Nephro-protective Effect of Aqueous Extract of *Syzygium cumini* Seed on Streptozotocin Induced Diabetes in Rats

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Introduction

Diabetes mellitus (DM) is a metabolic disorder. Hyperglycemia, hyperlipidemia, polyuria, glycosuria, polyphagia, polydipsia, weight gain, negative nitrogen balance and ketonemia are the major symptoms of DM. It can develop due to damage to beta-cells of pancreas, insulin resistance, down regulation of insulin receptors on cells.1,2 DM increase risk of several serious health problems include nephropathy, retinopathy, hepatopathy, cardiomyopathy. These major complications can increase the risk of death.3 Early control and regular medication is recommended to improve patient life span and prevent the complications. Different classes of drugs available to control plasma glucose level in diabetic patients. This drugs cause development of several adverse effects and cannot cure DM permanently.4 Several plant extracts are used in the treatment of DM. *Syzygium cumini* (*S. cumini*) is a medicinal plant, used for the treatment of diabetics in different parts of India. According to literature *S. CUMINI* seed powder is used for diabetes, diarrhoea, ringworm, teeth and gum problems, liver problems and infections and hyperlipidemia.5,6,7 The present study conducted to evaluate the neproprotective activity of aqueous extract of *S. cumini* in STZ induced diabetes rats.

Abstract: To evaluate the neproprotective effect of aqueous extract of *Syzygium cumini* (*S.C*) seed in diabetic rats. Wister Albino male rats weighing 230-250gm selected in for study. 30 rats were divided into 5 groups. G-I (Control) normal saline was administered. 24 rats were administered streptozotocin to induce diabetes. After 72hr blood glucose was measured. Rats has more 300mg/dl glucose level selected and divided in to 4 groups. G-II-diabetic control, G-III- Streptozotocin (45mg/kg/i.p/0day) + Glibenclamide (5mg/kg/orally/120days), G-IV-Streptozotocin (45mg/kg/i.p/0day) + Aqueous extract of *Syzygium cumini* seeds (250mg/kg/orally/120 days) and G-V- Streptozotocin (45mg/kg/i.p/0day) + Aqueous extract of *Syzygium cumini* seeds (500mg/kg/orally/120days). Before inducing diabetes all groups’ rats’ serum Creatinine and urea levels were measured. Drugs were administered 120 days. On 120th day serum Creatinine and urea were measured and compared. Histopathological changes in kidney also observed for all the groups. Diabetic group rats showed increased in serum Creatinine and urea levels compared to other groups. High dose seed extract and standard oral hypoglycemic drugs showed significant decrease in Creatinine and urea levels compared to diabetic control and low dose seed extract administered groups. It was observed that aqueous extract of *S.C* seed extract showed significant neproprotective effect compared to other groups. The results are equal to standard drug. This seed powder extract can use to treat patients having kidney problems along with diabetes. But more studies required arriving therapeutic dose and other pharmacological effects.

Key Words: Aqueous extract, Creatinine, Neproprotective, Streptozotocin, *Syzygium cumini*, Urea

Materials and Methods

Animals

Wister Albino male rats weighing of 230-250gm of rats was included in the study. The animals was maintained at temperature of 25±1°C and provided diet and water ad libitum. Animal house and care of animals maintained as per CPCEA guide lines. The study carried out after getting ethical clearance from Institutional Animal Ethical Committee, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamil Nadu.8

Preparation of aqueous extract of *S. cumini* seed extract

The *S. cumini* fruits were collected from local market. Fruits were cleaned and seeds were separated. The seeds were authenticated with the help of botanist at the Department of Botany, Annamalai University. Dried seeds were grinded in electrical grinder to make fine powder. 200gm of powder was distilled in 400ml of water. It was stirred intermittently overnight at room temperature. Whole volume filleted by using filter paper. The filtrate was evaporated to dryness under reduced pressure in a rotary evaporate. At the end dark brown semi solid extract was stored and used for study.9

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Study settings and design

Study was done in department of pharmacology, Annamalai University.

Group-I: Normal control (Normal Saline)
Group-II: Diabetic control (Streptozotocin 45mg/kg/i.p)\textsuperscript{10}
Group-III: Diabetic control (Streptozotocin 45mg/kg/i.p/0day) + Glibenclamide (5mg/kg/orally/120 days)\textsuperscript{11}
Group-IV: Diabetic control (Streptozotocin 45mg/kg/i.p/0day)+ Aqueous extract of Syzygium cumini seeds (250mg/kg/orally/120 days)\textsuperscript{11}
Group-V: Diabetic control (Streptozotocin 45mg/kg/i.p/0day)+ Aqueous extract of Syzygium cumini seeds (500mg/kg/orally/120 days)\textsuperscript{12}

