Assessment of hypoglycemic and antidiabetic effects of *Caesalpinia bonduc* (L.) Roxb. seeds in alloxan induced diabetic rat and its phytochemical, microscopic, biochemical and histopathological evaluation

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ABSTRACT

*Caesalpinia bonduc* (Lin.) Roxb. widely distributed throughout the coastal region of India and used ethnically by the tribal people of India for controlling blood sugar. So present study was undertaken to rectify the traditional claim of the *Caesalpinia bonduc* (L.) Roxb. seeds in treatment of type 2 diabetes mellitus in Wistar Albino male rats and in future to use the extract in development of polyherbal formulations for treatment of diabetes. This study also includes biochemical examination of blood serum along with histopathological studies of pancreas, kidney and liver in diabetic control, extract treated and standard drug glibenclamide treated rats. Powder microscopy and preliminary phytochemical screening also done. Effect of hydro-alcoholic extracts of *Caesalpinia bonduc* (L.) Roxb. seeds on fasting blood glucose level in normal, glucose loaded and alloxan induced hyperglycemic rats were evaluated and compared with that of reference drug, glibenclamide. The blood glucose was measured using one touch glucometer (Bayer), while histology of pancreas, kidney and liver was investigated after staining with heamatoxylin- eosin dye in normal, diabetic control, extract treated and standard drug glibenclamide treated rats. The extract at all tested doses (250mg and 500mg /kg of b.w.) significantly (p<0.05) lowered fasting blood glucose level in the treated rats compared with the diabetic but untreated rats (test control). The pancreas, kidney and liver histology indicates significant recovery with the extract administration. The efficacy of the extract in reducing blood glucose level, improving body weight and rejuvenating the damaged pancreas of alloxan induced diabetic rats favorably compared with that of reference drug, glibenclamide. Morphological and anatomical studies of the seed will enable to identify the crude drug. Preliminary phytochemical screening of hydro-alcoholic extract of seeds showed presence of flavonoids, triterpenoids and steroids.

**Key words:** Blood glucose, diabetics, glibenclamide, *Caesalpinia bonduc* (Lin.) Roxb. Seeds, pancreas.
INTRODUCTION

Diabetes mellitus is a metabolic disease as old as mankind and its incidence is considered to be high (4–5%) all over the World [1]. In spite of the introduction of hypoglycemic agents, diabetes and related complications continue to be a major medical problem. Since time immemorial, patients with non-insulin requiring diabetes have been treated orally in folk medicine with a variety of plant extracts. In India a number of plants are mentioned in ancient literature (Ayurveda) for the cure of diabetic conditions known as ‘Madhumeha’ and some of them have been experimentally evaluated and the active principles isolated [2-7].

Caesalpinia bonduc (Lin.) Roxb. commonly known as Nata Karanja, a prickly shrub found throughout the hotter parts of India, Myanmar and Sri Lanka, has grey, hard, globular shaped seeds with a smooth shining surface. Seeds consist of a thick, brittle shell with a yellowish white bitter fatty kernel [8]. The chemical constituents of the plant include flavonoids, triterpenoids, diterpenoids, and steroids [9-11]. Several reports on flavonoids, triterpenoids and steroids showed that these molecules have the multiple biological effects due to their antioxidant and free radical scavenging abilities. Recently, antibacterial and antifungal activities of diterpene bondenlind from the plant were reported by Simin et al. (2000)[12]. The aqueous solution of the outer shell of the seeds of Caesalpinia bonduc is traditionally used by the tribal people of Andaman and Nicober Islands for the relief of the symptoms of diabetes mellitus. Blood sugar lowering activity of Caesalpinia bonduc has been primarily evaluated with significant result in rabbit[13] and rat models [14-15]. Knowing the facts that seed of this plant contains flavonoids, triterpenoids and steroids which act as an antioxidant and free radical scavengers and are responsible for anti diabetic action. So the present work was undertaken to evaluate the hypoglycemic and anti diabetic activity of the hydroalcoholic seeds extract of Caesalpinia bonduc (Lin.) Roxb. Also powder microscopy of seeds and phytochemical evaluation of hydroalcoholic seeds extract of Caesalpinia bonduc (Lin.) Roxb was carried out. Concurrent histological studies of the pancreas of these animals showed comparable regeneration by hydroalcoholic extract and kidney and liver showed normal structure in the rats which were earlier, necrosed by alloxan. Total cholesterol (TC), low density lipoprotein (LDL), albumin and triglyceride (TG) levels also decreased in severely diabetic rats whereas, cardio protective, high density lipoprotein (HDL) and protein level in serum were increased.

