Phytochemical And Pharmacological Investigation
On Glinus Oppositifolius Linn And Mollugo
Oppositifolia Linn.
For Anti-Diabetic Activity

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Abstract

In the analysis of its potential to prevent diabetes, the effects of petroleum ether, chloroform and methanol, fast blood glucose and blood biochemical analyzes in streptozotocin diabetic rats were investigated. Of all three extracts, oral administration of 500 mg/kg of methanol extract significantly reduced glucose overload and streptozotocin in normal, normal rats after 15 days of treatment. Petroleum ether and chloroform extract did not show any significant effect on three groups of rats at an oral dose of 500 mg/kg. Simultaneous histological studies on the pancreas of these animals first demonstrated the comparative regenerative capacity of methanol extracts necrotic by streptozotocin. The results showed that it had a positive effect on diabetes in the experimental diabetes model. The plant material were collected dried and extracted. Among these ethanolic extract of Mollugo oppositifolius linn, and hydro-alcoholic extract of Glinus oppositifolius linn, were taken for studies. Glinus oppositifolius linn was examined for its anti-inflammatory activity also. The in-vitro analysis of both extracts were carried out and compared with standard drug. After screening the anti-diabetic property was confirmed by in- vivo methods. Both the plant extracts found to have a moderate to high capacity to regulate the hyperglycemia and associated complications.

Keywords: Glinus oppositifolius, oral glucose, Hypoglycemic, Streptozotocin
INTRODUCTION

As the old adage goes, if you only have one disease, make it diabetes because it is the only one over which you have control. "Diabetes is a terrific illustration of how, by providing the patient the tools, you could manage yourself very well," Clayton M. Christensen stated. The statement "eradicating the excess" is thought to date back to 1500BC in the Egyptian writings Ebers Papyrus. Around the same time, Indian physicians recognised the condition and classified it as madhumeha, or "honey urine," since the urine attracted ants. Sushruta and Charaka, two Indian physicians, defined two forms of diabetes for the first time in 400–500 CE, one of which was linked to age and the other to being overweight. Despite the fact that modern medicine and therapeutic agent development have progressed a long way from insulin therapy to stem cell therapy or various oral antihyperglycaemic drugs such as insulin secretagogues to the recently introduced incretins, the World Health Organization still lists diabetes as one of the top ten causes of death worldwide.

According to the World Health Organization, over 422 million people worldwide have diabetes, with the majority living in low and middle income countries. Diabetes is directly responsible for 1.6 million deaths per year. According to the International Diabetes Federation, the number of cases and prevalence of diabetes have consistently increased in most countries over the last several decades, to the point that by 2045, the total number of diabetics could reach 700 million. Though diabetes is becoming more common worldwide, the greatest increase is expected in India, where the numbers of diabetics will increase from 41 million in 2009 to 70 million by 2040. As a result, India is known as the world's diabetic capital. According to WHO, India will have the biggest rise (48 percent) in diabetics in the entire population by 2040. A big contributor to the increase is the constant growth in body weight & obesity throughout many parts of the world. Economic prosperity & technological growth encourage obesity in European and South Asian countries. Environmental factors, as well as social trends toward higher energy use and lower energy expenditure, play a role.

![Figure 0. Statistical report on Deaths attributable to diabetes](image)

Figure 0. Statistical report on Deaths attributable to diabetes
Diabetes Mellitus, also known as Type II Diabetes, is characterised by hyperglycemia as a result of impaired glucose absorption, dietary nutrient metabolism (carbohydrates, proteins, and lipids) as a result of insulin secretion abnormalities, or both. These metabolic changes contribute to the development of macro and microvascular disorders such as retinopathy, nephropathy, neuropathy, and coronary heart disease over time. Symptoms of diabetes include increased appetite (polyphagia), increased urination (polyuria), increased thirst (polydypsia), weight loss, lethargy, decreased vision, fatigue, nocturia, nausea, headache, mood swings, and irritability.  

### 1.2 Classification of diabetes mellitus and other categories of glucose regulation:

The type of diabetes that a person has is typically determined by the conditions that exist at the time of diagnosis, and many diabetics do not simply fit into a single category.

1. **Type 1 Diabetes Immune-Mediated Diabetes**

Insulin deficiency and hyperglycemia are hallmarks of type 1 diabetes, a chronic autoimmune disease. Despite the fact that the disorder has a significant genetic component, which would be inherited primarily through the HLA complex, the elements that allow clinical disease to emerge remain unknown. Even while symptoms are more common in childhood and adolescence, they can occur at any age. T1DM pathogenesis is thought to also include T cell-mediated apoptosis of -cells, despite the fact that the disease’s cause is unknown.

