SYNERGISTIC ACTIVITY OF BARK EXTRACTS OF *PONGAMIA GLABRA* AND *FICUS GLOMERATA* IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Polyherbal therapy is said to be a better choice in the treatment of diabetes mellitus having the advantage of producing maximum therapeutic efficacy with minimal side effects. In the present study the combination of low doses of methanolic extracts of *Pongamia glabra* (PBME) and *Ficus glomerata* (FBME) was subjected for determination of its antihyperglycemic effects in comparison with their individual treatments and a standard hypoglycemic agent, glibenclamide. The effect of combination of extracts was evaluated in normal and alloxan-induced diabetic rats using the parameters such as glucose tolerance test, acute and sub-acute levels of antidiabetic study and body weight estimations at various intervals. Glibenclamide was administered orally at 600 µg/kg. PBME and FBME were individually administered at 100 (low dose) and 200 mg/kg (high dose) respectively. Combination of 100 mg/kg (low doses) of PBME and FBME was also administered. Significant (P<0.01) hypoglycemic effect was noticed with the combination therapy of low doses of PBME and FBME compared to their individual treatments at both doses and glibenclamide in the parameters studied and it was also able to produce more percentage increase in body weight than the individual treatments. It is concluded from the study that the combination therapy of low doses of PBME and FBME showed synergistic antihyperglycemic effect. This could provide an opportunity to reduce the dose of antidiabetic herbal drugs to achieve an enhanced therapeutic effect with minimal side effects.
Key words: Polyherbal therapy, *Pongamia glabra*, *Ficus glomerata*, glibenclamide, hypoglycemic effect.

INTRODUCTION

Medicinal plants have been used traditionally to treat diabetes mellitus owing to their therapeutic efficacy with lesser side effects compared to synthetic drugs. The antidiabetic activity rendered by them may be credited to their vital roles such as correcting altered carbohydrate metabolism, maintaining integrity and function of β-cells, insulin-secreting activity, enhancing glucose uptake and utilization \[^1\]. Based on the recommendations of WHO, plant origin hypoglycemic agents used in traditional medicine are important. Plant drugs and formulations are considered to be less toxic and free from side effects than synthetic ones \[^2\]. Polyherbal therapy or combination of either different parts of plants or their extracts containing vital phytoconstituents widely used in treatment of many diseases owing to their synergistic properties. Polyherbal therapies have the advantages of synergistic, potentiative, agonistic/antagonistic pharmacological properties within themselves \[^3\]. Many ayurvedic formulations are polyherbal preparations. Many researchers have also reported the synergistic activities of polyherbal formulations against different diseases including diabetes \[^4,5\]. A number of traditionally used medicinal plants such as *Azadirachta indica* \[^6\], *Allium satium*\[^7\], *Momordica charantia*\[^8\], *Ocimum sanctum*\[^9\], *Pongamia glabra*\[^10\], *Ficus glomerata*\[^11\], etc. have been reported to have hypoglycemic effects. In the management of severe stages of diabetes, combination therapy of two or more herbs can be beneficial instead of single drug treatment.

*Pongamia glabra* Vent. (Syn. *Pongamia pinnata*, *Derris indica*), family: fabaceae, commonly known as, “Karanja” in regional languages of India and “Indian beech” in English. The tree found all over India bearing imparipinnate leaves and pinkish white flowers. It is used medicinally in India, China, Australia and Phillipine Island. Different parts of the plant have been largely used in the traditional Indian system of medicine (Ayurveda) for bronchitis, whooping cough, rheumatic arthritis and diabetes \[^12\]. Previous studies have reported the rich content of flavonoids and related compounds. Seeds and seed oil, flowers and stem bark reported to contain karanjin, pongapin, pongaglabrone, kanugin, desmethoxy kanugin and pinnatin\[^13\]. Literature survey indicates the stem bark and flavonoids, viz.- pongamol and karanjin isolated from the fruits of the plant have been reported for antihyperglycemic activity\[^14,15\].
**Ficus glomerata** Roxb. (Syn. *Ficus racemosa*) family: Moraceae, commonly known as, “Cluster fig”, an evergreen tree of 15-18 m height with aerial roots, glabrous and pubescent young shoots, ovate-oblong leaves and reddish, obovoid figs[16]. Literature survey shows presence of lupeol, β-sitosterol glucoside, stigmasterol, leucoanthocynin, tannin and wax. The plant has been reported to contain a glycoside, tetracyclic triterpene-glauanol[17]. Traditional system of medicine claims the use of various parts of the plant, viz. – bark, root, leaves, fruits and latex in dysentery, diarrhea, diabetes, bilious affections, stomach ache, menorrhagea, haemoptysis and piles. Methanolic extract of stem bark has been reported to have the glucose lowering effect at the doses of 200 and 400 mg/kg p.o. in normal and alloxan-induced diabetic rats[18]. β-sitosterol isolated from stem bark has also been reported to possess potent hypoglycemic activity when compared to other isolated compounds[19].

