Antidiabetic and Antihyperlipidamic Activity of the Ethanolic leaf Extract of Glycosmis pentaphylla against Alloxan Induced Diabetes in Albino Rats

N.Lal Mahammed  
Department of pharmacology, KLR Pharmacy college, KLR Coloney, Paloncha-507115, Khammam (Dist),Andhra Pradesh, India

G.Jyothi  
Department of pharmacology, KLR Pharmacy college, KLR Coloney, Paloncha-507115, Khammam (Dist),Andhra Pradesh, India

P.Inamul Hasan  
Department of pharmacology, KLR Pharmacy college, KLR Coloney, Paloncha-507115, Khammam (Dist),Andhra Pradesh, India

ABSTRACT

Leaf powder of Glycosmis pentaphylla was subjected to hot extraction (soxhlet) with ethanol. After preliminary phytochemical investigation ethanolic extract of the leaves of Glycosmis pentaphylla was evaluated for anti diabetic and antihyperlipidemic activity against alloxan-induced diabetes in albino rats. The ethanolic extracts showed significant activity as compare to standard glipizide.

INTRODUCTION:
Glycosmis pentaphylla (Rutaceae) is commonly called as orange berry, juice of leaves, is a shrub or small (1.5–5 m) tree widely distributed from India, Malaysia and southern China to the Philippine Islands where it occurs in tropical forests at low altitudes. It has been used as a folk medicine in the treatment of fever, liver complaints and certain other diseases (Sastri, 1956). In Hindu medicines, it has been used traditionally in bilious complaints, cough, worms, jaundice and fever. This drug has been proved by CCHR, monograph of which has also been published. This drug is proved to be effective in bilious complaints like nausea, vomiting, bitter taste in the mouth, heart burn with desire for lime juice, which ameliorates and gastric symptoms are aggravated by eating. Migraine-pain in temples. Juice of leaves is used in fever, liver complaints and as a vermifuge, while leaves are considered good antidote for eczema and other skin troubles. Here, the experiment of leaves has been evaluated for anti diabetic activity and antihyperlipidemic against alloxan induced model using standard drug glibenclamide.

MATERIALS AND METHODS:
The leaves of Glycosmis pentaphylla (Retz) DC were collected from the local forest areas around the Tirupati, A.P, India, and authenticated by the botanist Dr. Madhava Chetty K., Department of botany, Sri Venkateswara University, Tirupati, A.P, India. All the solvents and chemicals were of analytical grade and procured from Merck Ltd., Ambarnath and Alloxan monohydrate was obtained from SD Fine Chemicals Pvt. Ltd. Biosar.

PREPARATION OF EXTRACT:
The leaves were dried under shade and then powdered and stored in airtight container. The shade dried leaves of Glycosmis pentaphylla was powdered (1000g) and extracted with ethanol (95.99%), in 1:3 ratios in soxhlet apparatus exhaustively for 20-24 hours. The extract was concentrated to dryness under reduced pressure and controlled temperature (40-50°C) using flash evaporator and a semisolid extract was obtained, which was used in the study.

ANIMALS:
Male Sprague Dawley rats, aged 4 months (body weight: 160 ± 10g) were used for the present study, procured from Mahaveer enterprises, Hyderabad, India. The animals were housed in poly acrylic cages (30 cm × 23 cm × 10 cm) with not more than six animals per cage, and maintained under standard conditions (27±2°C, relative humidity 44 - 56% and light and dark cycles of 10 and 14 hours respectively) and fed with standard rat diet and purified drinking water ad libitum for 1 week before and during the experiments. All experiments and protocols described in present study were approved by the Institutional Animal Ethical Committee (IAEC) of KLR Pharmacy College, Paloncha, Khammam (No.1516/P0/a/11/CPCEA).

ANTIDIABETIC ACTIVITY
Hyperglycemia / diabetes was induced by single Intrapertoneal injection of freshly prepared aqueous solution of alloxan monohydrate 150 mg/kg, to overnight fasted rats. After 48 hrs of alloxan injection, the animals which did not developed hyperglycemia i.e glucose level >200mg/dl, were rejected/replaced with new animals. Immediately after confirmation of diabetes, rats were classified into five groups of six rats each. Standard drug used for treatment, Glipizide, 10 mg/kg, ethanolic test extract were prepared, 400mg/kg and 800mg/kg in 2% Carboxy Methyl Cellulose (CMC) and were given orally. Taking six rats in each five groups did evaluations of antidiabetic effect. Treatment was continued for 28 consecutive days, with twice a day dose (morning and evening). Before the treatment (0day) and at the end of 7th,14th 21st and 28th day, blood samples were collected from the tip of the tail of each rat under mild ether anesthesia in 1 ml Eppendorf tubes containing 50μl of anticoagulant (heparin)1,2,3,4. Serum separated by centrifugation of blood at 4000 rpm for 10mins was subjected for estimating glucose by Glucose oxidase method using semi auto analyzer. It was done with 1 ml of blood withdrawn on 28th day, from all five groups rats (normal, diabetic control, extracts of 400, 800mg/kg and standard treated) and stored in a refrigerator until analyzed. And the serum was subjected for the estimation of triglyceride (TGL), HDL, LDL, VLDL and total cholesterol level 5,6,7,8,9,10.

