ANTIHYPERTERGLYCEMIC AND BIOCHEMICAL EVALUATION OF HYDROALCOHOLIC EXTRACT OF CAESALPINIA BONDCUCELLA LEAVES

Fahad I.Al-Sai Khan¹, Sudarsini Saravanabhanav¹²

¹College of Pharmacy, Salman Bin Abdulaziz university, Al-Kharj, K.S.A
²King Saud bin Abdulaziz University for Health Sciences National Guard Health Affairs (NGHA), Jeddah, K.S.A.

ABSTRACT

Diabetes mellitus is a common disorder associated with markedly increased morbidity and mortality rates, which affect a large number of people around the globe, can be defined as a group of metabolic diseases characterised by chronic hyperglycaemia that results from defects in insulin secretion, insulin action, or both, and causes impaired function in carbohydrate, lipid, and protein metabolism. Diabetes was induced in male Wistar albino rats by alloxan monohydrate. Animals were randomly divided into 6 groups (1, normal control, 1 diabetic control and 4 treated groups). Control animals received saline (5 mL/kg) orally, whereas the treatment groups received different doses of Caesalpinia bonducella leaves (100, 200, and 400 mg/kg), Glibenclamide (4 mg/kg) was administered orally once a day for 14 days. Blood was withdrawn for glucose determination on the 1st, 6th, 10th, and 14th days. The rats were fasted overnight and then sacrificed on the 14th day; blood was collected for biochemical evaluation, including the levels of blood glucose. Administration of Caesalpinia bonducella leaves in alloxan-induced diabetic rats significantly reduced blood glucose when compared with the control group. The results suggested that Caesalpinia bonducella leaves exhibits antidiabetic effects in alloxan-induced diabetic rats.

Keywords: Caesalpinia bonducella, Diabetes mellitus, alloxan monohydrate Glibenclamide.

INTRODUCTION

Diabetes mellitus, or simply diabetes, is a group of metabolic diseases in which a person has
high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced\(^1\). Untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, chronic renal failure, and diabetic retinopathy (retinal damage). Adequate treatment of diabetes is thus important, as well as blood pressure control and lifestyle factors such as stopping smoking and maintaining a healthy body weight\(^2\). All forms of diabetes have been treatable since insulin became available in 1921, and type 2 diabetes may be controlled with medications. Insulin and some oral medications can cause hypoglycemia, which can be dangerous if severe. Both types 1 and 2 are chronic conditions that cannot be cured \(^3\). Pancreas transplants have been tried with limited success in type 1 DM; gastric bypass surgery has been successful in many with morbid obesity and type 2 DM. Gestational diabetes usually resolves after delivery.

Caesalpinia bonduc (L.) Roxb (Syn. Caesalpinia bonducella (L.) Fleming, Syn. Caesalpinia crista (Linn.), belonging to the family Febaceae/ caesalpiniaceae, is a prickly shrub widely distributed all over the world specially, in India, SriLanka and Andaman and Nicobar Islands, in India specially found in tropical regions. All parts of the plant have medicinal properties so it is a very valuable medicinal plant, which is utilized in traditional system of medicine. The plant has been reported to possess several activities\(^4\).

**MATERIALS AND METHODS**

**Preparation of hydroalcoholic extracts of Caesalpinia bonducella**

The leaves was separated from plant and it was washed with absolute ethanol to avoid the microbial growth, the leaves were dried at open air under the shade, cut in to small pieces and powdered mechanically, then 50 gm of powder *Caesalpinia bonducella* was extracted with 250 ml of aqueous alcohol (Ethanol + water, 3:1 ratio) in a soxhlet apparatus for 72 hrs. The extract obtained was concentrated by recovery of ethanol. The concentrated product was used as hydroalcoholic extract of *Caesalpinia bonducella* leaves.

