Hypoglycaemic effect of methylene chloride/methanol root extract of *Ceiba pentandra* in normal and diabetic rats

P. D. Djomeni Dzeufiet¹, L. Tédong¹, E. A. Asongalem², T. Dimo¹, S. D. Sokeng³, P. Kamtchouing¹

ABSTRACT

Objective: The current study examined the effects of the methylene chloride/methanol extract of root bark of *Ceiba pentandra* (L) in normal and streptozotocin-induced diabetic rats.

Materials and Methods: Diabetes was induced by intravenous streptozotocin (55 mg/kg) in adult male albino Wistar rats. Single and multiple dose studies were carried out. Blood glucose levels were determined after oral administration of graded doses of *C. pentandra* (40, 75, 150 and 300 mg/kg) in fasting normal and diabetic groups for the single dose study; and before and at the end of day 3 of the treatment period for the multiple dose study.

Results: In both the groups, the extract (40 and 75 mg/kg) significantly reduced the blood glucose 5 hours after administration, in a consistent and time-dependent manner. *C. pentandra* at the lower dose (40 mg/kg) produced 40% and 48.9% lowering of blood-glucose in normal and diabetic rats, respectively compared to the initial values. In the multiple dose studies, the diabetic rats were treated orally by gavage, twice a day for three days. On day 3, *C. pentandra* (40 and 75 mg/kg) significantly decreased blood and urine glucose, compared to initial values. With 40 and 75 mg/kg of drug, the 14 h fasting blood glucose concentration was reduced by 59.8% and 42.8% with corresponding reductions of urine glucose levels by 95.7% and 63.6%, respectively.

Conclusion: These results indicate that *C. pentandra* possesses a hypoglycaemic effect. The plant extract is capable of ameliorating hyperglycaemia in streptozotocin-induced diabetic rats and is a potential source for isolation of new orally active agent(s) for diabetes mellitus.

KEYWORDS: Diabetes mellitus, plant extract, oral hypoglycaemic, silk cotton.

Introduction

Diabetes mellitus is a group of disorders with different aetiologies. It is characterised by derangements in carbohydrate, protein and fat metabolism, caused by the complete or relative insufficiency of insulin secretion and/or insulin action. Approximately, 140 million people worldwide suffer from diabetes. The disease becomes a real problem of public health in developing countries, where its prevalence is increasing steadily. In those countries, adequate treatment is often expensive or unavailable.

Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries.

Plants used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the control of this disease. *Ceiba pentandra* (L) Gaertner known as silk cotton tree and locally as “dum” belongs to the Bombacaceae family. Various parts of this plant are widely reputed in African traditional medicine. The plant has been reported to be a useful diuretic and effective remedy against diabetes, hypertension, headache, dizziness, constipation, mental trouble, fever, peptic ulcer, rheumatism, leprosy. The chronic hypoglycaemic activity of the stem bark aqueous extract of *C. pentandra* at high doses has already been reported by Olusola, et al. The present study was undertaken to determine the hypoglycaemic effect of the root bark methylene chloride/methanolic extract of *C. pentandra* in normal and streptozotocin-induced diabetic rats.
Materials and Methods

Animals
Male albino Wistar rats (175-225 g, body weight), raised in the Animal House of the Faculty of Science, University of Yaounde I, were used for the study. They were fed standard chow and given tap water ad libitum. The study was approved by the institutional animal ethics committee.

Preparation of the plant extract
The roots of *C. pentandra* were collected in Yaounde (Centre Province, Cameroon) and a voucher specimen (HNG 43623) was deposited at the National Herbarium, Yaounde after botanical identification. The barks were removed from roots, sliced into small pieces, air dried at ambient temperature and ground into powder form. A kilogram of the dried powder was macerated in a 1:1 (V/V) mixture of methylene chloride/methanol for two days, with occasional stirring, at room temperature. The solution obtained after filtration was concentrated using a rotavapor at 80°C to obtain 106 g (10.6% yield) of solid extract of *C. pentandra*.

Induction of diabetes
Streptozotocin (STZ), purchased from Sigma Chemical Co., (Saint Louis, MO), was dissolved in 0.9% ice-cold saline immediately before use. The rats, which were made to fast overnight, were anaesthetised by diethyl ether and STZ (55 mg/kg) was administered through the dorsal vein of the penis. Fasting blood and urine glucose were estimated to confirm the diabetic state. The rats were maintained for a period of 15 days to stabilise the diabetic condition.[10] Only rats with a fasting blood glucose of at least 200 mg/dl and positive urine glucose were used in the experiment. Rats with similar severity of diabetes were used in each set of experiments to avoid large variability of response to test compounds.

