Effect of Solanum surattense on mitochondrial enzymes in diabetic rats and in vitro glucose uptake activity in L6 myotubes

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ABSTRACT

Background: S. surattense is widely used in Siddha medicine for various ailments. Objective: The aim was to evaluate the impact of alcoholic leaf-extract of S. surattense on mitochondrial enzymes in streptozotocin (STZ) induced diabetic rats and to study the in vitro muscle glucose uptake activity on L6 myotubes. Materials and Methods: The male albino Wistar rats were randomly divided into five groups of six animals each. Diabetes was induced by intraperitoneal injection of STZ (40 mg/kg body weight). After being confirmed the diabetic rats were treated with alcoholic leaf-extract of S. surattense (100 mg/kg body weight) for 45 days. The biochemical estimations (liver mitochondrial enzymes, antioxidants, thiobarbituric acid reactive substances [TBARS]) and histopathological studies were performed. Further, the in vitro muscle glucose uptake activity in L6 myotubes and messenger RNA (mRNA) expression of glucose transporter-4 (GLUT-4) was performed. Results: In diabetic rats, the activities of liver mitochondrial enzymes were found to be significantly lowered. The mitochondrial TBARS level increased, whereas the activities/level of enzymatic and non-enzymatic antioxidants decreased in diabetic rats. Administration of S. surattense to diabetic rats significantly reversed the above parameters toward normalcy. Furthermore in diabetic rats, the histopathological studies showed growth of adipose tissue and shrinkage of islets in the pancreas, liver showed fatty change with mild inflammation of portal triad, and kidney showed mesangial capillary proliferation of glomeruli and fatty infiltration of tubules. Treatment with S. surattense brought back these changes to near normalcy. The extract was analyzed for in vitro muscle glucose uptake activity in L6 myotubes and mRNA expression of GLUT-4 by semi-quantitative reverse transcription-polymerase chain reaction. One nano gram per millilitre of S. surattense leaf-extract gave 115% glucose uptake on L6 myotubes. It also showed elevated levels of GLUT-4 mRNA transcripts, when compared with control cells. Conclusion: These studies strongly support the anti-diabetic nature of S. surattense.

Key words: Glucose uptake, glucose transporter-4, mitochondrial enzymes, Solanum surattense, streptozotocin, thiobarbituric acid reactive substances

INTRODUCTION

Reactive oxygen species (ROS) generated by mitochondria are responsible for the activation of major, independent, but interrelated, pathogenic mechanisms for diabetic complications as modeled in endothelial cells exposed to hyperglycemia in vitro. Further, evidence associates...
diabetic pathology to mitochondrial dysfunction and oxidative stress. In recent years, the basic concept has emerged that mitochondrial dysfunction, often including redox disturbances, plays a fundamental and underlying role in many metabolic and degenerative diseases, including diabetes, cancer, and aging. Indeed, alterations of mitochondrial function have been strongly implicated as a critical early step in the development of renal tubular cell damage that results from diabetes.

Glucose transport is the rate-limiting step in glucose utilization, especially in insulin targeted skeletal muscle, mediated by major glucose transporter (GLUT) proteins, GLUT-4 and GLUT-1. Moreover, skeletal muscle is the primary tissue responsible for the postprandial uptake of glucose from the blood. GLUT-4 expressed in adipocytes and myocytes plays an important role in the regulation of whole body glucose homeostasis. A reduction in GLUT-4 gene expression and GLUT-4 protein level in pathophysiological states represents a decreased glucose clearance.

Current therapeutics for diabetes is often associated with undesirable side effects and in many cases the precise mechanism of action remains to be completely clarified. Therapeutic approaches with natural products provide a fruitful source for searching safe, effective and relatively inexpensive new remedies for diabetes mellitus and associated metabolic disorders. Herbal drugs have potential therapeutic applications because of their effectiveness, less side effects and relatively low cost. Solanum surattense (family - Solanaceae, synonym - Solanum xanthocarpum) (Indian nightshade) is commonly used in Indian traditional system for curing various ailments. We have reported the effect of S. surattense leaf-extract on glycemic control, antihyperlipidemic, erythrocyte and tissue antioxidant activity in streptozotocin-diabetic rats. In the present study, we analyzed the effect of S. surattense on mitochondrial enzymes in STZ-diabetic rats, and in vitro muscle glucose uptake and GLUT-4 expression in L6 myotubes.

