Antidiabetic Potential of *Gmelina asiatica* Linn. on alloxan induced diabetic rats

G. Jothi, D. Ramachandran and P. Brindha.

Department of Biochemistry, Srimad Andavan Arts and Science College, No.7, Nelson Road, Thiruvanaikovil, Tiruchirappalli, Tamil Nadu-600 005, India.

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Corresponding author:
Dr. P. Brindha
E-mail: brindhajana@yahoo.co.in

Abstract

**Background & Objectives:** The present study was aimed to evaluate the antidiabetic potential of *Gmelina asiatica* Linn, against alloxan induced diabetes mellitus in albino rats.

**Methods:** Diabetes mellitus was induced by intraperitoneal injection of alloxan monohydrate (150mg/Kg body weight). After induction of diabetes, animals were treated with aqueous extract of *Gmelina asiatica* L. (200, 300 and 400mg/kg body weight orally) and glibenclamide (600ng/Kg body weight orally) for a period of 45 days. After the experimental period animals were sacrificed, blood and tissue samples were collected and analyzed to various biochemical, enzymatic and histopathological parameters viz, blood sugar, glycosylated haemoglobin, insulin, urea, uric acid, creatinine, lipid profile, glucokinase, glucose-6-phosphatase and glucose-6-phosphate dehydrogenase.

**Results:** Aqueous extract of the plant decreases the blood sugar, glycosylated haemoglobin, urea, uric acid & creatinine level significantly and it also restored the lipid profile. Beta cells secretory activity was also found to be improved as evidenced by an increase in serum insulin concentration. Light microscopic study of pancreatic tissue showed significant rejuvenation and regeneration.

**Interpretation & Conclusion:** In the present work, antidiabetic potential of *Gmelina asiatica* L. is validated scientifically by evaluating the biochemical profiles of drug treated as well as diabetic groups. It revealed a significant increase in serum insulin level and had the potential of reverting the glucose metabolizing enzyme levels as well as lipid profile.

**Key words:** Antidiabetic, Glucokinase, Glycosylated haemoglobin, *Gmelina asiatica*, Triglycerides.

Introduction

Diabetes mellitus is a chronic metabolic disorder, characterized by profound hyperglycemia (1). Prolonged treatment of diabetes mellitus with synthetic hypoglycemic agents and insulin can produce side effects. Therefore, remedies by products have been tried to treat diabetes (2), as they are considered to be less toxic and free from side effects than synthetic drug.

Many herbal formulations are mentioned for the treatment of diabetes mellitus in Indian Materia medica as well as in traditional systems of medicines belonging to various parts of the world (3). *Gmelina asiatica* Linn, commonly called in tamil as 'Nilakkumzh' or 'Mulkumizh', belonging to the family Verbenaceae is selected for the present work. It is a scandent shrub, with small ovate leaves and bright yellow flowers. Fruit is a drupe obvoid, or ovoid. It is widely distributed in all districts of Tamil Nadu and South Western India (4). This is also used as 'Gambhari', one of the ingredient in 'Dasamoola' by the Indian medical practitioners of Tamil Nadu (5). 'Gambhari' is also used by Ayurvedic practitioner to reduce cholesterol and triglyceride (6). Hypoglycemic and antihyperglycemic effect of alcoholic extract of *Gasiaticus* root has been evaluated by analyzing the blood glucose level in 1, 2, 4, 6, 8 and 16 hrs after treatment (7). Though alcoholic root extract of *Gmela asiatica* Linn. has been studied for its antidiabetic potential by Kasivisvanath R et al there is no detailed reports available regarding the antidiabetic potential of aerial parts. Hence, the present work was carried out to evaluate scientifically the antidiabetic potential of aqueous extract of aerial parts of *Gmelina asiatica* Linn.
Materials and Methods

Plant material
Fresh plants of *Gmelina asiatica* L. were collected from in and around Tiruchirappalli, Tamilnadu, during the month of September, identified and authenticated with the herbarium specimen of RAPINAT Herbarium of St. Joseph's College, Tiruchirappalli, Tamilnadu.

Preparation of aqueous extract
The aerial parts of authenticated drug were collected, shade dried and powdered coarsely. The coarse powder was boiled in five times of water (200g/1000ml water) till the decoction was reduced to one third. Reduced decoction thus obtained was filtered and the filtrate was evaporated to dryness. The residue obtained was stored and used for pre clinical experiments. The percentage yield of the aqueous extract is 28%.

