ABSTRACT

Objective: This study aims to investigate the therapeutic effect of hypoglycemic activities of Coccinia indica fruits extract in streptozotocin induced diabetic rats. Method: Sprague Dawley rats of either sex were used for the experimental bioassay. Animals were made diabetic by a single intra-peritoneal dose of streptozotocin 60 mg/kg body weight. After 72 h of streptozotocin administration, blood glucose level was examined to confirm diabetes. All animals were randomly divided into seven groups with six animals in each. A single oral administration daily for three weeks of ethanolic extract at doses 200 and 400 mg/kg and triterpenoid fraction at doses 50 and 100 mg/kg was done. Glibenclamide 5 mg/kg was used as reference drug.

Results: Phytochemical study revealed the presence of triterpenoids, flavonoids, sterols, carbohydrates. The ethanolic extract and triterpenoid fraction showed significant glucose lowering activity along with good lipid lowering activity in streptozotocin induced diabetic rats. The histo-pathological findings suggest that the architecture of pancreas was retained. Conclusion: The findings suggest that alcoholic extract of Coccinia indica and triterpenoid fraction serve as good oral hypoglycemic agents and seem to be promising for the development of phytomedicines for hypoglycemic, hypolipidemic, renal-protective and hepatoprotective effects in diabetes mellitus.

Keywords: Streptozotocin, Coccinia indica, Glibenclamide, Ethanolic extract, Anti-diabetic.
INTRODUCTION

Plants have always been an exemplary source of drugs and many drugs currently available have been derived directly or indirectly from them. A vast majority of population particularly those living in villages depend largely on medicinal plants for treating and curing diseases. One such medicinal plant Ivy Gourd (Syn. Coccinia indica, Cephalandra indica Wight & Arnott.) is indigenous plant of Central Africa, India and Asia. This plant has been wildly used in traditional Indian medicinal system (Ayurvedic, Unani, and Siddha). Every part of the plant exhibit pharmacological activities, and is annual employed for treating various human ailments. Ivy Gourd (Coccinia indica) is extensively used as vegetable and grown wildly throughout Indian subcontinent. It is commonly known as ‘Kundru’ in India. Its taxonomic position, botanical synonyms and various vernacular names as follows: Order: Cucurbitaceae Family: Cucurbitaceae Class: Mangoliopsida Genus: Cephalandra Specific Epithet: indicanoudin Botanical name: Cephalandra indicanoudin.

Botanical Synonyms: Coccinia indica, Coccinia cordifolia, Coccinia grandis. Vernacular Names: English: Scarlet-fruited gourd, tindora, kovaifruit, Hindi: parval, tindora (tindori or tindola), tinda, tendus, kundru, kunduzi, Ayurveda: Bimbi, Kunduru, Raktaphala, Piluparni. Ivy plant has been used in traditional medicine as a household remedy for various diseases, including biliary disorders, anorexia, cough, diabetic wounds, and hepatic disorders. For the last few decades, some extensive work has been done to establish the biological activities and pharmacological actions of Ivy Gourd and its extracts. Anti-inflammatory, antioxidant, antimutagenic, antidiabetic, antibacterial, antiprotozoal, antiulcer, hepatoprotective, expectorant, analgesic, anti-inflammatory are the reported pharmacological activities of Ivy Gourd. Main objective of present study was to investigate the phytoconstituents present and to evaluate anti-diabetic potential of Coccinia indica\(^1\)[2].

MATERIALS AND METHODS

Approval of Experimental protocol

All the experimental procedures and protocols used in this study were reviewed and approved (SCOP/IAEC/2013-14/153) by the Institutional Animal Ethics Committee (IAEC) of Sinhgad College of Pharmacy, Pune, constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical guidelines were strictly followed during all the experiments.
Chemicals and Instruments
Streptozotocin (Siaco Research Labs Pvt.Ltd, Mumbai), Glibenclamide (Gem pharma, Pune), Biochemical estimation kits (Coral labs), Laboratory grade reagents (Loba chemie Pvt.Ltd, Mumbai), Rotary evaporator (Evator), Cooling centrifuge (Remi Instruments), Tissue Homogenizer (Biolabs Instruments), Auto-analyzer (Chemstar).

