Effect of Pongamia Bark Extract on antihyperglycemic effect of glibenclamide for possible herb-drug interaction

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ABSTRACT

Use of herbs or herbal medicines with prescription allopathic medicines may lead to beneficial or harmful herb-drug interaction, which needs a scientific substantiation. The present study was intended to determine the interaction between glibenclamide and methanolic extract of bark of *Pongamia glabra* Vent. (PBME), a herb with reported antidiabetic effect. The interaction 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 two different doses of 300 μg/kg (low dose) and 600 μg/kg (high dose) and PBME was co-administered at the dose of 200 mg/kg. Individual treatments of above drugs were also carried out. Significant ($P<0.001$) hypoglycemic effect was observed with the combination therapy of glibenclamide and PBME compared to their individual treatments in the above studied parameters. The combination therapy showed more percentage increase in body weight than the individual treatments. The study indicates that the present herb-drug interaction was beneficial as PBME showed synergistic effect with glibenclamide. This could provide an opportunity to reduce the dose of glibenclamide to achieve an enhanced therapeutic effect with minimal side effects.

Key words: *Pongamia glabra,* glibenclamide, herb-drug interaction, hypoglycemic effect.

INTRODUCTION

Diabetes mellitus is a chronic, progressive condition of impaired carbohydrate and lipid metabolism.[1] It is also associated with secondary complications including retinopathy, nephropathy, neuropathy, angiopathy, etc. leading to increased risk of morbidity and mortality rate.[2] According to WHO forecast the number of diabetic patients is expected to rise to 300 million by 2025 from 135 million in 1995.[3]

The conventional approach of treatment with insulin and oral hypoglycemic agents like sulfonylureas and biguanides cause many adverse effects on prolonged administration.[4] The use of herbal medicines for the treatment of diabetes has been a common traditional practice in countries like India and China. Herbal preparations treated alone or in combination with oral hypoglycemic agents sometimes produce a good therapeutic response in some resistant cases where modern medicines alone fail.[5] At the same time the increased use of herbal medicinal products along with prescription medicines may lead to adverse herb-drug interactions due to the reason that the herbal remedies contain multiple, biologically active constituents.[6]

Interactions between herbs and drugs may increase or decrease the pharmacological or toxicological effects of either component. The synergistic effect may also precipitate hypoglycemia thereby complicate the therapy as evidenced by previous reports.[7,8]

Pongamia glabra Vent. (Syn. *Pongamia pinnata, Derris indica*), family: fabaceae, commonly known as, "Indian beech" is used in India,

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China, Australia and Philippine Island. The tree found all over India bearing imparipinnate leaves and pinkish white flowers. 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. Seeds, flowers and stem bark have been reported to contain fixed oil, traces of essential oil, bitter alkaloid, resin, mucilage, steroids, saponins and the rich content of flavonoids and related compounds, viz.- karanjin, pongapin, pongaglabrone, kanugin, desmethoxy kanugin and pinnatin.[9] Literature survey indicates the stem bark and the flavonoids, viz.- pongamol and karanjin isolated from the fruits of the plant have been reported for antihyperglycemic activity.[10,11]

The present study was intended to find out the influence of methanolic extract of stem bark of *Pongamia glabra* Vent. on antihyperglycemic activity of glibenclamide at two different doses to evaluate the safety of combination.

MATERIALS AND METHODS

Extraction of Plant material

Pongamia glabra Vent. bark was collected from local areas of North Karnataka and a voucher specimen has been deposited at the departmental herbarium (GUG/BOT/Herbarium/2008-09/09). The mentioned part of the plant was dried and pulverized to particle size (#) 40 and then was first defatted with petroleum ether (40-60^{0}C) and extracted with methanol by continuous hot percolation method using Soxhlet apparatus at 40^{0}c for 48 h to obtain methanolic extract of bark of the plant. The filtrate of the extract was concentrated to dryness at 40^{0}C under reduced pressure in a rota flash evaporator. The yield of the methanolic extract of bark was 37.76 g (22.19%w/w).

