Eleven antidiabetic Indian medicinal plants were investigated in streptozotocin induced diabetic rat model and provided scientific validation to prove their antihyperglycemic activity. Antidiabetic principles from five plants were isolated. All the compounds isolated were evaluated for antihyperglycemic activity in streptozotocin induced diabetic rat model and activities were compared with standard drug metformin. Some compounds were also screened in db/db mice. Two compounds (PP-1 and PP-2) inhibited significantly the activity of PTPase-1B in an in vitro system. This might be the underlying mechanism of antihyperglycemic activity of these compounds.

**Keywords**: Antihyperglycemic activity, Normoglycemic rat model, PTPase-1B, STZ

Diabetes mellitus is characterized by group of metabolic disorders. Deficiency or insensitivity of insulin causes glucose to accumulate in the blood, leading to various complications. When breakdown of glucose is stopped, body uses fat and protein for producing the energy. Due to this polydipsia, polyuria, polyphagia, and excessive weight loss occur. High blood sugar harms organs and increases risk of heart disease. Cardiovascular disease, retinopathy, neuropathy and nephropathy are the complications associated with type 2 diabetes. Clinically diabetic patients are characterized by marked increase in blood glucose level followed by mild hyperlipidemia. Effective treatment includes controlling hyperglycemia as well as secondary complications.

For centuries, plants have been used to treat human diseases. In Ayurveda various herbs are reported for treating and preventing diabetes. Herbal drugs have lesser or no side effects and are less expensive as compared to synthetic drugs. Therefore, identification of antihyperglycemic leads from the plants has become more important. In the present study, We investigated antihyperglycemic activity in the extracts of *Aegle marmelose*, *Coccinia indica*, *Dodecadenia grandiflora*, *Ficus bengalensis*, *Ocimum sanctum*, *Pongamia pinnata*, *Pterocarpus marsupium*, *Tectona grandis*, *Tinospora cordifolia*, *Withania coagulans* and *Zingiber officinale*, and isolated anti diabetic principals from *F. bengalensis*, *P. pinnata*, *P. marsupium*, *T. cordifolia* and *W. coagulans*.

**Materials and Methods**

Plant materials—Authenticated leaves of *Aegle marmelose* (collected in the month of March from Lucknow, CDRI plant code no. 4499), *Coccinia indica* (collected in the month of July from Lucknow, CDRI plant code no. 4536), *Dodecadenia grandiflora* (collected in the month of October from Almora, CDRI plant code no. 4699), *Ocimum sanctum* (collected in the month of December from Lucknow, CDRI plant code no. 38), *Tectona grandis* (collected in the month of February from Sirumalai, Tiruchirapalli, CDRI plant code no. 4483) and fruits of *Pongamia pinnata* (collected in the month of April from Lucknow, CDRI plant code no. 3200), *Withania coagulans* (collected in the month of November from Lucknow, CDRI plant code no. 4554) as well as aerial roots of *Ficus bengalensis* (collected in the month of November from Lucknow, CDRI plant code no. 4554) as well as aerial roots of *Ficus bengalensis* (collected in the month of November from Lucknow, CDRI plant code no. 4617), heartwood of *Pterocarpus marsupium* (collected in the month of November from forest of Madhya Pradesh, CDRI plant code no. 4500), stems of *Tinospora cordifolia* (collected in the month of March from Palampur, CDRI plant code no. 4434) and rhizomes of *Zingiber officinale* (collected in the month of January from Lucknow, CDRI plant code no. 4735) were taken for the study.
Isolation of compounds—Compound FB (α-amyrin acetate) was obtained from the hexane fraction of the ethanolic extract of the aerial roots of *Ficus bengalensis* (Fig. 1).

The ethanolic extract of fruits of *Pongamia pinnata* was triturated successively with n-hexane and chloroform. The concentrated chloroform fraction was suspended in distilled water and then extracted with n-butanol (n-BuOH) saturated with water. The chloroform fraction was subjected to repeated column chromatography furnishing compounds PP-1 (pongamol) and PP-2 (karanjin)²⁻³.

Compound PM (pteroside) was isolated from n-BuOH fraction of the aqueous extract of heartwood of *Pterocarpus marsupium* through repeated flash chromatography⁴.

The n-BuOH fraction of the ethanolic extract of the stem of *Tinospora cordifolia* was subjected to repeated column chromatography affording compound TC (Tinosporaside)⁵.

