ANTIDIABETIC POTENTIAL OF SOLANUM XANTHOCARPUM SCHRAD. AND WENDL. IN STZ-NICOTINAMIDE INDUCED DIABETIC RATS

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ABSTRACT

Solanum xanthocarpum is a plant that belongs to solanaceae family with many therapeutic applications in traditional systems of medicine especially Ayurveda. The ability of the plant to promote glucose uptake was studied by the communicating author in an in vitro model using L6 skeletal muscle cell lines. The aerial parts and fruit of the plant were found to augment uptake of glucose by GLUT-4, which was indicative of increased insulin sensitivity or insulin-like activity or both. The present study was aimed at investigating the in-vivo antidiabetic potential of Solanum xanthocarpum Schrad.& Wendl, and to elucidate its probable mechanism of action. The ethanolic extracts of the aerial parts and fruit of the plant, at the dose of 400mg/kg produced significant reduction in elevated blood glucose level by the 28th day of STZ-induced diabetes. Intraperitoneal administration of nicotinamide in the dose of 230mg/kg prior to the administration of STZ produced partial destruction of beta cells that resembled type 2 diabetes. Administration of test extracts produced a corresponding increase in insulin level and reduction in HbA1c, CK and LDH values. The animals gained weight during the course of treatment.

Key words: aerial parts, fruit, Solanum xanthocarpum, STZ –NAD.

INTRODUCTION

Intravenous administration of streptozotocin in the dose of 65mg/kg produces partial destruction of beta cells when nicotinamide in the dose of 230mg/kg was administered intraperitoneally, 15 minutes prior to the administration of STZ, in adult rats (1). The highly reactive carbonium ions formed from STZ cause degeneration of beta cells of islets of
Langerhans and induce a state of stable long-lasting hyperglycemia as in type 2 diabetes. STZ produces depletion of nicotinamide adenine dinucleotide (NAD) and cause histopathologic effects in beta cells, which intermediates induction of diabetes. Induction of diabetes produces a decrease in body weight and an increase in blood glucose and HbA1C values. It also changes normal metabolism in comparison to normal rats. Nature’s treasure-trove has provided us with a number of plants with antidiabetic potential, which are being used in traditional systems of medicine, but without adequate scientific validation. Proper elucidation of their pharmacodynamic and pharmacokinetic characteristics would give us momentum in the right direction. *Solanum xanthocarpum* is one such plant used for different therapeutic purposes. An antidiabetic agent could exert a beneficial effect either by increasing insulin secretion and/or by improving insulin sensitivity. The studies were carried out to investigate the probable presence of active ingredients in the ethanolic extract of *Solanum xanthocarpum*, in different doses, which may have insulinogenic activity, insulin-like activity or may improve insulin sensitivity.

**MATERIALS AND METHODS**

**Plant material**

The whole plant of *Solanum xanthocarpum* was collected from the wastelands in Tiruvalla, Kerala, in the month of April when the fruits were yellow in colour. The plant materials were identified and authenticated by Professor Thomas Mathew, Head, Department of Botany, MarThoma College, Tiruvalla. The sample specimens were deposited at the herbarium of the college with voucher numbers DBH-2942-CS/2012 and DBH-2943-SX/2012. Different parts of the plant like aerial parts with fruit and fruit were separated and air dried in shadow. The dried materials were made into fine powder and packed in filter paper and loaded into the thimble. The solvent, 2.5 litres of 80% ethanol, was poured into the flask (distilling pot) and soxhlet extraction was performed for 10 hours. Later the extracted solvent was evaporated under reduced pressure to get a waxy material and the extractive values were found out.

**Animals**

Healthy, adult male Sprague-Dawley rats of body weight between 220-225 were procured from the animal house of the institute and conditioned at room temperature and natural photoperiods for 1 week before the commencement of study. All the rats were provided with commercially available balanced diet and tap water ad libitum. The guidelines of CPCSEA for
the proper conduct of animal experiments were strictly followed and permission of the institutional animal ethics committee was sought for conducting the work.\(^{(8)}\)

**Acute toxicity study**

Acute toxicity study was performed as per the guidelines of OECD. The ethanolic extracts of aerial parts and fruit of *Solanum xanthocarpum* were administered to rats from low-dose to high-dose (50mg/kg-2000mg/kg) and the animals were observed individually during the first 30 minutes and at regular periods during the first 24 hours with careful attention during the first 4 hours and daily thereafter for a total of 14 days. The highest dose of 2000mg/kg was found to be safe and no toxicity was observed during the entire course of 14 days study. One-fifth and one-tenth of the upper limit dose were selected as the dose for screening and evaluation of antidiabetic activity.\(^{(9)}\)

**Chemicals and Kits**

Streptozotocin was procured from Sigma Aldrich, St. Louis, USA. Nicotinamide was obtained as a gift sample from Apex Laboratories Private Ltd, Chennai. Ready to use Citrate buffer was purchased from Hi-media, Mumbai. Rodent Insulin Chemiluminescent ELISA kit by ALPCO diagnostics, Salem, NH 03079, United States, was supplied by Pro Lab Marketing Pvt Ltd, New Delhi. All other biochemical kits were procured from ERBA diagnostics, India.