Experimental animals

Swiss albino mice and rats of sex, weighing 25-30 g and 150-200 g respectively housed in standard conditions of temperature, humidity and light were used. They were fed with standard rodent diet and water *ad libitum* (IAEC Ref. No. HKECOP/IAEC/45/2011-12).

Acute Toxicity Studies

Acute toxicity studies were conducted as per OECD guideline by 425 methods (#26). The animals did not show any mortality at the dose of 2000 mg/kg and hence its 1/10th dose i.e. 200 mg/kg was used as the therapeutic dose for the methanolic extract of the study.

Test Samples

Weighed quantities of test extracts were suspended in 1% w/v sodium carboxy methyl cellulose to prepare a suitable dosage form. 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[12-14]

Oral glucose tolerance test

Overnight fasted rats were divided into six groups of six in each. The rats were administered orally with the respective treatment as follows.

- Group I – Normal Control – equal volume of vehicle.
- Group II – Glibenclamide low dose – 300 µg/kg
- Group III – Glibenclamide high dose – 600 µg/kg
- Group IV – *Pongamia glabra* bark methanolic extract (PBME) – 200 mg/kg
- Group V – PBME – 200 mg/kg and Glibenclamide low dose – 300 µg/kg
- Group VI – PBME – 200 mg/kg and Glibenclamide high dose – 600 µg/kg

After 30 min of the respective administration, the rats of all the groups were orally treated with 2 g/kg of glucose. Blood samples were collected from tail vein just prior to glucose administration and at 30, 60 and 90 min 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% w/v 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 – 300 µg/kg
- Group IV – Glibenclamide high dose – 600 µg/kg

- Group V – *Pongamia glabra* bark methanolic extract (PBME) – 200 mg/kg
- Group VI – PBME – 200 mg/kg and Glibenclamide low dose – 300 µg/kg
- Group VII – PBME – 200 mg/kg and Glibenclamide high dose – 600 µg/kg

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 21 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 <0.05, <0.01 and <0.001 were considered as statistically significant.

RESULTS

The effect of herb-drug combinations in normal and diabetic rats is shown in the following tables. 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 individually treated groups of glibenclamide low and high doses and PBME showed significant (*P<0.001*) reduction in blood glucose level when compared to control group. The combination therapy of both the doses with PBME showed significant (*P<0.05, P<0.01 and P<0.001*) blood glucose reduction when compared to the glibenclamide high dose treated group (Table 1).

The acute and sub-acute studies of PBME and both the doses of glibenclamide at 1, 3 and 5 h after the dose administration (Table 2) and on 7th, 14th and 21st day of treatment (Table 3) respectively, reduced the alloxan-induced sugar level very significantly (*P<0.001*) compared to the diabetic control group. The combination therapy produced significant (*P<0.05, P<0.01 and*

Table 1: Effect of PBME and glibenclamide combinations on blood glucose levels in Oral glucose tolerance test in normal rats

Groups	Blood glucose (mg/dl)			
	0 min	30 min	60 min	90 min
I-Control	76.33 ± 2.044	135.6 ± 1.913	120.3 ± 0.988	98.00 ± 1.317
II-GI-300	78.83 ± 1.167	121.4*** ± 1.806	112.8*** ± 0.945	82.67***± 1.706
III-GI-600	79.50 ± 1.727	115.0***± 1.703	107.0***± 0.930	74.17***± 2.725
IV-PBME 200	77.67 ± 1.542	123.0***± 1.140	115.3** ± 0.881	91.17* ± 1.195
V-PBME + GI-300	77.00 ± 1.155	114.6 ± 1.806	99.00** ± 1.633	68.83 ± 0.945
VI-PBME+ GI-600	77.50 ± 1.607	108.0* ± 0.707	97.33***± 0.707	65.50** ± 1.258

PBME- Pongamia glabra Bark Methanolic Extract (in mg/kg), GI- Glibenclamide (in µg/kg) Values are expressed as Mean ± SEM.; * = P<0.05, ** = P<0.01, ** = P<0.001 Individual drug treated groups (Gr. II, III and IV) were compared with control group (Group I) Combination drugs treated groups (Gr. V and VI) were compared with GI-600 µg/kg Gr. (Gr. III)