**MATERIALS AND METHODS**

**Method for mitochondrial enzymes assay**

**Plant materials**

Leaves of S. surattense were collected from local areas of Chidambaram, Tamil Nadu, and was botanically identified and authenticated in the Department of Botany, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India. A voucher specimen (AU 189) was deposited at the herbarium of botany.

**Preparation of leaf extract**

The plant leaf was shade dried at room temperature (32°C ± 2°C) and the dried leaf was ground into a fine powder using pulverizer. The powdered part was sieved and kept in the deep freezer until the time of use. A total of 100 g of dry fine powder was suspended in 400 mL of ethanol for 72 h. The extract was filtered using a muslin cloth and concentrated at 40°C ± 5°C to get solid substrate.

**Drugs and chemicals**

Streptozotocin was obtained from Sigma-Aldrich Company (St. Louis, Missouri, USA). All other chemicals used were of analytical grade obtained from E. Merck and HIMEDIA, Mumbai, India.

**Animals**

Male albino Wistar rats (weighing 180–200 g) was procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and maintained in an air-conditioned room (25°C ± 1°C) with a 12 h light/12 h dark cycle. Feed and water were provided *ad libitum*. All the experiments were conducted in Department of Biochemistry, Faculty of Science, Annamalai University, in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and the experimental study was approved by the Ethical Committee of Rajah Muthiah Medical College and Hospital (Reg No. 160/1999/CPCSEA, Pro. No. 360), Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.

**Experimental induction of diabetes**

The animals were made diabetic by intraperitoneal injection of STZ (40 mg/kg b.wt.) in freshly prepared citrate buffer (0.1 M, pH 4.5) after an overnight fast. STZ injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality. The animals exhibited massive glycosuria (determined by Benedict’s qualitative test) and hyperglycemia within a few days. Diabetes was confirmed by measuring the fasting blood glucose concentration, 96 h after induction. Albino rats with blood glucose level above 220 mg/dL were considered diabetic and were used in the experiment.

**Experimental protocol**

The animals were randomly divided into five groups of six animals each. S. surattense leaf extract was suspended in 2% gum acacia (vehicle solution) and fed by intragastric tube daily for 45 days.

- **Group I: Normal control**
- **Group II: Normal control + S. surattense (100 mg/kg b.wt.)**
- **Group III: Diabetic control**
- **Group IV: Diabetic rats + S. surattense (100 mg/kg b.wt.)**
- **Group V: Diabetic rats + glibenclamide (600 μg/kg b.wt.).**

After 45 days of treatment, the 12 h fasted animals were anesthetized between 8:00 am and 9:00 am, using
kemamine (24 mg/kg b.wt.) (intramuscular injection) and sacrificed by decapitation. Blood was collected in the tubes with ethylene diamine tetraacetic acid and erythrocytes were separated by washing with 0.15M sodium chloride solution. Liver tissue was collected for the measurement of mitochondrial enzymes.

Biochemical determinations
The liver mitochondria were isolated from cell debris, nuclei, microsomes, soluble components and contaminant red blood cell using differential centrifugation by the method of Johnson and Lordy.\textsuperscript{[10]} The activities of succinate dehydrogenase,\textsuperscript{[19]} isocitrate dehydrogenase,\textsuperscript{[12]} α-ketoglutarate dehydrogenase,\textsuperscript{[13]} malate dehydrogenase,\textsuperscript{[14]} nicotinamide adenine dinucleotide (NADH) - dehydrogenase,\textsuperscript{[15]} cytochrome-C-oxidase\textsuperscript{[16]} were assayed. The levels of mitochondrial thioarbutic acid reactive substances (TBARS),\textsuperscript{[17]} reduced glutathione (GSH),\textsuperscript{[18]} and the activities of superoxide dismutase (SOD)\textsuperscript{[19]} and glutathione peroxidase (GPx)\textsuperscript{[20]} were estimated. All the readings were recorded using spectrophotometer.

