Evaluation of anti-Diabetic Activity for Ethanoic extract of Syzygium cumini leaf in Dexamethasone induced diabetic rats

Chattu Maheswararao
Department of Pharmacology, Yalamarty Pharmacy College, Yalamarty Nagar, Tarluvada, Visakhapatnam-530052, India

ABSTRACT
Syzygium cumini is commonly known as insulin plant in India. Consumption of leaves of this plant are believed to lower blood glucose level in healthy normal and diabetic individuals. The present study was planned to evaluate the effect of the leaves of syzygium cumini leaves on dexamethasone induced hyperglycemic rats. Male Wistar rats (n=6) were treated with 10mg/kg of dexamethasone subcutaneously for 20 days from 11th day to 20 days. Different groups received 100mg/kg plant extract in distilled water and glibenclamide 500µg/kg per orally on plasma blood glucose level, serum total cholesterol, triglyceride level, HDL, LDL and Serum VLDL were observed. Dexamethasone caused an increase blood glucose level, serum total cholesterol, serum triglyceride level, Serum HDL, Serum LDL and Serum VLD the and with compare with normal control [*P<0.01]. In the dexamethasone model 100mg/kg p.o. of Ethanolic extract of syzygium cumini leaf showed significant decrease in blood glucose level, serum total cholesterol, serum triglyceride level, Serum HDL, Serum LDL and Serum VLD when compared to dexamethasone control[l.*P<0.01]. The study results concluded Syzygium cumini proved to be effective in treatment of Type-II Diabetes mellitus owing to its ability to decrease insulin resistance.

Keywords: diabetic mellitus, Dexamethasone, Glibenclamide, syzygium cumini leaf

INTRODUCTION
The Diabetogenic effect of exogenous or endogenous Glucocorticoid excess results in part from the development of peripheral insulin resistance [1], a wherein insulin fails to normally stimulate glucose uptake into skeletal muscle, the main site of insulin-mediated glucose disposal [2]. Glucocorticoid induced insulin resistance is attributed mainly to a post receptor defect of insulin action [3,4]. About 90% of type 2 diabetes is attributable to excess weight. Further-more, approximately 197 million people worldwide have impaired glucose tolerance, most commonly because of obesity and the associated metabolic syndrome [4]. It is projected that one in three American adults will have diabetes in 2050 if this trend continues [5]. This form of diabetes is characterized by insulin resistance and at least initially, a relative lack of insulin secretion. Most individuals with type 2 diabetes exhibit abdominal obesity which itself causes insulin resistance. In addition, hypertension, dyslipidemia (high triglyceride levels and low HDL-cholesterol levels), and elevated inhibitor plasminogen activator-1 (PAI-1) levels are often present in these individuals. This clustering of abnormalities is referred to as the “insulin resistance syndrome” or the “metabolic syndrome.” Because of these abnormalities, patients with type 2 diabetes are at increased risk of developing macro vascular complications [6]. Syzygium cumini (L.) Skeels, a polyembryonic species (family-Myrtaceae), is a tropical fruit tree of great economic importance [7]. It is a large evergreen tree up to 30 meters height and girth of 3.6 meters with a bole up to 15 meters. S. cumini has been widely used forth treatment of various diseases in traditional and folk medicine. Unani system of medicine describes the use of the plant in liver tonic, enrich blood, strengthen teeth and gums and form good lotion for removing ringworm infection of the head [8]. The leaves are antibacterial and used to strengthen the teeth and gums. The leaves have also been extensively used to treat diabetes, constipation, leucorrhoea, stomachaalgia, fever, gastropathy, stranger, dermopathy and to inhibit blood discharge in the feces [9]. It has been also showed before that the leaf, bark, stem and pulp of S. cumini plants possess potent antidiabetic activity [10]. The major phytoconstituents are reported to contain vitamin C, gallic acid, tannins, anthocyanins, includes cyanidin, petunidin, malvidinglucoside and other components [11]. Preliminary phytochemical analysis also showed the presence of phenols, terpenoids, tannins, saponins, phytosterols, carbohydrates, flavonoids, amino acids in stem bark of S. cumini [12]. Previous investigations also revealed the bark of S. cuminis contains butulnic acid, β-sitosterol, friedelin, epi-friedelanol [13]. It also contains new esters of epifriedelanol(eugenin), D-glucoside, kaempertor-3-O-glucoside, quercetin, myricetin, astragalin and Gallic acid [14]. The present study was designed to investigate the phytochemical...
bioactive compounds of the Ethanolic extract of S. cumini leaves to establish its antidiabetic activity.