Principal Constituents

The seeds of Caesalpinia bonduc (Lin.) Roxb. are found to contain various chemical constituents [16 such as furanoditerpenes-caesalpin-α, caesalpin-γ, caesalpin-δ, caesalpin-ε, caesalpin, and caesalpin-F. Fatty acids-palmitic, stearic, octadeca-4-enoic and octadeco-2-4-dienoic, lignocenic, oleic and linoleic acids, phytosterinin, β-sitosterol, homoiso flavone, bonducellin, amino acids-aspartic acid, arginine, and citrulline. Carbohydrates starch, sucrose, β-carotene, glycoside-bonducin, gums and resins.

MATERIALS AND METHODS

Plant material

The seeds of Caesalpinia bonduc were collected from the farms of Digras Dist.Yavatmal (M.S., India) [17-19]. Plant materials were authenticated by Dr.Mrs.P.Y.Bhogaonkar, Ex. Head of the Botany Department, Amravati (Maharashtra, India). The voucher specimen has been deposited in the Department of Pharmacognosy, Government College of Pharmacy, Amravati, India.
Chemicals
Alloxan monohydrate (S D Fine-Chem, India), glibenclamide (Sun Pharma, India) were used in this study. Other chemicals used were of analytical grade and were obtained from Qualigen, India.

Powder Microscopy
Some of the major diagnostic microscopic characters of the powder are columnar palisade cells, bone shaped thick walled parenchymatous cells with brown content and cells filled with starch grains [20].

Preparation of Extracts
The seed coats were broken and the kernels and the seed coat were separated and kernels were size reduced to a coarse powder. The seed powder (250 g) was extracted with petroleum ether (60-80°C), chloroform, and ethanol (90%) successively using Soxhlet apparatus and later extracted using 50% ethanol and water by maceration. All the above extracts were also tested for the identification of phytoconstituents. The semisolid hydro-alcoholic extract was suspended in distilled water and employed for anti-diabetic activity [21].

Identification of phytochemical constituents
Chemical tests were carried out on the hydro-alcoholic extracts for the qualitative determination of phytochemical constituents as described by Harborne (1998)[22], Trease and Evans (1983) [23].

Animals
Male Wistar Albino male rats (160 – 200 g) were used in the experiment. Animals maintained under standard environmental conditions, were fed with a standard diet and water ad libitum. The animals were fasted for 16h before experimentation but allowed free access to water. The Institutional Animal Ethical Committee of S.N.Institute of Pharmacy, Pusad Dist.Yavatmal, Maharashtra, India (SNIOP/IAEC/10-11/01-10), approved the study.

Acute toxicity studies
Healthy adult Wistar albino rats of either sex, starved overnight were divided into four groups (n = 6) and were orally fed with the hydro-alcoholic extract in increasing dose levels of 100, 500, 1000 and 3000 mg/kg body weight (Ghosh,1984)[24]. The rats were observed continuously for 2 h for behavioral, neurological and autonomic profiles and after 24 and 72 h for any lethality (Turner, 1965)[25].

Glucose tolerance test
The method of V. Babu et al. was used. Five groups of 6 rats each were used for the study. Group 1 served as normal (Vehicle: 2% acacia suspension), Group 2 animals were administered standard drug glibenclamide (10 mg/kg b.w.) Group 3 animals were administered with ayurvedic marketed formulation, Group 4 animals were administered with Caesalpinia bonduc hydro-alcoholic extract 250 mg, and Group 5 animals were administered with Caesalpinia bonduc hydro-alcoholic extract 500 mg. The rats of all the groups were loaded with 2 g/kg glucose p.o 30 min after extract administration. Blood samples were collected from the tail prior to drug administration and at 0, 30, 60 and 90 min after glucose loading. Blood glucose levels were measured using one touch Glucometer (Bayer)[26].
Effect of *Caesalpinia bonduc* hydro-alcoholic extract in normal rats

Thirty rats were fasted overnight for 16 h, but water was allowed. Using aseptic precautions, blood was collected from their tails by pricking method and blood glucose level measured. Immediately afterwards, these rats were divided randomly into 5 groups and treated orally (n = 6/group) in the following manner. Group 1 (1ml of 2% acacia suspension), 2 (10 mg/kg b.w. of glibenclamide), 3 (Ayurvedic marketed preparation 500 mg/kg b.w.), 4 (250 mg/kg of CBE) and 5 (500 mg/kg of CBE). For acute hypoglycemic study blood samples were collected from tails 0, 1, 3, 5 and 7 h and for sub-acute study blood samples were collected from tails 0, 7 and 14 days of post-treatment by tail prick method for the determination of serum glucose levels [27].