Statistical analysis
For \textit{in vivo} study statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT) using Statistical Package for the Social Science (SPSS, Chicago, IL) software version 11.5. The significance level was set at \( P < 0.05 \).

Method for \textit{in vitro} glucose uptake analysis in L6 myotubes
\textbf{Preparation of leaf-extract}
One milligram of concentrated alcoholic leaf-extract was reconstituted to 1 ml with 5% dimethyl sulfoxide and diluted to attain the final concentrations 1 pg, 10 pg, 100 pg 1 ng, 10 ng, 100 ng and 1 μg and 10 μg/ml, for the glucose uptake studies.

\textbf{Culture of L6 cells}
L6 cells (American Type Culture Collection, PO Box 1549, Manassas, VA 20108, USA) were maintained in Dulbecco’s modified Eagles medium (DMEM) with 10% fetal calf serum (FCS) supplemented with penicillin (120 units/ml), streptomycin (75 mg/ml), gentamycin (160 mg/ml) and amphotericin B (3 mg/ml) at 37°C humidified with 5% CO\textsubscript{2}. For the differentiation, L6 cells were transferred to DMEM with 2% FCS, 4-6 days postconfluence. The extent of differentiation was established by observing multinucleation of cells and approximately 90% fusion of myoblasts into myotubes were considered for our study. Differentiated myotubes were incubated with insulin and rosiglitazone for 30 min and 24 h, respectively, wherever indicated.\textsuperscript{[22]}

\textbf{Measurement of 2-deoxy-D-(1-\textsuperscript{3}H) glucose}
L6 cells grown in a 12-well plate (Corning, NY, USA) were subjected to glucose uptake as reported.\textsuperscript{[23]} Fully differentiated myotubes serum starved for 5 h were incubated with plant extracts. After incubation, cells were rinsed once with N-2-hydroxy ethyl piperazine-N0-2-ethanesulfonic sulfonic acid (HEPES) - buffered Krebs-Ringer phosphate solution (118 mM NaCl, 5 mM KCl, 1.3 mM CaCl\textsubscript{2}, 1.2 mM MgSO\textsubscript{4}, 1.2 mM KH\textsubscript{2}PO\textsubscript{4} and 30 mM HEPES- pH 7.4) and further incubated for 15 min in the HEPES-buffered solution containing 0.5 mCi/ml 2-deoxy-D-(1-\textsuperscript{3}H) glucose. The uptake was terminated by aspiration of media. Then the cells were washed thrice with an ice-cold HEPES-buffered solution and lysed in 0.1% SDS. An aliquot was used to measure the radioactivity. Glucose uptake values were corrected for nonspecific uptake in the presence of 10 mM cytochalasin B (5-10% of total uptake). All the assays were performed in triplicate.

\textbf{Reverse transcriptase-polymerase chain reaction}
Reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out as described previously Hall et al.\textsuperscript{[24]} After incubation, cells were lysed in TRIzol, proteins were extracted with chloroform, and total RNA was precipitated with isopropanol. The RNA precipitate was washed with 70% ethanol and resuspended in 50 ml of diethylpyrocarbonate-treated water. Reverse transcription was carried out using 200 units of avian RT and 200 ng/ml oligo d (T) 18. The primers used were as follows. GLUT-4: Sense, 50-CGG GAC GTG GAG CTG GCC GAG GAG-30; anti-sense, 50-CCC CCT CAG CTG GCC GAG GAG-30; anti-sense, 50-TCT AGA CGG CAG GTC AGG TCC ACC-30 (588-bp, Buhl et al., 1999).\textsuperscript{[25]} For PCR reaction, 1 ml of cDNA mixture was added to the PCR reaction mix containing 10X PCR buffer, 2 mM dNTP, 10 μM of paired primers, 2 units of Taq polymerase. PCR products were run on 1.5% agarose gels, stained with ethidium bromide and photographed.

