Original Research Article

In-vivo antidiabetic activity of *Saccharum spontaneum* on STZ-induced diabetic mice

Abstract

**Objectives:** *Saccharum spontaneum* is traditionally used in Arunachal Pradesh, India for the treatment of diabetes mellitus. Literature survey report no scientific investigation on this claim therefore, this study aim to evaluate the antidiabetic effect of ethanolic extract on STZ induced diabetic mice.

**Material and methods:** Diabetes was induced in male albino mice by single intraperitoneal injection of streptozotocin (100mg/ kg bodyweight). Initially Starch tolerance test was performed, blood glucose level and area under the curve (AUC) was evaluated. The test extract was administered for three weeks and the antidiabetic activity was evaluated by means of monitoring the change in blood glucose level, body weight and biochemical parameters.

**Results:** The extract showed a significant decrease in the blood glucose level, furthermore the decreased body weight was slightly improved after the treatment. Daily oral treatment of extract also showed a significant reduction in the Serum ALT, creatinine and triglyceride when compared to the diabetic control.

**Conclusion:** The results provide supports to the traditional claim made on *Saccharum spontaneum* and provide evidence that this plant might be a potential source of antidiabetic agent.

**Keyword:** Diabetes, streptozotocin, hyperglycemia, *Saccharum spontaneum*
Graphical abstract

Keyword: Diabetes, streptozotocin, hyperglycemia, Saccharum spontaneum
Introduction

Diabetes mellitus (DM) is one of the most prevalent disease. It is a chronic metabolic derangement of protein, fats and carbohydrate caused by an absolute or relative lack of insulin secretion resulting in hyperglycemia which is defined as a clinical characteristic of diabetes [1, 2]. Alteration in lipid and protein metabolism in long term may accelerate the development of microvascular and macrovascular complication [3]. Many clinical anti-diabetic drugs are available which do not restore normal glucose homeostasis and are associated with adverse side effect [4]. Herbal medicines are gaining importance as they are less expensive and free from side effects when compared to the synthetic drug. Therefore, medicinal plants with blood glucose lowering effects represent a promising alternative for the treatment of diabetes [5].

Saccharum spontaneum L. (Family: Poaceae) is a tall perennial grass with deep root and rhizome growing upto a height of 3-4 m [6], grows in the banks of water bodies or along roadsides. It is widely distributed throughout the tropical countries of Asia, Africa, America and Australia [7]. The roots of the plant are astringent, emollient, diuretic, tonic and areuse for treating dyspepsia, burning sensation, piles, sexual weakness, gynecological troubles and respiratory troubles [8]. Leaves and whole plant are reported to possess activities like antioxidant [9, 10], antidiarrhoeal [11], CNS depressant [12] and antiurolithiatic activity [13]. Traditionally, the plant is reported to use for treatment of diabetes [14] however no scientific evaluation has been reported on its claimed antidiabetic effect. Therefore the present work was aimed to evaluate the antidiabetic activity of ethanolic extract of Saccharum spontaneum on streptozocin (STZ) induced diabetic mice.

Material and methods

Collection of plant materials

The young shoot of Saccharum spontaneum was collected from Arunachal Pradesh, India. The collected plant were identified and authenticated by Dr. Manish Kandwal, Scientist D, Botanical Survey of India (BSI), Itanagar, Arunachal Pradesh. The sample specimen was deposited as herbarium and voucher number was preserved for future reference (HAG-008).

Preparation of plant extract
The air-dried plant material was grinded, extracted with ethanol and kept at rotary shaker for 24 h. The filtrate obtained were concentrated in a rotary vacuum evaporator to give solid residue. The residue was stored in an airtight container in refrigerator for subsequent experiments.

**Chemicals**

Streptozotocin (STZ) was purchased from SRL Pvt. Ltd, India. Total serum alanine transaminase, creatinine and triglyceride, standard commercial kit purchased from Glucometer (ACCU-CHEK active). All the chemical used in the study was of analytical grade.

**Experimental animals**

Adult male Swiss albino mice weight ranging from 25-35g were procured from stock animal facility of the Dept. of Zoology, Rajiv Gandhi University. The animals were kept in polypropylene cages at a temperature of 24±2°C with 12h light/dark cycle and were fed with standard diet and water ad libitum [30]. All the animals were maintained in accordance with NIH guidelines for care and use of laboratory animals (NIH, 1985). The protocol of the animal experiment was reviewed and approved by the Institutional Animal Ethical Committee, vide letter no. IAEC/RGU/17/12, dated 02/06/2017.