Alloxan induced hyperglycemia

Hyperglycemia was induced by injecting alloxan monohydrate at a dose of 120 mg/kg intraperitonially. The animals were kept under observation and after 48 h were tested for hyperglycemia using glucometer. The animals showing hyperglycemia were then grouped in 5 groups of 6 animals each. Another 6 normal animals served as normal control—

*Group 1 received 2% acacia suspension along with the vehicle. In diabetes induced,*

*Group 2 served as the negative control untreated (diabetes induced),*

*Group 3 were administered with glibenclamide (10 mg/kg in 2% acacia suspension)*

*Group 4 were administered with marketed herbal antidiabetic preparation*

*Group 5 were administered with CB hydro-alcoholic extract 250 mg/kg b.w,*

*Group 6 were administered with CB hydro-alcoholic extract 500 mg/kg b.w,*

For acute antidiabetic study blood samples were collected from tails after 0, 1, 3 and 5 h. For sub-acute study blood samples were collected from tails on 0, 1, 3, 5 and 7th days post-treatment by tail prick method for the determination of serum glucose levels [28]. The animals were treated for 7 days and were given free access to food and water ad libitum. On the 7th day animals were killed by decapitation and blood was collected from the arterial jugular and serum was separated [29].

Biochemical parameters

Blood glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, total protein and albumin levels in serum were measured spectrophotometrically by methods prescribed by the manufacturer [30-31].

Histopathological studies

Animals were sacrificed on 7th day during prolonged treatment. Pancreas, liver and kidney were removed, washed with cold saline and preserved in 10% formalin in buffered form. Blocks from tissues were routinely processed and embedded in paraffin. Thin sections were cut using rotary microtome and stained with hematoxilin and eosin for histomorphology evaluation [32-33].

Statistical analysis

Results were presented as mean±S.D. for fasting plasma glucose level and biochemical parameters of six observations. Statistical analysis was made using two-ways analysis of variance using the statistical software program, Graph Pad Prism 4.0. Statistical significance was considered at P<0.05.
RESULTS AND DISCUSSION

The phytochemical screening of *Caesalpinia bonduc* (Roxb.) seeds hydro-alcoholic extract revealed the presence of carbohydrates, proteins, amino acids, glycosides, flavonoids, saponins, steroids and triterpenoids etc.

Major diagnostic microscopic characters of the powder are columnar palisade cells, bone shaped thick walled parenchymatous cells with brown content and cells filled with starch grains. Powder does not show any fluorescence when exposed to ultraviolet light. However, the extract in 1% NaOH solution ethyl alcohol and solvent ether emitted a green fluorescence under ultraviolet light [Fig.1].

In acute toxicity studies oral administration of the extracts in doses from 1000 to 3000 mg/kg/day did not produce significant changes in behaviors. In a single dose administration no adverse effects was observed for the crude *Caesalpinia bonduc* (Roxb.) seeds hydro-alcoholic extract, indicating that the extracts are non toxic under the observable condition.

In oral glucose tolerance test in non-diabetic rats, hydro-alcoholic extract (500mg/kg b.w.) showed a significant reduction in blood glucose levels from 30 min onwards [Table 1]. In order to choose the optimum dose for the diabetic animals, different doses of hydro-alcoholic extract (250 and 500 mg/kg) were evaluated on glucose tolerance in diabetic rats along with the standard drug glibenclamide (10 mg/kg). The rats were treated with the extract and improvement in GTT was assessed by comparing the blood glucose level (BGL) before and after the treatment. A dose of 250 and 500 mg/kg of hydro-alcoholic extract reduced FBG by 1.94% and 4.08% within 90 min of the extract administration (0 h of GTT). The higher dose of 500 mg/kg had better effect as that of 10 mg/kg of standard drug glibenclamide. It therefore appears that 500 mg/kg of the hydro-alcoholic seeds extract of *Caesalpinia bonduc* is the effective dose on FBG.