\textbf{Statistical analysis}
For \textit{in vitro} glucose uptake ANOVA was conducted, and the effect of mean between the independent groups was found to be significant, \( P < 0.05 \). The Tukey’s honestly significant difference procedure revealed that all pair-wise differences among means were significant, \( P < 0.05 \). All data are expressed as mean ± standard error (SE).

\textbf{RESULTS}
\textbf{Effect of Solanum surattense on mitochondrial enzymes in streptozotocin-diabetic rats}
Tables 1 and 2 represent the activities of tricarboxylic
**Table 1: Effect of S. surattense on activities of mitochondrial tricarboxylic acid cycle enzymes in liver of normal and diabetic rats**

<table>
<thead>
<tr>
<th>Name of the group</th>
<th>Isocitrate dehydrogenase (U/mg protein)</th>
<th>α-ketoglutarate dehydrogenase (U/mg protein)</th>
<th>Succinate dehydrogenase (U/mg protein)</th>
<th>Malate dehydrogenase (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>753.87±68.75\textsuperscript{a}</td>
<td>172.04±15.50\textsuperscript{a}</td>
<td>33.87±2.09\textsuperscript{a}</td>
<td>362.76±33.08\textsuperscript{a}</td>
</tr>
<tr>
<td>Normal+ S. surattense (100 mg/kg b.wt.)</td>
<td>757.27±69.26\textsuperscript{a}</td>
<td>173.44±15.81\textsuperscript{a}</td>
<td>34.14±2.0\textsuperscript{a}</td>
<td>368.43±33.60\textsuperscript{a}</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>589.49±53.76\textsuperscript{b}</td>
<td>113.86±10.33\textsuperscript{c}</td>
<td>17.11±1.56\textsuperscript{d}</td>
<td>232.39±21.19\textsuperscript{b}</td>
</tr>
<tr>
<td>Diabetic+S. surattense (100 mg/kg b.wt.)</td>
<td>668.85±54.01\textsuperscript{c}</td>
<td>141.70±12.92\textsuperscript{c}</td>
<td>24.94±2.37\textsuperscript{c}</td>
<td>306.08±27.92\textsuperscript{c}</td>
</tr>
<tr>
<td>Diabetic+glibenclamide (600 µg/kg b.wt.)</td>
<td>736.87±67.20\textsuperscript{d}</td>
<td>158.71±14.47\textsuperscript{d}</td>
<td>29.47±1.68\textsuperscript{d}</td>
<td>328.75±29.98\textsuperscript{d}</td>
</tr>
</tbody>
</table>

SD=Standard deviation, DMRT=Duncan’s multiple range test, S. surattense=Solanum surattense, STZ=Streptozotocin, NADH=Nicotinamide adenine dinucleotide. *U=nmol of α-ketoglutarate formed/h, \( \Delta U=\)nmol of ferrocyanide formed/h, \( \Delta U=\)nmol of succinate oxidized/min, \( \Delta U=\)nmol of NADH oxidized/min. Values are expressed as means ± S.D. for six rats in each group. \( \Delta \Delta U=\)Values not sharing a common superscript (a,b,c,d) differ significantly at \( P < 0.05 \) (DMRT).