Experimental animals
Wistar strain of albino rats weighing 100g-150g were obtained from Tamilnadu Veterinary and Animal Sciences University, Chennai. They were fed with standard pellet diet and water *ad libitum*. Animals were maintained in a standard animal house. The experiments were designed and conducted according to the ethical guidelines of CPCSEA after obtaining the necessary clearance from the committees (Approval No: 790/03/ac/CPCSEA).

Induction of diabetes in rats
Animals were allowed to fast for 24 hours prior to the induction of diabetes mellitus. Freshly prepared alloxan monohydrate was injected to a batch of normoglycemic albino rats intraperitoneally at a dose level of 150mg/Kg of body weight (8). After two days of alloxan induction, animals with blood glucose level >250mg/dl were chosen for the studies.

Experimental Design
Rats were divided into six groups of six rats each.

- **Group-I**: Normal untreated rats
- **Group-II**: Diabetic control
- **Group-III**: Diabetic rats treated with 200mg/Kg bw of aqueous extract of *G. asiatica*L for 45 days daily.
- **Group-IV**: Diabetic rats treated with 300mg/Kg bw of aqueous extract of *G. asiatica*L for 45 days daily.
- **Group-V**: Diabetic rats treated with 400mg/Kg bw of aqueous extract of *G. asiatica*L for 45 days daily.
- **Group-VI**: Diabetic rats treated with 600µg/Kg bw of glibenclamide for 45 days daily.

At the end of the experimental period, the animals were sacrificed by cervical decapitation. Blood was collected and used for various biochemical estimations. Pancreas was excised, weighed and processed for histological studies and liver was collected for biochemical analysis.

Biological assays
Blood glucose was analyzed by Glucose oxidase / peroxidase method (9). Serum insulin was assayed by Chemiluminescent immunometric method obtained from Diagnostic Product Corporation, Los Angeles, USA. Glycosylated Haemoglobin was estimated by the method of Wang and Yang (10). Glucose -6- phosphate dehydrogenase was analyzed by the method of Baquer (11). Glucose -6- phosphatase was estimated by King method (12,13) and glucokinase by the method of ATP : D-hexose-6-phosphotransferases (14). Serum total cholesterol and HDL cholesterol were estimated using cholest erol oxidase / peroxidase method (15) while triglycerides were estimated by glycerol phosphate oxidase / peroxidase method (16). Serum urea (17), uric acid (18) and creatinine (19) were also analyzed.

Light microscopic studies
Small slices of the pancreas were fixed in Bouin’s fluid (20), dehydrated using graded alcohols and embedded in paraffin wax. Sections (4-5μm) were cut and stained with haematoxylin and eosin. The slides were then viewed under light microscope.

Statistical analysis
All the results were expressed as mean± SD. The data were statistically analyzed by one-way ANOVA. The p values <0.05 were considered as significant.

Results
The data of the results obtained, proved the antidiabetic potential of the aqueous plant extract of *Gmelina asiatica* L. Peritoneal injection of alloxan monohydrate produced severe diabetes in rats. Table 1 shows the levels of blood glucose, glycosylated hemoglobin and serum insulin. Group-II animals, which served as disease control, showed significant (p<0.05) increase in blood glucose and glycosylated hemoglobin (HbA1C) and a decrease in serum insulin level. In contrast, groups of animals treated with aqueous extract of *Gmelina asiatica* for 45 days showed a profound decrease in blood glucose and HbA1C level (p<0.05). Serum insulin level improved significantly in the highest dose given (400mg/Kg bw) compared to lower dose (200mg/Kg bw). Animals treated with reference drug also showed a significant reduction in blood glucose and HbA1C level compared to group V (p<0.05).

The effect of *Gmelina asiatica* L. on glucose metabolizing enzymes in liver viz glucokinase, glucose-6-Phosphatase and glucose-6-phosphate dehydrogenase are summarized in Table 2. A marked increase (p<0.05) in the activity of glucokinase and glucose-6-phosphate
dehydrogenase was seen in plant extract treated animals when compared to diabetic control. Glucose-6-phosphatase activity has decreased in *Gmelina asiatica* L. treated animals and is dose dependent.

Table 3 and 4 showed that the test drug *Gmelina asiatica* L. decreased serum cholesterol, triglyceride, urea, uric acid and creatinine. HDL cholesterol level was found to be decreased in disease control group of animals and on treatment with test drug, the HDL level was found to be elevated significantly (p<0.05).