In this connection the present work was designed to investigate the antidiabetic efficacy of combined extracts from two traditionally used plants possessing antihyperglycemic properties, *Pongamia glabra* Vent. and *Ficus glomerata* Roxb.

**MATERIAL AND METHODS**

**Extraction of Plant Material**

*Pongamia glabra* Vent. and *Ficus glomerata* Roxb. bark were collected from local areas of North Karnataka and a voucher specimen have been deposited at the departmental herbarium (Ref. No. GUG/BOT/Herbarium/2008-09/09). The mentioned part of the plants were dried and pulverized to particle size (#) 40 and then were first defatted with petroleum ether (40-60°C) and extracted with methanol by continuous hot percolation method using Soxhlet apparatus at 40°C for 48 h to obtain methanolic extracts of bark of the plants respectively. The filtrates of the extract were concentrated to dryness at 40°C under reduced pressure in a rotam flash evaporator. The yields of the methanolic extracts of bark were 37.76 g (22.19% w/w) and 37.76 g (22.19% w/w) respectively.

**Experimental animals**

Swiss albino mice and rats of either sex, weighing 25-30 g and 150-200g respectively housed in standard conditions of temperature, humidity and light were used. They were fed with standard rodent diet and water *ad libitum*. The study was approved by Institutional Animal Ethical Committee, Ref. No. HKECOP/IAEC/45/2011-12.
**Acute toxicity studies**
Acute toxicity studies were conducted as per OECD guidelines by 425 method (26). The animals did not show any mortality at the dose of 2000mg/kg and hence its 1/10th dose i.e. 200mg/kg and 1/20th dose i.e. 100mg/kg were used as the therapeutic doses for the methanolic extracts of the study.

**Test samples**
Weighed quantities of test extracts were suspended in 1% w/v sodium carboxy methyl cellulose to prepare suitable dosage forms. The control animals were given an equivalent volume of sodium CMC vehicle.

**Drugs**
Glibenclamide (Yashica Pharmaceutical Pvt. Ltd., Thane, India)
Alloxan (Prachi Enterprises, Pune, India)

**Experimental design** [20, 21]

**Oral glucose tolerance test**
Overnight fasted rats were divided into six groups of six in each (n=6). The rats were administered orally with the respective treatment as follows.
Group I – Normal Control – equal volume of vehicle.
Group II – Glibenclamide low dose – 600 µg/kg
Group III – *Pongamia glabra* bark methanolic extract (PBME) – 100 mg/kg
Group IV – *Pongamia glabra* bark methanolic extract (PBME) – 200 mg/kg
Group V – *Ficus glomerata* bark methanolic extract (FBME) – 100 mg/kg
Group VI – *Ficus glomerata* bark methanolic extract (FBME) – 200 mg/kg
Group VII – PBME and FBME – 100mg/kg each

After 30 m of the respective administration, the rats of all the groups were orally treated with 2g /kg of glucose. Blood samples were collected from tail vein just prior to glucose administration and at 30, 60 and 90 m after glucose loading. Blood glucose levels were measured immediately by using glucometer (One-Touch Horizon).