2. **Type 2 Diabetes**

Diabetes mellitus is a chronic disease characterised by hyperglycemia and the late onset of vascular and neuropathy complications. The pathophysiology of type 2 diabetes is complex, with many different elements interacting to cause the illness to develop. In both type 1 and type 2 diabetes, fasting hyperglycemia is produced by a disruption to normal in hepatic glucose production. The rapid mobilisation of fat storage raises fasting free fatty acid levels in diabetes, in addition to hyperglycemia. According to research, hyperglycemia-induced intracellular overproduction of reactive oxygen species may be the common trigger for a variety of pathways linked to diabetes-related cell damage. Microvascular and neuropathy complications of diabetes have a complicated and poorly understood pathophysiology.
Plant Material

.1 Introduction to Molluago oppositifolia linn.- Mollugenacae

Mollugo cerviana is a warm temperate to tropical annual blooming plant native to southern and south-eastern Europe, Africa, Asia, and Australia. It grows in practically all sections of India, but is most commonly found as a weed in the sandy and arid ponds of southern India. [62]

Common name: Slender carpet weed

Vernacular name:
Sanskrit: Grishma sundara, Phanya
Hindi: Taph-jhad
Malayalm/Kannada: Parpadaka
Telugu/Tamil: Parpadagum
Bengal: Ghima sak
Maharashtra: Pada

Taxonomy
Kingdom: Plantae
Order: Caryophyllales
Genus: Mollugenacae

Clade: Angiospermae
Family: Mollugenacace
Species: Molluago oppositifolia linn
2. Introduction of Glinus oppositifolia linn - Molluginace

Figure 3: Glinus oppositifolia linn

Glinus Lotoides is a prostrate annual or short-lived perennial herb that can be found across the tropics and subtropics, but especially in Africa, Asia, Australia, and South Europe. It can be found up to a height of 800 metres in India's warmer plains and highlands. The sensitive young leaves are used for both eating and fishing.

**Common name:** Lotus sweet juice

**Vernacular names**

**Bengali:** Duserasag

**Hindi:** Gandibudi

**Marathi:** Kothuk, Bhisata

**Oriya/Punjabi:** Gandhibuti

**Telugu:** Chandrasi koora

**Taxonomy**

**Kingdom:** Plantae

**Order:** Caryophyllales

**Genus:** Glinus

**Binomial name:** Glinus oppositifolia linn.
Extraction of Plant Material

Phytochemical Evaluation of Plant Extracts

Preliminary Phytochemical screening

The results of preliminary phytochemical screening are depicted

Drugs and chemicals

The following drugs and chemicals were used with their sources: streptozotocin Sigma-Aldrich Co USA, glucose kit Ranbaxy Diagnostics, India, Gliclazide Sun Pharma, India, petroleum ether SD Fine, Mumbai, chloroform SD Fine, Mumbai, and methanol SD Fine, Mumbai.

### Table 1: Phytochemical Evaluation of Plant Extracts:

<table>
<thead>
<tr>
<th>Phytochemical Constituent</th>
<th><em>Mollugo oppositifolius linn.</em> (Ethanolic extract)</th>
<th><em>Glinus oppositifolius linn.</em> (Hydro-Alcoholic Extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pet ether Extract</td>
<td>Pet ether Extract</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Proteins</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Tannins</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Phenols</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Saponins</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Glycosides</td>
<td>--</td>
<td>++</td>
</tr>
<tr>
<td>Terpenoides</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Steroids</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

(++) Present; (--) Absent

The phyto-constituents in the petroleum ether extracts of both plants were lower. The ethanolic extract of *Mollugo cerviana* was found to contain the highest concentration of powerful phyto components such as alkaloids, flavonoids, steroid glycosides, while hydro-alcoholic extract of *Glinus oppositifolius linn*, was found to contain mostly flavonoids and saponins

Effect of different solvent extract of *Glinus oppositifolius linn* on oral glucose tolerance:

To perform glucose tolerance test, overnight fasted rats were used. Rats were divided into five groups, each of six animals. Group I was kept as control which received 1 mL of 2.5% Tween 80 per oral and Group V received gliclazide 25 mg/kg, per oral sus- pended in vehicle. A dose 500 mg/kg a petroleum ether, chloroform, methanolic extracts of *Glinus oppositifolius linn* was administered orally, to the Groups II, III and IV respectively. All the animals were given glucose 3 g/kg orally, 30 min after dosing. Blood was
collected by retro-orbital puncture for glucose estimation 0 min and at 30, 90 and 150 min after drug administration.