Table 1: Glucose tolerance test of hydro-alcoholic seeds extract of Caesalpinia bonduc in normal Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Blood glucose concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Control</td>
<td>2% Acacia Suspension</td>
<td>89.6±5.9</td>
</tr>
<tr>
<td>C.b. (HA)</td>
<td>250 mg/kg</td>
<td>82.1±4.4</td>
</tr>
<tr>
<td>C.b. (HA)</td>
<td>500 mg/kg</td>
<td>80.8±5.3</td>
</tr>
<tr>
<td>Ayurvedic Marketed Preparation</td>
<td>500 mg/kg</td>
<td>90.4±2.6</td>
</tr>
<tr>
<td>Std.drug Glibenclamide</td>
<td>10mg/kg</td>
<td>83.1±5.1</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E, n=6.
Table 2: Acute hypoglycemic studies of hydro-alcoholic seeds extract of Caesalpinia bonduc in normal rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>0 hours</th>
<th>1 hour</th>
<th>3 hours</th>
<th>5 hours</th>
<th>7 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>82.5±2.5</td>
<td>82±2.0</td>
<td>77.5±2.5</td>
<td>75±2.6</td>
<td>74±2.6</td>
</tr>
<tr>
<td>C.b. (HA) 250 mg/kg</td>
<td>79.2±5.0</td>
<td>76.8±1.2</td>
<td>72.6±1.9</td>
<td>73±3.5</td>
<td>73.5±2.4</td>
<td></td>
</tr>
<tr>
<td>C.b. (HA) 500 mg/kg</td>
<td>82.4±2.9</td>
<td>77.3±2.9</td>
<td>70.3±1.9</td>
<td>71.8±3.5</td>
<td>71.9±2.4</td>
<td></td>
</tr>
<tr>
<td>Ayurvedic Marketed Preparation 500 mg/kg</td>
<td>74±2.6</td>
<td>66.6±2.4</td>
<td>60.8±2.3</td>
<td>63.1±2.1</td>
<td>64.8±2.4</td>
<td></td>
</tr>
<tr>
<td>Std.drug Glibenclamide 10mg/kg</td>
<td>74.5±4.7</td>
<td>60±4.0</td>
<td>49±3.9</td>
<td>50.8±4.0</td>
<td>53.8±3.5</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents mean±S.E, n=6.

Table 3: Sub-acute hypoglycemic studies of hydro-alcoholic seeds extract of Caesalpinia bonduc

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>0 day</th>
<th>7 day</th>
<th>14 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>83.1±3.0</td>
<td>81.6±2.7</td>
<td>82.6±2.7</td>
</tr>
<tr>
<td>C.b. (HA) 250 mg/kg</td>
<td>84.8±3.4</td>
<td>79.8±3.4</td>
<td>76.1±3.4</td>
<td></td>
</tr>
<tr>
<td>C.b. (HA) 500 mg/kg</td>
<td>82.5±4.3</td>
<td>72.5±3.3</td>
<td>65.1±3.2</td>
<td></td>
</tr>
<tr>
<td>Ayurvedic Marketed Preparation 500 mg/kg</td>
<td>85.1±2.2</td>
<td>77.3±3.0</td>
<td>72.8±2.9</td>
<td></td>
</tr>
<tr>
<td>Std.drug Glibenclamide 10mg/kg</td>
<td>85.0±3.2</td>
<td>75.7±3.1</td>
<td>69.1±2.9</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents mean±S.E, n=6.

Table 4: Acute antidiabetic studies of hydro-alcoholic seeds extract of Caesalpinia bonduc in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>0 hours</th>
<th>1 hour</th>
<th>3 hour</th>
<th>5 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>82.5±2.5</td>
<td>82.1±2.03</td>
<td>77.5±2.5</td>
<td>75.0±2.6</td>
</tr>
<tr>
<td>C.b. (HA) 250 mg/kg</td>
<td>232.1±30.1</td>
<td>234.8±30.0</td>
<td>235.8±30.0</td>
<td>236.6±29.7</td>
<td></td>
</tr>
<tr>
<td>C.b. (HA) 500 mg/kg</td>
<td>257.5±30.9</td>
<td>234.5±30.0</td>
<td>204.3±24.9</td>
<td>188.0±24.7</td>
<td></td>
</tr>
<tr>
<td>Ayurvedic Marketed Preparation 500 mg/kg</td>
<td>259.3±19.3</td>
<td>243.8±19.1</td>
<td>222.5±18.7</td>
<td>244.6±18.2</td>
<td></td>
</tr>
<tr>
<td>Std.drug Glibenclamide 10mg/kg</td>
<td>220.0±22.0</td>
<td>213.66±18.2</td>
<td>204.5±17.4</td>
<td>192.5±19.4</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents mean±S.E, n=6.
Table 5: Sub-acute antidiabetic studies of hydro-alcoholic seeds extract of Caesalpinia bonduc