**Induction of experimental diabetes**

Diabetes were induced in overnight fasted mice by single intraperitoneal (i.p) injection of freshly prepared STZ (100mg/kg) dissolved in 0.1 M citrate buffer (pH = 4.5). To avoid hypoglycemic shock 10% sucrose solution were given immediately to the mice. After 72 h mice with marked hyperglycemia (fasting blood glucose level greater than 200mg/dl) were selected for the experimental study [31, 32]. Fasting blood glucose level were monitored using blood glucose test strips with elegance glucometer (ACCU-CHEK active).

**Effect of plant extract on oral starch tolerance in normal mice**

This test evaluates the starch tolerance of ethanol extract of *Saccharum spontaneum* (SS) on non-diabetic mice. The overnight fasted non-diabetic mice were divided into four experimental groups of 6 animals (n=6) each. The normal untreated mice (Group I) received distilled water. Group II were administered with acarbose (10mg/kg, P.O) which serve as positive control. Group III and Group IV were administered with 100mg/kg and 1000 mg/kg of
ethanol extract of SS. After thirty minutes of respective treatment, the mice were administered orally with starch (3g/kg). The blood glucose level from each group were determined at 0, 30, 60, 120, 150 minutes after starch treatment via tail puncture \[33\]. The peak blood glucose (PBG) and area under the curve (AUC) for each group were determined. The maximum blood glucose concentration was taken as PBG and AUC was calculated using the following equation:

\[
\text{AUC (mmol/L)} = \frac{BG_0 + BG_{30} \times 0.5}{2} + \frac{BG_{30} + BG_{60} \times 0.5}{2} + \frac{BG_{60} + BG_{120} \times 1}{2} + \frac{BG_{120} + BG_{150} \times 0.5}{2}
\]

Where BG represents the blood glucose concentration at the time intervals 0, 30, 60, 120 and 150 minutes.

**Effect of plant extract on oral starch tolerance in diabetic mice**

This test evaluates the starch tolerance of ethanol extract of SS on diabetic mice. The normal untreated mice (Group I) received distilled water. The diabetic mice were divided into four experimental groups (n=6). Group II serve as diabetic control without any treatment. Group III were administered with acarbose (10mg/kg, P.O) which serve as positive control. Group IV and Group V were administered with 100mg/kg and 1000mg/kg of ethanol extract of SS. After thirty minutes of respective treatment, the mice were administered orally with starch (3g/kg). The blood glucose level from each group were determined at 0, 30, 60, 120, 150 minutes after starch treatment via tail puncture \[33\]. The peak blood glucose (PBG) and area under the curve (AUC) for each group were determined similarly as described above.

**Effect of long term/continuous plant extracts treatment on diabetic mice**

The long term (21 days) experiment was conducted to examine the efficacy of plant extract on diabetic mice. The overnight fasted male mice were divided into five groups (n=6). Normal untreated mice serve as Group I were fed with distilled water. STZ induced mice with confirmed diabetes were further divided into four groups. Group II serve as the diabetic control. Group III received standard drug acarbose (10mg/kg/day, P.O). Group IV and group V were treated with 100mg/kg and 1000mg/kg of ethanol extract of SS (P.O) for consecutive 21 days. During the study period, animals in all groups had free access to normal diet and water. Changes
in body weight were noted and blood glucose level from each group were estimated on initial day, 7th, 14th and 21st day.

Biochemical parameters

All the biochemical analysis were carried out using commercial kits following the manufacturer standard protocols in a semi-auto analyzer. Serum alanine transaminase (ALT) was estimated using Reitman and Frankel method [34], serum Creatinine level was measured using Jaffe’s reaction method [35] and triglycerides (TGL) was estimated using Foster and Dunn method [36].

Statistical analysis

All generated data were expressed as mean ± standard error of mean (SEM) and variation among the groups will be subjected to one way ANOVA followed by Tukey’s multiple comparison tests using Graph pad Prism 5.0 software. The level of significance was determined at P<0.05.

