Anti Diabetic Effect of *Rhinacanthus nasutus* Leaf Extract in Streptozotocin Induced Diabetic Rats

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Abstract: The effect of the leaf extract of *Rhinacanthus nasutus* in normal and Streptozotocin (STZ) induced diabetic rats was studied. Blood glucose levels were determined after oral administration of graded doses of *R.nasutus* (200 mg/kg) in fasted normal and diabetic groups. In both groups, 200 mg/kg of the extract, significantly reduced blood glucose levels 8 h after administration, which was consistent and time-dependent. The higher doses of 100 and 300 mg/kg did not affect significantly the blood glucose levels as preliminary experiments conducted earlier. The plant extract (methanolic) is given to the diabetic rats as well as normal rats orally by gavages. The blood glucose levels were increased when diabetes is induced in all the experimental rats. The methanolic extract of *R. nasutus* action was moderate in reducing the higher blood glucose levels in diabetic when compare with the normal rats. The normal rats' blood glucose levels did not change much when treated with plant extract alone. The plant extract was capable of ameliorating at the dosage of 200 mg/kg, hyperglycaemia in streptozotocin-induced diabetic rats and could be a potential source for isolation of new orally active agent(s) for anti-diabetic therapy.

**Key words:** *Rhinacanthus nasutus* %Streptozotocin %Hypoglycemia %Diabetes

**INTRODUCTION**

Diabetes mellitus is a group of disorders with different aetiologies and it is characterized by derangements in carbohydrate, protein and fat metabolism caused by the complete or relative insufficiency of insulin secretion or insulin action [1]. Diabetes mellitus is also associated with long-term complications, including retinopathy, nephropathy, neuropathy and angiopathy and several others [2]. Medicinal plants are frequently considered to be less toxic and free from side effects than the synthetic ones. The world health organization has also recommended that this should be encouraged, especially in countries where conventional treatment of diabetes seems insufficient [3]. *Rhinacanthus nasutus* (L.) CURZ (Acanthaceae), a shrub with a height of 1 - 2 meters and widely distributed in South China and India. It is commonly called as Nagamalli, Kaligai, Anichi in Tamil, Nagamalli in Telugu, Doddapatika in kannada, Jupani in Hindi, Yuthikaparni in Sanskrit and Gajakarni in Marathi [4]. The plant has been recently cultivated for use in the treatment of hepatitis, diabetes, hypertension and skin diseases in Taiwan [5, 6]. The decoction of its roots or whole plant is drunk for treatment of some cancer forms [7]. It has long been used in Thai traditional medicine for treatment of many diseases like pruritic rash, ringworm, abscess pain, skin diseases [8] and the plant extracts possess inhibitory activity against different species of bacteria and fungi [9]. The aim of our study was to investigate the hypoglycemic effect of Methanolic extract of *Rhinacanthus nasutus* leaves in streptozotocin (STZ) induced diabetic rats.

**MATERIALS AND METHODS**

**Plant Materials:** The fresh leaves of *Rhinacanthus nasutus* were collected from Tirumala Hills, Tirupati, Chittoor district of Andhra Pradesh in the month of July - October and identified by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V.University, Tirupati.

**Preparation of Extract:** Fresh leaves of *Rhinacanthus nasutus* (L) were shade dried and milled to fine powder using a mechanical grinder. The powdered plant material was macerated and shaken in methanol for 48 h using a
bath shaker. The extract was then filtered with filter paper (Whatman No. 1) and concentrated to dryness under reduced pressure using rota evaporator at 40°C. The concentrate was then layered on aluminum foil and freeze dried for further use [10].

**Chemicals:** Streptozotocin was purchased from Sigma (Germany) and all other chemicals and reagents used in this study were of analytical grade. Glibenclamide was purchased from a local drug store.

**Experimental Design:** 90 Days old male albino Wistar rats (150-180 g) obtained from Sri Venkateshwara Traders, Bangalore and maintained in Animal House, Department of Biotechnology, Sri Venkateswara University, Tirupati. The animals were fed on a standard pellet diet and water ad libitum. They were maintained in a controlled environment (12 h/12 h light/dark) and temperature (30±2°C). The animals were acclimatized to the laboratory conditions for one week before starting the experiment.

The rats were divided into 5 groups comprising of 6 animals in each group as follows:

- **Group I:** Normal rats (Controls).
- **Group II:** Diabetic untreated rats.
- **Group III:** Normal + Plant extract treated rats.
- **Group IV:** Diabetic+ Plant extract treated rats.
- **Group V:** Diabetic+ Glibenclamide treated rats.