**Materials and methods**

**Drugs and chemicals**

Dexamethasone and all other reagents used were of analytical grade. Diagnostic kits used in this study were procured from Span Diagnostics Ltd., India, NR Chemicals, Mumbai and Excel diagnostics Ltd., India.

**Plant material**

The leaves of Syzygium cumini were collected from Guntur and were shade dried. Powdered and extracted in Macronization process successively with Ethanol respectively due to their nature of polarity. After extraction, the ethanol extracts were filtered through Whatman No.1 filter paper and stored for further use.

**Preparation of Extraction**

The powdered plant material (400 g) was first defatted with Ethanolic solvent and then macerated at room temperature (24-26°C) with Ethanolic (850 mL) for 4 days with occasional shaking, followed by re-maceration with the same solvent for 3 more days. The macerates were combined, filtered and distilled off in reduced pressure. The resulting concentrate was vacuum dried at 40°C. The dry extract was kept in a vacuum desiccators until use. Preliminary phytochemical studies on Ethanolic extract of syzygium cumini leaf revealed the presence of alkaloids, triterpenoids, steroids and tannins. The percentage yield of the extract was calculated by using the formula below:

\[
\% \text{ yield} = \frac{\text{weight of extract}}{\text{weight of plant material}} \times 100\%
\]

**Phytochemical screenings**

The leaf extracts of Syzygium cumini were analyzed for the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoids, cardiac glycosides and tannins according to standard methods [15].

**Experimental design**

The antidiabetic activity of Ethanolic extract of S. cumini leaf was assessed in normal, glucose loaded and Dexamethasone induced diabetic rats. In all studies, the animals were fasted overnight for 16 h with free access to water throughout the duration of the experiment.

**Animals used**

Experiments were performed with male wistar rats procured from Albino Research & Training institute (Hyderabad, Andhra Pradesh, India), weighing about 200-230 g. The animals were housed in individual polypropylene cages under standard laboratory conditions of light, temperature (23 ± 1°C) and relative humidity for at least one week before the beginning of experiment, to adjust to the new environment and to overcome stress possibly incurred during transit. Animals were given standard rat pellets and drinking water ad libitum. The animals were fasted 12 hours before the conduct of experiment and during the experiment they were withdrawn from food and water. The experiments were planned after the approval of Institutional Animal Ethical Committee (Approval number is 1722/RO/ERe/S/13/CPCSEA).

**Acute toxicity studies**

Acute oral toxicity study was performed as per OECD-421 guidelines (acute toxic class method). Albino rats (n=6) of either sex selected by random sampling techniques were employed in this study. The animal were kept fasting for overnight providing only water. Then the extracts (Leaf) were administered orally at the dose of 800 mg/kg by intragastric tube and observed for 2 days for the gross behavioral changes and mortality [12].

**Methodology**

**Methods**

1. **Dexamethasone – induced diabetes mellitus [16]**

Thirty Male Wistar rats weighing 200-250 gm were randomly divided into 4 groups of 5 each and kept in their cages for 10 days prior dosing to allow for acclimatization to the laboratory conditions.

Group 1 served as normal control; group 2, 3, 4, received Dexamethasone 10mg/kg/day subcutaneously for 10 days; on day 11, after overnight fasting, retro-orbital puncture was performed to obtain blood sample for estimation of lasting and postprandial blood sugar. Only those rats whose fasting and postprandial blood glucose levels were higher than those of the normal controls were utilized for further study. From day 11 to day 20, group 2, 3, 4, continued to receive dexamethasone 10mg/kg/day subcutaneously. Group 3 received 100mg/kg/day of Syzigium cumini pant leaf powder in 1ml of distilled water per oral, in addition to dexamethasone in addition to dexamethasone. Group 4 received Glibenclamide 50µg/kg per oral, in addition to dexamethasone.