Result

Effect of SS on oral starch tolerance test in non-diabetic mice

The effect of ethanol extract of SS on starch tolerance test in overnight fasted non-diabetic mice is presented in Table 1 and Figure 1. In this study, the peak blood glucose (PBG) and area under curve (AUC) were estimated at different interval of time from 0min to 150 min. The administration of SS showed reduction in PBG after 30 minutes of oral starch load. The doses 100 and 1000 mg/kg bw showed 4.15% and 7.55% reduction in PBG level. There was also reduction in AUC, 4.51% in 100mg/kg and 8.71% in 1000mg/kg treated group. However, SS treated group exerted no significant (P<0.05) reduction when compared with the diabetic control. Whereas the standard drug acarbose exhibited a significant reduction in PBG level (19.38%) and AUC (21.51%).

Effect of SS on oral starch tolerance test in diabetic mice
The treatment of SS on diabetic mice for starch tolerance test showed decrease in PBG and AUC level after 30 minutes of oral starch load. The diabetic control showed significantly \((P<0.05)\) higher blood glucose level than the normal control. The \%\ reduction of SS in PBG level at doses 100 mg/kg (4.06 \%) and 1000 mg/kg (10.75 \%) and AUC were 5.04 \% (100 mg/kg) and 11.48 \% (1000 mg/kg) (Table 2 and figure 2). However, SS treated group exerted no significant \((P<0.05)\) reduction when compared with the diabetic control. While significant reduction was observed in acarbose treated group PBG level (21.99 \%) and AUC (23.79 \%).

**Effect of long term treatment with SS on body weight**

The effect of ethanol extract of SS on body weight of STZ induced diabetic model is presented in Table 4. The body weight of normal untreated control group almost remained the same throughout the study. Whereas in diabetic control slight decrease in body weight was observed when compared to the normal control. The diabetic group treated with SS and AC showed slight increase in the body weight. However, no significance difference was observed in the body weight of mice till the end of 21 days treatment.

**Effect of long term treatment with SS on blood glucose level**

The effect of ethanol extract of SS on blood glucose level in STZ induced diabetic model is presented in Table 3. The blood glucose level of diabetic untreated group continued to increase gradually in the 21 days study and were significantly \((P<0.05)\) higher than the normal group. The SS extract at doses of 100 and 1000 mg/kg showed no significant difference in the blood glucose level in initial (Day 0) and Day 7 of the treatment. However, dose of 1000 mg/kg showed significance difference in the blood glucose level on the 14\textsuperscript{th} day but 100 mg/kg showed no significance difference when compared with the diabetic control. Whereas the 21\textsuperscript{st} day of the treatment showed the maximum significance difference in both the dose of SS. Similarly the standard drug acarbose also showed significance difference on 14 and 21 day of the treatment. Although the SS extract did not show a better result than acarbose but SS treated group exhibited marked reduction in the blood glucose levels as compared to the diabetic control group.

**Biochemical parameters**
The effect of ethanol extract of SS on various biochemical parameters such as ALT (hepatic markers), creatinine (renal marker) and TG (lipid profile) were assessed in all control and experimented group (Table 5). The Serum ALT, creatinine and TG activity were significantly (P<0.05) elevated in the diabetic control group. After the daily dose of SS and standard drug acarbose for 21 days, the ALT, creatinine and TG level were restored to normal level. A significant decrease in the creatinine and TG level was observed in SS treated diabetic group. However, ALT level showed no significant difference when compared with both normal and diabetic control.

**Discussion**

Diabetes is one of the most prevalent metabolic disorder which has become a greater health concern all over the world. The role of medicinal plant as an alternative source in treatment of diabetes is gaining more popularity [15]. In the present study, the ethanol extract of young shoot of Saccharum spontaneum was selected for the evaluation of antidiabetic activity on the STZ-induced diabetes mice. Initially the ethanolic extract were screened for acute hyperglycemic effect using oral test of starch tolerance method [16]. The result obtained showed that the young shoot extract of SS suppressed the elevated blood glucose level in both non-diabetic and STZ induced diabetic mice. However, the reduction level of PBG and AUC in group treated with AC was more pronounced when compared to the SS treated group.