Treatment of animals
Two set of experiments were carried out: single and multiple dose studies.

Single dose studies
The experiment involved testing for hypoglycaemic effect of the plant extract after single oral administration in normal and diabetic rats. Six groups of 5 normal and 6 groups of 5 diabetic rats each were used. Normal and diabetic control groups received 1.5% dimethyl sulphoxide/distilled water (DMSO/H₂O). Four groups each of normal and diabetic rats received 40, 75, 150 and 300 mg/kg of *C. pentandra* extract. Their positive control group received 5 mg/kg glibenclamide (Daonil®) as the standard oral hypoglycaemic for comparison. Blood glucose levels were determined before (0.0) and then at 0.5, 1, 2, 3, 5 and 8 h after drug administration.

Multiple dose studies
The experiment consisted of two groups of 5 diabetic rats given by gavage. 40 and 75 mg/kg of methylene chloride/methanol extract twice daily, for three consecutive days. Normal and diabetic control groups received 1.5% DMSO/H₂O (vehicle) twice daily, whereas the positive control rats received 2 IU of insulin once daily at 7 am, by the subcutaneous route. After 14 hours of fasting, blood and urine glucose levels were assessed before and at the end of day 3 of the treatment period. Daily body weight, food and water intake were monitored.

Blood and urine glucose estimation
Before testing for blood and glucose levels, the rats were kept fasting overnight (at least 12 h), but were allowed free access to water. Blood samples (20 µl) for glucose determination were obtained from the tail tips of fasting rats. Blood glucose level was determined using a glucometer ACCUTREN GC (Boehringer Mannheim, Germany) while glucose indicator sticks (Boehringer Mannheim Germany) were used to assess glucose in fresh urine, before and after the treatment.

Statistical analyses
All the data are presented as mean±SEM. The differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by the Tukey multiple comparisons test. *P*<0.05 was considered significant.

Results

Effect of single dose *C. pentandra* extract on blood glucose in normal fasting rats
A single dose of 40 mg/kg of the extract exhibited a significant hypoglycaemic effect (*P*<0.01) after 2 h. [Table 1] This effect was remarkable for lower doses, whereas at 300 mg/kg, the effect was felt 8 h post-dose (*P*<0.01). Glibenclamide had significant effect 2 h post-dose (*P*<0.05).

Effect of single dose *C. pentandra* extract on blood glucose in fasting diabetic rats
Oral administration of 40 and 75 mg/kg of *C. pentandra* significantly reduced hyperglycaemia in streptozotocin-induced diabetic rats. The maximum effect was observed with 40 mg/kg, 8 h after dosing, with a slight decrease thereafter. [Table 2] The plant extract was less effective in reducing blood glucose at doses of 150 and 300 mg/kg. Glibenclamide had a significant effect on blood glucose in diabetic rats, but was less effective than *C. pentandra* extract (40 mg/kg).

Effect of repeated administration of *C. pentandra* extract on the blood and urine glucose levels of diabetic rats
When administered twice daily for three days, the extract decreased the blood glucose in diabetic rats compared to untreated diabetic rats. [Table 3] *C. pentandra* (40 and 75 mg/kg) significantly reduced blood glucose levels by 59.8% (*P*<0.01) and 42.8% (*P*<0.01), respectively, compared to insulin (26.8%, *P*<0.05). A corresponding reduction (*P*<0.01) in urine glucose by 95.7% (40 mg/kg), 63.6% (75 mg/kg) and 45.8% (insulin), respectively, was observed.

Discussion
At a single dose of 40 mg/kg, the extract produced significant reduction in the blood glucose concentration of fasting normal and diabetic rats by 40.0% and 48.9%, respectively, after 8 hours. These results were in line with those of Olusola, *et al.*[10] which showed that the aqueous stem bark of *C. pentandra* exhibited hypoglycaemic action. Our results demonstrated that methylene chloride/methanol extract of *C. pentandra* root bark was able to reduce blood glucose levels of diabetic rats at a low dose (40 mg/kg) contrary to findings of Olusola, *et al.*[10] who used higher doses of the stem bark aqueous extract. These observations may suggest that active principle (s) may be more lipid soluble.
Table 1