**Table 2: Effect of S. surattense on activities of mitochondrial respiratory chain enzymes in liver of normal and diabetic rats**

<table>
<thead>
<tr>
<th>Name of the group</th>
<th>NADH dehydrogenase (U/mg protein)</th>
<th>Cytochrome c-oxidase (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>31.74±2.89\textsuperscript{a}</td>
<td>6.80±0.62\textsuperscript{a}</td>
</tr>
<tr>
<td>Normal+ S. surattense (100 mg/kg b.wt.)</td>
<td>32.87±2.99\textsuperscript{a}</td>
<td>6.91±0.63\textsuperscript{a}</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>19.27±1.75\textsuperscript{b}</td>
<td>3.74±0.34\textsuperscript{c}</td>
</tr>
<tr>
<td>Diabetic+S. surattense (100 mg/kg b.wt.)</td>
<td>26.07±2.37\textsuperscript{b}</td>
<td>5.10±0.45\textsuperscript{d}</td>
</tr>
<tr>
<td>Diabetic+glibenclamide (600 µg/kg b.wt.)</td>
<td>30.60±2.79\textsuperscript{d}</td>
<td>6.00±0.54\textsuperscript{d}</td>
</tr>
</tbody>
</table>

SD=Standard deviation, DMRT=Duncan’s multiple range test, STZ=Streptozotocin, S. surattense=Solanum surattense, NADH=Nicotinamide adenine dinucleotide. *U=nmol of NADH oxidized/min. Values are expressed as means ± S.D. for six rats in each group. \( \Delta \Delta U=\)Values not sharing a common superscript (a,b,c,d) differ significantly at \( P < 0.05 \) (DMRT).

acid (TCA) cycle enzymes and respiratory chain enzymes in the liver mitochondria of normal and diabetic rats. The activities of these enzymes were found to be significantly lowered in diabetic rats and treatment with S. surattense and glibenclamide brought back these parameters towards normalcy.

Table 3 depicts the levels of TBARS, enzymes of enzymic antioxidants (SOD and GPx) and the levels of nonenzymic antioxidant (GSH) in the liver mitochondria of normal and diabetic rats. The level of TBARS increased, whereas the activities/level of mitochondrial antioxidants decreased in diabetic rats. S. surattense and glibenclamide administration decreased the levels of lipid peroxidation and increased the mitochondrial antioxidant status toward normalcy.

In our study, histopathological examination of diabetic pancreas [Figure 1a-e] showed growth of adipose tissue and shrinkage of islets in the pancreas. Administration of S. surattense showed expansion in pancreatic islet cells and glibenclamide treatment showed normal islet cells supporting the biochemical analysis.

Histopathological examination of diabetic liver [Figure 2a-e] showed fatty change with mild inflammation of portal triad. Administration of S. surattense rat liver showed normal hepatocytes with mild fatty changes; whereas glibenclamide treated rat showed normal hepatocytes.

Histopathology of diabetic kidney [Figure 3a-e] showed diabetic rat kidney showed messangial capillary proliferation of glomeruli and fatty infiltration of tubules. Administration of S. surattense showed mild fatty infiltration and mild dilation of tubules. Glibenclamide treated kidney showing normal tubules and congested glomeruli.

The effect of Solanum surattense on in vitro glucose uptake analysis in L6 myotubes

Figure 4 shows the glucose uptake assay on L6 myotubes at different concentrations of leaf-extract. One nano gram per millilitre of leaf-extract gave 115% uptake, while 2 µL of rosiglitazone at 50 µM gave 145.7% uptake.

Figure 5(a) shows the analysis of GLUT-4 transcripts in L6 myotubes at 18 h. L6 myotubes incubated with insulin (100 nM) or rosiglitazone (50 µM) or S. surattense (1 ng/ml) showed elevated levels of GLUT-4 transcripts (318 bp), when compared with control cells. GAPDH was used as internal control (597 bp). Figure 5b shows the semi-quantification analysis of GLUT-4 transcripts using scanning densitometry. Signals of GLUT-4 in agarose gel were quantified arbitrarily. Bars represent means ± SE of three independent experiments and a representative agarose gel is shown here. *\( P < 0.05 \) when compared with untreated control group.