**Phytochemical investigation** \(^5,6,7\)

*Caesalpinia bonducella* extract was subjected to various phytochemical tests, viz., saponins, amino acids, proteins, glycosides, cardiac glycosides, alkaloids, carbohydrates and flavanoids.
Acute toxicity study
This study was carried out as per OECD guideline 425. In the assessment and evaluation of the toxic characters of the substance, determination of acute oral toxicity is usually an initial step. It provides information of health hazards likely to arise from a short-term exposure by the oral route. Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24h. Data from an acute study may serve as a basis for classification and labeling. LD (medium lethal 50 doses), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50% of animals when administered by the oral route. The LD$\text{50}$ value expressed in terms of test substance per unit weight of test animal (mg/kg). It is initial step in establishing a dosage regimen in sub chronic and other studies and may provide initial information on the mode of toxic action of a substance.

The concept of the up and down (UDP, stair case method) was first designed by Dixon and Mood. In this method animals of a single sex, usually females, with the first animal receiving a dose just below the best estimate of the LD$\text{50}$. Depending on the outcome for the previous animal, the dose for the next is increased or decreased, usually by the factor of 3.2. This sequence continues until there is a reversal of the initial outcome (the point where an increasing dose results in death rather than survival or decreasing dose result in survival rather than death) then, additional animals are dosed following the up-down principle until a stopping criterion is met. If there is no reversal before reaching the selected upper (2000 or 5000 mg/kg) limit dose, then a specific number of animals are dosed at the limit dose. The option to use an upper limit dose of 5000 mg/ kg should be taken only when justified by a specific regulatory need.

Statistical analysis of the results was done using the statistical functions of the Graphpad Prism 5.0 software. The results were expressed in terms of mean ± SD. The significance of difference between mean values for the various treatments were tested using one way analysis of variance test (ANOVA test) followed by Dunnett Multiple Comparisons Test and the p values less than 0.05 were considered significant.

Experimental animals and alloxan-induction of experimental diabetes
Male Wistar albino rats weighing 180-200 g were housed in polycarbonated cages at a temperature regulated (22°C) and humidity (55%) controlled room with a 12 h light/12 h dark cycle. A water and standard pellet diet were available ad libitum throughout the experimental
period. The rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 120 mg kg⁻¹ b.wt. Two weeks after treatment, rats with moderate diabetes having glycosuria (indicated by Benedict’s qualitative test) and hyperglycemia (i.e. with blood glucose of 200-300 mg dL⁻¹) were used for the experiment.

**Experimental design**
The rats were divided into six groups of six rats each after the induction of alloxan diabetes.

- **Group 1:** Normal control rats
- **Group 2:** Diabetic control rats.
- **Group 3:** Diabetic rats given extract of 100 mg *Caesalpinia bonducella* leaves.
- **Group 4:** Diabetic rats given extract of 200 mg *Caesalpinia bonducella* leaves.
- **Group 5:** Diabetic rats given extract of 400 mg *Caesalpinia bonducella* leaves.
- **Group 6:** Diabetic rats given Glibenclamide 4mg.

An oral administration was conducted extract with 100 mg, 200mg, 400mg kg⁻¹ b.wt. Blood glucose was measured at 1st, 2nd, 10th, 14th day. Blood was withdrawn from the tail vein each day. To measure lipids and some enzymes, oral administration was conducted daily for 14 days. At the end of 14th days, all the rats were anaesthetized by pentobarbitone sodium (60 mg kg⁻¹) and opened at the abdomen. Blood was withdrawn from the abdominal aorta and centrifuged at 3000 rpm for 10 min to obtain the plasma. Blood glucose was determined such as GOT (Glutamic oxaloacetic transaminasse), GPT (glutamic pyruvic transaminase), LDH (Locate dehydronase), TC (total cholesterol) and TG (triglyceride).