**Induction of Experimental Diabetes:** Streptozotocin was freshly dissolved in citrate buffer (0.01M, pH 4.5) and maintained on ice prior to use. The overnight fasted rats were made type 2 diabetes with a single intraperitoneal injection of STZ (50 mg/kg) [11]. Rats with plasma glucose levels above 250 mg/dL were considered as severe diabetic and were used in the experiment.

**Measurement of Blood Glucose Levels:** The blood was drawn from the tail and measured the blood glucose levels by using Accuchek glucometer. The body weights of the rats were also recorded.

**Statistical Analysis:** All values are presented as the means ± SD. Statistical comparisons of the differences were performed using one way analysis of variance for repeated measures combined with the Newman-Keuls post hoc test. P values below 0.05 were considered statistically significant.

**RESULTS**

A significant decrease in body weight (p<0.05) was observed in diabetic rats (Group II) when compared to the control rats (Group I). In Group III, the normal animals given plant extract showed slight changes in body weights and Group IV diabetic animals significantly decreased in body weight and recovered with the treatment of methanolic extract of *Rhinacanthus nasutus* leaf. Group V animals were given with a standard drug Glibenclamide and compared all the results. The blood glucose levels also significantly increased in Group II animals whereas we have considered all the rats above 250 mg/dL were diabetic and the blood glucose levels were nearer to the normal when they are treated with plant extract and Glibenclamide. The body weights and the blood glucose levels of all the groups are expressed and showed in Table 1 and Table 2 respectively.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 15</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>190.83±4.35</td>
<td>193.16±4.35</td>
<td>197.33±3.77</td>
<td>206.66±5.57*</td>
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<tr>
<td>Diabetic</td>
<td>197.83±2.31</td>
<td>194.66±3.26</td>
<td>185.16±3.54</td>
<td>163.25±1.70</td>
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<td>Normal + PE</td>
<td>194.33±5.39</td>
<td>196.33±3.38</td>
<td>201.66±3.66</td>
<td>211.50±3.93</td>
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<tr>
<td>Diabetic + PE</td>
<td>194.50±4.84</td>
<td>191.50±4.59</td>
<td>185.50±3.93</td>
<td>192.16±3.92</td>
</tr>
<tr>
<td>Diabetic + Gli</td>
<td>195.16±6.04</td>
<td>193.16±6.17</td>
<td>186.83±7.98</td>
<td>193.50±4.37</td>
</tr>
</tbody>
</table>

Values are calculated as mean ± SD for six rats in each group.

<table>
<thead>
<tr>
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<th>Day 7</th>
<th>Day 15</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>81.33±5.88</td>
<td>82.83±6.08</td>
<td>86.16±5.19</td>
<td>87.83±2.130</td>
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<td>Diabetic</td>
<td>251.5±4.99</td>
<td>275.16±8.79</td>
<td>295.66±7.47</td>
<td>293.75±8.220</td>
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<td>Normal + PE</td>
<td>93.83±4.07</td>
<td>92.83±4.11</td>
<td>91.5±3.720</td>
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<td>Diabetic + PE</td>
<td>247.33±5.64</td>
<td>240.16±5.34</td>
<td>216.16±4.07</td>
<td>178.83±12.62</td>
</tr>
<tr>
<td>Diabetic + Gli</td>
<td>242.5±4.28</td>
<td>221.33±4.88</td>
<td>204.5±3.670</td>
<td>158.83±5.110</td>
</tr>
</tbody>
</table>
**DISCUSSION**

Diabetes is one of the leading diseases around the globe. Management of Diabetes is being a tough task with the organic medicines as they have many side effects. The interest has been increased on the medicinal plants used for remedy or reducing the risk of diseases. In the recent scenario the scientists have glance into the herbal extracts to observe their effective and protective role in the diseased animal models. The present results indicate significant decrease in body weight and raise in blood glucose levels in diabetic rats and they became normal when treated with the plant extract. This suggests that the plant *Rhinacanthus nasutus* protective role in reducing glucose levels as well as in increasing body weight. This study confirms the earlier findings that *R. nasutus* leaf extract has anti-diabetic effect [5, 6]. The anti-hyperglycemic effect of the extract on the fasting blood sugar levels of diabetic rats is shown in Table 2. Administration of Streptozotocin (50 mg/kg, i.p.) led to 1.5-fold elevation of fasting blood glucose levels, which was maintained over a period of 4 weeks. Four weeks of daily treatment of methanolic extract of *Rhinacanthus nasutus* led to a dose-dependent fall in blood sugar levels by 25-68%. Effect seems to reach maximum after 15 days of treatment and remains constant in third week and fourth week effect is also exponential. Vehicle control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 30 days (Table 1).

**REFERENCES**