STZ, an antibiotic produced by *Streptomyces achromogenes* is known to specifically destroy pancreas by reducing the size and number of functionally active b-cells, the cell which produce hormone insulin to regulate the normal blood glucose level [17, 18]. As a result of pancreatic b-cells destruction, STZ is widely used as a diabetogenic agent to induce diabetes in experimental animals [19, 20]. The long term 21 day treatment with SS in the STZ-induced diabetic mice showed a significant reduction in their blood glucose levels at the end of the treatment, when compared with the diabetic control. STZ-induced diabetes is also characterized by severe weight loss and this reduction might be due to the degeneration of muscle and loss of tissue protein which is the major contributor to body weight [21]. A significant loss in the bodyweight was observed in diabetic group which was slightly improved in the group treated with SS and AC.
Diabetes mellitus is usually associated with lipid abnormalities and increase the risk for coronary heart disease [22]. A variation in the metabolic and regulatory mechanism due to insulin resistance or deficiency are accountable for lipids accumulation [23]. The characteristic features of dyslipidemia in diabetes are hypercholesterolemia and hypertriglyceridemia [24]. STZ-induced diabetes also developed hyperlipidemia [25]. The present study showed a marked increase in the total triglyceride level in diabetic control group. The reduced ratio of TGL shows a potential improvement in insulin sensitivity while increased TGL ratio is associated with insulin resistant in diabetes [26]. Our study showed a signification reduction in TGL level after the 21 day treatment with SS. The elevation of transaminase such as ALT indicate liver dysfunction resulting from hepatic damage which may increase diabetes complication such as gluconeogenesis and ketogenesis [27, 28]. The repeated administration of SS results in significant reduction of ALT when compared to the diabetic control group. Diabetes may also lead to impaired kidney function and serum level of creatinine can be a useful marker for renal damage [29]. The SS extract attenuated the elevated level of creatinine observed among diabetic control group.

Conclusion

This study highlights the potential of ethanol extract of Saccharumspontaneum in preventing hyperglycemia. The prolong treatment with extract also showed improvement in bodyweight and other biochemical parameters associated with diabetes. This study provide a scientific validation of the safe use of young shoot of Saccharumspontaneum by traditional healers in the treatment of diabetes. Hence Saccharumspontaneum can be a prospective source of antidiabetic agent.
Reference


Table and Figure:

Table 1: Effect of ethanol extract of *Saccharum spontaneum* (SS<sub>EtOH</sub>) on peak blood glucose (PBG) and area under the curve (AUC) after starch loading in non-diabetic mice. Data are represented as Mean ± SEM (n=6), p<0.05 vs. control. NC: Negative control, AC: Acarbose, SS: Saccharum spontaneum.

<table>
<thead>
<tr>
<th>Group</th>
<th>PBG (mg/dl)</th>
<th>% Reduction of PBG</th>
<th>AUC (mg/dl)</th>
<th>% Reduction of AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: Normal control</td>
<td>139.28 ± 5.67</td>
<td></td>
<td>355.55 ± 7.93</td>
<td></td>
</tr>
<tr>
<td>II: Acarbose</td>
<td>113.57 ± 8.33</td>
<td>19.38</td>
<td>282.21 ± 19.17</td>
<td>21.51</td>
</tr>
<tr>
<td>III: S$\text{S}_{\text{EtOH}}$(100 mg/Kg)</td>
<td>135.03 ± 2.21</td>
<td>4.15</td>
<td>343.33 ± 4.91</td>
<td>4.51</td>
</tr>
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<td>-----------------------------------------</td>
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</tr>
<tr>
<td>IV: S$\text{S}_{\text{EtOH}}$(1000 mg/Kg)</td>
<td>130.23 ± 3.24</td>
<td>7.55</td>
<td>328.25 ± 3.560</td>
<td>8.71</td>
</tr>
</tbody>
</table>

![Graph showing blood glucose level over time for different conditions.](image-url)
Figure 1: Effect of ethanol extract of SS on Starch tolerance test after oral starch administration in non-diabetic mice. Values are represented as Mean ± SEM (n=6). NC: Negative control, AC: Acarbose, SS: Saccharumspontaneum.

Table 2: Effect of SS EtOH on peak blood glucose (PBG) and area under the curve (AUC) after starch loading in diabetic mice. Data are represented as Mean ± SEM (n=6).

