Antidiabetic and hypolipidemic activities of *Dillenia indica* extract in diabetic rats

Sunil Kumar, Vipin Kumar, Om Prakash
Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra 136119, Haryana, India

**Objective:** The present study was carried out to investigate the antidiabetic and hypolipidemic activities of bioactive fraction (ethyl acetate fraction) of *Dillenia indica* methanolic extract in experimental diabetic Wistar rats.

**Methods:** Type-1 diabetes was induced by single intraperitoneal injection of streptozotocin (60 mg/kg body weight), and type-2 diabetes was induced by single intraperitoneal injection of streptozotocin (60 mg/kg body weight), 15 min after the intraperitoneal injection of 120 mg/kg nicotinamide. The rats were treated by administering graded oral doses of isolated ethyl acetate fraction of methanolic extract of *D. indica* (DIEE), 200 and 400 mg/kg body weight, respectively, for 21 d. The blood glucose level was estimated at weekly intervals by glucometer. Serum cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were also evaluated in normal and diabetic rats by an autoanalyzer.

**Results:** In both experimental models, daily oral treatment with DIEE for 21 d resulted in a significant reduction in blood glucose ($P < 0.01$), serum cholesterol ($P < 0.05$) and triglycerides ($P < 0.05$) levels whereas HDL-C level was found to be increased ($P < 0.05$) as compared with the diabetic control group.

**Conclusion:** DIEE at 400 mg/kg body weight has prominent antidiabetic effect in experimental type-1 and type-2 diabetes models in rats. It may therefore be used as an alternative remedy for the treatment of diabetes mellitus.

**Keywords:** Dilleniaceae; plant extracts; diabetes mellitus; experimental; hypoglycemic agents; antilipemic agents; rats

Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrates, fat and protein metabolism\(^{[1]}\). Worldwide, total number of people with diabetes is projected to increase from 171 million in 2000 to 366 million in 2030\(^{[2]}\). The pathogenesis of diabetes mellitus is
managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides[13]. Unfortunately, having numbers of side effects, they are not suitable for use during pregnancy[4, 5]. Plants are well-known in traditional herbal medicine for their hypoglycemic activities, and available literature indicates that there are more than 800 plant species showing hypoglycemic activity[4]. Plants used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the control of this disease. Therefore, plant materials are continuously scrutinized and explored for their effect as hypoglycemic agents.

Dillenia indica belongs to the family Dilleniaceae, commonly called “Elephant tree”. The fruit shows laxative properties and is used for relieving abdominal pain. The bark and leaves have astringent effects[7]. The juice of D. indica leaves, bark and fruits are mixed and given orally for the treatment of cancer and diarrhea[7]. Fruit and leaf extracts of D. indica are reported to have antioxidant activity[8, 9]. Central nervous system depressant[10] and anti-inflammatory activity[11] in mice and antimicrobial activity[12] were found from the alcoholic extract of the leaves of D. indica. D. indica extract revealed significant cytotoxic activity when tested by brine shrimp lethality bioassay and showed antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria and fungi[4]. The seed extract showed hepatoprotective effect[13]. Earlier phytochemical studies showed that D. indica contains triterpenoids and flavonoids such as lupeol, betulin, betulinic acid, β-sitosterol, stigmastanol and myricetin[8, 14, 15]. The fruits also showed significant antileukemic activity in human[16]. Traditionally, the plant is also used in treatment of diabetes[10, 17].

There is no previous research work available on antidiabetic effect of the plant. So, the present work was performed to explore the antidiabetic and hypolipidemic activities of isolated ethyl acetate fraction of methanolic extract of D. indica (DIEE).

1 Materials and methods

1.1 Drugs and reagents Streptozotocin (STZ; Lot Number: 000091422) was purchased from Sigma-Aldrich, India and nicotinamide was purchased from Hi-Media, India. Total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triacylglycerol were assayed by an autoanalyzer (Erba Chem 7, Mannheim, Germany) by using standard kits from Erba Diagnostics Mannheim Gmbh, Germany and blood glucose level was measured by using a glucose meter (Elegance CT-X10) of Convergent Technologies, Germany. All the reagents used in this study were of analytical grade.

