EVALUATION OF ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF SECHIUM EDULE FRUITS IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

The objective of this study was to investigate the effect of ethanolic extract of Sechium edule fruits on alloxan induced diabetes in wistar rats. Oral administration of Sechium edule (200 mg/kg, p.o. and 100mg/kg, p.o.) prevent the body weight loss and significantly (p<0.01) decrease the blood glucose level on 0, 7, 14, and 21 day. The lipid profile of diabetic rats was also improved on administration of ethanolic extract of Sechium edule fruits.

Keywords: Sechium edule, diabetes, alloxan, lipid profile.

INTRODUCTION

Diabetes Mellitus (DM) is one of the most prevalent metabolic disorders characterized with increased blood sugar level and improper primary metabolism. It is characterized by alterations in the metabolism of carbohydrate, fat and protein, which are caused by a relative or absolute deficiency of insulin secretion and different levels of insulin resistance ¹. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia². The number of people with diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity. According to recent estimate, the greatest absolute increase in the number of people with diabetes will be in India and the total number of people with diabetes is projected to rise from 31.7 million in 2000 to 79.4 million
in 2030. Globally the prevalence of diabetes was estimated to be 2.8% in 2000 and 4.4% in 2030. Worldwide the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. Therefore, the human population worldwide appears to be in the midst of an epidemic of diabetes.

The plant kingdom serves as a food and medicinal source, and thus maintains the vitality of human beings as well as animals without causing any toxicity. Herbal plants have been used for medicinal applications from earliest time, when man began caring for his body and health. India has a rich history of using plants for medicinal purposes. *Sechium edule* is an edible plant that belongs to the family cucurbitaceae also known as sayote, choko, chocho, chow-chow, and vegetable pear. The chayote is a herbaceous, perennial, monoecious, vigorous creeper or climbing plant. The fruits grow either individually or in pairs on a shared peduncle. They are fleshy or fleshy-fibrous, may have longitudinal ridges or furrows, and come in many different shapes (globose, ovoid, subovoid, pyriform) and colours (dark or light green). The fruits and the seed especially, are rich in several important amino acids. A lectin from the exudate of *Sechium edule* was purified. Eight flavonoids, including three C-glycosyl and five O-glycosyl flavones, were detected. Twenty known Gibberellins’ have been identified in extracts of the seeds of *Sechium edule*. The leaves and fruits have diuretic, cardiovascular and anti-inflammatory properties, the leaves has been used in the treatment of arteriosclerosis and hypertension, and to dissolve kidney stones. It has been reported that the ethanolic extracts of dried leaves and water extracts of seeds were found to possess higher radical-scavenging, reducing power and antioxidant activity and hepatoprotective activity.

Several management strategies have been proposed for the early stages of hyperglycemia, with the aim of preventing the development of diabetes and associated complications. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. So, in the present study an attempt has been made to evaluate the antidiabetic activity of ethanolic extract of *sechium edule* fruits in experimental rats.

**MATERIAL AND METHODS**

**Plant material**

The fresh fruits of *Sechium edule* were collected from Reliance Fresh, Secunderabad and also from Bangalore. The fruit material was taxonomically identified and authenticated at
Regional Research Institute (Ay.), Bangalore, where the voucher specimen is conserved under the reference number (RRCBI/MCW/7/2008) for future reference.

**Preparation of extract**
The fruits of *Sechium edule* were first thoroughly washed under running tap water and then washed with distilled water. Then the fruits were chopped and air dried under shade. Then it was powdered using a mechanical grinder to obtain a coarse powder. The powder was used for the preparation of ethanolic extract. The powder was then subjected to maceration with sufficient volume of ethanol (99.9%) for 72 hrs with intermittent shaking. Then the extract was filtered and subjected to distillation to remove the solvent. The product so obtained was reduced to a dark coloured mass by keeping in boiling water bath for further solvent elimination. This part of the sample was named ethanolic extract. The extracts were preserved in a refrigerator.

**Phytochemical screening**
Preliminary phytochemical screening of ethanolic extract of *Sechium edule* fruits and its ethyl acetate and n-butanol fractions were performed for the presence of alkaloids, phenolics, flavonoids, saponins, carotenoids, carbohydrates and glycosides.

**Drugs and chemicals**
Alloxan was procured from Loba Cheme, India, glibenclamide were obtained from Aventis Pharma, India. Total cholesterol diagnostic kit, triglycerides diagnostic kit and glucose estimation kit were obtained from Span Diagnostics, India. All other chemicals used in this study were obtained commercially and were of analytical grade.

**Experimental animals**
Studies were carried out using male Wistar albino rats (120–150 gm) and swiss albino mice (20-25gm) of either sex were used. They were obtained from the animal house, Indian Institute of Chemical Biology (IICB), Kolkata, India. All the animals were housed in polypropylene cages maintained in controlled temperature (27 ± 2°C) and light cycle (12 h light and 12 h dark). They were provided with standard rat pellet diet and water ad libitum. All the animals were given a week time to get acclimatized with the laboratory conditions. The experiments were carried out according to guidelines of Committee for Prevention and Control of Scientific Experimentation on Animals (CPCSEA).
Acute toxicity studies
Mice were kept overnight fasting prior to drug administration. Animals were received a single oral dose (2000 mg/kg, b.w.) of ethanolic extract of Sechium edule fruits and its ethyl acetate and n-butanol fractions. After the administration of Sechium edule fruit extract and its different fractions food was withheld for further 3–4 h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of 2 weeks.