The aqueous extract of *W. coagulans* was subjected to extensive column chromatographic procedure, leading to isolation of compound WC (coagulin L). WC was further derivatized to WC-1⁶ and WC-2⁷, out of which WC-1 is a new compound. WC (1 equivalent) was refluxed with hydroxylamine hydrochloride (31 equivalent) and crystallized sodium acetate for 1 at 100°C. The product oxime (WC-1) was purified by column chromatography over flash silica gel using methanol:chloroform (1:4) eluate. Compound WC-2, which is aglycon of WC, was formed by enzymatic hydrolysis of compound WC by using culture of *Aspergillus ochraceus*.

Animals—Male albino rats of Sprague–Dawley strain (body weight 140 ± 20 g) was selected for the study. C57BL/KsJ-db/db mice were taken as type 2 diabetes model.

Assessment of antihyperglycemic activity in normoglycemic rat model—Male albino rats of Sprague Dawley strain were selected for this study having body weight 140±20g. After 14-16 h starvation fasting blood glucose of each animal was measured and the animals showing blood glucose level between 60 to 80 mg/dl were finally selected

![Fig. 1—Isolated compounds and derivatives](image-url)
and divided into groups of 5 animals each. Rats of experimental group were orally administered the suspension of the desired compounds at a dose of 100 mg/kg body weight prepared in 1.0% gum acacia. Suspension of the standard drug metformin was given at 100 mg/kg dose level. Animals of control group received vehicle (1.0% gum acacia). An oral sucrose load of 10 g/kg body weight was given to rats of all group post 30 min administration of the test sample/vehicle. Blood glucose level was measured at 30, 60, 90 and 120 min post administration of sucrase

Assessment of antihyperglycemic activity in streptozotocin (STZ) induced diabetic rat model—Male albino rats of Sprague Dawley strain were selected for this study having body weight 140±20g. Streptozotocin was dissolved in 100 mM citrate buffer, pH 4.5, and calculated amount of the fresh solution was injected to overnight fasted rats (60 mg/kg) intraperitoneally. Blood glucose was checked 48 h later by glucometer by using glucostrips and animals showing blood glucose values between 144 and 270 mg/dl were selected and divided into groups of 5 animals each. Rats of experimental groups were administered suspension of the desired test samples orally (made in 1.0% gum acacia) at a dose of 100 mg/kg body weight. Animals of control group were given an equal amount of 1.0% gum acacia. A sucrose load of 2.5 g/kg of body weight was given after 30 min of drug administration. After 30 min of post-sucrose load, blood glucose level was again checked at 1, 2, 3, 4, 5 and at 6 h, respectively. Comparing the AUC of experimental and control groups determined the percent antihyperglycemic activity.

Assessment of antihyperglycemic activity in Type 2 diabetes model (C57BL/KsJ-db/db mice)—Hyperglycemic animals were divided into four groups having five animals each. Animals of one group were regarded as control group (orally administered 1% gum acacia) and other groups were treated as experimental groups (treated with suspension of the desired test substances at a dose of 50 mg/kg body weight). The treatment was continued for 10 consecutive days. Animals were dosed daily at a fixed time (10.00 to 11.00 AM). All animals had free access to fresh water and normal diet. Blood glucose profile of each animal was measured by Glucometer using glucostrips (Boehringer Mannheim). An oral glucose tolerance test (OGTT) of each individual was performed on day 10 after an overnight fast (10 h). Blood was sampled from the tail vein at time 0 min. (baseline), followed by 30, 60, 90 and 120 min after an oral glucose load of 3.0 gm/kg of body weight. Quantitative glucose tolerance of each animal was calculated by area under curve (AUC) method using Prism Software. Comparing the AUC of experimental and control groups determined the percentage antihyperglycemic activity.

Protein tyrosine phosphatase-1B assay—Protein tyrosine phosphatase-1B inhibitory activity of the compounds was determined by comparing the activity of the enzyme in the control, with a sample containing pure compound. The assay was performed by adding compound to the reaction mixture containing 10 mM pNPP in 50 mM HEPES buffer (pH 7.0) with 1 mM DTT, 2 mM EDTA and defined unit of enzyme protein. The reaction was terminated after 10 min of incubation at 37°C by the addition of 0.1N NaOH and the absorbance was determined at 405 nm. A molar extinction coefficient of 1.78×104 M⁻¹ cm⁻¹ was utilized to calculate the concentration of the p-nitrophenolate ion produced in the reaction mixture. IC₅₀ and Kᵢ values were determined by measuring the inhibitory activity of compounds at different concentrations.