RESULTS AND DISCUSSION:
Diabetes induction caused significant (P<0.001) hyperglycemia in all diabetic groups (Table 1). Oral administration of the extract and Glipizide for 28 days significantly (P<0.001) lowered the hyperglycemia of the experimental groups, when compared to diabetic control group. Fasting blood glucose levels of untreated diabetic rats were significantly higher than those in normal rats. Over production of glucose by means of excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental bases of hyperglycemia in diabetes mellitus. The fasting blood glucose of the groups treated with plant extract (400mg/kg, 800mg/kg body weight) lowered the glucose level from 191 mg/dl to 152 mg/dl and 197 mg/dl to 138 mg/dl and Glipizide from 192 mg/dl to 116 mg/dl representing 20.67%, 29.94% and 39.32% reductions respectively. The effect on the fasting blood glucose is dose dependent (Table 1 & 2). The proposed mechanism of action may be by promoting regeneration of β-cells or by protecting the cells in pancreas from destruction, by restricting glucose load as well as by promoting unrestricted endogenous inulin action and further effect β-cells to release insulin and activate the inulin receptors to absorb the blood sugar. Regeneration of islet β-cells following
### Table No: 1
The effect of 28th day treatment with ethanolic extract of Glycosmis pentaphylla on blood glucose levels (mg/dl) in Alloxan induced diabetes in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose levels (mg/dl) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>I- Normal control</td>
<td>91.33 ± 3.50</td>
</tr>
<tr>
<td>II- Diabetic control (Alloxan 130mg/kg)</td>
<td>292.66 ± 7.75a</td>
</tr>
<tr>
<td>III- Diabetic + STD (Glibizide 10mg/kg)</td>
<td>116.50 ± 0.20a</td>
</tr>
<tr>
<td>IV- Diabetic + GP (400mg/kg)</td>
<td>159.50 ± 4.10a</td>
</tr>
<tr>
<td>V- Diabetic + GP (800mg/kg)</td>
<td>139.83 ± 7.94a</td>
</tr>
</tbody>
</table>

All values given in average blood glucose levels (mg/dl) shown are Mean ± SEM and n=6  Data was analyzed using one way ANOVA and Dunnett’s t-test. *P<0.05, **P<0.01, ***P<0.001 ns=non significance.

### Table No: 2
The effect of ethanolic extract of Glycosmis pentaphylla on oral glucose tolerance in normal and alloxan induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Triglycerides (mg/dl) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day</td>
</tr>
<tr>
<td>I- Normal control</td>
<td>87.50 ± 3.06</td>
</tr>
<tr>
<td>II- Diabetic control (Alloxan 130mg/kg)</td>
<td>198.16 ± 3.47a</td>
</tr>
<tr>
<td>III- Diabetic + STD (Glibizide 10mg/kg)</td>
<td>192.00 ± 4.30a</td>
</tr>
<tr>
<td>IV- Diabetic + GP (400mg/kg)</td>
<td>191.83 ± 6.87a</td>
</tr>
<tr>
<td>V- Diabetic + GP (800mg/kg)</td>
<td>197.00 ± 6.24a</td>
</tr>
</tbody>
</table>

All values given in average triglyceride levels (mg/dl) shown are Mean ± SEM and n=6  Data was analyzed using one way ANOVA and Dunnett’s t-test. a=P<0.001,b=P<0.01

### Table No: 3
Showing triglyceride levels (mg/dl) (Mean ± SEM) (n=6) in different groups of rats compared to normal control.

### Table No: 4
Showing total cholesterol levels (mg/dl) (Mean ± SEM) (n=6) in different groups of rats compared to normal control.