1.2 Experimental animals Wistar rats of either sex, weighing about 150 to 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% to 55% relative humidity for 12 h, each of dark and light cycle and fed with a standard pellet rat’s 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 Kurukshetra University, India.

1.3 Plant materials D. indica leaves were collected from the campus of Kurukshetra University, Kurukshetra, India and were identified by Dr. H.B. Singh, Scientist F & Head, Raw Material Herbarium & Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. A voucher specimen of the plant is preserved in the herbarium (NISCAIR/RHMD/Consult/-2009-10/1381/182/1).

1.4 Extract preparation and fractionation The collected leaves were washed with distilled water and dried in shade. The dried leaves were powdered by using dry grinder and passed through a sieve. The powdered material was packed into Soxhlet apparatus and extracted with methanol below 60 °C. The extract was evaporated to dryness under reduced pressure at 45 °C to yield solid residues. The solid extract was suspended in water and successively extracted with hexane, ethyl acetate and n-butanol in separating funnel. Each fraction was stored in airtight containers in a refrigerator below 10 °C for subsequent experiments.

1.5 Induction of type-1 diabetes model (T1DM): insulin-dependent diabetes mellitus Animals were made diabetic by single intraperitoneal injection of STZ (60 mg/kg body weight) dissolved in 0.1 mol/L citrate buffer, pH 4.5. The blood glucose level was checked before and 72 h after STZ injection to confirm the development of diabetes. Only those animals which showed hyperglycemia (blood glucose level > 250 mg/dL) were used in the experiment.

1.6 Induction of type-2 diabetes model (T2DM): non-insulin-dependent diabetes mellitus T2DM was induced in overnight fasted animals by a single intraperitoneal injection of 60 mg/kg body weight STZ, 15 min after the intraperitoneal injection of 120 mg/kg body weight nicotinamide. Hyperglycemia was confirmed by the elevated blood glucose level determined at 72 h after and then on day 7 of the injection. Only rats confirmed with permanent T2DM were used in the antidiabetic study[18].

1.7 Biochemical parameters Blood samples were taken from the tip of tail of each rat of different groups under mild ether anesthesia, and glucose levels were determined by using blood glucose test strips with Elegance glucometer (Frankenberg, Germany) at weekly intervals i.e., 0, 7, 14 and 21 d after daily administration of the extract orally. Serum cholesterol, triacylglycerol and HDL-C
were also evaluated in normal and diabetic rats by
an autoanalyzer using Erba diagnostic kits[18, 39].

1.8 Experimental design Overnight fasted normoglycemic and diabetic rats were divided into
seven groups with six rats each and treated orally
daily for 21 d as follows. Group I: normal healthy
control receiving only vehicle (Tween 80,
2% v/v); group II: type-1 diabetic control
receiving only vehicle (Tween 80, 2% v/v);
group III: type-2 diabetic control receiving only
vehicle (Tween 80, 2% v/v); group IV: type-1
diabetic rats were treated with DlEE5 (200 mg/kg
body weight); group V: type-1 diabetic rats treated
with DlEE5 (400 mg/kg body weight); group VI:
type-2 diabetic rats treated with DlEE5 (200 mg/kg
body weight); group VII: type-2 diabetic rats
treated with DlEE5 (400 mg/kg body weight).

1.9 Statistical analysis All the data were presented
as $\bar{x} \pm s_x$. The statistical analysis involving
two groups was evaluated by means of one way analysis
of variance followed by Dunnett-t multiple comparison
post-test which was used for statistical comparison
between control and various treatment groups.
Statistical significance was accepted at $P<0.05$.

2 Results

2.1 Effect on T1DM STZ induced T1DM in rats
with fasting blood sugar level more than 250 mg/dL.
Daily oral administration of DlEE at 200 and
400 mg/kg significantly ($P<0.01$) reduced blood
glucose level in diabetic rats (Table 1).

2.2 Effect on T2DM STZ-nicotinamide induced
T2DM diabetes in rats. Blood glucose level was
increased in diabetic control group. Significant
reduction ($P<0.01$) in the blood glucose levels
was brought about in diabetic rats by daily adminis-
tration of DlEE at 400 mg/kg (Table 1).