Procedure

Total 30 rats were included in the study. 6 rats were kept in control groups received normal saline. 24 rats received freshly prepared Streptozotocin (45mg/kg) in 0.1ml citrate buffer pH 4.5 solution intra-peritoneal route in a volume of 0.1ml/kg. To avoid hypoglycemia rats allowed drink glucose (5%) solution given over night. Blood samples were collected after 72 hours. Animals have glucose level 300mg/dl were selected and divided in to 4 groups of each of 6 rats. G-II serves as diabetic control, G-III serves standard group, G-IV and V serves test-I and test-II. All drugs were administered to respective groups for 120 days (See study design). 0 day and 120\textsuperscript{th} day blood was collected and serum was separated and stored at 4\textdegree C.\textsuperscript{13} The stored serum was used to estimate creatinine and urea levels. All the groups’ rats were sacrificed under anesthesia and kidney was isolated stored in 10% formalin solution. Small piece of kidney used for histopathological examination. Kidney histopathology slides were prepared according standard procedure.

Statistical analysis

The data analysed by SPSS (0.6 version) to find statistical significant between the groups. ANOVA (Post hoc test) followed by Sheff's t test applied to find statistical significant at 95% confidence interval. P value less than 0.05 considered statistically significant.

Results

Control rats showed less creatine and urea levels compared to standard and test groups. STZ administered group showed maximum increase in creatine and urea levels compared to other groups. STZ treated groups showed significant difference compared to Glibenclamide and aqueous extract of S. cumini seed powder (500mg/kg). Graded doses of plant extract do not show any significant difference. Administration high dose of seed extract showed equality with standard drug. Administration of standard and high dose plant extract significantly decreased creatine and urea levels by 120\textsuperscript{th} day compared to other groups (Table-1). Histopathological observations showed almost normal kidney cells in group-I, III and V. Hypertrophic, necrotic cells in II and IV group rats.

Table 1: Effect of aqueous extract of S.C seed extract on Creatinine and urea levels in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Creatinine (µmol/L) (MEAN±SEM)</th>
<th>Serum Urea (µmol/L) (MEAN±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day 120\textsuperscript{th} day</td>
<td>0 day 120\textsuperscript{th} day</td>
</tr>
<tr>
<td>Group-I</td>
<td>42.33±2.80 45.00±2.45</td>
<td>35.33±4.13 32.50±3.80</td>
</tr>
<tr>
<td>Group-II</td>
<td>44.17±2.86 46.33±2.73</td>
<td>31.83±5.74 35.00±4.47</td>
</tr>
<tr>
<td>Group-III</td>
<td>45.00±1.79 44.00±1.79*</td>
<td>32.17±5.81 32.33±5.61*</td>
</tr>
<tr>
<td>Group-IV</td>
<td>43.50±3.94 45.17±2.14</td>
<td>33.83±5.42 33.83±5.31</td>
</tr>
<tr>
<td>Group-V</td>
<td>43.67±2.58 44.67±2.58*</td>
<td>34.00±5.55 33.17±4.22*</td>
</tr>
<tr>
<td>(*P&lt;0.05 significant compared group-II with other groups)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Histopathological study of group-I kidney
Normal kidney showing glomeruli (\textsuperscript{\rightarrow}) and tubules (H & E 10X)

Figure 2: Histopathological study of group-II group kidney
Diabetic kidney showing increased epithelial and mesangial cell proliferation and cloudy swelling (\textsuperscript{\rightarrow}) and tubules (H & E 10X)
Figure 3: Histopathological study of group-III kidney

Diabetic + glibenclamide treated kidney showing normal glomeruli (→) with normal tubules (H & E 10X)

Figure 4: Histopathological study of group-IV kidney

Diabetic + Syzygium cumini (250mg/kg) treated kidney showing normal glomeruli (→) with normal tubules (H & E 10X)

Figure 5: Histopathological study of group-V kidney

Diabetic + Syzygium cumini (500mg/kg) treated kidney showing normal glomeruli (→) with normal tubules (H & E 10X)

Discussion

The present study aimed to evaluate the nephroprotective effect of aqueous extract of *S. cumini* seed powder in STZ diabetic rats. STZ administered rats had acute dysfunction as evidence by increase in Creatinine and urea levels. Treatment with aqueous extract of *S. cumini* seed powder 500mg/kg for 120days significantly decrease the blood levels of Creatinine and urea when compared to STZ, low dose plant extract treated group. It was supported by histopathological observations. *S. cumini* seed extract may conation antioxidants can neutralize the STZ induce oxidative stress in beta cells. The study results suggest that the aqueous extract of *S. cumini* seed powder showed nephroprotective activity and the dose level. Extensive and multiple animal studies required to elucidate the exact mechanism of action on active ingredients in plant extract.

References


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**Conflict of interest:** None Declared