**Grouping and drug treatment**

The animals (42 rats) were initially divided into two groups, the first group of 6, received neither STZ nor any drug, but 0.01M citrate buffer and was kept as normal control (Group I). The second group (36 rats) was fasted overnight and injected with nicotinamide in normal saline, 230mg/kg, intraperitoneally followed by a single intravenous dose of streptozotocin (STZ) at the dose of 65 mg/kg of body weight, dissolved in 0.01 M citrate buffer, pH adjusted to 4.5, immediately before use. STZ was administered 15 minutes after NAD treatment.

Three days later blood samples were collected from the tip of the tail to confirm the diabetic condition and blood glucose levels were determined in this group. Animals with a fasting blood sugar level of 250-300mg/dl were considered to be diabetic. Diabetic animals (36 rats) were further divided into six groups of 6 rats each. The second group of 36 rats (n = 6) were randomly divided into six experimental groups (Group II to Group VII) as follows
Group I: Normal rats treated with water orally once in a day for 4 weeks served as normal control.

Group II: Diabetic rats treated with water orally once a day for 4 weeks served as diabetic control.

Group III: Diabetic rats treated with Test extract SXA (200 mg/kg b.wt.) orally once a day for 4 weeks.

Group IV: Diabetic rats treated with Test extract SXA (400 mg/kg b.wt.) orally once a day for 4 weeks.

Group V: Diabetic rats treated with Test extract SXF (200 mg/kg b.wt.) orally once a day for 4 weeks.

Group VI: Diabetic rats treated with Test extract SXF (400 mg/kg b.wt.) orally once a day for 4 weeks.

Group VII: Diabetic rats treated with Gliclazide(GLI) 25 mg/kg b.wt orally once a day for 4 weeks.

All the animals were on food and water ad libitum once they were confirmed to be diabetic. Body weights were monitored and recorded weekly during the 4 weeks of study (10).

**Blood collection**

Three days after STZ treatment blood samples were collected from the tail tips to confirm the diabetic state and for the estimation of blood glucose level using GOD-POD method. Animals with a blood glucose value above 250-300mg/dl were considered to be diabetic. The blood samples were collected on the 0, 7, 14 and 28th day of administration of STZ, during the course of treatment with test extracts. At the end of the experiment, rats were fasted overnight and anesthetized with sodium pentothal (intraperitoneally) and 4 ml of blood was withdrawn through the retro-orbital plexus using a glass capillary and collected in tubes. The animals were sacrificed and organs were separated and subjected for biochemical and histopathological studies (11).

**Preparation of hemolysate**

The collected blood was centrifuged for 10 minutes at 3000 rpm to separate the serum. Tubes containing EDTA were used for collection and separation of plasma. The plasma thus obtained was used for glucose and glycosylated hemoglobin (HbA1c) analysis, using commercial kits (ERBA diagnostics). The serum samples were used for the estimation of creatine kinase (CK), lactate dehydrogenase (LDH), using commercial kits (ERBA.
The serum insulin level was estimated using Chemiluminescent ELISA method\(^{12,13}\).

**RESULTS**

**Biochemical findings**

Treatment of rats with STZ produced a significant rise in blood glucose level on day 0 itself. The blood sugar value was maximum with the diabetic control group on the 28\(^{th}\) day (342.17±23.67). (fig-1) There was a gradual but significant reduction in blood sugar level in the SXA 400mg/kg treated group, 294.33±12.45 on day 0, got reduced to 160.67±24.65 on the 28\(^{th}\) day. SXF 400mg/kg too produced comparable results with a value of 173.83±31.68. The blood glucose level was higher in the positive control group on the 28\(^{th}\) day than in the extract treated groups. By the 28\(^{th}\) day of study, diabetic control animals had showed a gradual reduction in the body weight compared to their body weights on day 0. But supplementation of SXA 400mg/kg showed considerable improvement in body weight, 217.67±6.47 versus 242.00±9.63 in the gliclazide treated group. The body weight was only 159.17±9.35 in the STZ treated diabetic control group. The plasma glucose level in the positive control group was 147.17±5.12 whereas the SXA 400mg/kg produced a comparable result of 179.67±9.00. HbA1c value, indicative of glycosylated haemoglobin, was 2.89±0.42 percent in the SXA 400mg/kg group which was comparable to the positive control value of 2.83±0.24 percent. SXF 400mg/kg produced a better value of 2.78±0.29 percent. The serum sample of SXA 400mg/kg treated group showed a significant reduction in the creatine kinase levels with 237.33±17.51 IU/L which was comparable to SXF 400mg/kg group with a value of 252.83±19.92 IU/L. These values are lower than that of the gliclazide treated group. The lactate dehydrogenase level in the SXA 400mg/kg group is lesser than that of the positive control, 115.67±11.40 versus 134.67±8.80 (fig - 2). The serum insulin level, on the day of sacrifice, was found to be 0.227±0.001ng/ml in the diabetic control group whereas it was found to be elevated to 0.379±0.012ng/ml and 0.312±0.002ng/ml in the SXA 400mg/kg and SXF 400mg/kg treated groups. (Table3)
Fig 1: Effect of test extracts on blood glucose levels during treatment.