Table 2: Effect of PBME and glibenclamide combinations on blood glucose levels in acute antidiabetic study in alloxan-induced diabetic rats

Groups	Blood glucose (mg/dl)			
	Basal value			
	(O h)	1 h	3 h	5 h
I-Control	78.33 ± 2.171	78.67 ± 2.525	79.83 ± 2.227	79.67 ± 2.486
II-Diabetic Control	335.8 ± 2.725	345.0 ± 1.461	350.2 ± 0.792	365.7 ± 1.498
III-GI-300	307.0 ± 6.658	254.2** ± 1.352	219.3** ± 1.382	177.7** ± 1.453
IV-GI-600	311.7 ± 7.500	238.7**± 0.918	207.8** ± 1.797	160.7** ± 2.231
V-PBME 200	307.0 ± 6.382	265.5** ± 1.565	243.3** ± 1.874	187.7b**± 2.472
VI-PBME + GI-300	318.0 ± 11.45	225.0** ± 2.380	199.8*± 1.195	150.3* ± 1.256
VII-PBME + GI-600	310.2 ± 9.833	219.3** ± 1.333	194.0**± 1.528	145.7** ± 2.140

PBME- Pongamia glabra Bark Methanolic Extract (in mg/kg), GI- Glibenclamide (in µg/kg) Values are expressed as Mean ± SEM.; * = P<0.01, ** = P<0.001 Individual drug treated groups were compared with diabetic control group (Group II) Combination drugs treated Gr. (VI and VII) were compared with GI-600 µg/kg Gr. (Gr. IV)

Table 3: Effect of PBME and glibenclamide combinations on blood glucose levels in sub-acute antidiabetic study in alloxan-induced diabetic rats

Groups	Blood glucose (mg/dl)			
	Basal value			
	(O day)	7th day	14th day	21st day
I-Control	78.33 ± 2.171	78.83±3.038	76.83±2.496	75.83±2.344
II-Diabetic Control	335.8 ± 2.725	379.2 ± 3.679	398.0 ± 3.357	415.0 ± 2.366
III-GI-300	307.0 ± 6.658	163.8***±1.956	125.2***± 1.851	95.33***± 2.231
IV-GI-600	311.7 ± 7.500	142.0***±2.352	113.5*** ± 2.446	81.67*** ± 1.994
V-PBME 200	307.0 ± 6.382	180.3***±1.926	140.0*** ± 2.324	103.3*** ± 3.756
VI-PBME+ GI-300	318.0 ± 11.45	130.8** ± 1.302	105.2* ± 1.922	74.00** ± 1.366
VII-PBME+GI-600	310.2 ± 9.833	125.2***±1.851	102.7** ± 1.745	64.67*** ± 0.954

PBME- Pongamia glabra Bark Methanolic Extract (in mg/kg), GI- Glibenclamide (in µg/kg) Values are expressed as Mean ± SEM.; * = P<0.05, ** = P<0.01, *** = P<0.001 Individual drug treated groups were compared with diabetic control group (Group II) Combination drugs treated Gr. (VI and VII) were compared with GI-600 µg/kg Gr. (Gr. IV)

Table 4: Effect of PBME and Glibenclamide combinations on body weight in alloxan-induced diabetic rats

Groups	Body weight (in g)			
	Basal value			
	(O day)	7th day	14th day	21st day
I-Control	164.8 ± 2.372	168.7 ± 2.186	174.2 ± 2.212	176.7 ± 5.011
II-Diabetic Control	157.5 ± 1.784	141.0 ± 2.113	124.3 ± 2.305	111.2 ± 1.662
III-GI-300	170.3 ± 2.565	174.2*** ± 2.023	181.0*** ± 1.770	190.0*** ± 1.826
IV-GI-600	162.0 ± 2.352	176.2*** ± 2.428	187.3*** ± 1.687	192.7*** ± 1.606
V-PBME 200	159.3 ± 3.442	168.3*** ± 1.358	171.3*** ± 1.333	181.8*** ± 1.249
VI-PBME+GI-300	168.2 ± 2.330	181.2 ± 1.167	190.8 ± 1.014	194.5 ± 1.408
VII-PBME+GI-600	157.0 ± 4.640	184.8* ± 2.120	194.2** ± 1.376	200.3** ± 1.358

PBME- *Pongamia glabra* Bark Methanolic Extract (in mg/kg), GI- Glibenclamide (in µg/kg) Values are expressed as Mean ± SEM.; * = P<0.05, ** = P<0.01, *** = P<0.001 Individual drug treated groups were compared with diabetic control group (Group II) Combination drugs treated Gr. (VI and VII) were compared with GI-600 µg/kg Gr. (Gr. IV)

P<0.001) reduction compared to the glibenclamide high dose treated group.