**RESULTS AND DISCUSSION**

**Effect of extract on plasma glucose levels**
The preliminary phytochemical screening like Saponins, Tannins, Amino acids, Proteins, Glycosides, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids was done with the hydroalcoholic extract of *Caesalpinia bonducella* leaves according to the procedure. In the above chemical test the hydroalcoholic extract of *Caesalpinia bonducella* leaves showed positive results for Saponins, Tannins, Amino acids, Proteins, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids except glycosides. The results of preliminary test of hydroalcoholic extract of *Caesalpinia bonducella* leaves were shown in table no 1. Acute toxicity test at 3000mg/kg extracts of *Caesalpinia bonducella* leaves produced no mortality after 24 hours of observation. The median lethal dosage (LD₅₀) of the hydroalcoholic leaves extract was greater than 3000 mg/kg body weight. The extract did not produce any grossly
negative behavioral changes such as excitement, restlessness, respiratory distress, convulsions or coma. However, a reduction in body weights of rats was observed. The reduction in body weight may be due to reduced fluid and water intake, which may be secondary to feeling of fullness and loss of appetite after administration of the extract.

The effect of hydroalcoholic extracts *Caesalpinia bonducella* leaves on the blood glucose levels of experimental animals was determined from 1, 6, 10, 14 day after oral administration of 100 mg, 200mg, 400mg doses kg-1 b.wt. There was a significant elevation in the blood glucose level by 10\textsuperscript{th} and 14\textsuperscript{th} day during experimental time period in alloxan-induced diabetic rats, when compared to normal rats. The administration of *Caesalpinia bonducella* leaves extract caused the blood glucose levels of diabetic rats to 83.4, 67.6, 75.1, 81.1 and 74.3\% at the time interval of 1st, 6\textsuperscript{th}, 10\textsuperscript{th} and 14\textsuperscript{th} day respectively (p<0.05). Maximum reduction of 32.4\% was observed 14\textsuperscript{th} day after treatment. The administration of *Caesalpinia bonducella* leaves extract produced the most significant reduction (p<0.05) in the blood glucose levels of 34, 41, 33 and 35\% at 1, 6, 10 and 14 day respectively was shown in table no 2 and figure no 1.

**Effect of extract on plasma GOT, GPT and LDH**

Table 3 shows the activities of GOT, GPT and LDH of experimental rats. Compared with diabetic rats showed significantly more activities of serum GOT, GPT and LDH by 3.9, 2.6 and 27.1 times, respectively. Treatment with *Caesalpinia bonducella* leaves extracts significantly reduced the activity of GOT, GPT and LDH in the diabetic control rats (p<0.05). The administration of *Caesalpinia bonducella* leaves extract brought down GOT, GPT and LDH values in alloxan-induced diabetic rats to 52.8, 85.7 and 6.5\%, respectively\textsuperscript{13} was shown in table no 3 and figure no 2.

Alloxan induces “chemical diabetes” in a wide variety of animal species by damaging the insulin secreting pancreatic beta-cell, resulting in a decrease in endogenous insulin release\textsuperscript{14-16}. Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose level in alloxan-induced diabetic animals\textsuperscript{17-19}. In the present study, the hydroalcoholic extracts of *Caesalpinia bonducella* leaves effectively decreased the blood glucose in alloxan-induced diabetic rats, which is even better than glibenclamide. Hyperglycemia increases the generation of free radicals by glucose auto-oxidation and the increment of free radicals may lead to liver cell damage. The increase in oxygen free radicals in diabetes could be primarily due to the increase in blood glucose levels and secondarily due
to the effects of the diabetogenic agent alloxan\textsuperscript{20,21}. In our previous study, \textit{Caesalpinia bonducella} leaves extract showed strong free radical scavenging and antioxidant activities and also showed a protective effect on DNA damage caused by hydroxyl radicals\textsuperscript{22, 23}.