**DISCUSSION**

Mitochondria undergo rapid fragmentation with a concomitant increase in ROS formation after exposure to high-glucose concentrations.[27] Disturbance of mitochondrial bioenergetics due to mutations in mitochondrial DNA and altered electron flow through respiratory complexes has been reported due to increased reactive active species formation in mitochondria.[18,29] The toxic reactive intermediates generation from these macromolecules has been concerned in...
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**Table 3: Effect of *S. surattense* on TBARS and the activities of enzymic and level of GSH antioxidants in mitochondrial fraction of liver of normal and diabetic rats**

<table>
<thead>
<tr>
<th>Name of the group</th>
<th>TBARS (nmol/mg protein)</th>
<th>SOD (Ua/mg protein)</th>
<th>GPx (Ub/mg protein)</th>
<th>GSH (µg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.47±0.13</td>
<td>2.59±0.23</td>
<td>7.36±0.67</td>
<td>12.01±1.09</td>
</tr>
<tr>
<td>Normal+S. <em>surattense</em> (100 mg/kg b.wt.)</td>
<td>1.45±0.12</td>
<td>2.56±0.23</td>
<td>7.42±0.65</td>
<td>11.56±1.00</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>3.74±0.33</td>
<td>1.24±0.11</td>
<td>4.30±0.39</td>
<td>7.02±0.64</td>
</tr>
<tr>
<td>Diabetic+S. <em>surattense</em> (100 mg/kg b.wt.)</td>
<td>2.72±0.24</td>
<td>1.92±0.17</td>
<td>5.44±0.47</td>
<td>8.50±0.77</td>
</tr>
<tr>
<td>Diabetic+glibenclamide (600 µg/kg b.wt.)</td>
<td>2.04±0.18</td>
<td>2.15±0.10</td>
<td>6.12±0.55</td>
<td>10.08±0.92</td>
</tr>
</tbody>
</table>

SD=Standard deviation, NBT=Nitroblue tetrazolium, STZ=Streptozotocin, *S. surattense=Solanum surattense*, TBARS=Thiobarbituric acid reactive substances, GSH=Glutathione, SOD=Superoxide dismutase, GPx=Glutathione peroxidase, DMRT=Duncan’s multiple range test. a=Enzyme concentration required for 50% inhibition of NBT reduction/min, b=µmol of reduced glutathione consumed/min. Values are expressed as means ± S.D. for six rats in each group. a-d Values not sharing a common superscript (a,b,c,d) differ significantly at *P* < 0.05 (DMRT)

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**Figure 1:** Histopathological changes. (a) Pancreas of normal rat showing duct(D), acini and islet cells, (b) Pancreas of normal rat treated with *S. surattense* showing normal islet cells and acini, (c) Pancreas of diabetic rat showing growth of adipose tissue and shrinkage of islets, Pancreas (H and E, ×100), (d) Pancreas of diabetic rat treated with *S. surattense* showing expanded pancreatic islets, (e) Pancreas of diabetic rat treated with glibenclamide showing normal islets with reduction in adipose tissue

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A number of relatively common disorders, including diabetes. Previous reports exemplified that alloxan and STZ-induced diabetic rodent's exhibit increased mitochondrial oxidative stress and dysfunction. Diabetes mellitus has been found to profoundly alter mitochondria, including the selective depletion of mitochondrial SOD, GPx and GSH. Treatment with *S. surattense* and glibenclamide reversed these changes towards normalcy. It has been reported that normalizing superoxide mitochondrial production blocks the pathways of hyperglycemic damage. Further phytochemical analysis of the leaf-extract showed the presence of alkaloids, flavonoids, tannins, glycosides, triterpenoids and sterols, which may exert antilipidperoxidative effect thereby improving the antioxidant defense.

In diabetic rats, decreased activities of mitochondrial TCA cycle enzymes such as isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, and respiratory chain enzymes such as cytochrome-C oxidase and NADH dehydrogenase were seen in STZ-induced diabetic rats. Severe impairment of mitochondrial functions results in a dramatic fall of oxidative phosphorylation and ATP content. Abnormalities of mitochondrial enzymes may impair the glucose metabolism. Previous reports also suggest that other herbs such as *Rhinacanthus nasutus* and *Pimpinella tirupatiensis* can reduce the activities of mitochondrial enzymes. The administration of *S. surattense* and glibenclamide improved the activities of these enzymes, probably by insulin secretion and by improving the mitochondrial antioxidant defense system.