<table>
<thead>
<tr>
<th>Group</th>
<th>PBG (mmol/L)</th>
<th>% Reduction of PBG</th>
<th>AUC (mmol/L)</th>
<th>% Reduction of PBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: Normal control</td>
<td>128.17 ± 8.79</td>
<td></td>
<td>328.54 ± 20.64</td>
<td></td>
</tr>
<tr>
<td>II: Diabetic control</td>
<td>328.26 ± 29.58</td>
<td>21.99*</td>
<td>832.50 ± 71.86</td>
<td>23.79*</td>
</tr>
<tr>
<td>III: Acarbose</td>
<td>256.06 ± 11.88</td>
<td></td>
<td>634.41 ± 29.00</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>Blood Glucose Level (mg/dL)</td>
<td>Time (min)</td>
<td></td>
<td></td>
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<tr>
<td>------------------------------</td>
<td>-----------------------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>IV: SS&lt;sub&gt;EIOH&lt;/sub&gt;(100 mg/Kg)</td>
<td>314.93± 19.60</td>
<td>4.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>790.50 ± 46.89</td>
<td>5.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V: SS&lt;sub&gt;EIOH&lt;/sub&gt;(1000 mg/Kg)</td>
<td>292.97± 17.04</td>
<td>10.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>736.96 ± 41.39</td>
<td>11.48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig 2: Effect of ethanol extract on Starch tolerance test after oral starch administration in non-diabetic mice. Values are represented as Mean ± SEM (n=6). NC: Negative control, AC: Acarbose, SS: Saccharumspontaneum.

Table 3: Effect of SS on body weight of control and experimental group. NC: Negative Control, AC: Acarbose, DC: Diabetic Control, SS: Saccharumspontaneum. Data are represented as Mean ± SEM (n=6). * p<0.05 significance between normal control and diabetic control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g) at different days during the experiment period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>I: Normal control</td>
<td>33.96 ± 2.86</td>
</tr>
<tr>
<td>II: Diabetic control</td>
<td>31.47 ± 0.532</td>
</tr>
</tbody>
</table>
### III: Acarbose

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>27.31± 1.084</td>
<td>27.65±1.201</td>
<td>28.17 ± 1.023</td>
<td>29.04±0.955</td>
</tr>
</tbody>
</table>

### IV: SS\textsubscript{EtOH}(100 mg/Kg)

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30.32± 1.609</td>
<td>30.15 ±1.331</td>
<td>29.99 ± 1.001</td>
<td>29.96±1.041</td>
</tr>
</tbody>
</table>

### V: SS\textsubscript{EtOH}(1000 mg/Kg)

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>27.82± 1.259</td>
<td>27.80 ±1.616</td>
<td>27.94 ± 1.651</td>
<td>28.36±1.561</td>
</tr>
</tbody>
</table>

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**Table 4:** Effect of SS on blood glucose level of control and experimental group. NC: Negative Control, AC: Acarbose, DC: Diabetic Control, SS: Saccharumspontaneum. Data are represented as Mean ± SEM (n=6); ns - not significance; ###p<0.001 when compared to the normal control; *p<0.05, **p<0.001 when compared to the diabetic control.
### Table 5: Effect of SS on biochemical parameters.
NC: Negative Control, AC: Acarbose, DC: Diabetic Control, SS: Saccharum spontaneum. Data are represented as Mean ± SEM (n=6). ns represent no significance difference; ###p<0.001 when compared to the normal control, **p<0.01, ***p<0.001 when compared to the diabetic control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Triglyceride (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>ALT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: Normal control</td>
<td>115.427±7.404</td>
<td>0.308±0.12</td>
<td>29.818±0.314</td>
</tr>
<tr>
<td>II: Diabetic control</td>
<td>182.527±10.018###</td>
<td>0.621±0.076###</td>
<td>40.053±0.880#</td>
</tr>
<tr>
<td>III: Acarbose</td>
<td>115.427±5.902***</td>
<td>0.412±0.063***</td>
<td>33.969±0.262ns</td>
</tr>
<tr>
<td>IV: SS&lt;sub&gt;E&lt;sub&gt;OH&lt;/sub&gt;(100 mg/Kg)</td>
<td>129.491±7.100**</td>
<td>0.390±0.041***</td>
<td>38.787±5.157ns</td>
</tr>
<tr>
<td>V: ( \text{SS}_{\text{EtOH}}(1000 \text{ mg/Kg}) )</td>
<td>124.163 ± 10.328***</td>
<td>0.369 ± 0.046***</td>
<td>34.141 ± 4.782NS</td>
</tr>
</tbody>
</table>