Collection of plant material
The whole plant of *Coccinia indica* was obtained in October 2013, from the vicinity of Pune. The plant material was authenticated at Botanical Survey of India, Pune and voucher specimen was deposited.

Extraction and isolation
The *Coccinia indica* fruits were dried at 40°C so as to produce a fine powder on grinding. The powder was placed in a thimble and extracted using a soxhlet apparatus. Various solvents used for extraction were: Petroleum ether, Ethanol, Methanol, Chloroform, Water. The extraction was continued until the powder was exhausted. The obtained extract was concentrated using rotary evaporator and the hot plate.

Isolation of triterpenoids from ethanolic extract of *Coccinia indica* was done by suspending extract in distilled water and then fractionating successively with petroleum ether, chloroform, n-butanol and methanol. All the fractions were then washed with distilled water, dried over anhydrous sodium sulphate and freed of solvent by distillation. The petroleum ether soluble extract was dissolved in CHCl₃ and loaded onto TLC plate. Mobile phase used was benzene: ethyl acetate (9.7: 0.3) and visualization was done by vanillin- sulphuric acid reagent heated at 110°C.

Phytochemical studies of extracts
All extracts were subjected to the phytochemical tests to detect the presence of various phytoconstituents.

Experimental Design
All the rats were fasted overnight before administration of streptozotocin (STZ). STZ was prepared in citrate buffer (pH 4.4, 0.1 M). Streptozotocin (60 mg/kg, i.p.) was given to different groups of rats. Control rats were injected with citrate buffer only. Two days after STZ injection, blood samples were collected, and plasma glucose levels estimated by using
Glucometer. Surviving rats with fasting serum glucose level higher than 250 mg/dl were used for the study.

Animal grouping
Group 1 - Normal Control (vehicle, p.o)
Group 2 – Diabetic control (DC + vehicle, p.o)
Group 3 – Standard treatment (Glibenclamide 5mg/kg, p.o)
Group 4 - Coccinia indica Ethanolic extract (DC + EECI 200 mg/kg, p.o)
Group 5 - Coccinia indica Ethanolic extract (DC + EECI 400 mg/kg, p.o)
Group 6 - Triterpenoid fraction (DC + triterpenoid fraction of EECI 50 mg/kg, p.o)
Group 7 - Triterpenoid fraction (DC + triterpenoid fraction of EECI 100 mg/kg, p.o)

The study protocol was of 21 days. Each group received the respective treatment orally once daily for 3 weeks after induction of diabetes. Body weight and biochemical parameters were estimated on 0th, 7th, 14th, and 21st day. On 21st day, the animals were sacrificed to evaluate the tissue glycogen content and histopathological study of pancreas was performed.

Statistical analysis
All statistical analyses were made using the software Graph Pad Prism. All results were expressed as mean ± SEM, and compared with those of the control and diabetic groups using Tukey’s test and statistical significance was determined. The values were considered statistically significant when p<0.05.

RESULTS
The preliminary phytochemical screening showed presence Carbohydrates, Flavanoids, Cardiac glycosides, Tannins, Steroids in ethanolic extract of Coccinia indica fruits. The % yield was found to be 20.28%, 14.32%, 8.12%, 9.40% and 12.40% for Aqueous, Ethanolic, Petroleum ether, Chloroform and Methanolic extracts respectively (Table 1).