Results
We have investigated eleven Indian medicinal plants for their antihyperglycemic activity (Table 1). Ethanolic extracts of Pongamia pinnata fruits, Tinospora cordifolia stems, Ficus bengalensis aerial roots and aqueous extracts of Pterocarpus marsupium heartwood, Withania coagulans fruits were further taken for isolation of active compounds.

Compound FB isolated from the hexane fraction of ethanolic extracts of F. bengalensis aerial roots exhibited 19.4% blood glucose lowering in normoglycemic model at the dose of 100 mg/kg. Administration of compound FB in sucrose challenged STZ-induced diabetic rats at the dose of 50 mg/kg caused fall in their blood glucose by 35.6% (P<0.01), comparable to metformin (37.8%, P<0.01). In db/db mice compound FB significantly lowered blood glucose level on day 3 by 18.7%, day 5 by 27.1% (P<0.05) and on days 7–10 by 40.0 and 51.6% (P<0.001), respectively, in comparison to control group. The same dose of metformin, i.e. 50 mg/kg p.o. significantly lowered the blood glucose level on day 3 by 17.8%, day 5 by 30.5% (P<0.05) and on days 7–10 by 39.4 and 52.5% (P<0.001). Compound PP-1 isolated from active chloroform fraction of ethanolic extracts of P. pinnata fruits
exhibited 12.8 (%<0.05) and 22.0 (%<0.01), whereas compound PP-2 exhibited 11.7 (%<0.05) and 20.7% (%<0.01) antihyperglycemic activity in STZ-model at the dose of 50 and 100 mg/kg respectively. Metformin showed 19.4% (%<0.01) antihyperglycemic activity at 100 mg/kg.

In db/db mice at 100 mg/kg dose, compounds PP-1 and PP-2 showed 35.7 (%<0.01) and 30.6% (%<0.01) cure in postprandial blood glucose level as well as 18.6 (%<0.01) and 15.0% (%<0.01) improvement in oral glucose tolerance test (OGTT), respectively. At the same dose metformin exhibited 32.3% (%<0.01) cure in postprandial blood glucose level and 19.3% (%<0.01) improvement in OGTT.

Both compounds were found to inhibit the activity of Protein Tyrosine Phosphatase 1B (PTPase-1B) in an in vitro system to a significant level. In PTPase-1B inhibitory activity evaluation, compounds PP-1 and PP-2 were found to possess significant activity (−66.8 and −64.34%) at 100 µM concentration with IC_{50} values of 75.0µM and 84.5 µM, respectively. The Ki values of the compounds were calculated to be 58 and 76 µM, respectively. The standard sodium orthovanadate showed 56.2% inhibition at 100 µM concentration. This may be the underlying mechanism of antihyperglycemic activity of these compounds.

Compound PM (pteroside) purified from n-butanol fraction of the aqueous extract of P. marsupium heartwood showed significant 23.8 % (%<0.01) antihyperglycemic activity in STZ-induced diabetic rats at a dose of 100 mg/kg body weight when compared to metformin which showed 18.5% (%<0.05) activity at the same dose.

Compound WC (coagulin L) isolated from the aqueous extract of the fruits of W. coagulans was found to improve glucose tolerance up to 29.8% in SLM and 23.3% in STZ-induced diabetic rats at a dose of 100 mg/kg body weight. In db/db mice compound WC at a dose of 50 mg/kg body weight for 10 consecutive days, significantly lowered the postprandial blood glucose level by 22.7% (%<0.01), whereas reference standard drug metformin lowered the postprandial blood glucose by 18.6% (%<0.05), when compared to vehicle-treated control group.

Compound WC was further derivatized to compounds WC-1 and WC-2, out of which compound WC-1 is a new compound. Compounds WC, WC-1 and WC-2 were tested for their % glucose uptake stimulatory effect at 10 µg/ml dose. WC, WC-1 and WC-2 exhibited 67.6, 38.5 and 27.1% glucose uptake stimulatory effect, when compared to metformin which showed 57.0 % activity.

In dyslipidemic db/db mice compound WC showed significant improvement in plasma lipid profiles at a dose of 50 mg/kg body weight, after 10 days of consecutive treatment. The treatment with compound WC lowered the level of plasma triglycerides (TG) by 14.7%, total cholesterol (TC) by 25.7%, low-density lipoprotein cholesterol (LDL-C) by 21.2% and very low density lipoprotein cholesterol (VLDL-C) by 15.6%, when compared to standard drug fenofibrate which lowered the level of TG, TC, LDL-C and VLDL-C by 16.2, 30.8, 24.5 and 21.6%, respectively.

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Table 1—Plant extracts investigated for their antihyperglycemic activity in STZ-induced diabetic rats at the dose of 250 mg/kg.