Effect of single dose of methylene chloride/methanol extract of *Ceiba pentandra* on blood glucose levels in normal rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose (mg/dL)</th>
<th>0 h</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>5 h</th>
<th>8 h</th>
<th>12 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>99.8±3.4</td>
<td>98.8±5.2</td>
<td>97.2±2.3</td>
<td>98.4±2.0</td>
<td>94.0±1.7</td>
<td>91.0±1.8</td>
<td>89.4±2.0</td>
<td>88.0±3.5</td>
</tr>
<tr>
<td>Extract (40 mg/kg)</td>
<td></td>
<td>97.0±4.7</td>
<td>90.4±3.6</td>
<td>83.6±2.6</td>
<td>77.2±2.5</td>
<td>73.0±1.9</td>
<td>61.6±3.2</td>
<td>57.6±2.5</td>
<td>65.0±3.1</td>
</tr>
<tr>
<td>Extract (75 mg/kg)</td>
<td></td>
<td>99.2±5.7</td>
<td>92.8±3.9</td>
<td>83.6±4.5</td>
<td>78.2±5.5</td>
<td>79.4±2.9</td>
<td>63.2±3.1</td>
<td>70.8±2.1</td>
<td>72.0±3.3</td>
</tr>
<tr>
<td>Extract (150 mg/kg)</td>
<td></td>
<td>104.6±4.7</td>
<td>98.4±6.0</td>
<td>88.0±4.1</td>
<td>79.4±3.2</td>
<td>75.4±2.8</td>
<td>64.0±3.1</td>
<td>61.4±2.4</td>
<td>63.0±5.5</td>
</tr>
<tr>
<td>Extract (300 mg/kg)</td>
<td></td>
<td>102.8±1.9</td>
<td>104.4±4.5</td>
<td>104.4±3.9</td>
<td>96.0±2.8</td>
<td>96.0±3.9</td>
<td>77.0±3.4</td>
<td>68.6±5.6</td>
<td>70.0±6.0</td>
</tr>
<tr>
<td>Gilbenclamide (5 mg/kg)</td>
<td></td>
<td>98.1±2.5</td>
<td>93.0±3.1</td>
<td>92.0±1.5</td>
<td>86.4±2.9</td>
<td>75.8±2.0</td>
<td>71.2±2.7</td>
<td>59.2±1.5</td>
<td>57.0±3.0</td>
</tr>
</tbody>
</table>

One-way ANOVA: F = 1.91, P = 0.1288

ANOVA F = 1.91, P = 0.1288

Values are mean±SEM; n=5 in each group.  *P<0.05;  **P<0.01;  ***P<0.001;  ****P<0.0001, with respect to control. df = 5, 24.

Table 2

Effect of single dose of methylene chloride/methanol extract of *Ceiba pentandra* on blood glucose levels in diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose (mg/dL)</th>
<th>0 h</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>5 h</th>
<th>8 h</th>
<th>12 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td>474.8±21.4</td>
<td>471.6±20.9</td>
<td>465.4±18.5</td>
<td>467.0±20.8</td>
<td>443.8±20.3</td>
<td>466.8±21.7</td>
<td>451.6±19.0</td>
<td>445.0±17.1</td>
</tr>
<tr>
<td>Extract (40 mg/kg)</td>
<td></td>
<td>449.2±21.9</td>
<td>450.6±21.9</td>
<td>410.5±16.8</td>
<td>390.5±18.8</td>
<td>364.8±21.0</td>
<td>350.0±12.3</td>
<td>230.2±15.6</td>
<td>225.0±16.1</td>
</tr>
<tr>
<td>Extract (75 mg/kg)</td>
<td></td>
<td>449.8±20.8</td>
<td>475.5±29.3</td>
<td>469.6±20.6</td>
<td>425.8±16.4</td>
<td>421.0±18.1</td>
<td>354.2±11.8</td>
<td>307.6±29.6</td>
<td>302.0±20.4</td>
</tr>
<tr>
<td>Extract (150 mg/kg)</td>
<td></td>
<td>477.0±22.6</td>
<td>506.2±26.9</td>
<td>513.2±20.2</td>
<td>454.4±15.9</td>
<td>436.4±16.3</td>
<td>422.8±14.0</td>
<td>401.0±12.6</td>
<td>399.0±14.5</td>
</tr>
<tr>
<td>Extract (300 mg/kg)</td>
<td></td>
<td>434.2±29.5</td>
<td>489.8±18.5</td>
<td>503.6±21.7</td>
<td>485.8±20.1</td>
<td>482.0±22.7</td>
<td>467.8±21.0</td>
<td>408.2±20.2</td>
<td>402.0±12.2</td>
</tr>
<tr>
<td>Gilbenclamide (5 mg/kg)</td>
<td></td>
<td>465.6±20.9</td>
<td>450.6±16.8</td>
<td>441.4±20.8</td>
<td>413.8±16.0</td>
<td>392.2±19.1</td>
<td>372.8±26.0</td>
<td>352.2±21.4</td>
<td>350.2±17.3</td>
</tr>
</tbody>
</table>

One-way ANOVA: F = 2.69, P = 0.045

ANOVA F = 2.69, P = 0.045

Values are mean±SEM; n=5 in each group.  *P<0.05;  **P<0.01;  ***P<0.001;  ****P<0.0001, with respect to control. df = 5, 24.