**Alloxan-induced diabetic model**
Diabetes mellitus was induced by intraperitoneal injection of freshly prepared solution of alloxan monohydrate (150 mg/kg) dissolved in physiological saline in overnight fasted rats. After 1 h of alloxan administration, the animals were given feed *ad libitum* and 5% dextrose
solution was also given in feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation and after 48 h blood glucose was measured by glucometer. Threshold value of blood glucose was taken between 250–300 mg/dl. One group (Group I) served as normal control, which received vehicle alone. The diabetic animals were grouped and received the following treatment for 21 days.

Group II – Diabetic Control - equal volume of vehicle.
Group III – Glibenclamide low dose – 600 µg/kg
Group IV – *Pongamia glabra* bark methanolic extract (PBME) – 100 mg/kg
Group V – *Pongamia glabra* bark methanolic extract (PBME) – 200 mg/kg
Group VI – *Ficus glomerata* bark methanolic extract (FBME) – 100 mg/kg
Group VII – *Ficus glomerata* bark methanolic extract (FBME) – 200 mg/kg
Group VIII - PBME and FBME – 100mg/kg each

The acute study involved measuring the blood glucose levels at 0, 1, 3 and 5 h after administration of respective treatment.

The sub-acute study involved measuring the blood glucose levels on 0, 7, 14 and 21st days, 1 h after daily administration of respective treatment for 21 days.

**Body weight determination**

Weight of rats was recorded on 0, 7, 14 and 21st days during the study period of 21 days. Mean change in body weight was calculated and tabulated.

**Statistical analysis**

Data were expressed as mean ± SEM and differences between the groups were statistically determined by analysis of variance (ANOVA) followed by Dunnet’s test. P-values <0.5 were considered as statistically significant.

**RESULTS**

The effect of combination herb therapy on glucose tolerance test in normal rats is shown in Table 1. At 30 min after glucose administration, the peak of blood glucose level increased rapidly from the initial value at 0 min and then subsequently decreased at 60 and 90 min. The combination therapy of low doses of PBME and FBME showed more significant (P<0.001) reduction in blood glucose level compared to the individual treatment of PBME, FBME at low and high doses and similar level of significance of standard drug, glibenclamide.

The acute studies of the combination herb therapy of low doses of PBME and FBME at 1, 3 and 5 h after the dose administration reduced the alloxan-induced sugar level very
significantly (P<0.001) compared to the individual treatment of PBME and FBME at low and high doses (Table 2).

The sub-acute studies of the combination herb therapy of low doses of PBME and FBME on 7th, 14th and 21st day of treatment showed very significant (P<0.001) reduction of elevated blood glucose compared to the individual treatment of PBME, FBME at low and high doses and the standard drug, glibenclamide. It was observed that the combination herb therapy brought back the alloxan-induced diabetes to normal state after 21 days treatment. Glibenclamide showed significant (P<0.01, P<0.5) antihyperglycemic activity. However PBME and FBME at 100 and 200 mg/kg individual treatments were able to reduce the elevated blood glucose level significantly but to a lesser extent than the combination herb therapy. On the 21st day percentage reduction in blood glucose level of low dose and high doses PBME and FBME were 63.52%, 66.45% and 51.23%, 64.55% respectively. While the combination of low doses with PBME and FBME was 74.37%. The reduction observed with the standard drug, glibenclamide was 72.03%. Results revealed that the highest percentage reduction in blood glucose level in alloxan-induced diabetic rats was exhibited by the combination of low doses of PBME and FBME (Table 3).

The body weight of diabetic control rats was decreased by 29.3% during the period of study. Combination therapy of low doses of PBME and FBME not only prevented weight loss of diabetic rats but also brought a gradual increase in weight compared to the individual treatment of both doses PBME and FBME (Table 4). Combination herb therapy gave comparative results with the standard drug, glibenclamide.