2.3 Effect on lipid profile Daily treatment with
DlEE at 200 and 400 mg/kg for 21 d caused
significant improvement in the HDL-C level as
compared with the diabetic control groups. Serum
cholesterol and triglyceride levels were decreased
significantly ($P<0.01$) by DlEE in T1DM and
T2DM rats (Table 2).

| Table 1 Effect of DlEE on the blood glucose levels in diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose level ($\bar{x} \pm s_x$, mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>I: normal control</td>
<td>102.3±56.0</td>
</tr>
<tr>
<td>II: type-1 diabetic control</td>
<td>2616.5±32.2</td>
</tr>
<tr>
<td>III: type-2 diabetic control</td>
<td>780.0±35.2</td>
</tr>
<tr>
<td>IV: type-1 diabetic+DlEE (200 mg/kg body weight)</td>
<td>652.3±34.8</td>
</tr>
<tr>
<td>V: type-1 diabetic+DlEE (400 mg/kg body weight)</td>
<td>745.8±37.6</td>
</tr>
<tr>
<td>VI: type-2 diabetic+DlEE (200 mg/kg body weight)</td>
<td>892.3±35.7</td>
</tr>
<tr>
<td>VII: type-2 diabetic+DlEE (400 mg/kg body weight)</td>
<td>853.5±32.5</td>
</tr>
</tbody>
</table>

* $P<0.05$, ** $P<0.01$, vs group III; $\Delta P<0.05$, $\Delta\Delta P<0.01$, vs group II. DlEE: isolated ethyl acetate fraction of methanolic extract of D. indica.

| Table 2 Effect of DlEE on lipid profile in diabetic rats after 21 d of treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cholesterol</th>
<th>Triacylglycerol</th>
<th>High-density lipoprotein-cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: normal control</td>
<td>872.8±38.0</td>
<td>824.2±51.6</td>
<td>373.2±29.0</td>
</tr>
<tr>
<td>II: type-1 diabetic control</td>
<td>547.3±76.0</td>
<td>505.2±47.1</td>
<td>282.3±22.0</td>
</tr>
<tr>
<td>III: type-2 diabetic control</td>
<td>575.4±54.0</td>
<td>564.3±32.5</td>
<td>243.7±25.0</td>
</tr>
<tr>
<td>IV: type-1 diabetic+DlEE (200 mg/kg body weight)</td>
<td>185.8±45.0</td>
<td>1048.7±25.4</td>
<td>321.3±34.4</td>
</tr>
<tr>
<td>V: type-1 diabetic+DlEE (400 mg/kg body weight)</td>
<td>132.4±34.0</td>
<td>984.7±32.5</td>
<td>354.4±24.4</td>
</tr>
<tr>
<td>VI: type-2 diabetic+DlEE (200 mg/kg body weight)</td>
<td>274.4±34.0</td>
<td>1072.4±44.6</td>
<td>303.4±39.0</td>
</tr>
<tr>
<td>VII: type-2 diabetic+DlEE (400 mg/kg body weight)</td>
<td>174.3±49.0</td>
<td>1023.0±22.8</td>
<td>244.4±43.0</td>
</tr>
</tbody>
</table>

* $P<0.05$, ** $P<0.01$, vs group II; $\Delta P<0.05$, $\Delta\Delta P<0.01$, vs group III. DlEE: isolated ethyl acetate fraction of methanolic extract of D. indica.

3 Discussion

STZ possesses diabetogenic properties mediated
by destruction of pancreatic $\beta$-cells and has been
widely used to induce diabetes in experimental animals[21]. STZ-nicotinamide could induce type-2
diabetes in rats. The present research work was
carried out to evaluate antidiabetic and hypolipidemic activities of DlEE in both T1DM and T2DM
rat models. DlEE brought significant reduction in
blood glucose level in both types of diabetic rats.
Lipid abnormality accompanying with atherosclerosis
is the major cause of cardiovascular disease in
diabetes. Ideal treatment for diabetes, in addition
to glycemic control, should have a favorable effect
on lipid profiles. Abnormalities in lipid profile are
found in about 40% of diabetics[31]. Untreated
diabetes is also associated with hypercholesterolemia
and hypertriglyceridemia[31]. In the present work,
DlEE reduced the total cholesterol and triglycerides
levels as compared with the diabetic control groups. There was also beneficial effect on HDL-C. This hypolipidemic effect may be due to inhibition of fatty acid synthesis\cite{1}. Therefore, DIEE could be useful in managing both types of diabetes as well as abnormal lipid profile. The antidiabetic activity of the plant may be due to the presence of bioactive constituents such as triterpenoids and flavonoids.