**Histological analysis**

In eosin stained sections, the islets of disease control showed a profound distortion and vacuolation (Fig. 2), compared to normoglycemic rats (Fig. 1). Islets of plant drug treated animals showed marked regeneration and improvement in the architecture of the islets tissues. The nuclei of cells of treated islets were also clear when compared to diseased animals (Figs. 3, 4 & 5). There is a mild variation in the histoarchitecture of glibenclamide treated diabetic rats (Fig. 6).

**Table 1:** Blood glucose, glycosylated haemoglobin and serum insulin levels of untreated and treated rats after oral administration of plant aqueous extract for 60 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Blood Sugar (mg/dl)</th>
<th>Serum insulin (iu/ml)</th>
<th>Glycosylated haemoglobin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>87.56±0.4</td>
<td>7.4 ±1.4</td>
<td>2.43 ± 0.36</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>305±1.1a*</td>
<td>11.8 ± 0.4 a*</td>
<td>5.6 ± 0.1 a*</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic+Plant extract treated (200mg/Kg bw)</td>
<td>194.9 ± 1.4b*</td>
<td>15.3 ± 0.1b*</td>
<td>4.5 ± 0.12b*</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic+Plant extract treated (300mg/Kg bw)</td>
<td>108 ± 0.6b*</td>
<td>19.4 ± 0.8b*</td>
<td>3.7 ± 0.5b*</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic+Plant extract treated (400mg/Kg bw)</td>
<td>89 ± 1.2b*</td>
<td>26.4 ± 0.3b*</td>
<td>2.98 ± 0.7b*</td>
</tr>
<tr>
<td>VI</td>
<td>Diabetic+Glibenclamide treated (600µg/Kg bw)</td>
<td>79 ± 1.3c*</td>
<td>14.2 ± 0.4c*</td>
<td>3.08 ± 0.5c*</td>
</tr>
</tbody>
</table>

**Values are mean ±SD (n = 6); a- Group I vs. Group II, b- Group II vs. Group III, IV & V, c- Group V & VI. *P < 0.05.**

**Table 2:** Activity of glucokinase, glucose-6-phosphatase and glucose-6-phosphate dehydrogenase in untreated and treated rats after oral administration of aqueous plant extract for 60 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Glucokinase (µmoles of Glucose-6-phosphate formed min/mg protein)</th>
<th>Glucose-6-Phosphatase (µmoles of Pi liberated/min/mg protein)</th>
<th>Glu-6-phosphate dehydrogenase (µmoles of NADPH formed/min/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>130 ± 1.03</td>
<td>7.76 ± 0.5</td>
<td>11.4 ± 1.02</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>96 ± 1.2 a*</td>
<td>14.72 ± 1.3 a*</td>
<td>9.23 ± 0.7 a*</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic+Plant extract treated (200mg/Kg bw)</td>
<td>109 ± 0.6c*</td>
<td>11.32 ± 0.4c*</td>
<td>9.97 ± 0.10c*</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic+Plant extract treated (300mg/Kg bw)</td>
<td>119 ± 0.98c*</td>
<td>9.45 ± 0.12c*</td>
<td>9.46 ± 0.12c*</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic+Plant extract treated (400mg/Kg bw)</td>
<td>132 ± 0.89h*</td>
<td>8.04 ± 0.2b*</td>
<td>11.32 ± 0.5b*</td>
</tr>
<tr>
<td>VI</td>
<td>Diabetic+Glibenclamide treated (600µg/Kg bw)</td>
<td>135 ± 0.9c*</td>
<td>7.05 ± 0.2c*</td>
<td>11.01 ± 0.3c*</td>
</tr>
</tbody>
</table>

**Values are mean ±SD (n = 6); a- Group I vs. Group II, b- Group II vs. Group III, IV & V, c- Group V & VI. *P < 0.05.**
Table 3: Serum cholesterol, HDL Cholesterol and triglyceride levels of untreated and treated rats after oral administration of plant aqueous extract for 60 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Cholesterol (mg/dl)</th>
<th>HDL Cholesterol (mg/dl)</th>
<th>TGL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>58.6 ± 1.4</td>
<td>60.5 ± 0.6</td>
<td>68 ± 1.8</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>140.3 ± 0.9 a*</td>
<td>22.5 ± 0.5 a*</td>
<td>168 ± 1.5 a*</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic + Plant extract treated</td>
<td>117.4 ± 0.6b*</td>
<td>31.4 ± 1.1b*</td>
<td>102 ± 0.9b*</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic + Plant extract treated</td>
<td>94.7 ± 1.3b*</td>
<td>49.5 ± 0.7b*</td>
<td>84.6 ± 0.25b*</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic + Plant extract treated</td>
<td>69.8 ± 0.14b*</td>
<td>56.7 ± 0.9b*</td>
<td>65.7 ± 1.2b*</td>
</tr>
<tr>
<td>VI</td>
<td>Diabetic + Glibenclamide treated</td>
<td>59.6 ± 0.10c*</td>
<td>52.3 ± 0.6c*</td>
<td>64.7 ± 1.3c*</td>
</tr>
</tbody>
</table>