**Effect of different solvent extract of Glinus oppositifolius on blood glucose level** in normal fasted rats: Overnight fasted rats were divided into five groups of six rats of each. Group I received only vehicle Tween 80 in distilled water 2.5% v/v, per oral. Group V received gliclazide 25 mg/kg per oral. Group II, III and IV received dose 500 mg/kg petroleum ether, chloroform, methanolic extracts of Glinus oppositifolius suspended in vehicle was administered to the animals. Blood was collected by retro-orbital puncture for glucose estimation just prior to and at 1, 2, and 3 hours after dos.

**Effect of different solvent extract of Glinus oppositifolius on streptozotocin-induced diabetes in rats**: Diabetes was induced in overnight fasted rats by intraperitoneal injection of streptozotocin 50 mg/kg, i.p., dissolved in 0.1M citrate buffer, pH 4.5. One group of 6 identical rats was kept without streptozotocin administration as normal control, Group I. Forty eight hours after streptozotocin administration blood samples were drawn by retro orbital puncture and glucose levels determined to confirm diabetes. The diabetic rats exhibiting blood glucose levels in the range of 200 and 300 mg/100 mL were selected for the studies. These diabetic rats were subdivided into 5 groups as follows: Group II, untreated rats, given 0.5 mL of 5% Tween 80; Group III, diabetic rats given 500 mg/kg Glinus oppositifoliusL petroleum ether extract in 0.5 mL 5% Tween 80; Group IV, diabetic rats given 500 mg/kg Glinus oppositifolius chloroform extract in 0.5 mL 5% Tween 80; Group V diabetic rats given Gliclazide 25 mg/kg.

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose mg/dl, concentration at different time day,</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Vehicle</td>
<td>243.92±1.56</td>
</tr>
<tr>
<td>Ether extract 500 mg/kg</td>
<td>216.48±3.08</td>
</tr>
<tr>
<td>Chloroform extract 500 mg/kg</td>
<td>224.59±2.73</td>
</tr>
<tr>
<td>Methanol extract 500 mg/kg</td>
<td>227.06±2.54</td>
</tr>
<tr>
<td>Gliclazide 25 mg/kg</td>
<td>228.68±2.56</td>
</tr>
</tbody>
</table>

n = 6; *, ** Values are statistically significant compared to normal Group at p < 0.05, p < 0.01 respectively

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose mg/dl, concentration at different time min,</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Vehicle</td>
<td>87.61±3.58</td>
</tr>
<tr>
<td>Ether extract</td>
<td>86.23±2.43</td>
</tr>
<tr>
<td>Chloroform</td>
<td>87.57±2.06</td>
</tr>
<tr>
<td>Methanol</td>
<td>86.48±2.48</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>87.13±2.16</td>
</tr>
</tbody>
</table>

n = 6; *, ** Values are statistically significant compared to normal Group at p < 0.05, p < 0.01 respectively
Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose mg/dl, concentration at different time hour,</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td>73.25±2.53</td>
<td>80.19±2.09</td>
<td>82.73±3.45</td>
<td>73.81±2.68</td>
</tr>
<tr>
<td>Ether extract 500 mg/kg</td>
<td></td>
<td>75.61±2.06</td>
<td>74.53±3.46</td>
<td>72.19±3.19</td>
<td>76.93±2.47</td>
</tr>
<tr>
<td>Chloroform extract 500 mg/kg</td>
<td></td>
<td>80.13±3.17</td>
<td>76.18±2.39</td>
<td>72.49±2.58</td>
<td>78.26±2.83</td>
</tr>
<tr>
<td>Methanol extract 500 mg/kg</td>
<td></td>
<td>73.20±2.29</td>
<td>71.39±2.41</td>
<td>62.57±2.32*</td>
<td>56.38±2.95*</td>
</tr>
<tr>
<td>Gliclazide 25 mg/kg</td>
<td></td>
<td>73.25±2.46</td>
<td>61.75±2.38*</td>
<td>23.64±2.42*</td>
<td>46.16±2.73*</td>
</tr>
</tbody>
</table>

n = 6; * Values are statistically significant compared to normal Group at p < 0.01 respectively

Effect of extracts on estimation of blood glucose in normal and experimental rats

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by Dunnnett’s ***P< 0.001, **P < 0.01, *P < 0.05 calculated by comparing treated group with CONTROL group.

