Evaluation of antidiabetic activity of bioactive constituent of *Sauropus androgynus* in alloxan induced diabetic Rats and effect on inhibition of α-glucosidase enzyme

Sujila V, Dr. Biju CR and Dr. G Babu

Abstract

The antidiabetic effect of bioactive constituent isolated from alcoholic extract of the leaves of *S. androgynus* investigated in alloxan induced diabetic rats. The antidiabetic potential tested by docking studies using isolated compound as ligand and intestinal α-glucosidase as receptor. The isolate and the standard hypoglycaemic drug glibenclamide (10mg/kg) were dissolved in isotonic saline solution (NaCl, 0.9%w/v) and were administered orally. Diabetes was induced in by a single intraperitoneal injection of 150mg/kg alloxan monohydrate. Hypoglycaemia was confirmed by the elevated glucose levels determined at 72 hr after alloxan injection. The rats having blood glucose levels above 200mg/dL were used for the antidiabetic study. The isolate and standard drug were administered orally for 14 days. The fasting blood samples were collected by retro orbital sinus puncture under mild ketamine xylazine anaesthesia. Fasting blood glucose estimation was measured on day 0, 7 and 14 of the study using glucometer. The result reveal that the isolate at both dose levels (200 and 400mg/kg) cause a significant (p < 0.001) decrease in the blood glucose levels after oral administration at 7 and 14 days when compared to the diabetic control group.

The isolated compound tested for their antidiabetic potential by docking studies using intestinal α-glucosidase as receptor. Chemically the isolated compound is a bound flavonoid which binds with the α-glucosidase with docking score of -9. The drug receptor interaction studies also shows excellent hydrogen bond and hydrophobic interactions. The result indicates that the isolated compound is potent α-glucosidase inhibitor with good antidiabetic potential.

Keywords: Antidiabetic activity, *Sauropus androgynus*, Alloxan induced Diabetic Rats, α-glucosidase enzyme, Docking

1. Introduction

Diabetes means a siphon or running through, mellitus means sugar. Therefore, diabetes mellitus is a clinical state which is associated with flow of sugar. In 2014, the International Diabetes Federation estimated that diabetes resulted in 4.9 million deaths. The WHO estimated that diabetes resulted in 1.5 million deaths in 2012, making it the 8th leading cause of death. The greatest increase in rates was expected to occur in Asia and Africa, where most people with diabetes will probably live in 2030. Chemical agents are available in the market to control and to treat diabetic patients but are unsafe. Present trend manages this deadly disorder through nutraceutical approaches as it is safe without any serious side effects (Sincy et al., 2016) [20].

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Diabetes cause wide spread pathological changes such as thickening of capillary basement membrane, increase in vessel wall matrix and cellular proliferation, resulting in vascular complications like lumen narrowing, early atherosclerosis, sclerosis of glomerular capillaries, retinopathy, neuropathy and peripheral vascular insufficiency.

Currently available treatment of Diabetes includes lifestyle modifications, medications and surgery. Presently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycaemic agents for the treatment of diabetes. In the light of the literature on *S. androgynus*, we made an attempt for the first time to study the effect of bioactive constituent isolated from *S. androgynus* alcoholic leaf extract in antidiabetic activity by alloxan induced diabetes rat model. The isolated compound was tested for antidiabetic potential by docking studies using intestinal α-glucosidase enzyme as receptor.
S. androgyrus endemic to India, many people use this plant as traditional medicine to relieve fever and treat urinary problems. The anti-inflammatory and antioxidant activity of S. androgyrus plant was report by Ida Christi, 2014 [19]. The S. androgyrus is a tropical shrub, used as a leafy vegetable. The people in southern regions of India believed that this plant has good potential to resist diabetes. Hence, an attempt has been made to explore the antidiabetic potential of leaves of S. androgyrus.

Materials and methods
Plant materials and extraction
S. androgyrus (Euphorbiaceae) leaves were collected. The leaves (plant material) were dried, coarsely powdered and extracted (2kg) separately with ethanol (70%) in a soxhlet extractor for 2hr. The extracts were concentrated to dryness. The nature and yields of the extract were noted and stored in a refrigerator at 4 °C for further studies.