Inter-group comparison:

I vs II, P<0.05
II vs III, IV & V, P<0.05
V vs VI, P<0.05

Table 4: Serum urea, uric acid and creatinine levels of untreated and treated rats after oral administration of plant aqueous extract for 60 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Urea (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>38 ± 0.3</td>
<td>0.4 ± 0.01</td>
<td>0.1 ± 0.04</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>95.6 ± 1.1 a*</td>
<td>0.78 ± 0.05 a*</td>
<td>4.0 ± 0.5 a*</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic + Plant extract treated</td>
<td>76 ± 0.6b*</td>
<td>0.55 ± 0.06b*</td>
<td>2.2 ± 0.2b*</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic + Plant extract treated</td>
<td>52.4 ± 0.3b*</td>
<td>0.49 ± 0.02b*</td>
<td>0.8 ± 0.5b*</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic + Plant extract treated</td>
<td>41.4 ± 1.0b*</td>
<td>0.41 ± 0.09b*</td>
<td>0.44 ± 0.09 b*</td>
</tr>
<tr>
<td>VI</td>
<td>Diabetic + Glibenclamide treated</td>
<td>40.2 ± 1.1c*</td>
<td>0.38 ± 0.07c*</td>
<td>0.4 ± 0.08c*</td>
</tr>
</tbody>
</table>

Inter-group comparison:

I vs II, P<0.05
II vs III, IV & V, P<0.05
V vs VI, P<0.05

Values are mean ±SD (n = 6); a- Group I vs. Group II, b- Group III vs. Group IV & V, c- Group V & VI, *P < 0.05.

Discussion

Alloxan as a diabeticogenic agent caused severe diabetes by its necrotic free radicals (8). Due to the damage in pancreas, group II animals had decreased level of serum insulin which caused hyperglycemia. Treatment with aqueous extract of Gmelina asiatica L caused regeneration of α-cells of pancreas (Fig 3, 4, 5). Hence, there was increased production and secretion of insulin, which regulate the blood glucose level.

In the present investigation, diabetic rats showed an increase in glycosylated hemoglobin level and decrease in serum insulin level (21). On treatment with plant extract, HbA1C and insulin level resumed to normal (Table-I ). Administration of plant extract maintains the level of glucose by decreasing the formation of glucose as shown by the profound decrease in the activity of glucose-6-phosphatase and elevated activity of glucokinase and glucose-6-dehydrogenase. (Table II). This confirmed the antidiabetic potential of Gmelina asiatica L. The plant extract showed 70% activity, whereas standard hypoglycemic drug—glibenclamide showed 75% activity. Effect of standard hypoglycemic drug on blood glucose and glucose metabolizing enzymes were found to be higher when compared to the plant drug. But prolonged usage of allopathic drug may cause some serious side effects (22).

From the lipid profile of plant extract treated group, it is evident that Gmelina asiatica L. aqueous extract is...
possessing hypocholesteremic and hypotriglyceridemic activity. Kidney function is altered in severe diabetes (23) and is reversed in plant extract treated animals (Table-III). At a dose level of 400mg/Kg body weight of plant extract for 60 days, there was a significant reduction in the urea, uric acid and creatinine levels (Table-IV). This also further confirmed the improved kidney function in plant drug treated animals.

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Degenerative changes were observed (24) in the histology of pancreas (Fig-2) due to alloxan administration. The plant drug treated islets showed visible improvement in architecture, compared to diabetic untreated rats and reference drug treated rats (Fig- 6). Rejuvenation of β−cells of pancreas helps in maintaining blood glucose homeostasis (Figs. 3, 4 & 5).

**Conclusion**

In the present study, one of the plant drug sources - *Gmelina asiatica* L. is selected based on books of Indian Materia medica and is screened for its antidiabetic potential. The results of the scientific investigations were encouraging. The plant drug also contributed significantly towards the regeneration and rejuvenation of β−cells of langerhans. The plant drug also helps to maintain normal lipid profile and normal kidney function. In depth studies on this herbal drug source can result in the development of a safe efficacious and cost effective antidiabetic herbal drug from this natural source.

**References**