Table 1 Effect of PBME, FBME and their combination on blood glucose levels in oral glucose tolerance test in normal rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose (mg/dl)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>O min</td>
<td>30 min</td>
<td>60 min</td>
<td>90 min</td>
</tr>
<tr>
<td>Control</td>
<td>86.33±6.716</td>
<td>119.7±3.575</td>
<td>109.2±2.286</td>
<td>91.00±3.425</td>
</tr>
<tr>
<td>Gl-600 µg/kg</td>
<td>89.67±6.464</td>
<td>122.3±1.745</td>
<td>100.7±4.709</td>
<td>71.33*±6.075</td>
</tr>
<tr>
<td>PBME 100mg/kg</td>
<td>82.83±5.199</td>
<td>130.7±4.088</td>
<td>100.0±5.837</td>
<td>83.33±4.624</td>
</tr>
<tr>
<td>PBME 200mg/kg</td>
<td>64.67±2.716</td>
<td>131.7±6.622</td>
<td>74.50**±3.181</td>
<td>60.50**±3.374</td>
</tr>
<tr>
<td>FBME 100mg/kg</td>
<td>73.00±2.620</td>
<td>125.2±3.736</td>
<td>98.17±4.012</td>
<td>80.83±2.257</td>
</tr>
<tr>
<td>FBME 200mg/kg</td>
<td>70.33±2.472</td>
<td>156.7±13.26</td>
<td>113.2±7.867</td>
<td>89.67±6.103</td>
</tr>
</tbody>
</table>
Table 2  Effect of PBME, FBME and their combination on blood glucose levels in acute anti-diabetic study in alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal value (O hr.)</td>
</tr>
<tr>
<td>Control</td>
<td>78.33±2.171</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>335.8±2.725</td>
</tr>
<tr>
<td>Gl-600 µg/kg</td>
<td>311.7±7.500</td>
</tr>
<tr>
<td>PBME 100mg/kg</td>
<td>318.3±10.16</td>
</tr>
<tr>
<td>PBME 200mg/kg</td>
<td>307.0±6.382</td>
</tr>
<tr>
<td>FBME 100mg/kg</td>
<td>324.0±9.395</td>
</tr>
<tr>
<td>FBME 200mg/kg</td>
<td>330.3±9.545</td>
</tr>
<tr>
<td>PBME + FBME 100mg/kg each</td>
<td>316.3±9.472</td>
</tr>
</tbody>
</table>

PBME- *Pongamia glabra* Bark Methanolic Extract
FBME- *Ficus glomerata* Bark Methanolic Extract, Gl- Glibenclamide

Values are expressed as Mean ± SEM.; *= P<0.01, **= P<0.001

Table 3  Effect of PBME, FBME and their combination on blood glucose levels in sub-acute anti-diabetic study in alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal value (O day)</td>
</tr>
<tr>
<td>Control</td>
<td>78.33±2.171</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>335.8±2.725</td>
</tr>
<tr>
<td>Gl-600 µg/kg</td>
<td>311.7±7.500</td>
</tr>
<tr>
<td>PBME 100mg</td>
<td>318.3±10.16</td>
</tr>
</tbody>
</table>

PBME- *Pongamia glabra* Bark Methanolic Extract
FBME- *Ficus glomerata* Bark Methanolic Extract, Gl- Glibenclamide

Values are expressed as Mean ± SEM.; *= P<0.01, **= P<0.001
### Table 4  Effect of PBME, FBME and their combination on body weight in alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (in g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal value (O day)</td>
</tr>
<tr>
<td>Control</td>
<td>164.8±2.372</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>157.5±1.784</td>
</tr>
<tr>
<td>Gl-600 µg/kg</td>
<td>162.0±2.352</td>
</tr>
<tr>
<td>PBME 100mg/kg</td>
<td>172.0±3.055</td>
</tr>
<tr>
<td>PBME 200mg/kg</td>
<td>159.3±3.442</td>
</tr>
<tr>
<td>FBME 100mg/kg</td>
<td>168.8±5.528</td>
</tr>
<tr>
<td>FBME 200mg/kg</td>
<td>185.0±4.282</td>
</tr>
<tr>
<td>PBME + FBME 100mg/kg each</td>
<td>164.0±4.171</td>
</tr>
</tbody>
</table>

PBME- *Pongamia glabra* Bark Methanolic Extract
FBME- *Ficus glomerata* Bark Methanolic Extract, Gl- Glibenclamide
Values are expressed as Mean ± SEM.;*= P<0.05, **= P<0.01, ***= P<0.001

Test drug treated groups were compared with control group (Group I)
DISCUSSION

Treatment employing two or more herbs in combination known as, “polyherbal therapy” has the advantage of producing maximum therapeutic efficacy than the single herb treatment. Polyherbal therapy may provide synergistic, potentiative, agonistic/antagonistic pharmacological properties within themselves because of presence of vast range of phytobioactive constituents \[^{22}\].