Table No.1: Phytochemical screening results of \textit{Caesalpinia bonducella} leaves

<table>
<thead>
<tr>
<th>S.NO</th>
<th>PHYTOCONSTITUENT</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SAPONINS</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>TANNINS</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>AMINO ACIDS</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>PROTEINS</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>GLYCOSIDES</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>CARDIAC GLYCOSIDES</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>ALKALOIDS</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>CARBOHYDRATES</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>FLAVONOIDS</td>
<td>+</td>
</tr>
</tbody>
</table>

Table No.2: Anti Diabetic Activity of hydroalcoholic Extract of \textit{Caesalpinia bonducella} leaves on Alloxan Induced Diabetic Rats

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Day1</th>
<th>Day 6</th>
<th>Day10</th>
<th>Day14</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>242±2.0</td>
<td>250±3.02</td>
<td>230±0.6</td>
<td>240±1.2</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic control</td>
<td>463±20.2</td>
<td>472±18.0</td>
<td>498±16.02</td>
<td>508±14.08</td>
</tr>
<tr>
<td>3</td>
<td>\textit{Caesalpinia bonducella} 100mg</td>
<td>424±14.02</td>
<td>390±12.0</td>
<td>360±12.00</td>
<td>340±22.04</td>
</tr>
<tr>
<td>4</td>
<td>\textit{Caesalpinia bonducella} 200mg</td>
<td>418±8.06</td>
<td>350±14.56</td>
<td>300±16.80</td>
<td>280±13.06</td>
</tr>
<tr>
<td>5</td>
<td>\textit{Caesalpinia bonducella} 400mg</td>
<td>388±4.04</td>
<td>324±8.10</td>
<td>298±10.2</td>
<td>240±12.00</td>
</tr>
<tr>
<td>6</td>
<td>Glibenclamide</td>
<td>390±16.2</td>
<td>344±14.13</td>
<td>286±4.20</td>
<td>248±12.00</td>
</tr>
</tbody>
</table>

Values are mean concentration of blood glucose ±SE (n=6)
Extract produced the most significant reduction (p<0.05)

Table No.3: Effect of \textit{Caesalpinia bonducella} leaves on plasma GOT, GPT and LDH

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>GOT</th>
<th>GPT</th>
<th>LDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>68±2.2</td>
<td>72±6.2</td>
<td>53±4.2</td>
</tr>
<tr>
<td>1</td>
<td>Diabetic control</td>
<td>269±4.0</td>
<td>158±3.4</td>
<td>167±12.2</td>
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<tr>
<td>2</td>
<td>\textit{Caesalpinia bonducella} 100mg</td>
<td>158±2.0</td>
<td>132±0.2</td>
<td>112±4.8</td>
</tr>
<tr>
<td>3</td>
<td>\textit{Caesalpinia bonducella} 200mg</td>
<td>92±2.8</td>
<td>94±12.2</td>
<td>82±6.3</td>
</tr>
<tr>
<td>4</td>
<td>\textit{Caesalpinia bonducella} 400mg</td>
<td>76±0.4</td>
<td>68±8.2</td>
<td>61±2.3</td>
</tr>
<tr>
<td>5</td>
<td>Glibenclamide</td>
<td>72±5.6</td>
<td>67±2.3</td>
<td>48±7.8</td>
</tr>
</tbody>
</table>

Values are mean concentration of blood glucose ±SE (n=6)
Significantly reduced the activity of GOT, GPT and LDH in the diabetic control rats (p<0.05).
CONCLUSION
Based on above-mentioned reports, we suggest that the possible mechanism of action by hydroalcoholic extracts of *Caesalpinia bonducella* leaves could be related to antioxidants that
aid to recover from impaired metabolism of glucose. The activities of GOT and GPT are cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage. Therefore, we used the activities of GOT, GPT and LDH in the circulation as indicators of hepatic damage. In the present study, all treatment groups with experimental *Caesalpinia bonducella* leaves extracts effectively reduced plasma GOT, GPT and LDH activities in diabetic rats, suggesting that the hydroalcoholic extracts of *Caesalpinia bonducella* leaves may prevent hepatic injury associated with diabetes.

**REFERENCE**