Table 1: Phytochemical Constituents and % yield of various extracts of Coccinia indica

<table>
<thead>
<tr>
<th>EXTRACT</th>
<th>% YIELD</th>
<th>CONSTITUENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>20.28%</td>
<td>Carbohydrates, Flavanoids, Saponins, Cardiac glycosides,</td>
</tr>
<tr>
<td>Ethanolic</td>
<td>14.32%</td>
<td>Carbohydrates, Flavanoids, Cardiac glycosides, Tannins, Steroids</td>
</tr>
<tr>
<td>PET ether</td>
<td>8.12%</td>
<td>Cardiac glycosides, Steroids</td>
</tr>
<tr>
<td>Chloroform</td>
<td>9.4%</td>
<td>Carbohydrates, Cardiac glycosides, Steroids, Saponins</td>
</tr>
<tr>
<td>Methanolic</td>
<td>12.48%</td>
<td>Carbohydrates, Cardiac glycosides, Steroids, Saponins</td>
</tr>
</tbody>
</table>
The glucose tolerance test is a medical test in which glucose is given and blood samples taken afterward to determine how quickly it is cleared from the blood. The oral glucose tolerance test is one of the form of glucose tolerance testing recommended for the diagnosis of diabetes.

Table 2: Effect of single dose treatment of various extracts of C.indica fruits on Antihyperglycaemic activity in glucose-loaded hyperglycaemic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Blood Glucose level (mg/dl) at time t (Hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Glu.</td>
<td>-</td>
<td>85.50±2.41</td>
</tr>
<tr>
<td>Std.</td>
<td>5</td>
<td>84.67±2.60 ** Glucose Load</td>
</tr>
<tr>
<td>AECI</td>
<td>400</td>
<td>64.50±1.65</td>
</tr>
<tr>
<td>EECI</td>
<td>400</td>
<td>70.00±1.53</td>
</tr>
<tr>
<td>PtECI</td>
<td>400</td>
<td>68.67±1.61</td>
</tr>
<tr>
<td>CECI</td>
<td>400</td>
<td>72.67±1.61</td>
</tr>
<tr>
<td>MECI</td>
<td>400</td>
<td>69.17±1.30</td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>100</td>
<td>79.00±0.08</td>
</tr>
</tbody>
</table>

Glu- Glucose Control,
Std- Glibenclamide
AECI- Aqueous extract of Coccinia indica,
EECI- Ethanolic extract of Coccinia indica,
PtECI- Petroleum ether extract of Coccinia indica,
CECI- Chloroform extract of Coccinia indica,
MECI- Methanolic extract of Coccinia indica

n = 6, values are expressed as mean ± SEM, *p<0.05, **p<0.01 as compared to Glucose control. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test
Glucose lowering ability of ethanolic extract and triterpenoid fraction was comparable to standards used. Three weeks repeated dose treatment of EECI showed significant (p<0.001) restoration of body weight in diabetic animals (Table 3).
Table 3: Effect of 3 weeks repeated treatment of EECI and Triterpenoid fraction on Body Weight

<table>
<thead>
<tr>
<th>GROUP</th>
<th>BODY WEIGHT (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAY 0</td>
</tr>
<tr>
<td>Normal Control</td>
<td>225.8±1.786</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>221.6±4.72</td>
</tr>
<tr>
<td>Glibenclamide (5mg/kg)</td>
<td>235.7±4.196</td>
</tr>
<tr>
<td><em>C.indica</em> (200mg/kg)</td>
<td>232.8±4.574</td>
</tr>
<tr>
<td><em>C.indica</em> (400mg/kg)</td>
<td>222.7±3.991</td>
</tr>
<tr>
<td>Triterpenoid (50mg/kg)</td>
<td>196.4±34.55</td>
</tr>
<tr>
<td>Triterpenoid (100mg/kg)</td>
<td>230.8±5.67</td>
</tr>
</tbody>
</table>

n = 6, values are expressed as mean ± SEM. *p<0.001 as compared to Normal control @p<0.001 #p<0.01 as compared to DC. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.

Biochemical parameters were significantly affected in diabetes. There was elevation in serum glucose, cholesterol, triglycerides, creatinine and alkaline phosphatase whereas there was reduction in levels of serum HDL, albumin and tissue glycogen. Treatment with extract (400mg/kg) and triterpenoid fraction (100mg/kg) was found to be comparable with standard treatment, glibenclamide (5mg/kg) and could restore the altered levels.