Thus, the present study was focused to establish the therapeutic efficacy and probable benefit associated with the combination therapy of low doses of methanolic extracts of bark of *Pongamia glabra* (PBME) and *Ficus glomerata* (FBME) in comparison to their individual treatments at two doses and standard antidiabetic drug, glibenclamide.

Alloxan-induced hyperglycemia has been described as one of the experimental methods to study the activity of hypoglycemic agents. Alloxan, a β-cytotoxin cause a massive destruction of β-cells of islets of Langerhans, resulting in reduced synthesis and release of insulin. The function of the insulin system is suppressed leading to hyperglycemia. Alloxan-induced diabetes is characterized by loss in body weight and increased food intake. Body weight loss might be the result of protein wasting due to defect in carbohydrate metabolism and excessive breakdown of tissue protein \[^{23}\].

Oral administration of glibenclamide, PBME, FBME at low and high doses and the combination therapy of low doses of PBME and FBME showed significant (P<0.01) reduction in blood glucose level at 60 and 90 m after glucose load. The reduction in blood glucose level was more significant (P<0.001) with the combination therapy than the single treatment. This suggests that the administration of combination of low doses of PBME and FBME can more significantly reduce the postprandial hyperglycemia.

The acute and sub-acute studies of glibenclamide, both doses of PBME, FBME and the combination of low doses of the above showed significant decrease in glycemia. The percentage reduction in glycemia after 21 days treatment was more with the combination therapy than the single drug treatment, suggesting the advantage of combination in long term treatment.

Oral daily administration of glibenclamide, both doses of PBME, FBME and the combination of low doses of the above not only sustained the weight loss due to alloxan but also exhibited improvement in body weight, which may be due to improvement in glycemic control.
Combination therapy was found to be more beneficial in long term treatment and brings the hyperglycemia to normal comparable to that of the standard drug, glibenclamide.

The hypoglycemic potency of the glibenclamide, both doses of PBME, FBME and the combination of low doses of the methanolic extract of bark of *Pongamia glabra* and *Ficus glomerata* may be attributed to the vital phytoconstituents contained in both the plants, viz. – flavonoids, furanoflavonoids, sterols, saponins, glycosides, glaunol, tannins and other polyphenol compounds. The anti-oxidant and free radical scavenging properties of flavonoids and other polyphenolic compounds of the two plants \(^{[24, 25]}\) might be responsible for the antidiabetic activity of the combination therapy.

Glibenclamide, a 2\(^{nd}\) generation sulfonyleurea antidiabetic agent, lowers blood glucose acutely by stimulating the release of insulin from the pancreas. With chronic administration of glibenclamide in Type II diabetic patients the blood glucose lowering effect persists, but there is a gradual decline in the insulin secretory response to the drug, i.e. the long-term use of these oral hypoglycemic agents does not enhance insulin secretion in response to metabolic stimuli in patients with Type II diabetes \(^{[26]}\). Besides this special precautions are to be taken for administering the glibenclamide in patients with decreased kidney function, liver function and with severe thyroid and adrenal gland problems \(^{[27]}\). In this regard the combination herbal therapy can be used as an alternative approach of treatment of hyperglycemia in order to avoid the untoward effects of the synthetic drugs.

**CONCLUSION**

The results observed suggest the enhanced synergistic hypoglycemic effect of the combination therapy of low doses of *Pongamia glabra* (PBME) and *Ficus glomerata* (FBME). This also gives an opportunity to reduce the dose of herbs used for glycemic control in order to avoid the burden of herbal over dosing. At the same time proper precaution and care should be exercised as the combination herbal therapy may pose the condition of severe hypoglycemia.

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