Antidiabetic, Antihyperlipidemic and Antioxidant Activities of *Cansjera Rheedii* in Alloxan Induced Rats

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Summary

*Cansjera rheedii* is widely used traditional medicine to treat so many ailments. It is also used as folk medicine to treat diabetes in India. The present study was carried out to identify the anti-diabetic activity of the leaf extract of chloroform extract of the plant Cansjera rheedii. The chloroform extract of the leaf was evaluated for its effect on blood sugar against the alloxan induced diabetic rats and compared it with standard drug, Glibenclamide. The result of this experimental study indicates that the ECR possess a significant antidiabetic effect (hypoglycemic effect). It also showed that the ECR possess significant antihyperlipidemic and antioxidant effect.

Key words: Cansjera rheedii, hypoglycemic effect, antioxidants, antihyperlipidemic effect, alloxan.

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Introduction

Diabetes is a complex heterogeneous group of disorder characterized by Hyperglycemia. It is a chronic disorder of carbohydrate, fat and protein metabolism characterized by allegation of both fasting and post-paradinal blood sugar levels. Usage of oral hypoglycemic agents and insulin being reported to posses serious side effects. This leads to increase in demand for herbal products with antidiabetic activity. A large number of plants have been recognized to be effective in the treatment of Diabetes mellitus. It is a multi-factorial disease in which increased oxidative stress plays an important pathogenic role. Oxidative stress represents a shift towards the pro-oxidant/Antioxidant balance that can occur as a result of an increase in oxidative metabolism. Biochemical defects related to all diabetic complications may arise from overproduction of reactive oxygen species/nitrogen species. ROS(reactive oxygen species) induced by elevation of glucose and free fatty acid levels, directly damage DNA, proteins, lipids, decrease insulin mRNA, cytosolic ATP, mitochondria, calcium influx into cytosol, and causes apoptosis. Oxidative stress participates not only in beta cell dysfunction and insulin resistance but also in the genesis of late complications of diabetes. Effect of advanced glycation end products on vascular structures is important in the pathogenesis of diabetic micro and macro vascular complications. Oxidative stress involves altering mitochondrial function, ion channel alteration, and abnormal growth factor signaling. Involvement of oxidative stress and advanced glycation end products in diabetes complications is the basis of the development of adjunct therapies with antioxidant and/or anti)advanced glycation end products molecules\textsuperscript{[1]}. 
Cansjera rheedii (Family: Opilliaceae) is a climbing shrub commonly known as Kalimanakeeri in Tamil is generally found in India\textsuperscript{[2,3]}. The plant is reported to show hepatoprotective effect\textsuperscript{[4]}, anthelmintic activity\textsuperscript{[5]}, and membrane stabilizing properties and antipyretic activity\textsuperscript{[6]} and diuretic activity\textsuperscript{[7]}. It has been reported that the plant contains saponins, flavonoids, glycosides, phenolic compounds, alkaloids and tannins\textsuperscript{[8]}. In folklore practice, some of the tribes of Andhra Pradesh, India use the decoction of the leaf part of the plant for the treatment of diabetes mellitus\textsuperscript{[9]}. Hence, in the present study, we report the antihyperglycemic activity, in vitro antioxidant effect of ECR.

**Materials and Methods**

**Plant collection**

The leaf of *Cansjera rheedii* have been collected from Sri Venketeswara University near Tirupati, Andhra Pradesh during the month of December 2009 and dried under shade. The plant was authentified by Mr. K. Madhava chetty, Assistant Professor, Department of Botany of S. V. University, Tirupati. The voucher specimen (IT-P-08-S5) of the plant was deposited at the college for further reference.

**Preparation of extracts**

Leaves of *Cansjera rheedii* were shade dried and the dried leaves were powdered to get coarse granules. The coarse powder was subjected to continuous hot extraction in Soxhlet apparatus using different solvents like ethanol, methanol and chloroform. The solvents were removed by distillation under reduced pressure, which produced a greenish sticky residue (yield 30\%w/w with respect to dried plant material). The concentrated crude extract were stored and used for the further study.
Preliminary phytochemical screening

The extracts were investigated for various phytochemical constituents such as alkaloids, carbohydrates, Phyto sterols, Flavonoids, Saponins and gums. These extract were used for the further studies\cite{10, 11}.

Animals used

Adult male wistar rats weighing about 180-200g were taken for the study. Male rats were chosen to avoid fluctuations due to estrous cycle (Sugioka et al., 1987). The rats were housed in under standard laboratory conditions with 12 h light/dark cycle. The rats were fed with standard laboratory chow (Hindustan Lever Ltd., Mumbai) and water ad libitum. Animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were carried out between 900 and 1200h. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref No. IAEC / XIII / 05 / SVCP /2009 - 2010).

