Effect of *Sesbania grandiflora* Methanolic leaf extract on *In vitro* studies of α-amylase, glucose uptake in muscle and adipose tissue of male Sprague Dawley rat model.

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**ABSTRACT** The aim of the present study was to investigate the anti diabetic activity by determining the alpha α-amylase inhibitory activity and in vitro glucose uptake in isolated psoas muscle and adipose tissue of male Sprague Dawley (SD) rats. Sesbania grandiflora is used in traditional knowledge of ayurveda for various diseases and infections. 150 g male SD rats (n=3) were sacrificed for isolation of psoas muscle and adipose tissue. 250 mg of tissue was used for this study. Sesbania grandiflora methanolic leaf extract (SGL) (5-25 mg/mL) showed 11.68–63.16% α-amylase inhibitory activity. In vitro glucose uptake studies were carried out in both psoas muscle and adipose tissue in four sets i.e tissue alone, tissue with (Plant extract : 50μg, insulin : 25 mU/L and Plant extract: 50μg + insulin : 25 mU/L). SGL stabilized the rate of glucose uptake via insulin action by tissues, which is an indication of synergetic activity of insulin and S. grandiflora extract.

**Keywords:** α-amylase, Glucose uptake, Insulin, Sesbania grandiflora

**Introduction:**

Previous scientific reports denoted that *Sesbania grandiflora* one of the medicinal plants is used in ethnomedicine (Padal et al. 2014). Syonym with *Sesban grandiflora - Agati grandiflora* and *Coronilla grandiflora* are widely used as “Traditional Medicinal Plant” in Asia to alleviation symptoms of various diseases (Kashyap and Mishra, 2012). It is commonly known as a humming bird, flamingo or the butterfly tree; it belongs to the family Fabaceae. *S. grandiflora* is traditionally used for anti-inflammation (Patil et al. 2012), antimicrobial activities (Hasan et al. 2012; Jothi Karumari et al. 2014), anticancer (Sreelatha et al. 2011), antidiabetic activities (Panigrahi Ghanshyam et al. 2012 ; Nandi et al. 2014), antioxidant activities (Radhika et al. 2014), anti-ulcer activity (Shyamala Gowri and Vasabtha, 2010 ; Bhalke et al. 2010), an immunomodulatory activity (Arunabha and Satish, 2015) and associated diseases such as hepatic diseases (Roy et al. 2014; Pari and Uma, 2003), respiratory diseases (Tathe et al. 2010), and renal diseases (Ramesh and Begum, 2007) *S. grandiflora* leaves and pods were reported palatable and non-toxic to cattle (Kumaravel et al. 2011). In the present study we used white flower variety of *S. grandiflora due to its non-toxic nature, the purple flower type is highly toxic (NAS, 1979). α-amylase is involved in the breakdown of long chain carbohydrates. Alpha α-amylase is the potential targets in the development of lead compounds for the treatment of diabetes (Prashant Agarwal and Ritika Gupta, 2016). *Cinnamon Extract Enhances Glucose Uptake in 3T3-L1 Adipocytes*, (Yan et al. 2014). Diabetes mellitus is associated with insulin deficiency and decreased glucose uptake in skeletal muscles (Annie Shirwaikar, 2006). By considering these scientific reports we have intended to study the anti diabetic activity by determining the alpha α-amylase inhibitory activity and *in vitro* glucose uptake in isolated psoas muscle and adipose tissue of male SD rats.

**Materials and Methods**

**Chemicals:**
All the chemicals used were of analytical grade. Glucose, NaCl, Sodium potassium tartrate, Starch, NaOH, 3, 5–Dinitrosalicylic acid, were obtained from Sisco Research Laboratories Pvt Ltd, Hyderabad, India.

**Plant extract:**
Methanolic extract of *Sesbania grandiflora* (brown, dry powder with Batch Number SSB/15001) aerial part of leaf was a gift from Green-Chem Herbal Extracts and Formulations, Bangalore, Karnataka, India. The procedure followed by the firm for the preparation of the extract is as follows: Sesbania grandiflora leaves are charged to extractor along with methanol. It is extracted by heating the mass for 5-6 hours, in a closed system by re pumping the extract to the herb bed. This process is repeated. The extracts
are combined and filtered. Then concentrated under vacuum. This is charged to spray drier unit to dry and separate the product in a powder form. This is further powdered in a multi mill to a fine mesh size. It is sieved using a sifter to make uniform particle size. The extract was dissolved in distilled water prior to use.