Selection of dose of the extract and its fractions
LD$_{50}$ was done as per OECD guidelines for fixing the dose for biological evaluation. The LD$_{50}$ of Sechium edule fruit extract and its different fractions as per OECD guidelines falls under class four values with no signs of acute toxicity at 2000 mg/kg. The biological evaluation was carried out at doses of 100 and 200 mg/kg body weight.

Alloxan induced diabetic in rat
Thirty male wistar rats were divided into five groups of 6 animals in each group as follows:

Group 1: Control (distilled water p.o)
Group 2: Alloxan monohydrate (150 mg/kg, i.p).
Group 3: Glibenclamide (5 mg/kg, p.o.) + Alloxan monohydrate (150 mg/kg, i.p).
Group 4: Ethanolic extract of Sechium edule (200 mg/kg) + Alloxan monohydrate (150 mg/kg, i.p).
Group 5: Ethanolic extract of Sechium edule (100 mg/kg) + Alloxan monohydrate (150 mg/kg, i.p).

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg, i.p). Alloxan was first weighed individually for each animal according to the weight and then solubilized with 0.2 ml saline (154 mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection. Fasting blood glucose estimation and body weight measurement were done on day 0, 7, 14 and 21 day of the study.
Biochemical Assessment:
On 21st day Blood was drawn from retro orbital vein under mild ether anaesthesia from overnight fasted rats and and serum was separated by centrifugation and utilized for the estimation of various bio-chemical parameters namely: Serum glucose, Total cholesterol, Serum Triglycerides, LDL- cholesterol, HDL- cholesterol and VLDL- cholesterol.

Statistical Analysis
Results were expressed as Mean ± SEM. Differences between diabetic control and treated groups were statistically analyzed by ANOVA (Dunnette’s test) using Graph Pad Prism version 4. p<0.05, p<0.01 were considered as statistically significant.

RESULTS
Preliminary phytochemical investigation
The preliminary phytochemical investigation of the ethanolic extract of Sechium edule showed that it mainly contains carbohydrates, tannins, flavonoids and saponins.

Acute toxicity studies
In LD50 studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity and mortality were observed. The biological evaluation was carried out at doses of 100 and 200 mg/kg body weight.

Effect of sechium edule on alloxan induced diabetic in rats
Figure.1 depicted the effect of glinbenclamide (5 mg/kg, p.o.) and sechium edule (200 mg/kg, p.o. and 100 mg/kg, p.o.) on body weight changes in experimental groups of rats at 0, 7, 14, 21 days. During the experimental period, there was a significant (p<0.05) decrease in the body weight of diabetic control rats when compared with that of normal control. However, this significant weight loss is prevented in diabetic rats treated with glinbenclamide (5 mg/kg, p.o.) and Sechium edule (200 mg/kg, p.o. and 100 mg/kg, p.o.).

In animals treated with alloxan, a significant increase in the blood glucose levels was observed on 0, 7, 14, 21 day when compared to normal control (Group-I). Group-III treated rats with standard drug showed a significant (p<0.01) decrease in blood glucose levels on 0, 7, 14, 21 day when compared to diabetic control. On administration of sechium edule (200
mg/kg, p.o. and 100 mg/kg, p.o.) the blood glucose levels were significantly (p<0.01) decreased on 0, 7, 14, 21 day when compared to diabetic control (Table 1).

A significant increase in serum cholesterol, triglyceride, LDL-C and VLD-C in diabetic control rats was observed. The HDL-C level was significantly decreased in diabetic control rats. On administration of *Sechium edule* (200mg/kg, p.o. and 100mg/kg, p.o.) the serum cholesterol, triglyceride, LDL-C and VLD-C levels were significantly (p<0.01) decreased day when compared to diabetic control rats. *Sechium edule* (200 mg/kg, p.o. and 100 mg/kg, p.o.) significantly (p<0.01) increased the HDL-C level (Table 1).

![Figure 1: Effect of *Sechium edule* on changes in body weight of diabetic rats](image)

Group I- Normal control, Group II- Diabetic control, Group III- Diabetic rats treated with Glinbenclamide (5 mg/kg), Group IV- Diabetic rats treated with *sechium edule* (200 mg/kg, p.o.), Group V- Diabetic rats treated with *sechium edule* (100 mg/kg, p.o.).

Values are given as mean ± S.E.M for groups of six animals each.