Acute Toxicity Study

The acute toxicity of ECR was determined as per the OECD guideline no.423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, 1/20th (100mg/kg) and 1/10th (200mg/kg) of this dose were selected for further study\cite{12}.
Induction of Diabetes mellitus with Alloxan

After fasting for 18 hrs the rats were injected with alloxan (1000mg/kg) intraperitonally in saline which resulted in decrease of endogenous insulin release and caused less utilization of glucose by tissues \cite{13, 14}. Diabetic rats with plasma glucose level above 150mg/dl were included in the study.

Experimental design

The rats were segregated into five groups of six rats in each group. All the groups were induced with diabetes except the control group. The standard used drug was Glibenclamide and was given to another group. Rats were considered diabetic when the blood glucose level was raised beyond 250 mg/dl of blood. This condition was observed at the end of 48 h after Alloxanisation.

Group I : Normal control

Group II : Diabetic Control and rats received only vehicle (2 ml/kg oral)

25% Tween 80

Group III : Rats received Ethanol Extract of *Cansjera rheedii*

(200 mg/kg/day oral) suspended in 2% v/v Tween 80

Group IV : Rats received Ethanol Extract of *Cansjera rheedii*

(400 mg/kg/day oral) suspended in 2% v/v Tween 80

Group V : Rats received Glibenclamide (2.5 mg/kg oral) suspended in

2% v/v Tween 80
**Invivo antidiabetic activity of Cansjera rheedii**

The Invivo Antidiabetic method ([Dash et al., 2001]^[15^] was followed. The test samples were suspended in 2% v/v Tween 80 in distilled water. Glibenclamide (2.5 mg/kg) was used as reference control during the study. All the test samples were administered through oral route.

**Single dose study**

**In normoglycaemic rats**

The rats were fasted for 18 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn (0.1 ml) from the tip of the tail (Caudal Vein) of each rat under mild ether anaesthetic condition. Plasma was separated following centrifugation the glucose was estimated by using Glucose estimation kit from One touch ultra, Life Scan, Johnson and Johnson, Milpitas, C.A., U.S.A. The normal rats were then divided into three groups of six rats each. Groups III and IV received the test extract at a dose of 200 and 400 mg/kg, respectively, through oral route. Group V received Glibenclamide (2.5 mg/kg) and served as reference control. All the test samples were administered in a similar manner. Blood glucose levels were examined after 3, 7, 14 and 24 hr of administration of single dose of test samples.

**In Alloxan induced diabetic rats**^[16^]

The acclimatized rats were kept fasting for 24 h with water *ad libitum* and injected intraperitonally a dose of 120 mg/kg of Alloxan monohydrate in normal saline.
After 1 h, the rats were provided feed *ad libitum*. The blood glucose level was checked before Alloxanisation and 24 h after Alloxanisation.

**Multidose study**

**In Alloxan induced diabetic rats.**

The selected rats were treated with similar kind of test samples as above, but the blood glucose level was measured on 0, 5, 10, and 15 days of treatment.

**Estimation of lipid profile**[17]

Estimation of Lipid profile such as Total Cholesterol, Triglycerides, and HDL & LDL levels.

**Invivo antioxidant activity of Cansjera rheedii**

**Scavenging of nitric oxide radical**[18]

Nitric oxide radical inhibition can be estimated by the use of Griess Ilosvay reaction. The procedure is based on the method, where sodium nitroprusside in aqueous solution at physiological p$^\text{H}$ spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM, 4ml) in phosphate buffered saline p$^\text{H}$ 7.4 (1ml) was mixed with different concentration (100 – 500 µg/ml) of drug were dissolved in ethanol and incubated at 25$^0\text{C}$ for 150min. The same reaction mixture without the drug but the equivalent amount of ethanol serves as control. After the incubation period, add
0.25ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotization. Then, 0.25ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink colored chromophore was formed. The absorbance of chromophore was read at 540nm. Inhibition of nitrite formation by the drug and standard ascorbic acid were calculated with respect to control. Inhibition data (% inhibition) were linearized against the concentration of drug and standard antioxidant. IC\text{50} is an inhibitory concentration of each sample required to reduce 50% nitric oxide formation was determined. The absorbance for different concentrations of ECR was determined using UV spectrophotometer at 540 nm.

**Statistical Analysis**

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet’s test p values less than 0.05 were considered as significance.