Animal and Ethical clearance:

Our University, obtained ethical clearance for conducting experiments on animals from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Regd. No: 1889/GO/Re/S/16/CPCSEA, dt.30th May 2016), and the work was approved by the IAEC protocol No: SKU/Biochem/02/2016. In the present study, we used three male Sprague Dawley rats (6 to 8 weeks of age with an average weight of 140 ± 5g) acclimatized in animal house before experimentation.

Preparation of enzyme

10 mL of the saliva was collected and diluted to 100 mL with cold phosphate buffer pH 7.0. The solution was centrifuged at 8000 rpm for 20 min and the clean supernatant was used α-amylase assay. Clear supernatant resulted after completion of 10% saliva in phosphate buffer (0.1 M, pH 7.0, containing 2 N NaCl) was used as α-amylase enzyme source.

α-amylase inhibitory assay:

α-amylase activity was determined by the method out lined by Jayaraman, (1981). Different concentrations of plant extracts (5–25 mg/ml) was pre incubated with α-amylase (1 U/ml) and 2 ml of phosphate buffer (pH 6.9) containing 2 N NaCl and thereafter 1 ml starch solution was added. The mixture was incubated for 20 min. Then the reaction was stopped by adding 0.5 ml of DNS reagent (12.0 g of sodium potassium tartrate in 8 ml of 0.25 M NaOH and 96 mM 3, 5-dinitrosalicylic acid) and the contents were heated in a boiling water bath for 5 min. A blank was prepared without plant extracts and another without the amylase enzyme, replaced by equal quantities of buffer (20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 20°C), and the absorbance was measured at 540 nm. A control was prepared using the same procedure except that the extract/standard drug was replaced with distilled water. A series of standard maltose (1.0 - 5.0 mg) was treated in a similar manner. The reducing sugar released from starch was estimated as maltose equivalent from a standard graph. All measurements were carried out in triplicate. The percentage of α-amylase activity inhibition was evaluated by the following formula:

\[ \% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \]

The concentrations of plant extract required to inhibit 50% of α-amylase activity under the conditions was defined as the IC \(_{50}\) value. The α-amylase inhibitory activity of plant extract was calculated and their IC \(_{50}\) value was determined.

In vitro glucose uptake activity in skeletal muscle and adipose tissue:

Glucose uptake study was carried out in skeletal muscle and adipose tissues by the method of Rajesh Kumar et al. (2005) In brief, four sets (triplets) were performed twice, including tissue alone (250 mg), tissue with insulin (50 μ/L), tissue with SGL (50μg), and tissue with both insulin and SGL(50μg). During the experimentation, aliquots of 10μl were removed from the incubation mixture at 0, 30, 60, 90, 120 and 150 min, and changes in glucose concentrations were measured.

Statistical analysis:

The experiments were repeated three times and the results were expressed as mean ± S.E.M. (P < 0.01).

Results:

α-amylase inhibitory effect of Sesbania grandiflora methanolic leaf extract:

The SGL showed α-amylase inhibitory activity in a concentration dependent manner with 11.68%, 23.52%, 37.82%, 49.08% and 63.16% inhibition at concentrations of 5, 10, 15, 20 and 25 mg/mL of the extract respectively, with an IC \(_{50}\) value of 20.4 ± 1.2 mg/mL. The results obtained in this study are represented in table 1.

<table>
<thead>
<tr>
<th>Conc. of SGL (mg/ml)</th>
<th>% Inhibition</th>
<th>IC (_{50}) Value (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>11.68 ±1.48</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>23.52 ±1.67</td>
<td></td>
</tr>
</tbody>
</table>
Data are presented as expressed as mean ± standard error of the mean (n = 3).

