ANTIHYPER GLYCEMIC ACTIVITY OF LEAF EXTRACTS OF SYZYGIUM ALTERNIFOLIUM (WIGHT) WALP. AND SYZYGIUM CUMINI (LINN.)SKEELS IN STZ INDUCED DIABETIC RATS

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INTRODUCTION

For a variety of reasons, the popularity of complementary medicine is increased. Traditional plant therapies coupled with dietary measures as prescribed in Ayurveda and other indigenous systems of medication. In Australia and in U.S., a sizable number of populations use at least one form of unconventional therapy including herbal medicine. The World Health Organization (1980) was also recommended the evaluation of the effectiveness of plants in conditions where there is lack of safe synthetic drugs (Iwe et al., 1999). To promote the ecological survival of plants, secondary products have evolved to interact with molecular targets affecting the cells, tissues, and physiological functions in competing with other plants, microorganisms and animals. These natural compounds formed the foundations of modern drugs. Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds and many more. The medicinal value of the plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Himal and Yogol, 2008).

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ABSTRACT

In recent years, several plant extracts have been scrutinized for their antidiabetic properties in an effort to identify alternative treatment strategies that pose less of a risk for diabetes. Phytochemicals play a vital role and showing multiple beneficial effects in combating diabetes and diabetic-related complications. *Syzygium alternifolium* is an endemic to Tirumala Hills commonly known as ‘Mogi’ and *Syzygium cumini* which is commonly called ‘Jamun’ were taken for the present study to isolate the phytochemicals which act against diabetes. Ethanolic leaf extracts (450mg/kg body weight) of *S. alternifolium* and *S. cumini* were given to the STZ induced diabetic rats for 21 days. Both the leaf extracts showed the significant decrease in blood sugar levels (276-140mg/dl, p< 0.005). The ethanolic leaf extract of *S.alternifolium* was more effective against diabetes than *S. cumini*.
Collection of plant material
Fresh leaves of *S. alternifolium* and *S. cumini* were collected in July 2007 from plants which grown in Tirumala regions. The leaves were washed neatly and air dried at room temperature (25°C) for 3 days and fine powdered with an auto-mix blender. This powder was kept in a deep freezer until the time of use.

Preparation of plant extract
1000 g of dry powder was suspended in 3 liters of ethanol, stirred magnetically and kept for overnight (24h) at room temperature. The extract was preserved and the process was repeated for three consecutive times with the residual powder, each time collecting the extract. The collected extract was pooled and passed through a fine cotton cloth. The filtrate upon evaporation at 40°C at low-pressure (Rotavapor apparatus) yielded 15% of semi solid extract. It was stored in a refrigerator at 0ºC- 4ºC until used in the diabetic studies.

Selection of experimental animals
Adult male albino rats of Wistar strain weighing approximately 180 ± 20g were collected from Indian Institute of Science (I.I.Sc.), Bangalore. The animals were kept in polycarbonate cages and maintained in an animal room with a 12h day-night cycle, at a temperature of 22º ± 2ºC and humidity upto 45-64%. During the whole experimental period, animals were fed with a balanced commercial diet manufactured by Lipton India Ltd, Bangalore.

Induction of experimental diabetes
Streptozotocin (Formula: C<sub>6</sub>H<sub>11</sub>N<sub>2</sub>O<sub>6</sub>, Mol.mass: 265.221 g/mol) Streptozotocin (Streptozocin, STZ and Zanosar) is a naturally occurring chemical that is particularly toxic to the insulin producing β-cells of the pancreas in mammals. It is used in medicine for treating certain cancers of the Islets of Langerhans and used in medical research to produce an animal model of type-1 diabetes.

Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptozotocin (45mg/kg) in 0.1 M citrate buffer (pH 4.5) in a volume of 1 mL/kg body weight. Normal rats received 1 mL citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After 48h of streptozotocin administration, blood glucose levels were estimated in rats fasted overnight. Rats with a blood glucose ranging between 200-300 mg/dl were considered as diabetic and used for the experiment.