**Results**

**Phytochemical screening**

The results of preliminary phytochemical screening of chloroform extract of *Cansjera rheedii* revealed that presence of alkaloids, saponins and triterpenoids. Ethanol extract revealed the presence of reducing sugar, steroids, saponins, tannins, glycosides, flavonoids, gums and mucilage. Methanol extract showed the presence of reducing sugar, steroids, tannins, flavonoids, gums and mucilage.
Effect of ECR on blood glucose level

There was an observable changes in BGL and lipid profile of treated and untreated rats. Treatment of diabetic rats with ethanol extract of *Cansjera rheedii* and Glibenclamide significantly decreased the BGL compared to untreated diabetic rats. Dose dependent reduction in BGL, TC and TG was observed in Alloxan induced diabetic rats treated with ethanol extract of *Cansjera rheedii*.

Single dose study

After single dose of the ECR (200 or 400 mg/kg, oral) on the Alloxan induced diabetic rats, there was a significant reduction (P>0.01) in BGL of the diabetic rats with in the period of acute study which was seven hours compared to the control. The effect was significant like the standard drug, Glibenclamide. ECR at the dose of 400 mg/kg body weight exhibited a better BGL reduction (70.74%) than 200 mg/kg body weight (68.7%) and that produced by the standard drug, Glibenclamide 2.5mg/kg (71.42%) at the same period.

Multidose study

During prolonged study (14 days), the ECR (200 or 400 mg/kg) produced a significant (P>0.01) in BGL of the diabetic rats compared to control. ECR at the dose of 400 mg/kg body weight exhibited better BGL reduction (74.39%) than 200 mg/kg body weight (65.74%) and that produced by the standard drug, Glibenclamide 2.5mg/kg (75.77%) at the same period.
Serum lipid profile

Beneficial effects of ECR on serum lipids, one of the major cardiovascular risk factors in type 2 diabetes mellitus, can be observed from lipid-related data (Table 6.5). Compared with the control values, the ECR (200 or 400 mg/kg) groups showed significant reduction (P>0.01) in the serum levels of total cholesterol and triglycerides.

Table: 1. Effect of Cansjera rheedii on blood glucose levels of Alloxan induced diabetic rats after a single dose

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drugs</th>
<th>Dose</th>
<th>Initial</th>
<th>3hr</th>
<th>5hr</th>
<th>7hr</th>
<th>24hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Diabetic control</td>
<td>2% Tween 80 w/v soln</td>
<td>326.33±1.26</td>
<td>275±1.77</td>
<td>276±1.39</td>
<td>281.5±2.03</td>
<td>294±1.37</td>
</tr>
<tr>
<td>Group II</td>
<td>Diabetic control + ECR</td>
<td>200 mg/kg</td>
<td>324.3±2.26**b</td>
<td>200.33±1.49**b</td>
<td>157.50±2.80**b</td>
<td>134.37±2.14**b</td>
<td>113.82±1.49**b</td>
</tr>
<tr>
<td>Group III</td>
<td>Diabetic control + ECR</td>
<td>400 mg/kg</td>
<td>333.3±1.83**b</td>
<td>194±1.77**b</td>
<td>149.17±2.10**b</td>
<td>109.17±2.94**b</td>
<td>92.12±2.17**b</td>
</tr>
<tr>
<td>Group IV</td>
<td>Diabetic control + Glibenclamide (2.5 mg/kg)</td>
<td>333.5±1.94**a</td>
<td>151.83±1.22**a</td>
<td>139.17±1.70**a</td>
<td>100.67±2.49**a</td>
<td>84.667±1.89**a</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01 and ns-non significant. Significance compared within the groups as follows: a. diabetic + ECR - 200 & 400 treated rats compared with diabetic control rats. b. diabetic + Glibenclamide treated rats compared with diabetic control rats.
Table: 2. Effect of Cansjera rheedii on blood glucose levels of diabetic induced rats, after a prolonged treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drugs</th>
<th>Dose</th>
<th>Initial</th>
<th>Fifth day</th>
<th>Tenth day</th>
<th>Fifteenth day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal control</td>
<td>1 ml of 2% Tween 80 w/v soln</td>
<td>91.5±1.73</td>
<td>91.5±1.33</td>
<td>90.5±1.31</td>
<td>90.83±1.42</td>
</tr>
<tr>
<td>Group II</td>
<td>Diabetic control</td>
<td>1 ml of 2% Tween 80 w/v soln</td>
<td>326.33±3.00</td>
<td>286.32±2.24</td>
<td>284.14±1.94</td>
<td>282.46±2.17</td>
</tr>
<tr>
<td>Group III</td>
<td>Diabetic control + ECR</td>
<td>200 mg/kg</td>
<td>324.3±3.31</td>
<td>254.6±9.30</td>
<td>198.66±7.96</td>
<td>116.83±1.56***</td>
</tr>
<tr>
<td>Group IV</td>
<td>Diabetic control + ECR</td>
<td>400 mg/kg</td>
<td>333.33±3.64**</td>
<td>265.16±5.83**</td>
<td>151.5±6.7**</td>
<td>106.83±0.87***</td>
</tr>
<tr>
<td>Group V</td>
<td>Diabetic control + standard Glibenclamide (2.5 mg/kg)</td>
<td>333.5±2.94**</td>
<td>106.66±1.82***</td>
<td>102.32±1.48***a</td>
<td>90.00±2.32**a</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01 and ***p<0.001 and ns non significant. Significance compared within the groups as follows: a. diabetic + ECR - 200 & 400 treated rats compared with diabetic control rats. b. diabetic + Glibenclamide treated rats compared with diabetic control rats.