4 Conclusion

The results indicate that the DIEE is effective in decreasing the blood glucose level in diabetic animals and also have beneficial effect on lipid profile. However, the nature of the active constituents responsible for such an effect requires further investigation. The possible mode of action of the plant extract might be by potentiation of the insulin effect by increasing the pancreatic secretion of insulin from β-cells of islet of Langerhans or its release from the bound form or regeneration of the cells.

5 Competing interests

The authors declare that they have no competing interests.

REFERENCES

15 Bate-Smith EC, Harborne JB. Differences in flavonoid content between fresh and herbarium leaf tissue in Dillenia. Phytochemistry. 1971; 10(5): 1055-1058.
五桠果提取物对糖尿病大鼠的降血糖及降血脂作用

Sunil Kumar, Vipin Kumar, Om Prakash
Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra 136119, Haryana, India

目的：使用实验性糖尿病 Wistar 大鼠模型验证五桠果（Dillenia indica）甲醇提取物的生物活性成分（乙酸乙酯馏分）的降血糖及降血脂作用。

方法：通过腹腔内注射链脲佐菌素（60 mg/kg）建立大鼠1型糖尿病模型，腹腔内注射烟酰胺（120 mg/kg）15 min 后腹腔内注射链脲佐菌素（60 mg/kg）建立大鼠2型糖尿病模型。在不同的实验模型中，用药组大鼠均分别给予 200 mg/kg 和 400 mg/kg 的五桠果甲醇提取物的乙酸乙酯馏分连续 21 d。使用血糖仪每周 1 次监测各组大鼠血糖水平。使用自动分析仪器分别测定各组大鼠血清总胆固醇、三酰甘油及高密度脂蛋白胆固醇水平。

结果：在两个实验模型中，与模型组比较，五桠果甲醇提取物的乙酸乙酯馏分均显著降低模型大鼠的血糖水平（P<0.01）以及血清总胆固醇（P<0.05）和三酰甘油（P<0.05）含量，升高高密度脂蛋白胆固醇水平（P<0.05）。

结论：五桠果甲醇提取物的乙酸乙酯馏分在 400 mg/kg 剂量时，对于 1 型及 2 型糖尿病模型大鼠具有显著的治疗作用。因此这种提取物有望被研究开发成为治疗糖尿病的又一替代药物。

关键词：五桠果科；植物提取物；糖尿病，实验性；降血糖药；降血脂药；大鼠

-------------------------------

2011 全国时间生物医学学术会议征文通知

由中国中西医结合学会时间生物医学专业委员会主办、苏州大学承办、山东省医学科学院山东省抗衰老研究中心协办的“2011 全国时间生物医学学术会议”将于 2011 年 9 月中旬在广西南宁市召开。现将有关会议事宜通知如下。

会议时间：2011 年 9 月 15～18 日，会期 4 天。

会议地点：南宁市天德商务酒店（南宁市兴宁区明秀东路 238 号）。

会议内容：（1）学术交流；①现代时间生物医学研究方法与技术；②生物节律基因控制；③时间生理、时间药理、时间毒理、时间治疗、时间护理、时间营养、睡眠、时差及轮班不适应症的防治等相关研究；②中医药时间医学与时间针灸。包括脏腑经络气血生理节律、疾病时间节律、疾病时间治疗、时间养生保健、传统时间针灸等相关研究；（2）国内外时间生物学著名专家作有关时间生物学研究进展学术报告；（3）时间生物医学专业委员会换届；（4）成立时间生物医学青年委员会。

征稿要求：论文全文（包括 400 字以内的中、英文摘要，研究性论文请按目的、方法、结果、结论四部分撰写），以电子邮件的附件形式（Word 文件）发送至会议联系人邮箱（联系人：赵子彦；电子信箱：ziyianzhao@163.com 或 ziyianzhao@sina.com），截稿日期：2011 年 7 月 31 日。详情参见中国时间生物医学网：http://www.chrono-biology.net。

会务费：800 元/人，学生 400 元/人。食宿及交通费用自理。