Fig 2: Effect of test extracts on STZ induced changes on the body weight and blood glucose, HbA1C, CK and LDH

Table 3: Effect of test extracts on serum insulin level

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Serum insulin ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>0.453±0.007</td>
</tr>
<tr>
<td>II</td>
<td>STZ treated control</td>
<td>0.227±0.001</td>
</tr>
<tr>
<td>III</td>
<td>STZ + SXA (200 mg/kg)</td>
<td>0.272±0.004</td>
</tr>
<tr>
<td>IV</td>
<td>STZ + SXA (400 mg/kg)</td>
<td>0.379±0.012</td>
</tr>
<tr>
<td>V</td>
<td>STZ + SXF (200 mg/kg)</td>
<td>0.265±0.002</td>
</tr>
<tr>
<td>VI</td>
<td>STZ + SXF (400 mg/kg)</td>
<td>0.312±0.002</td>
</tr>
<tr>
<td>VII</td>
<td>STZ + GLI (25 mg/kg)</td>
<td>0.441±0.006</td>
</tr>
</tbody>
</table>
DISCUSSION

Diabetes is a metabolic syndrome of hyperglycemia caused by relative or absolute deficiency of insulin or resistance to insulin at cellular level. STZ induced hyperglycemia is an experimental model to study the effect of antidiabetic agents against diabetes mellitus. Prior administration of NAD helped to produce partial destruction of beta cells leading to relative deficiency of insulin which resembles type 2 DM. STZ enters beta cell through GLUT2 transporter and causes DNA alkylation thereby inducing activation of polyADP-riboseylation, which leads to depletion of cellular NAD+ and ATP\(^{(14,15)}\). Free radicals and nitric oxide were formed that inhibits aconitase activity and results in DNA damage. Beta cells die by apoptosis. Pretreatment with NAD, protected beta cells from complete destruction, inducing a condition that resembles type 2 diabetes. The experimental rats developed all the characteristics of diabetes like polyuria, polydipsia, polyphagia and hyperglycemia when diabetes was induced with STZ\(^{(16,17)}\). The STZ alone treated rats showed significant reduction in body weight whereas animals treated with test extracts of the aerial parts of *Solanum xanthocarpum*, SXA, 400mg/kg, showed improvement in body weight and significant reduction in blood sugar levels as well as HbA1C values. The blood sugar lowering activity was found to be superior to that of positive control and other results were comparable to that of gliclazide, 25mg/kg. The animals treated with the ethanolic extracts of aerial parts as well as fruit showed significant increase in the serum insulin values which indicates the augmented insulin secretion on administration of plant extracts of *Solanum xanthocarpum*. The level of serum creatine kinase and lactate dehydrogenase were lowered in SXA 400mg/kg treated group than in the gliclazide treated test group, which was indicative of better protection of skeletal muscle health and protection against tissue breakdown. The fruit of *Solanum xanthocarpum* too possessed considerable hypoglycemic activity at the dose of 400mg/kg. It also reduced the CK and LDH values, showing good muscle and tissue protection.

CONCLUSION

*Solanum xanthocarpum* is a plant with great potential in the treatment of T2 DM as its aerial parts were found to possess good antihyperglycemic activity that is comparable to the established sulfonyl urea drug gliclazide. The elevated levels of serum insulin in group IV and VI were indicative of the insulinogenic action of *Solanum xanthocarpum*. The plant produced good muscle protective effect too. The activities may be attributed to the constituents found in the leaves, stem and fruit of the plant, that may act as insulin.
secretagogues. The active principles like flavonoids, sterols, terpenoids and phenolics may contribute to the antihyperglycemic effect of *Solanum xanthocarpum Schrad & Wendl*.

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