Table 3

Effect of *Ceiba pentandra* methylene chloride/methanol extract on blood and urine glucose levels after 3 days oral administration to streptozotocin-induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose (mg/dL)</th>
<th>0 h</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>5 h</th>
<th>8 h</th>
<th>12 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>99.0±1.3</td>
<td>98.8±1.9</td>
<td>0.2±0.0</td>
<td>(-0.2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
<td>343.0±13.6</td>
<td>347.4±11.2</td>
<td>4.4±0.9</td>
<td>(+1.3)</td>
<td>4.8±0.2</td>
<td>4.8±0.4</td>
<td>0.09±0.0</td>
<td>(+0.2)</td>
</tr>
<tr>
<td>Extract</td>
<td>40</td>
<td>330.0±39.6</td>
<td>132.6±18.6</td>
<td>197.4±8.5</td>
<td>(-59.8)</td>
<td>4.6±0.2</td>
<td>0.4±0.2</td>
<td>4.2±0.3</td>
<td>(-95.7)</td>
</tr>
<tr>
<td>Extract</td>
<td>75</td>
<td>339.2±14.4</td>
<td>192.2±12.3</td>
<td>147.0±8.1</td>
<td>(-42.8)</td>
<td>4.4±0.2</td>
<td>1.6±0.2</td>
<td>2.8±0.2</td>
<td>(-63.6)</td>
</tr>
<tr>
<td>Insulin</td>
<td>2 IU</td>
<td>379.2±24.2</td>
<td>277.4±35.2</td>
<td>59.8±9.3</td>
<td>(-26.8)</td>
<td>4.8±0.2</td>
<td>2.6±0.2</td>
<td>2.2±0.1</td>
<td>(-45.8)</td>
</tr>
</tbody>
</table>

One-way ANOVA: F = 57.92, P = 0.0001

ANOVA F = 57.92, P = 0.0001

Values are mean±SEM; n=5 in each group.  *P<0.05;  **P<0.01;  ***P<0.001;  ****P<0.0001, with respect to diabetic control initial values. df = 4, 20.
The oral administration of single dose of the plant extract at the higher doses (150 and 300 mg/kg) did not significantly affect the blood glucose levels. The lack of significant changes in blood glucose at higher doses may be due to antagonism. The extract may contain antagonistic molecules. Therefore, at low doses, the concentration of antagonistic molecule(s) are low, thus, offering no hindrance to the hypoglycaemic causative substance(s). A similar observation was reported by Kameswara, et al. [1,12] (bark extract of Pterocarpus santalinus) and Prince, et al. [13] (Tinospora cardifolia extract). The acute hypoglycaemic action of C. pentandra in streptozotocin-induced diabetic rats suggests that the extract remains active even when pancreatic β-cells are almost completely destroyed. Glibenclamide (5 mg/kg) was less effective in reducing blood glucose in STZ-diabetic rats as in normoglycaemic rats. It has been reported that glibenclamide is not effective when destruction of β-cells has occurred. [14] It is also known that glibenclamide is effective in moderate diabetic rats, not in severe diabetic animals. [15-16] The acute hypoglycaemic effect of glibenclamide results from the stimulation of insulin release and inhibition of glucagon secretion. [17] The extract may possess an insulin-like effect on peripheral tissues, either by promoting glucose uptake and metabolism, or by inhibiting hepatic gluconeogenesis. The phytochemical studies of C. pentandra have revealed the presence of naphthoquinone [18] and flavonoid (epicatechin). [19] Epicatechin, isolated from other plants, has been found to stimulate insulin secretion or possess an insulin-like effect. [12-20] In our study, we have found that administration of the C. pentandra extract to diabetic rats reversed, at lower doses, their blood glucose, which was also reflected in their urine sugar levels. The possible mechanism by which the plant extract brings about its hypoglycaemic action may be through enhancement of glucose uptake into skeletal muscle, and/or by inhibition of hepatic gluconeogenesis.

Conclusion

These results indicate that the root bark of C. pentandra is effective in decreasing the blood glucose level in normal and diabetic animals. However, the molecule(s) responsible for such an effect requires further investigation.

Acknowledgments

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References


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