Figure: 4 Analysis of *Mollugo oppositifolius* linn, on fasting blood sugar level

Table 5

Fasting blood sugar level on diabetes animals with standard and extract
<table>
<thead>
<tr>
<th>Group</th>
<th>Before Induction of STZ</th>
<th>After Induction of STZ</th>
<th>Fasting blood sugar level on diabetes animals with standard and extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial fasting blood sugar</td>
<td>Fasting blood sugar on 72 hr</td>
<td>Fasting blood sugar on 10th day</td>
</tr>
<tr>
<td>Control</td>
<td>86.5±3.95</td>
<td>88.5±4.05</td>
<td>88±3.63</td>
</tr>
<tr>
<td>ONLY ST</td>
<td>97±3.14ns</td>
<td>480±45.5***</td>
<td>493±49.9***</td>
</tr>
<tr>
<td>STZ+STD</td>
<td>89.3±1.89ns</td>
<td>473±82.6***</td>
<td>345±44.3***</td>
</tr>
<tr>
<td>STZ + Mollugo oppositifolius linn, EXT 250mg/kg</td>
<td>93.5±3.62ns</td>
<td>470±41.4***</td>
<td>320±33.4***</td>
</tr>
<tr>
<td>STZ + Mollugo oppositifolius linn, EXT 500mg/kg</td>
<td>90.8±2.39ns</td>
<td>463±51.1***</td>
<td>333±33.3***</td>
</tr>
<tr>
<td>STZ + Glinus oppositifolius linn, EXT 250mg/kg</td>
<td>92.5±2.09ns</td>
<td>474±11.04***</td>
<td>332±2.4***</td>
</tr>
<tr>
<td>STZ + Glinus oppositifolius linn, EXT 500mg/kg</td>
<td>89.8±3.12ns</td>
<td>465±17.21***</td>
<td>253±24.5***</td>
</tr>
</tbody>
</table>
Hyperglycemia is caused by high rates of glycogenolysis and gluconeogenesis, as well as impaired glucose use by peripheral tissues due to decreased glucose uptake from the circulation. Because carbohydrates cannot be used as fuel in diabetes, the activity of hyperglycemic hormones becomes more prominent due to a shortage of insulin. A high glucagon level lowers the hepatic fructose-2,6-bisphosphate level, lowering glucose consumption. Insulin-dependent enzymes are less active, resulting in glycolysis suppression and gluconeogenesis promotion, resulting in hyperglycemia.

The delivery of STZ+NIC resulted in a 5 times increase in fasting blood sugar level in the current study, as shown in table 5.9. For around three weeks, the hyperglycemic state was maintained. After 28 days of daily treatment with the usual medicine glibenclamide and various doses of Glinus lotoides, Mollugo cerviana (250 mg/Kg and 500 mg/Kg), blood sugar levels fell by 23–72 percent in a dose-dependent manner. The depletion was still rapid on the 15th day, and thereafter saw a steady and consistent decline in FBS.

The groups receiving 500mg/Kg weight of ethanolic extract of Mollugo cerviana showed the greatest drop in glucose levels. As evidenced by the considerable increase in insulin levels in diabetic rats. Increased pancreatic production of insulin from existing -cells could be one plausible route for antihyperglycemic effect in diabetic rats. Several researchers have observed that using a variety of plants with antihyperglycemic action has a similar stimulatory impact on insulin release.

**Conclusion:**

The plants that include in the study are *Glinus oppositifolius linn*, *Mollugo oppositifolius linn*, Ser, locally referred to as thread stem carpet weed. It is a member of the Molluginaceae family. Both plants are found in marshy wet land as a weed. that both plants has got immense pharmacological properties because of their richness in flavonoids class of secondary metabolites. Both the plant materials were reported to have anti-diabetic components in it.
The plant material were collected dried and extracted. Among these ethanolic extract of *Mollugo oppositifolius linn*, and hydro-alcoholic extract of *Glinus oppositifolius linn*, were taken for studies. *Glinus oppositifolius linn* was examined for its anti-inflammatory activity also. The in-vitro analysis of both extracts were carried out and compared with standard drug. After screening the anti-diabetic property was confirmed by in-vivo methods. Both the plant extracts found to have a moderate to high capacity to regulate the hyperglycemia and associated complications.

Then the extract was further purified to isolate the potent molecule with bioactive guided isolation method.

**Acknowledgments:**

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