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Blood glucose concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
<td>1 day</td>
</tr>
<tr>
<td></td>
<td>3 day</td>
<td>5 day</td>
</tr>
<tr>
<td></td>
<td>7 day</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2% Acacia Suspension</td>
<td>82.1±2.0</td>
</tr>
<tr>
<td>Control Diabetic</td>
<td>2% Acacia Suspension</td>
<td>303.6±26.6</td>
</tr>
<tr>
<td>C.b. (HA)</td>
<td>250 mg/kg</td>
<td>290±33.2</td>
</tr>
<tr>
<td>C.b. (HA)</td>
<td>500 mg/kg</td>
<td>290±8.8</td>
</tr>
<tr>
<td>Ayurvedic Marked formulation</td>
<td>500 mg/kg</td>
<td>243.8±21.0</td>
</tr>
<tr>
<td>Std.drug</td>
<td>10mg/kg</td>
<td>213.6±19.9</td>
</tr>
</tbody>
</table>

Each value represents mean± S.E, n=6.

Tables 6- Effect of aqueous extract of Caesalpinia bonduc on serum lipid profile in diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Triglyceride (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>HDL cholesterol (mg/dl)</th>
<th>LDL cholesterol (mg/dl)</th>
<th>Total protein (mg/dl)</th>
<th>Albumin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>121.01±7.20</td>
<td>143.06±4.38</td>
<td>31.66±1.34</td>
<td>54.76±3.20</td>
<td>7.11±0.14</td>
<td>3.80±0.39</td>
</tr>
<tr>
<td>Diabetic control Std.Drug Glibenclamide</td>
<td>64.83±5.78</td>
<td>140.5±5.1</td>
<td>32.01±0.56</td>
<td>81.33±5.11</td>
<td>6.09±0.19</td>
<td>3.41±0.17</td>
</tr>
<tr>
<td>Ayurvedic formulation CB(HA) extract (250mg/kg)</td>
<td>119.39±4.24</td>
<td>178.4±3.2</td>
<td>24.22±0.76</td>
<td>30.31±5.12</td>
<td>6.89±0.27</td>
<td>3.41±0.17</td>
</tr>
<tr>
<td>CB(HA) extract (500mg/kg)</td>
<td>79.0±2.22</td>
<td>149.0±4.5</td>
<td>30.01±1.22</td>
<td>85.23±1.55</td>
<td>7.38±0.16</td>
<td>3.5±0.39</td>
</tr>
<tr>
<td>CB(HA) extract (500mg/kg)</td>
<td>77.4±2.89</td>
<td>134.0±5.2</td>
<td>39.62±1.19</td>
<td>65.45±2.33</td>
<td>6.89±0.29</td>
<td>3.46±0.36</td>
</tr>
</tbody>
</table>

Each value represents mean± S.E, n=3

Fig 1: Powder Microscopy of Caesalpinia bonduc (Roxb.)
Fig 2: Histopathology of Kidney

Photomicrographs of the kidney in the diabetic rats after 7 days post-treatment (A) Normal control B) Diabetic control C) Photomicrograph of kidney treated with ayurvedic marketed preparation D) Photomicrograph of kidney treated with Caesalpinia bonduc (Roxb.) hydro-alcoholic extract 500 mg/b.w.
In acute hypoglycemic effects of CB HA extract in normal rats after a single dose of extract only the dose of 500 mg/kg showed a significant reduction in the blood sugar level after 1 h (21.20%), where the extract at a dose of 250 mg/kg showed a significant reduction in the blood glucose level only after 3 h [Table 2]. Where as standard drug glibenclamide showed reduction in the blood sugar level after 3 h (19.46%) and ayurvedic marketed antidiabetic preparation showed reduction in the blood sugar level after 7 h (12.40%). The sub-acute hypoglycemic effects of CB HA extract in normal rats orally treated with 250 and 500 mg/kg of the respective extracts for a period of 14 days daily shows that both extracts induced significant (P< 0.05) hypoglycemic effects in dose-dependent fashion when compared to the control group [Table 3]. The results showed that in fasted rats hydro-alcoholic *Caesalpinia bonduc* seeds extract (250 mg/kg) produced an 5.89% mg/dl fall in blood glucose level after 7 days daily treatment and 10.25% mg/dl fall in blood glucose level after 14 days treatment, however the dose of 500 mg/kg produces 15.27% and 21.09% fall in blood glucose level after 7th and 14th day respectively which is better than the ayurvedic marketed preparation (9.16% and 14.25% FBGL on 7th and 14th day) and standard drug glibenclamide (10.94% and 18.70% FBGL on 7th and 14th day).