The column chromatographic technique is widely used for separation, isolation and purification of natural compounds. The fractions were chromatographed over silica gel (100-200) mesh of column length 1 meter and diameter 1.5 inch. Elution was carried out with solvent mixtures of increasing polarities. The fractions were collected and monitored on TLC (silica gel G, absorbent with suitable solvent system) and similar fractions were pooled together based upon their TLC profile. The flavonoidal compound isolated from ethyl acetate: ethanol fraction give a single spot with Rf value and it was subjected to IR, 13C NMR, 1H NMR and Mass spectral analysis to confirm the structure of compound.

In-vivo studies
Experimental animals
Healthy Albino rats of wistar strain of either sex, weighing 180-220g, were purchased from the Animal house, Kerala Agriculture University College of Veterinary and Animal science, Mannuthy, Thrisur, Kerala and stock maintained in the Animal house of Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram, Kerala. The animal house was well ventilated and animals had 12±1h day and night schedule. The animals were housed in large spacious hygienic cages during the course of the experimental period and room temperature was maintained at 25±1 °C. The animals were fed with standard rat feed and water ad libitum. The experiments were conducted as per the guidelines of CPCSEA.

Alloxan induced diabetes (Shilali et al., 2015) [13].
Preparation of test samples
The isolated compound and the standard hypoglycaemic drug glibenclamide (10mg/kg) were dissolved in isotonic saline solution (NaCl, 0.9%w/v) and were administered orally.

Induction of diabetes in rats by Alloxan monohydrate
Diabetes was induced in overnight fasted adult Wistar albino rats weighing 180-220g by a single intraperitoneal injection of 150mg/kg alloxan monohydrate (CHEMCO) freshly dissolved in normal saline (0.9%w/v). Hyperglycemia was confirmed by the elevated glucose levels determined at 72hr after alloxan injection. The rats having blood glucose levels above 200mg/dL were used for the antidiabetic study. After induction of diabetes the experimental animals were divided in to 5 groups, each consisting of six rats.

Group I: Normal control (saline)
Group II: Diabetic control (Alloxan, 150mg/kg, ip)
Group III: Alloxan (150mg/kg, ip) + Glibenclamide, 10mg/kg
Group IV: Alloxan (150mg/kg, ip) + Isolated compound (200mg/kg, p.o)
Group V: Alloxan (150mg/kg, ip) + Isolated compound (400mg/kg, p.o)

The isolated compound and standard drug were administered orally for 14 days. The fasting blood samples were collected by retro orbital sinus puncture under mild Ketamine xylazine anaesthesia. Fasting blood glucose estimation was measured on day 0, 7, and 14 of the study using glucometer (Mark Inc).

Statistical analysis
The results are presented as mean ± SEM with n = 6. Statistical analysis was performed using one-way ANOVA followed by Dunnett’s method. The p value was reported to denote the degree of significance. Graph pad prism software was used for statistical analysis.

Drug-receptor interaction studies
The study was carried out with intestinal α-glucosidase as receptor and isolated compound as drug. Miglitol was selected as the standard drug for comparison of results.

Preparation of protein molecule
Crystallographic structure of intestinal α-glucosidase was obtained from PDB. The inhibitors and water molecules attached to protein were removed using Argus Lab software.

Preparation of ligand
The ligand compounds were drawn using ACD/Chemsketch as MDL MOL file and imported to Argus Lab after adding hydrogen bonds.

Docking
The docking of ligand and target were done in Argus Lab software. The docking score and best pose of isolated compound with targets were noted. The hydrogen bond interaction and hydrophobic interaction noted by Molegro molecular viewer.

Results
The leaves (plant material) were dried, coarsely powdered and extracted (2kg) separately with ethanol (70%) in a soxhlet extractor for 2hr. The extracts were concentrated to dryness and subjected to phytochemical identification tests to confirm the various phytoconstituents.