Figure 1: Effect of 3 weeks repeated treatment of EECI and triterpenoid fraction on blood glucose level in STZ induced diabetic rats

n = 6, values are expressed as mean ± SEM. *p<0.001 as compared to Normal control @p<0.001 #p<0.01 as compared to DC. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.
Figure 2: Effect of 3 weeks repeated treatment of EECI and triterpenoid fraction on blood Cholesterol level in STZ induced diabetic rats

\( n = 6 \), values are expressed as mean ± SEM. \( ^{c}p<0.001 \) as compared to Normal control \( ^{p}<0.001 \) \( ^{#}p<0.01 \) as compared to DC. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.

Figure 3: Effect of 3 weeks repeated treatment of EECI and triterpenoid fraction on Serum HDL level in STZ induced diabetic rats

\( n = 6 \), values are expressed as mean ± SEM. \( ^{c}p<0.001 \) as compared to Normal control \( ^{p}<0.001 \) \( ^{#}p<0.01 \) as compared to DC. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.
Figure 4: Effect of 3 weeks repeated treatment of EECI and triterpenoid fraction on Serum Albumin level in STZ induced diabetic rats

$n = 6$, values are expressed as mean ± SEM $^c p<0.001$ as compared to Normal control $^@ p<0.001$ # $p<0.01$ as compared to DC. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.

Figure 5: Effect of 3 weeks repeated treatment of EECI and triterpenoid fraction on Serum Triglycerides level in STZ induced diabetic rats

$n = 6$, values are expressed as mean ± SEM $^c p<0.001$ as compared to Normal control $^@ p<0.001$ # $p<0.01$ as compared to DC. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.
Figure 6: Effect of 3 weeks repeated treatment of EECI and triterpenoid fraction on Serum Alkaline Phosphatase level in STZ induced diabetic rats

$n = 6$, values are expressed as mean ± SEM $^c p<0.001$ as compared to Normal control $^@ p<0.001$ $^p<0.01$ as compared to DC. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.

Figure 7: Effect of 3 weeks repeated treatment of EECI and triterpenoid fraction on Serum Creatinine level in STZ induced diabetic rats

$n = 6$, values are expressed as mean ± SEM $^c p<0.001$ as compared to Normal control $^@ p<0.001$ $^p<0.01$ as compared to DC. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.
Table 4: Effect of 3 weeks repeated treatment of EECI and Triterpenoid fraction on Tissue Glycogen content

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver Glycogen content (glucose is equivalent to glycogen content)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>54.8400±0.710</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>23.2900±1.809</td>
</tr>
<tr>
<td>Glibenclamide (5mg/kg)</td>
<td>48.1300±1.708</td>
</tr>
<tr>
<td>Extract (200mg/kg)</td>
<td>38.32±1.17</td>
</tr>
<tr>
<td>Extract (400mg/kg)</td>
<td>39.8100±0.8846</td>
</tr>
<tr>
<td>Triterpenoid (50mg/kg)</td>
<td>41.7000±1.195</td>
</tr>
<tr>
<td>Triterpenoid (100mg/kg)</td>
<td>46.5600±2.081</td>
</tr>
</tbody>
</table>

\( n = 6 \), values are expressed as mean ± SEM \(*p<0.001\) as compared to Normal control \( \#p<0.01 \) as compared to DC. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test

Histopathological findings of pancreas of the diabetic rats showed necrosis, atrophy and fibrotic changes. Whereas, the pancreas of the rats treated with EECI, triterpenoid fraction showed improvement in necrosis (mild to moderate atrophy) and fibrotic changes (Figure 1).

Figure 8: Effect of 3 weeks repeated treatment of EECI and triterpenoid fraction on Histology of Pancreas

A- Normal Control,  
B- Diabetic Control (+++),  
C- Standard (+),  
D- Extract 200mg/kg (++),  
E- Extract 400mg/kg (+),  
F- Fraction 50mg/kg (++),  
G- Fraction 100mg/kg (+)
**DISCUSSION**

Diabetes is a group of chronic diseases characterized by hyperglycemia. The direct and indirect effects on the human vascular tree are the major source of morbidity and mortality in both type I and type II diabetes. Generally, the injurious effects of hyperglycemia are separated into macro-vascular complications (coronary artery disease, peripheral arterial disease, and stroke) and micro-vascular complications (diabetic nephropathy, neuropathy, and retinopathy) \[4\].