### Table No: 5
Showing HDL-c levels (mg/dl) (Mean ± SEM) (n=6) in different groups of rats compared to normal control.

### Table No: 6
Showing LDL-c levels (mg/dl) (Mean ± SEM) (n=6) in different groups of rats compared to normal control.

### Table No: 7
Showing SGPT (U/L), SGOT (U/L) levels (Mean ± SEM) (n=6) in different groups of rats compared to normal control.
Table No: 8 The effect of 28th day’s treatment with ethanol-ic extract of plant Glycosmis pentaphylla on body weight (g) after alloxan induced diabetes in rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>BODY WEIGHTS (gm)</th>
<th>% of increased body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>I- Normal control</td>
<td>170.33 ± 3.74</td>
<td>188.83 ± 3.67</td>
</tr>
<tr>
<td>II- Diabetic control (Alloxan 130mg/kg)</td>
<td>176.16 ± 1.55 ns</td>
<td>226.83 ± 2.52 a*</td>
</tr>
<tr>
<td>III- Diabetic + STD (Glipizide 10mg/kg)</td>
<td>167.83 ± 4.30 ns</td>
<td>200.50 ± 2.59 b***</td>
</tr>
<tr>
<td>IV- Diabetic + GP (400mg/kg)</td>
<td>165.66 ± 3.69 ns</td>
<td>175.66 ± 2.29 a**</td>
</tr>
<tr>
<td>V- Diabetic + GP (800mg/kg)</td>
<td>162.83 ± 2.31 b*</td>
<td>180.50 ± 2.39 b***</td>
</tr>
</tbody>
</table>

All values given in average body weight (g) shown are mean ± SEM and n=6 Data was analyzed using one way ANOVA and Dunnett’s t-test. a=P<0.001, b=P<0.01, ns=non significance.

Fig: 1 Showing blood glucose levels (mg/dl) (Mean ± SEM), (n=6) in different groups of rats compared to normal control.

Fig: 2 Showing OGGT blood glucose levels (mg/dl) (Mean ± SEM), (n=6) in different groups of rats compared to normal control.

Fig: 3 Showing triglyceride levels (mg/dl) (Mean ± SEM), (n=6) in different groups of rats compared to normal control.

Fig: 4 Showing total cholesterol levels (mg/dl) (Mean ± SEM), (n=6) in different groups of rats compared to normal control.

Fig: 5 Showing HDL-c levels (mg/dl) (Mean ± SEM), (n=6) in different groups of rats compared to normal control.

Fig: 6 Showing LDL-c levels (mg/dl) (Mean ± SEM), (n=6) in different groups of rats compared to normal control.

Fig: 7 Showing SGOT levels (U/L) (Mean ± SEM), (n=6) in different groups of rats compared to normal control.
Fig:8 Showing SGPT levels (U/L) (Mean ± SEM) (n=6) in different groups of rats compared to normal control.

Fig:9 Showing body weights (gm) (Mean ± SEM) (n=6) in different groups of rats

SGPT levels

![Graph showing SGPT levels](image1)

Body weight

![Graph showing body weight](image2)

**Figures:**

- **Fig:8** SGPT levels (U/L) (Mean ± SEM) (n=6) in different groups of rats compared to normal control.
- **Fig:9** Body weights (gm) (Mean ± SEM) (n=6) in different groups of rats.

**Discussion:**

Destruction by alloxan may be the primary cause of the recovery. As the ALX (130 mg/kg, i.p.) treated diabetic rats exhibited clear cut abnormalities in lipid metabolism as evidenced from the significant elevation of serum TG, TC, LDL-c, and reduction of HDL-c levels. Treatment with EEGP for 28 days was sufficient to produce a significant reduction in the TG, TC, LDL-c, and significant increase in HDL-c levels in diabetic rats. These results indicate that EEGP has a lipid lowering effect on the diabetic rats.

Loss in body weight was observed in alloxan-induced diabetic rats and was controlled by treatment with Glycosmis pentaphylla fraction. Administration of GP to diabetic (Group IV and V) rats resulted in an increase in body weight compared to diabetic rats (Group II). Results suggested that ethanolic extract of the Glycosmis pentaphylla treatment has positive effect on maintaining body weights in diabetic rats. The protective effect of plant fraction on body weight of diabetic rats may be due to its ability to reduce hyperglycemia. A gradual increase in body weights of Glipizide treated groups (Group III) was similar to that of normal control rats. Alloxan induced diabetes was characterized by severe loss of body weight due to increased muscle wasting in diabetes.

**Acknowledgement:**

The authors are thankful to the Management and Principal, KLR Pharmacy college, paloncha for providing the facilities to carry out this study.

**Reference:**