**Insulin sensitizing effect of *Sesbania grandiflora* extract:**
Effect of SGL on the uptake of glucose by psoas muscle and adipose tissue of rat with and without insulin was studied by measuring the decrease in glucose concentration in the incubation medium with time. When tested with 50, 100 and 150 µg concentration of the extract, maximum effect was observed with 50 µg of plant extract. Thus results are represented only with 50 µg of the extract. It is clear from the results (Table 2) that in the psoas muscle 50 µg of the plant extract by itself increased glucose uptake by 41.7% at 30 min, after which the effect decreased to 30.5% at 150 min. However, in combination with 25 U of insulin, enhances glucose uptake was 114.5% up to 30 min, but it decreased to 73.7% by 150 min. Similarly, in adipose tissue 50 mg of the plant extract by itself increased glucose uptake by about 146.5% in 30 min, after which the effect decreased to 62.7% by 150 min. However, in combination with 25 U of insulin, the extract increased the uptake of glucose by 162.5% in 30 min, which was further decreased to 52% by 150 min compared to corresponding values of controls (Table 3).

**Table 2: Effect of SGL on uptake of glucose by psoas muscle tissue isolated from rat. Fall in glucose concentration of the medium indicates glucose uptake by psoas muscle tissue.**

<table>
<thead>
<tr>
<th>Set</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>150 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle tissue (MT)</td>
<td>32.18 ± 0.38</td>
<td>33.92 ± 0.24</td>
<td>43.10 ± 0.18</td>
<td>48.26 ± 0.26</td>
<td>52.28 ± 0.12</td>
</tr>
<tr>
<td>MT + extract (50 µg)</td>
<td>45.58 ± 0.16</td>
<td>52.55 ± 0.21</td>
<td>57.46 ± 0.09</td>
<td>63.48 ± 0.27</td>
<td>68.21 ± 0.17</td>
</tr>
<tr>
<td>(41.7%)</td>
<td>(35.0%)</td>
<td>(33.3%)</td>
<td>(31.5%)</td>
<td>(30.4%)</td>
<td></td>
</tr>
<tr>
<td>MT + insulin (25mU/L)</td>
<td>38.85 ± 0.33</td>
<td>44.84 ± 0.23</td>
<td>48.92 ± 0.28</td>
<td>55.15 ± 0.06</td>
<td>38.75 ± 0.15</td>
</tr>
<tr>
<td>(14.5%)</td>
<td>(12.2%)</td>
<td>(13.3%)</td>
<td>(14.2%)</td>
<td>(12.3%)</td>
<td></td>
</tr>
<tr>
<td>MT + insulin (25mU/L) + extract (50 µg)</td>
<td>68.98 ± 0.18*</td>
<td>71.52 ± 0.04</td>
<td>80.24 ± 0.13</td>
<td>87.56 ± 0.08</td>
<td>90.82 ± 0.14</td>
</tr>
<tr>
<td>(114.3%)</td>
<td>(83.7%)</td>
<td>(86.1%)</td>
<td>(81.4%)</td>
<td>(73.7%)</td>
<td></td>
</tr>
</tbody>
</table>

* Values in brackets indicate percent increase of individual set when compared with psoas muscle tissue alone.
* P < 0.01 when compared to control (without extract).

**Table 3: Effect of SGL on uptake of glucose by adipose tissue isolated from rat. Fall in glucose concentration of the medium indicates glucose uptake by adipose tissue.**