Table: 3. Effect of Cansjera rheedii on lipid profile of Alloxan induced diabetic rats after a prolonged treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>Diabetic control</th>
<th>Diabetic control + ECR-200</th>
<th>Diabetic control + ECR-400</th>
<th>Diabetic control + standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>185.3±1.8</td>
<td>340±0.67**a</td>
<td>245.3±1.58**b</td>
<td>226.5±1.54**b</td>
<td>195±1.51**c</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>138.5±2.17</td>
<td>205.2±1.16**a</td>
<td>185.7±1.43**b</td>
<td>164.2±1.68**b</td>
<td>144.2±1.56**c</td>
</tr>
<tr>
<td>HDL</td>
<td>75.83±1.67</td>
<td>26.83±0.60**a</td>
<td>37.17±0.74**a</td>
<td>40.33±1.14**b</td>
<td>43.67±1.56**c</td>
</tr>
<tr>
<td>LDL</td>
<td>85±1.59</td>
<td>255.3±2.3**a</td>
<td>200.5±1.43**a</td>
<td>174.3±1.22**b</td>
<td>133.8±1.56**c</td>
</tr>
<tr>
<td>VLDL</td>
<td>27.5±0.76</td>
<td>65.3±1.95**a</td>
<td>37.33±0.88**b</td>
<td>34.3±0.66**b</td>
<td>30±0.88**c</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01 and ns non significant. Significance compared within the groups as follows: a. Normal control rats compared with diabetic control rats. b. diabetic + ECR - 200 & 400 treated rats compared with diabetic control rats. c. diabetic + Glibenclamide treated rats compared with diabetic control rats.
Table 4. Antioxidant activity of ECR by nitric oxide scavenging radical method

<table>
<thead>
<tr>
<th>Samples</th>
<th>Avg OD (nm)</th>
<th>Radical scavenging activity (%)</th>
<th>IC$_{50}$ values(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>0.312</td>
<td>79</td>
<td>18.9</td>
</tr>
<tr>
<td>ECR</td>
<td>0.297</td>
<td>53</td>
<td>28.3</td>
</tr>
</tbody>
</table>

Histopathological studies

The histopathological studies of isolated pancreas were conducted at Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh. The following photographs show the slides isolated pancreas.
Preliminary phytochemical screening of the ECR reveals the presence of reducing sugars, steroids, saponins, tannins, glycosides, flavonoids, gums and mucilage. The antihyperglycemic effect of ECR in alloxan induced diabetic rats was shown in Table 1, 2. A significant reduction (P < 0.05) in blood glucose levels was observed at 3, 5, 7 and 24th hr of post administration of ECR (200 mg/kg and 400 mg/kg b.w.) in the diabetic rats. The ECR also showed a significant decrease of total cholesterol levels and triglycerides as resulted in the Table 3. So, it was also found to exhibit antihyperlipidemic activity.

In the present study, ECR was also investigated for its antioxidant activity using nitric oxide radical scavenging method shown in Table 4. Increased absorbance of the extract indicates the increased reducing power. The percentage inhibition of free radicals by ECR was found to be effective when compared with the standard Ascorbic acid and hence the extract was found to possess the significant antioxidant activity. The IC50 values of ECR and reference standard were found to be 28.3µg/ml and 18.9µg/ml respectively.
Discussion

The possible sources of oxidative stress in the pathogenesis of diabetes and diabetic complications have been extensively studied for years both in animal models and in clinical setting. Certain studies have found increased lipid peroxides or ROS and oxidative stress (or both) in different animal models of diabetes. There are different models for inducing diabetes in animals. In the present study we selected alloxan induced model. Alloxan, a chemical diabetogen causes diabetes through its ability to destroy the insulin producing beta cells of the pancreas. In vitro studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis. During its cytotoxic action, alloxan is reduced into dialuric acid via alloxan radical formation in the presence of glutathione. During this redox cycling process, reactive oxygen species are formed that destroy beta-cells in islets of Langerhans.