**Table 1: Effect of ethanolic extract of *Sechium edule* on blood glucose level in experimental groups of rats**

<table>
<thead>
<tr>
<th>Blood glucose (mg/dl)</th>
<th>Group-I (Normal control)</th>
<th>Group-II (Diabetic control)</th>
<th>Group-III (Glinbenclamide 5mg/kg)</th>
<th>Group-IV (Sechium edule 200mg/kg)</th>
<th>Group-V (Sechium edule 100mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0(^{th}) day</td>
<td>96.5±3.06*</td>
<td>189.9±3.11</td>
<td>190.67±2.15^a</td>
<td>195±5.13^b</td>
<td>196.83±3.72^b</td>
</tr>
<tr>
<td>7(^{th}) day</td>
<td>98.8±1.89*</td>
<td>244.2±3.18</td>
<td>157.67±1.86^a</td>
<td>163.67±1.54^b</td>
<td>180±1.39^b</td>
</tr>
<tr>
<td>14(^{th}) day</td>
<td>94.5±1.61*</td>
<td>271±3.22</td>
<td>132.5±2.38^a</td>
<td>142.33±1.28^b</td>
<td>163±2.05^b</td>
</tr>
<tr>
<td>21(^{st}) day</td>
<td>94.3±2.95*</td>
<td>259.5±2.53</td>
<td>112±2.77^a</td>
<td>133.33±2.39^b</td>
<td>153±2.37^b</td>
</tr>
</tbody>
</table>

*P<0.01 When compared to Group II diabetic control.*
a P<0.01 When compared Group III diabetic rats with Group-II diabetic control.
b P<0.01 When compared Group IV and Group V diabetic rats with Group-II diabetic control.

Values are given as mean ± S.E.M for groups of six animals each.

**Table 2: Effect of ethanolic extract of *Sechium edule* on lipid profile of alloxan induced diabetic in rats on 21st day**

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Group I (Normal control)</th>
<th>Group II (Diabetic control)</th>
<th>Group III (Glinbenclamide 5 mg/kg)</th>
<th>Group IV (Sechium edule 200 mg/kg)</th>
<th>Group V (Sechium edule 100 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>63.72±1.29*</td>
<td>148.9±2.77</td>
<td>71.48±0.61*a</td>
<td>79.04±0.58*b</td>
<td>86.25±0.68*b</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dl)</td>
<td>52.68±0.73*</td>
<td>38.73±2.43</td>
<td>55.34±0.76*a</td>
<td>71.82±0.77*b</td>
<td>62.01±0.47*b</td>
</tr>
<tr>
<td>Serum LDL cholesterol (mg/dl)</td>
<td>51.75±1.89*</td>
<td>148.7±2.23</td>
<td>60.27±0.36*a</td>
<td>74.03±3.63*b</td>
<td>121.7±3.86*b</td>
</tr>
<tr>
<td>Serum VLDL cholesterol (mg/dl)</td>
<td>29.4±1.58*</td>
<td>36.97±0.27</td>
<td>32.53±0.72*a</td>
<td>31.43±0.47*b</td>
<td>35.63±0.43*b</td>
</tr>
<tr>
<td>Serum Triglycerides (mg/dl)</td>
<td>147.2±0.54*</td>
<td>242.1±0.76</td>
<td>155.9±2.02*a</td>
<td>165.1±0.54*b</td>
<td>170.5±0.56*b</td>
</tr>
</tbody>
</table>

* P<0.01 When compared to Group II diabetic control.
a P<0.01 When compared Group III diabetic rats with Group-II diabetic control.
b P<0.01 When compared Group IV and Group-V diabetic rats with Group-II diabetic control.

**DISCUSSION**

Pancreas is the primary organ involved in sensing the organism’s dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted 19. However, alloxan causes diabetes through a massive reduction in insulin release by the destruction of the β-cells of the islets of langerhans, inducing hyperglycaemia, which is mediated by reactive oxygen species 20, 21. When there are not enough available beta-cells to supply sufficient insulin to meet the needs of the body, decreased utilization of glucose by
body tissues and insulin-dependent diabetes results. The consequently elevation of blood glucose level, decreased protein content, increased levels of cholesterol and triglycerides.

In this present study, the ethanolic extract of *sechium edule* exhibited significant (p<0.01) decrease in blood glucose in alloxan induced hyperglycemic rats. It also significantly (p<0.01) decrease the total cholesterol, triglyceride, LDL-C, VLDL-C and improve the condition of diabetes. The extract also improved the HDL-C level significantly (p<0.01). It has been also found that there was a significant (p<0.05) decrease in the body weight of diabetic rats. This significant weight loss is prevented in diabetic rats treated ethanolic extract of *Sechium edule*.

Literature reviews showed that some flavonoids and saponins isolated from medicinal plants significantly reduce the blood glucose levels. Flavonoids and glycosides stimulate the secretion of insulin in β-cells of pancreas. The preliminary phytochemical investigation of the ethanolic extract of *Sechium edule* showed that mainly it contains flavonoids and saponin. So, on the basis of above evidence it is possible that the presence of flavonoids and saponins are responsible for their activity. Further study on flavonoids and saponins of *sechium edule* is required to establish the active antidiabetic principle.

REFERENCES