In present study, anti-diabetic effect of ethanolic extract of *Coccinia indica* and standard drug Glibenclamide was studied in STZ induced diabetic rats. The diabetogenic agent streptozotocin (STZ) inhibits insulin secretion and causes a state of insulin dependent diabetes mellitus through its ability to induce a selective necrosis of pancreatic beta cells. Both effects can be assorted to alkylating potency of streptozotocin. The common chemical denominator of two effects is selective cellular uptake and the accumulation of streptozotocin by beta cells \[5\].

The mechanism of action of *C.indica* extract is proposed to be through inhibition of Glucose-6-Phosphatase enzyme and by insulin secreting action \[6\]. In this study, the emphasis was given to evaluate the antidiabetic potential of triterpenoid fraction that was isolated. Triterpenoids are present abundantly in *C.indica* fruits and believed to possess anti-diabetic activity. Diabetes was induced in rats by injecting 60 mg/kg of Streptozotocin intra-peritoneally.

Diabetes causes failure to use of glucose for energy which leads to increased utilization and decreased storage of protein responsible for reduction of body weight \[7\]. The results of the present study indicated that restoration of body weight might be contributed by increased use of glucose as fuel by the tissues.

Significant hyperglycemia was observed before treatment in diabetic animals when compared to normal control animals \[8\]. The abnormal high concentration of serum lipids is mainly due to increase in the mobilization of free fatty acids from the peripheral fat deposits, because of
acute deficiency of insulin which is responsible for the inhibition of hormone sensitive lipase production \[9\].

HDL decrease was observed in the present study in diabetic rats which will increase the chances of atherosclerosis. Increase in HDL cholesterol is associated with a decrease in coronary risk \[9\].

Decreased levels of albumin were found in diabetic rats as compared to normal control. This may be due to the renal damage caused in diabetic condition. Induction of diabetes cause progressive renal damage which damage the glomeruli. This affects the filtration capacity. Thus, the albumin is excreted through urine and decreased levels are found in serum.

Diabetic condition increases the lipolysis and produces more free fatty acids (FFA). Increased release of FFA increases the production of ketone bodies and triglycerides synthesis. In the present investigation, triglycerides are increased significantly. Normally, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. But in diabetic state, lipoprotein lipase is not activated due to insulin deficiency, resulting in hyper-triglyceridemia \[10\] \[11\].

Diabetes mellitus is basically a disorder of carbohydrate metabolism but, with progression of disease, protein metabolism is also affected. Gluconeogenesis is a major biochemical process that produces glucose from protein and it is accelerated in diabetes \[14\]. The oral treatment with extract, Triterpenoid fraction and Glibenclamide for three weeks significantly increased serum total protein levels in diabetic rats which might be due to inhibition of gluconeogenesis in diabetic animals.

Induction of diabetes was associated with decreased hepatic glycogen, which could be attributed to decrease in the availability of the active form of enzyme glycogen synthetase, which might be due to low level of insulin \[15\]. In this study, the EECI and triterpenoid fraction restored the decreased hepatic glycogen levels possibly by increasing the level of insulin.

Histological evaluation of pancreas was carried out on 21st day post diabetes induction for the treated and untreated samples. It did not reveal the ß cell regeneration potential of extract. Thus, the anti-diabetic activity might be due to peripheral mechanisms.
CONCLUSION
The findings suggest that alcoholic extract of *C. indica* and triterpenoid fraction serve as good oral hypoglycemic agents and seem to be promising for the development of phytomedicines for hypoglycemic, hypolipidemic, and renal-protective effects in diabetes mellitus.

Conflict of interest statement
We declare that we have no conflict of interest.

ACKNOWLEDGEMENT
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