It was observed that the combination therapy brought back the alloxan-induced diabetes to normal state after 21 days treatment. Glibenclamide at low and high doses showed dose dependent antihyperglycemic activity. However PBME – 200 mg/kg individual treatment was able to reduce the elevated blood glucose level significantly but to a lesser extent than the combination therapy. On the 21st day percentage reduction in blood glucose level of low dose glibenclamide, high dose glibenclamide and PBME were 68.94%, 73.79% and 66.35% respectively. While the combination of low dose glibenclamide with PBME and high dose glibenclamide with PBME were 76.00% and 79.20% respectively. The results revealed that the highest percentage reduction in blood glucose level in alloxan-induced diabetic rats was exhibited by the high dose glibenclamide with PBME after 21 days of respective treatment.

The body weight of diabetic control rats was decreased by 29.39% during the period of study. Combination therapy of glibenclamide both doses with PBME not only prevented weight loss of diabetic rats but also brought a gradual significant (*P<0.05, P<0.*01) increase in weight compared to the glibenclamide high dose treated group (Table 4).

DISCUSSION

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.[15]

Oral administration of PBME, both doses of glibenclamide have shown significant (*P<0.001*) reduction in blood glucose level at 60 and 90 min after glucose load compared the control group. The reduction in blood glucose level was significant (*P<0.05, P<0.01 and*

P<0.001) with the combination therapy compared to glibenclamide high dose treated group. This suggests that the administration of PBME with low and high doses of glibenclamide can more significantly reduce the postprandial hyperglycemia than glibenclamide alone administration (Table 1).

The acute and sub-acute studies (Tables 2 and 3) of the single treatment of PBME, both doses of glibenclamide and the combination therapy of the above have shown significant decrease in glycemia. The percentage reduction glycemia after 21 days treatment was more with combination therapy than single treatment suggesting the advantage of combination in long term treatment.

Oral daily administration of PBME, glibenclamide low and high doses and the combination of the PBME and both doses of glibenclamide 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 (Table 4). The comparison of the activity of the combination therapy with the glibenclamide treated group indicates a higher level of significance than glibenclamide and hence the combination therapy was found to be more beneficial than glibenclamide in long term treatment. The hypoglycemic potency of the methanolic extract of bark of *Pongamia glabra* Vent. may be attributed to the vital phytoconstituents contained, viz.- flavonoids, furanoflavonoids, sterols, saponins and other polyphenolic compounds. The anti-oxidant and free radical scavenging properties of flavonoids and other polyphenolic compounds of the plant might be responsible for its antidiabetic activity.[16]

Glibenclamide, a 2nd generation sulfonylurea 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.[17] Besides special precautions are to be taken for administrating the glibenclamide in patients with decreased kidney function, liver function and with severe thyroid and adrenal gland problems.[18,19] In this regard the dosage of glibenclamide should be reduced to reduce the side effects in long-term treatment. The drug also has been claimed to have additive hypoglycemic effect with other antidiabetic drugs.[20,21]

CONCLUSION

The results observed suggest that the *Pongamia* bark extract (PBME) when combined with glibenclamide enhances the hypoglycemic activity of the latter. The results of the study indicate the beneficial herb-drug interaction of combining PBME with glibenclamide and also the combination could provide an opportunity to reduce the dose of glibenclamide for minimizing the adverse effects and achieving enhanced therapeutic effect. At the same time proper precaution and care should be taken as the combination therapy may pose the condition of severe hypoglycemia.

CONFLICTS OF INTEREST

We declare that we have no any conflicts of interest

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