*Solanum surattense* administration showed recovery from histopathological changes of pancreas, liver and kidney, which may be due to improved glycemic profile, decreased lipid peroxidation and improved antioxidants’ status. The increase in insulin level after treatment with the leaf extract
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may be due to the recovery/regeneration of damaged islets. Similar results have been shown by other medicinal plants like *Annona muricata* and *Merremia emarginata*.\(^{[36-38]}\)

Skeletal muscle, a key insulin sensitive tissue, is the principal site for postprandial glucose utilization and disposal. In skeletal muscle insulin stimulates glucose uptake
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primarily by increasing translocation and redistribution of the GLUT-4 from internal membrane to the plasma membrane. GLUT-4 is directly involved in whole body glucose homeostasis and peripheral tissue glucose uptake in response to insulin receptor stimulation. Insulin can induce a shift in GLUT-4 traffic leading to its accumulation on the cell surface and increased rate of glucose uptake.

Altered glucose transport associated with defective GLUT-4 translocation and impaired insulin signaling cascade was evidenced as one among the major defects in diabetes. L6 muscle cell line is a suitable *in vitro* model used to study the glucose transport activity since skeletal muscle is the major site for primary glucose disposal and glucose utilization. Our study evaluated the increased glucose uptake by *S. surattense* leaf-extract which gave 115% uptake at 1 ng/ml when compared with rosiglitazone, which was 145.7%. Similar, *in vitro* L6 muscle glucose uptake results by medicinal plants have also been reported with *Aegle marmelos, Syzygium cumini* extracts and *Helicteres isora.* Yonemitsu et al. also reported that the elevated glucose uptake in L6 cells was due to increased GLUT-4 level. Insulin sensitizing by *S. surattense* may be responsible for the glucose uptake. Further, it has been reported that GLUT-4 molecules are recruited at random for insertion within the plasma membrane upon insulin stimulation. Moreover, flavonoids present in the *S. surattense* may also be responsible for the glucose uptake. Since, GLUT transporters are involved in flavonoid incorporation into cells, indicating a direct interaction of flavonoids with GLUT-4, rather than by a mechanism related to protein-tyrosine kinase and insulin signaling inhibition. Similarly, our current findings evaluated that the concomitant elevation of GLUT-4 protein expression parallel with glucose uptake, reinforced the enhanced glucose transport by *S. surattense*.

Further, Mathews et al. evaluated the glucose uptake potential of ethanolic extract of different parts of *S. xanthocarpum* using L-6 cell lines. They evaluated cytotoxicity of extracts by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide assay. The results showed the extracts did not show any cytotoxicity and possessed effective glucose uptake potential, which supports the present study.

Glyceraldehyde-3-phosphate dehydrogenase is a key enzyme in glycolysis, catalyzing the conversion of D-glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate. GAPDH is constitutively expressed in almost all tissues at high

![Figure 4: Effect of *Solanum surattense* dose-dependent glucose uptake in L6 myotubes](image)

![Figure 5: Effect of *Solanum surattense* leaf extract on glucose transporter-4 (GLUT-4) messenger RNA (mRNA) expression at 18 H (a) agarose gel electrophorotogram of mRNA (b) effect of *S. surattense* leaf extract on GLUT-4 mRNA band intensities scanned by densitometer](image)
levels and used as the house keeping gene. There are however some physiological factors such as hypoxia and diabetes that increase GAPDH expression in certain cell types.

**CONCLUSION**

In the present study, we have demonstrated the *in vivo* and *in vitro* antidiabetic property of *S. surattense*. The leaf extract was also able to reduce the altered activities of the TCA cycle enzymes and respiratory chain enzymes, thereby reducing glucose level. The *in vitro* screening assays clearly validate the glucose transport by *S. surattense* via up-regulating the GLUT-4 messenger RNA expression. Purification of the above plant extracts towards the isolation of novel lead molecule is worth pursuing.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

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