On the 20th day, after overnight fasting retro orbital puncture was done on the left eye to obtain blood for estimation of fasting blood glucose using autoanalyzer. Immediately after this, a glucose load for estimation of postprandial blood glucose levels

Group 1: Administered vehicle serves as Normal control.
Group 2: Administered Dexamethasone (10mg/kg s.c.) Serves as diabetic control
Group 3: Diabetic rats treated with Dexamethasone (10mg/kg, s.c. once daily) [Dexamethasone (10mg/kg, s.c.) + Syzigium cumini 100mg/kg p.o.]
Group 4: Administered Reference standard, Glibenclamide (500µg/kg, p.o. once daily) [Dexamethasone (10mg/kg, s.c.) + Glibenclamide (500µg/kg, p.o.)]

On day 21, rats were sacrificed and serum was analyzed for serum triglycerides, serum cholesterol, serum HDL-cholesterol, serum LDL-cholesterol, serum VLDL-cholesterol, and serum glucose.

**Statistical analysis**
The results were represented as Mean ± SD. The statistical significance was computed using One Way ANOVA followed by Tukeys multiple comparison test and compared with diabetic control group with Standard drug, fig ;1.2 where the n=6 animals in each group were used.** P <0.01 was considered statistically significant.

**Result:**

Table 1: Effect of administration of Syzygium cumini leaf extract (100 mg/kg, p.o) + Glibenclamide (500µg/kg, p.o) for 21 days on Blood Glucose levels in diabetic rats

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT</th>
<th>GLUCOSE LEVEL AT 11th DAY</th>
<th>GLUCOSE LEVEL AT 16th DAY</th>
<th>GLUCOSE LEVEL AT 21st DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group – 1</td>
<td>Control (Normal control)</td>
<td>106 ±1.61</td>
<td>99.86 ± 1.56</td>
<td>95.58 ± 1.32</td>
</tr>
<tr>
<td>Group – 2</td>
<td>Diabetic control</td>
<td>287.53 ±5.51*</td>
<td>274.93± 9.31**</td>
<td>270.6 ± 9.16**</td>
</tr>
<tr>
<td>Group – 3</td>
<td>Syzigium cumini leaf extract (100 mg/kg , p.o)</td>
<td>294.13 ±15.41*</td>
<td>199.73±36.50**</td>
<td>150.06±4.48**</td>
</tr>
<tr>
<td>Group - 4</td>
<td>Glibenclamide (500 µg/kg , p.o)</td>
<td>285.43 ±5.48*</td>
<td>189.65±34.65**</td>
<td>145.58±5.49**</td>
</tr>
</tbody>
</table>

Value expressed in MEAN ±SEM ,n=6 Experimental groups statically compared with control groups where significant *p<0.05, moderately significant **p<0.01  All the values are compared with the Dexamethasone control group.

Fig -1: Effect of administration of Syzigium cumini leaf extract (100 mg/kg, p.o) + Glibenclamide (500 µg/kg, p.o), for 21 days on serum blood glucose levels in diabetic rats
Chattu et al., antidiabetic activity of Syzygium cumini

Table 2: Effect of administration of Syzygium cumini leaf extract (100 mg/kg, p.o) + Glibenclamide (500 µg/kg, p.o), for 21 days on serum Total cholesterol, Triglycerides, HDL, LDL VLDL levels in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SERUM CHOLESTEROL(mg/dl)</th>
<th>SERUM TRIGLYCERIDES(mg/dl)</th>
<th>SERUM HDL(mg/dl)</th>
<th>SERUM LDL(mg/dl)</th>
<th>SERUM VLDL(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>90.76±2.45</td>
<td>60.78±0.009</td>
<td>49.90±0.021</td>
<td>20.89±0.025</td>
<td>17.35±0.042</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>146.1±6.41**</td>
<td>156.45±0.171**</td>
<td>29.32±0.020*</td>
<td>84.23±0.026*</td>
<td>37.64±0.017*</td>
</tr>
<tr>
<td>Syzygium cumini leaf(100mg/kg)</td>
<td>100.05±3.57**</td>
<td>67.81±0.030**</td>
<td>54.90±0.013*</td>
<td>34.64±0.036*</td>
<td>22.58±0.014*</td>
</tr>
<tr>
<td>Glibenclamide (500µg/kg)</td>
<td>94.9±3.88**</td>
<td>62.91±0.018**</td>
<td>51.28±0.014*</td>
<td>30.66±0.035*</td>
<td>19.11±0.019*</td>
</tr>
</tbody>
</table>

Value expressed in MEAN ±SEM ,n=6 Experimental groups statically compared with control Groups Where *significant *p<0.05,  moderately significant **p<0.01   All the values are compared with the Dexamethasone control group.