Table 1: Preliminary phytochemical studies on Sauropus androgyrus

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Phytoconstituents</th>
<th>Hydro alcohol extract</th>
<th>Isolated compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carbohydrate</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>2.</td>
<td>Amino acids &amp; proteins</td>
<td>P</td>
<td>A</td>
</tr>
<tr>
<td>3.</td>
<td>Oils &amp; fat</td>
<td>P</td>
<td>A</td>
</tr>
<tr>
<td>4.</td>
<td>Flavonoids</td>
<td>P</td>
<td>A</td>
</tr>
<tr>
<td>5.</td>
<td>Tannins</td>
<td>P</td>
<td>A</td>
</tr>
<tr>
<td>6.</td>
<td>Saponins</td>
<td>P</td>
<td>A</td>
</tr>
</tbody>
</table>

P: Present, A: Absent

The structural elucidation of isolated compound carried out by IR, 13C NMR, 1H NMR and Mass spectral analysis. The structure of isolated compound found to be,
Effect of isolated compound on blood glucose level

The results of the blood glucose level are given in Table 2 and Figure 2. The data reveals that the isolated compound at both the dose levels (200 and 400mg/kg) show a significant ($p < 0.001$) decrease in blood glucose level after oral administration at 7 and 14 days when compared to the diabetic control group.

<table>
<thead>
<tr>
<th>Sl. no</th>
<th>Groups</th>
<th>Glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st day</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>58.83 ± 2.372</td>
</tr>
<tr>
<td>2</td>
<td>Alloxan induced</td>
<td>327.8 ± 19.29 ***</td>
</tr>
<tr>
<td>3</td>
<td>Alloxan induced+ Glibenclamide treated</td>
<td>302.3 ± 7.688 ns</td>
</tr>
<tr>
<td>4</td>
<td>Alloxan induced+ 200 mg/kg isolate treated</td>
<td>298 ± 16.07 ns</td>
</tr>
<tr>
<td>5</td>
<td>Alloxan induced+ 400 mg /kg isolate treated</td>
<td>2 85 ± 14.15 ns</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM (n=6). Values are significantly different from group 2, alloxan induced; ns: non significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (One way ANOVA followed by Dunnett’s test).

Drug-receptor interaction studies

The docking studies carried out using Intestinal α-glucosidase with PDB Id: 3l4w as receptor and isolated compound as drug.

The docking score revealed that isolated compound has better α-glucosidase inhibitory activity than Miglitol (docking score: -7.35)
Discussion
The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It is predicted that by 2030, India, China and the United states will have the largest number of people with diabetes (Wild et al., 2004) [16]. In the light of the literature on Sorghum vulgare, we made an attempt for the first time to study the effect of bound flavonoid compound isolated from ethanolic extract of S. androgynus in antidiabetic activity by Alloxan induced diabetes rat model and the isolated compound tested for antidiabetic potential by docking studies. Alloxan induces diabetes by damaging the insulin secreting pancreatic β cells, resulting in a decrease in endogenous insulin release. Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose level in alloxan induced diabetic animals (Ji Su Kim et al., 2006) [11]. The present study revealed that the isolated compound at both the dose levels (200mg/kg and 400mg/kg) showed a significant (p < 0.001) decrease in blood glucose level after oral administration at 7 and 14 days when compared to the diabetic control group. The docking of α-glucosidase enzyme and isolated compound showed docking score of -9 and good hydrophobic interaction as well as hydrogen bond interaction. The enzyme α-glucosidase is the final enzymes for the digestion of carbohydrates in the brush border of small intestine mucosa. It slows down and decreases digestion and absorption of polysaccharides and sucrose. The drug-receptor interaction studies confirmed that the isolated compound is a potent α-glucosidase inhibitor.

Conclusion
The present work revealed that the bound flavonoidal compound isolated from S. androgynus has good antidiabetic activity. The antidiabetic effect comparable to that of Glibenclamide in in vivo studies and Miglitol in drug-receptor interaction studies. I hope this study will help to develop flavonoid or flavonoid based semi synthetic compound as antidiabetic agent in future.

Reference