Experimental design
36 rats were divided into 6 groups each contained 6 and used as follows; Group 1: Normal Rats, Group 2: Normal Rats treated with *S. alternifolium* extract for 21 days, Group 3: Normal Rats treated with *S. cumini* extract for 21 days, Group 4: Diabetic Control Rats, Group 5: Diabetic Rats administered with *S. alternifolium* extract daily for 21 days. Group 6: Diabetic rats administered with *S. cumini* extract daily for 21 days.

Throughout the experimental period, the body weight, food and fluid intake were monitored. At the end of 21 days, all the rats were killed by cervical dislocation. Blood was collected in heparin-coated tubes and centrifuged at 1,000g for 15 min at 4ºC.

Estimation of blood glucose levels
Blood glucose levels were estimated by using Accu Chek Glucometer (Sensor Comfort) regularly for the experimental period of 21 days before and after giving the leaf extracts of *S. cumini* and *S. alternifolium*.

RESULTS
Since, diabetes is a chronic disorder requiring long-term therapy, there is a need to assess the effect of putative hypoglycemic/antihyperglycemic agents for a longer duration. In addition, this application would be beneficial to reveal the late onset activity profile of the agent (Sezik et al., 2005). Therefore, an experiment was planned to assess the effects of *S. alternifolium* and *S. cumini* leaf extracts for a period of 21 days in streptozotocin-induced diabetic rats. Drug treatment was started after 48h of streptozotocin injection. No detectable irritation or restlessness was observed after each drug or vehicle administration.

Blood glucose levels
Intraperitoneal injection of STZ raised the blood glucose levels up to 4 times of the normal range i.e. 80-300mg/dl. However, the administration of ethanolic extracts of *S. alternifolium* and *S. cumini* to diabetic groups decreased their blood glucose by

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**Table 1: Antihyperglycemic effects (mg/dl) of *S. alternifolium* and *S. cumini* extracts normal, diabetic and treated rats (Mean ± SD values) p < 0.005**

<table>
<thead>
<tr>
<th>Groups of experimental animals</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 15</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>80.4 ± 0.9</td>
<td>81.3 ± 1.8</td>
<td>80.2 ± 1.8</td>
<td>81.5 ± 1.9</td>
<td>82.4 ± 1.3</td>
</tr>
<tr>
<td>Normal + <em>S. alternifolium</em> treated</td>
<td>80.3 ± 1.6</td>
<td>79.3 ± 1.9</td>
<td>70.5 ± 1.9</td>
<td>68.3 ± 1.8</td>
<td>58.8 ± 1.9</td>
</tr>
<tr>
<td>Normal + <em>S. cumini</em> treated</td>
<td>92.3 ± 1.2</td>
<td>92.5 ± 1.5</td>
<td>88.7 ± 1.6</td>
<td>80.9 ± 1.4</td>
<td>76.9 ± 1.9</td>
</tr>
<tr>
<td>Diabetic</td>
<td>249.3 ± 8.5</td>
<td>251.3 ± 5.5</td>
<td>258.9 ± 7.1</td>
<td>276.6 ± 4.5</td>
<td>319.1 ± 3.8</td>
</tr>
<tr>
<td>Diabetic + <em>S. alternifolium</em> treated</td>
<td>276.1 ± 6.8</td>
<td>265.4 ± 7.9</td>
<td>202.5 ± 0.9</td>
<td>145.8 ± 4.1</td>
<td>140.2 ± 1.3</td>
</tr>
<tr>
<td>Diabetic + <em>S. cumini</em> treated</td>
<td>290.5 ± 2.7</td>
<td>290.3 ± 7.9</td>
<td>260.7 ± 3.2</td>
<td>247.7 ± 3.3</td>
<td>214.2 ± 3.1</td>
</tr>
</tbody>
</table>