DISCUSSION

In Dexamethasone control group there was significant increase in blood glucose levels (p<0.05) when compared to the vehicle control. Ethanol extract of syzygium cumini leaf (100mg/kg p.o) increased blood glucose level as compared with normal control. Glibenclamide (500µg/kg.p.o) increased blood glucose level. On 16th day collecting with blood sample retro orbital puncture in estimation of blood glucose and compare with normal control diabetic control increase in blood glucose level .plant extract of syzygium cumini leaf significantly reducing blood glucose level (**p<0.01),glibenclamide (5µg/kg p.o) show as significant action in reducing the blood glucose level (**P<0.01. )21st day collecting blood sample in retro orbital puncture and then centrifugation and collecting the plasma to estimation of blood glucose level. Plant extract of syzygium cumini leaf(100mg/kg p.o_ show as significant reducing the blood glucose level and compare in dexamethasone control **P<0.01)Glibenclamide (5µg/kg p.o) show as significant reducing blood glucose and compare to dexamethasone control group**P<0.01).The values of blood glucose levels are shown in the Table No.1

Fig -2: Effect of administration of Syzygium cumini leaf extract (100 mg/kg, p.o) Glibenclamide (5 µg/kg, p.o), for 21 days on serum Total cholesterol, Triglycerides, HDL, LDL VLDL levels in diabetic rats

Fig 2: Effect of administration of Syzygium cumini leaf extract (100 mg/kg, p.o) Glibenclamide (5 µg/kg, p.o), for 21 days on serum Total cholesterol, Triglycerides, HDL, LDL VLDL levels in diabetic rats

Fig -2: Effect of administration of Syzygium cumini leaf extract (100 mg/kg, p.o) Glibenclamide (5 µg/kg, p.o), for 21 days on serum Total cholesterol, Triglycerides, HDL, LDL VLDL levels in diabetic rats

Fig -2: Effect of administration of Syzygium cumini leaf extract (100 mg/kg, p.o) Glibenclamide (5 µg/kg, p.o), for 21 days on serum Total cholesterol, Triglycerides, HDL, LDL VLDL levels in diabetic rats

Fig -2: Effect of administration of Syzygium cumini leaf extract (100 mg/kg, p.o) Glibenclamide (5 µg/kg, p.o), for 21 days on serum Total cholesterol, Triglycerides, HDL, LDL VLDL levels in diabetic rats
Serum Triglycerides (mg/dl) level increase (156.45±0.017mg/dl) in the untreated dexamethasone induced rats compares to the normal control rats(60.78±0.009 mg/dl) after treatment with the (100mg/kg p.o) dose of syzygium cumini leaf. Shown a significant increase in the Triglycerides' level compared with the normal control animals .Glibenclamide( 5µg/kg p.o) a significant activity in increase the Triglycerides level. Serum HDL level decrease (29.32±0.02 mg/dl) diabetic control and compare to normal control rats (49.90±0.02 mg/dl) after treatment with the (100mg/kg p.o) dose of syzygium cumini leaf. Shown a significant increase in the serum HDL level compared with the normal control animals .Glibenclamide( 5µg/kg p.o) a significant activity in increase the Serum HDL level. The values of serum HDL levels are shown in the Table No 2.

Serum LDL (mg/dl) level increase 84.23±0.026mg/dl in the untreated dexamethasone induced rats compares to the normal control rats(17.35±0.042 mg/dl) after treatment with the (100mg/kg p.o) dose of syzygium cumini leaf. Shown a significant increase in the LDL level compared with the normal control animals .Glibenclamide( 5µg/kg p.o) a significant activity in increase the LDL level. Serum VLDL (mg/dl) level increase (37.64±0.017mg/dl) in the untreated dexamethasone induced rats compares to the normal control rats(156.45±0.017mg/dl) after treatment with the (100mg/kg p.o) dose of syzygium cumini leaf. Shown a significant increase in the serum VLDL level compared with the normal control animals .Glibenclamide( 5µg/kg p.o) a significant activity in increase the serum VLDL level. The values of serum VLDL levels are shown in the Table No 2.

Conclusion

These observations concluded that the grains extract of the plant Syzygium cumini leaf possesses insulin resistance activity. The Syzygium cumini showed significant and dose dependent decrease in blood glucose levels, triglyceride levels and Total cholesterol, HDL, LDL and VLDL.

References


