMFE on the body weight in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Change in body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial day</td>
</tr>
<tr>
<td>I</td>
<td>215.2 ± 2.3</td>
</tr>
<tr>
<td>II</td>
<td>225.23 ± 3.4</td>
</tr>
<tr>
<td>III</td>
<td>234.23 ± 2.5</td>
</tr>
<tr>
<td>IV</td>
<td>228.41 ± 2.2</td>
</tr>
<tr>
<td>V</td>
<td>225.34 ± 2.7</td>
</tr>
</tbody>
</table>

Data represent means ± S.E.M., *p<0.05, When groups III and IV compared with diabetic control i.e. group II

Effect on lipid profile

In the present study the total cholesterol and triglycerides was reduced in by 21 days treatment with HIMFE. HDL cholesterol level was significantly improved by treatment of HIMFE as compared to diabetic control group (Table 3).

Table 3. Effect of HTMFE on lipid profile (mg/dl)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Cholesterol</th>
<th>Triglycerides</th>
<th>HDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>87.28 ± 3.8</td>
<td>82.42 ± 5.16</td>
<td>37.32 ± 2.9</td>
</tr>
<tr>
<td>II</td>
<td>254.73 ± 7.6</td>
<td>150.52 ± 4.71</td>
<td>28.23 ± 2.2</td>
</tr>
<tr>
<td>III</td>
<td>124.31 ± 2.1*</td>
<td>116.42 ± 3.21*</td>
<td>35.32 ± 3.1*</td>
</tr>
<tr>
<td>IV</td>
<td>112.17 ± 2.5*</td>
<td>93.23 ± 4.13*</td>
<td>39.24 ± 2.3*</td>
</tr>
<tr>
<td>V</td>
<td>98.72 ± 5.3**</td>
<td>83.47 ± 4.5*</td>
<td>45.28 ± 4.8**</td>
</tr>
</tbody>
</table>

Data represent means ± S.E.M., *p<0.05, **p <0.01, When groups III and IV compared with diabetic control i.e. group II
The results of present study indicated that the methanolic flowers extract of *Hibiscus tiliaceus* possesses significant hypoglycemic activity.

**Discussion**

Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost (17). Therefore, investigation on such agents from traditional medicinal plants has become more important. *H. rosa sinensis* and *H. sabdariffa* from the same genus has antidiabetic effect (13-14). Keeping in view of this and traditional uses, the methanolic extract of *H. tiliaceus* flowers were investigated for its antidiabetic and hypolipidemic activities. The diabetic state was induced by intraperitoneal injection of streptozotocin. The animals having blood glucose levels above 250 mg/dl were selected for the experiment. Significant reduction of blood glucose levels is observed in diabetic rats treated with *H. tiliaceus* flowers 500 mg/kg ($p <0.01$).

The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (18-19) and contribute to coronary artery disease (20). The repeated administration the extract for a period of 21 days resulted in a significant decrease in lipid parameter levels when compared to the diabetic control. It was also observed that there was also significant weight gain in HTMFE treated diabetic rats compared with untreated diabetic animals. *H. tiliaceus* had showed *in vitro* antioxidant potential (7). Various studies have shown that *Diabetes mellitus* is also associated with increased formation of free radicals and decrease in antioxidant potential. It is accepted that oxidative stress results from an imbalance between the generations of oxygen derived radicals and the organism’s antioxidant potential (21) So, antidiabetic properties of *H. tiliaceus* might be due to antioxidant effect of the plant. Untill, the exact mechanism of action of reduction of blood glucose levels after administration (p.o.) of the extracts is not clear. The extracts should further be subjected to bioactivity guided drug discovery to isolate a lead compound responsible for this activity.

**Conclusion**

From this study, we can conclude that *Hibiscus tiliaceus* flowers extract has significant antidiabetic effects. The extracts also showed improvement in parameters like lipid profile and body weight. Further studies are required to identify the active constituents.
References

15. Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER. (Eds.), Tietz Textbook of