The hypoglycemic effect of the hydro-alcoholic extract of *Caesalpinia bonduc* seeds on the fasting blood sugar levels of diabetic rats [Tables 4]. After a single dose of extract only the dose of 500 mg/kg showed a significant reduction in the blood sugar level after 1 h, where the extract at a dose of 250 mg/kg showed a significant (P < 0.05) reduction in the blood glucose level after 3 h. In the subacute study at the end of the study the extract at dose of 500 mg/kg showed a significant (P< 0.01) reduction in the blood glucose comparable with that of glibenclamide (10...
mg/kg) treated group [Table 5]. It was suggested that the regeneration of β cells following destruction by alloxan might be the primary cause for the antidiabetic activity of the extracts³⁴.

Serum cholesterol, serum triglycerides, serum albumin, serum LDL levels were decreased significantly by glibenclamide and all the extracts of *Caesalpinia bonduc* (Roxb.) due to 7 days of treatment. HDL levels and total proteins were increased by glibenclamide and aqueous extracts. As shown in Table No.6.

Photomicrographs [Fig.3] of pancreas in slide no,(A) showed normal acini, and normal cellular population in the islets of langerhans in pancreas of vehicle-treated rats. In slide no. (B) extensive damage to the islet of langerhans and reduced dimensions of islets. In slide no. (C) and (D) restoration of normal cellular population size of islets with hyperplasia by *Caesalpinia bonduc* (Roxb.) extract 500 mg/kg b.w, (C) was also shown. The partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia was shown by aqueous extract.

Photomicrographs [Fig.2] of Kidney tissues in the control group showed normal renal corpuscles (Slide A). But some morphological and pathological changes occurred in the kidney tissues of diabetic control albino rats (Slide B) In slide C and D treated with aqueous extracts of *Caesalpinia bonduc* (Roxb.) extract 250 and 500 mg/kg b.w, (D) of the glomerular region of the kidney, some atrophic changes and haemolysis were seen but cellularity and basement membrane were normal. No inflammatory cells were found. The tubular portion also showed atrophic changes, shedding of epithelium and oedema.

Photomicrographs [Fig.4] of liver in normal animal showed normal hepatic cells with well preserved cytoplasm, nucleus, nucleolus and central vein. In diabetic control liver section showed that the lobular architecture was maintained but there was also severe fatty changes, sinusoidal dilation and congestion, mild perportal inflammation, fibrosis, severe feathery degeneration and necrosis. In diabetic rats treated with aqueous *Caesalpinia bonduc* (Roxb.) seeds extract 500 mg/kg b.w, liver section maintained lobular architecture and had mild fatty change, mild sinusoidal dilation and congestion, mild periportal inflammation and mild feathery degeneration.

From the above results, it was concluded that the present study seems to support the claims by traditional medicine practitioners about the usefulness of *Caesalpinia bonduc* seeds for the treatment of diabetes.
Photomicrographs of the liver in the diabetic rats at 1 week post-treatment (A) Normal control B) Diabetic control C) Photomicrograph of liver treated with ayurvedic marketed preparation D) Photomicrograph of liver treated with Caesalpinia bonduc (Roxb.) hydro-alcoholic extract 500 mg/b.w.

CONCLUSION

The hydro-alcoholic seeds extract of *Caesalpinia bonduc* have shown significant reduction in blood glucose levels in both glucose loaded, normal and alloxan induced diabetic rats. In glucose loaded animals, the drug has reduced the blood glucose to the normal levels. It is possible that the drug may be acting by potentiating the pancreatic secretion or increasing the glucose uptake. Repeated administration of *Caesalpinia bonduc* extracts had decreased the blood glucose, total cholesterol and triglycerides significantly. Histopathological examination of pancreas, liver and kidney showed the recovery of damaged tissues when section of treated groups compared with diabetic control.

In conclusion, *Caesalpinia bonduc* hydro-alcoholic seeds extract showed significant anti-diabetic effect in diabetic rats after oral administration. Thus the claim made by the traditional Indian systems of medicine regarding the use of seeds of this plant in the treatment of diabetes stands confirms.

In future studies the aqueous *Caesalpinia bonduc* seeds extract may be used along with other traditional antidiabetic plant extracts in development of polyherbal formulations and determination of its synergistic effects.

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