<table>
<thead>
<tr>
<th>Set</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>150 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose tissue (MT)</td>
<td>10.80 ± 0.06</td>
<td>14.52 ± 0.08</td>
<td>18.72 ± 0.04</td>
<td>22.60 ± 0.10</td>
<td>26.78 ± 0.14</td>
</tr>
<tr>
<td>AT + extract (50 µg)</td>
<td>26.62 ± 0.05*</td>
<td>30.28 ± 0.07</td>
<td>34.52 ± 0.03</td>
<td>35.92 ± 0.08</td>
<td>43.56 ± 0.09</td>
</tr>
<tr>
<td>(116.4%)</td>
<td>(108.5%)</td>
<td>(84.4%)</td>
<td>(59.9%)</td>
<td>(62.6%)</td>
<td></td>
</tr>
<tr>
<td>AT + insulin (25mU/L)</td>
<td>12.82 ± 0.13</td>
<td>16.32 ± 0.08</td>
<td>20.48 ± 0.11</td>
<td>24.52 ± 0.06</td>
<td>28.62 ± 0.08</td>
</tr>
<tr>
<td>(18.7%)</td>
<td>(12.4%)</td>
<td>(9.4%)</td>
<td>(8.5%)</td>
<td>(6.8%)</td>
<td></td>
</tr>
<tr>
<td>AT + insulin (25mU/L) + extract (50 µg)</td>
<td>28.35 ± 0.08*</td>
<td>31.75 ± 0.06</td>
<td>35.38 ± 0.05</td>
<td>37.46 ± 0.04</td>
<td>40.70 ± 0.03</td>
</tr>
<tr>
<td>(162.5%)</td>
<td>(118.6%)</td>
<td>(89.0%)</td>
<td>(63.7%)</td>
<td>(51.9%)</td>
<td></td>
</tr>
</tbody>
</table>

* Values in brackets indicate percent increase of individual set when compared with adipose tissue alone.
* P < 0.01 when compared to control (without extract).
Discussion:

For treating diabetes one of the therapeutic approaches is, controlling the postprandial glucose by inhibiting the alpha glucosidase which manages Diabetes mellitus (DM) and Insulin Resistance (IR). Both postprandial hyperglycemia and hyperinsulinemia are reported to be improved by treatment with a disaccharide inhibition in Type 2 diabetes patients; the possibility that the drug with this activity might improve Insulin resistance was suggested (Shinozaki et al., 1996). Acarbose, miglitol and voglibose are examples of such type of inhibitors which find application in the clinical practice for management of diabetes (bailey, 2003). Various side effects like abdominal pain, flatulence and diarrhea in patients are associated with these drugs (Sing et al., 2007). Therefore, it is the need of time to investigate and explore the α-amylase inhibitors from natural sources having limited side effects. Our present study indicates Sesbania grandiflora methanolic leaf extract exhibit α-amylase inhibitory activity (table 1) and this study was supported by earlier studies in our laboratory (Srinivasulu et al. 2016). In addition, SGF60 and SGF90 proteins isolated from the flowers of S. grandiflora have been shown to possess significant inhibitory effect on digestive enzymes, α-amylase and α-glucosidase which are responsible for the metabolism of carbohydrates (Sangeetha et al., 2014). Thus the effective α-amylase inhibitory activity of SGL may contribute for effective management of DM and IR.

Skeletal muscle is the pivotal site for the postprandial glucose utilization. In addition, it is the most abundant tissue in the whole body, and thus, proper function of skeletal tissue is restoration of blood glucose to the normal level (Končič MZ et al. 2010). Defects in insulin stimulated skeletal muscle glucose uptake are general pathological condition in non-insulin dependent diabetes mellitus (Defronzo RA et al. 1981). Decreased basal glucose uptake was observed in adipose tissue of obesity/overweight individuals (Stolic et al. 2002). Major glucose transporter GLUT4 is expressed in insulin responsive tissues such as skeletal muscle and adipose tissue, where they respond to an acute insulin challenge by translocating GLUT4 rapidly from an intracellular membrane storage site to the plasma membrane (Baron AD et al.1991). Cinnamon extract enhanced the translocation of GLUT4 to the plasma membrane and increased glucose uptake in 3T3-L1 adipocytes and C2C12 myocytes (Yan et al. 2014). Our results (Table 2 & 3) demonstrated increased glucose utilization in muscle and adipose tissues are correlated to above reports. Hence from above observations SGL may not only be used for treating the diabetes and IR but also for obesity/overweight management.

Conclusion:

Our present study showed that methanolic leaf extract of S.grandiflora has the ability to enhance the glucose uptake in skeletal muscle as well as adipose tissue may contribute to management of Diabetes Mellitus and IR as well as Overweight/Obesity.

Conflict of interest & Acknowledgement:

The authors declare no conflict of interest. The authors are thankful to CEO Rajendra Ramaswamy, M/s. Green Chem Herbal Extracts and Formulations, Bangalore, for supplying the plant extract.

References :