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Family</th>
<th>Parts used</th>
<th>Nature of extract</th>
<th>% Antihyperglycemic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aegle marmelose (L.)</td>
<td>Rutaceae</td>
<td>Leaves</td>
<td>Ethanolic</td>
<td>28.0</td>
</tr>
<tr>
<td>Coccinia indica (L.)</td>
<td>Cucurbitaceae</td>
<td>Leaves</td>
<td>Ethanolic</td>
<td>9.6</td>
</tr>
<tr>
<td>Dodendenia grandiflora</td>
<td>Lauraceae</td>
<td>Leaves</td>
<td>Ethanolic</td>
<td>16.5</td>
</tr>
<tr>
<td>Ficus bengalensis L.</td>
<td>Moraceae</td>
<td>Aerial roots</td>
<td>Ethanolic</td>
<td>10.6</td>
</tr>
<tr>
<td>Ocimum sanctum L.</td>
<td>Lamiaceae</td>
<td>Leaves</td>
<td>Ethanolic</td>
<td>27.0</td>
</tr>
<tr>
<td>Pongamia pinnata (L.)</td>
<td>Leguminosae</td>
<td>Fruits</td>
<td>Ethanolic</td>
<td>31.8</td>
</tr>
<tr>
<td>Pterocarpus marsupium Roxb.</td>
<td>Leguminosae</td>
<td>Heartwood</td>
<td>Aqueous</td>
<td>19.7</td>
</tr>
<tr>
<td>Tectona grandis Linn. F.</td>
<td>Verbenaceae</td>
<td>Leaves</td>
<td>Ethanolic</td>
<td>22.2</td>
</tr>
<tr>
<td>Tinospora cordifolia Miers.</td>
<td>Menispermaceae</td>
<td>Stem</td>
<td>Ethanolic</td>
<td>13.1</td>
</tr>
<tr>
<td>Withania coagulans Dunal</td>
<td>Solanaceae</td>
<td>Fruits</td>
<td>Aqueous</td>
<td>25.1</td>
</tr>
<tr>
<td>Zingiber officinale Roscoe</td>
<td>Zingiberaceae</td>
<td>Rhizome</td>
<td>Aqueous</td>
<td>14.6</td>
</tr>
<tr>
<td>Metformin (100 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td>25.5</td>
</tr>
</tbody>
</table>
Treatment with the compound WC increased the level of high-density lipoprotein cholesterol (HDL-C) by 24.7%, whereas fenofibrate increased the HDL-C level by 12.4%.

Discussion
Since the last two decades, traditional systems of medicine have become topic of global interest and importance. For decades, natural products have been a wellspring of drug leads and drugs. Evidences for the antihyperglycemic potential of the eleven plants studied have been given in Ayurveda. The present study was designed to investigate their activity in search of antihyperglycemic leads and to provide scientific validation to prove their antihyperglycemic activity. We have scientifically validated their antihyperglycemic activity in streptozotocin induced rats. Out of the eleven studied plants’ extracts, five plants’ extracts viz., Ethanolic extracts of *Pongamia pinnata* fruits, *Tinospora cordifolia* stems, *Ficus bengalensis* aerial roots and aqueous extracts of *Pterocarpus marsupium* heartwood, *Withania coagulans* fruits were further preceded to isolate their antidiabetic principles. Five leads with varied skeletons were isolated. All the compounds isolated were evaluated for in vitro antihyperglycemic activity in STZ-induced β-cell damaged diabetic male Sprague–Dawley rats and activities were compared with standard drug metformin. db/db Mice represent a good model for type 2 diabetes and display many of the characteristics of the human disease, including hyperphagia, hyperglycaemia, insulin resistance and progressive obesity. Compounds PP-1 (pongamol), PP-2 (karanjin), FB (α-amyрин acetate) and WC (coagulin L) isolated in large amount were also tested in db/db mice. Mode of action of compounds PP-1 and PP-2 was determined. They were found acting via inhibiting the activity of PTPase-1B. A new compound WC-1 and a known compound WC-2 were found by derivatizing the compound WC. WC, WC-1 and WC-2 exhibited glucose uptake stimulatory effect. WC was also found significantly antidislipidemic when treated in dyslipidemic db/db mice.

All the compounds showed promising antihyperglycemic potential when compared to standard drug metformin. Isolated lead compounds should be tested for their effect in secondary complications associated with diabetes such as diabetic retinopathy, neuropathy, nephropathy, cardiovascular diseases etc. Mode of action of leads should be studied in detail.

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References