One way Anova

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>49.71458</td>
<td>3</td>
<td>16.57153</td>
<td>10.8481</td>
<td>3.0983</td>
</tr>
<tr>
<td>within Groups</td>
<td>32.54167</td>
<td>20</td>
<td>1.627083</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>82.25625</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
10-33% in 10 days, 33-52% in 15 days and 40-82% in 21 days. Blood glucose levels decreased after feeding ethanolic extracts of S. alternifolium and S. cumini were highly significant (p<0.005) which indicates this antihyperglycemic effect of the ethanolic extracts. Normal rats are treated with extracts showed the decrease in blood glucose levels which indicate the hypoglycemic effect of the ethanolic extracts (Table 1).

### Changes in body weights

The body weights of the experimental rats there is no more weight gain in normal rats after 21 days of treatment with S. alternifolium and S. cumini leaf extracts (180-195 ± 5). The rats with high blood sugar (STZ induced) 20-45% loss in the body weight was observed, where as in diabetic rats treated with extracts for 21 days resulted in 12-15% increase in body weight (Table 2).

### DISCUSSION

Many traditional plant treatments for diabetes exist, a hidden wealth of potentially useful natural products for diabetes control. The conventional therapies for diabetes may have many shortcomings, such as side effects and high rate of secondary failure. On the other hand, herbal extracts are expected to have similar efficacy, without side effects, to that of conventional drugs.

Species of Myrtaceae family are often used for several medicinal purposes, including the treatment of diarrhea (Caceres et al., 1993) and pain. Experimental data also suggest the action of these species on inflammatory processes, respiratory diseases (Muruganandan et al., 2001) and allergic disorders (Kim et al., 1998). The seeds of S. cumini have been reported to be useful as astringents in diarrhea as well as dysentery (Chopra et al., 1958). Other parts of the plant have been reported to possess anti-diabetic (Chakraborty et al., 1986), bactericidal (Prince et al., 2004) and anti-mutagenic (Matsuo et al., 1994) properties. The ethanolic bark extract of S. cumini has been reported to have anti-inflammatory activity in carrageenan and formaldehyde paw edema (Muruganandan et al., 2001).

Hypoglycemic activity of different parts of E. jambolana seeds such as whole seed, kernel and seed coat on streptozotocin induced diabetic rats, with the ethanolic extract kernel at a concentration of 100 mg/kg/b.w significantly decreased the levels of blood glucose, blood urea and cholesterol, increased glucose tolerance and levels of total proteins, liver glycogen and decreased the activities of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase in experimental diabetic rats (Shankar et al., 2007).

The aqueous seed extracts of S. alternifolium at a dosage of 0.75/kg b.w. showed a maximum blood glucose lowering effect in both normal and alloxon induced diabetic rats (Rao and Apparao, 2001). The aim of the present study was to evaluate the anti diabetic effect of ethanolic leaf extracts of S. alternifolium and S. cumini against STZ induced diabetic rats. The continuous treatment of the extracts for a period of 21 days produced a significant decrease in blood glucose levels in diabetic rats. Comparatively the ethanolic extract of S. alternifolium showed a significant fall of glucose levels than the extract of S. cumini.

The characteristic loss of body weight, as revealed in present study in STZ induced diabetic animals, is due to the increased muscle wasting and loss of tissue proteins in diabetes. It appears that the administration of S. alternifolium and S. cumini extracts improve the body weight in diabetic rats by protective effect in controlling muscle wasting (i.e. reversal of gluconeogenesis and glycogenolysis). It may be due to the improved insulin secretion and glycemic control (Shirwaikar et al., 2004).

Streptozotocin is believed to destroy the beta cells of the islets and this leads to deficiency in circulating insulin levels leading to many pathological alterations. Generally, in the diabetic pancreas the islets number is reduced and there are individual variations in the number of islets. The treatment with S. alternifolium and S. cumini resulted in normalization of islets with increased secretory granular were observed.

### REFERENCES


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