The present study results suggested that the ECR exhibit significant antihyperglycemic activity in alloxan induced diabetic rats. Blood glucose level in diabetic rats is an important basal parameter for monitoring diabetes and it has shown that the ECR causes the antihyperglycemic effect by reducing the blood glucose level. The ECR showed a dose dependent reduction in blood glucose levels and this hypoglycemic effect was compared with that of standard oral hypoglycemic agent, glibenclamide. This decrease in blood glucose levels may be due to presence of flavonoids and phytosterols in the ECR as these constituents may be responsible for antidiabetic action as revealed in the previous literature.
The significant decrease in the levels of blood glucose in diabetic rats treated with the ECR may be, by increasing insulin level or by other mechanisms. It is also well known that in uncontrolled diabetes mellitus, always there is an increase in total cholesterol, triglycerides and LDL levels associated with decrease in HDL and contribute to coronary artery disease\cite{19}. In the present study, the total cholesterol, triglycerides and LDL levels was increased in diabetic control groups and it was reduced in 15 days treatment with ECR. After the treatment with ECR the HDL cholesterol levels was significantly increased. This suggests that the ECR may also inhibit the pathway of cholesterol synthesis and increases the HDL/LDL ratio.

The antioxidant activity reflected by the nitric oxide radical scavenging method was clearly observed in ECR in dose dependent manner. This suggests that the physico chemical nature of the flavonoids in the extract may be important in the antioxidant activity. The antioxidant property was well correlated with the concentration of the extract, which showed the presence of active principles in the extract.

In histopathological study, the light microscopic photograph of Islets from control rat appeared circular with the granulated beta cells appearing darker. Small and shrunken islets and destruction of beta cells were observed in the diabetic rats. Well formed islets and increased cell number were observed in diabetic rats, after ECR therapy. The data presented in electron micrograph of the beta cells of normal and treated rats showed an evidence for increased secretory granule synthesis and thereby increased insulin secretion after the administration of ECR suggesting the possible regeneration/repair of the Islets of Langerhans in Alloxan induced rats.
In diabetes, there is always a relationship between glucose homeostasis, lipid metabolism, renal function and enzyme activities. We found that a 15 day administration of *Cansjera rheedii* showed effectiveness in controlling diabetics when compared with diabetic rats treated with standard drug (Glibenclamide). Ethanolic extract of *Cansjera rheedii* was thus proved to have a hypoglycemic effect on Alloxan induced diabetic rats. As a result, there was an increase in insulin level, which brought about homeostasis in the above mentioned biochemical parameters such as Cholesterol, triglycerides, HDL and LDL levels. Diabetes mellitus is a global burden as its incidence is considered to be high (4–5%) all over the world. However, quest for the development of more effective antidiabetic agents is being pursued relentlessly\cite{12}. Recently, herbal products have started gaining importance as complementary and alternative medicine to treat diabetic mellitus [20, 21].

Hence a proper remedy for diabetes mellitus has to be found before the need reaches to its culmination. Though, many herbal products have been described for the treatment of diabetic mellitus, very few of them have been explored scientifically so far. The existing antidiabetic drugs encounter many adverse effects and need on prolonged treatment including questionable efficacy in the treatment. This forces the area of research to find improved treatments which will counteract the adverse effects and draw backs of the existing treatment. The study of such medicines might offer a natural key to unlock a diabetologist’s pharmacy for the future.

**Conclusion**

The present study indicated a significant dose dependent anti-diabetic effect for the Ethanolic extract of *Cansjera rheedii* (200 and 400 mg/kg) and supports its traditional
usage in the control of diabetes. It is also concluded that the extract have strong antioxidant potential activity by invitro studies. Although the present data do not clearly indicate the mechanism of action, the strong antihyperglycemic and antihyperlipidemic effect observed in Alloxan-induced diabetic rats justifies the use of Cansjera rheedii for the treatment of diabetes and related complications. Further studies are necessary to isolate and identify the active principles as well as identify possible links between ethanol extract of Cansjera rheedii Gmelin. and plant’s chemical composition and pharmacological investigations of the constituents, which are responsible for the pharmacological activity reported traditionally and its exact mechanism of action.

References

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