SHORT COMMUNICATION

EFFECT OF DOLICHOS BIFLORUS ON BLOOD SUGAR AND LIPIDS IN DIABETIC RATS

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Abstract: Diabetes mellitus is a chronic disease characterized by hyperglycemia and dyslipidemia, which leads to severe morbid complications. In view of the effectiveness of conventional herbal adjutants the present study was taken to assess the anti-diabetic and anti-lipidemic effect of Dolichos biflorus. 32 healthy albino rats of either sex were randomly divided into 4 equal groups (I–IV) each having 8 rats. Group I, control rats received only vehicular fluid while Group II received only Dolichos biflorus for the experimental period of 30 days. Diabetes was induced in Group II and Group IV rats by single intravenous dose of STZ and confirmed on 3rd day with fasting blood sugar (FBS) more than 250 mg/dl. Group III diabetic rats, received vehicular fluid while Group IV diabetic rats received Dolichos biflorus in single dose of 300 mg/kg body weight/day intra-gastrically up to the experimental period of 30 days. FBS levels were assessed on 1st, 8th, 15th, 22nd and 30th day. On last day overnight fasted rats were sacrificed for drawing the sample for lipid profile from beating heart and harvested for pancreatic tissue histology.

Data obtained showed that FBS levels were decreased significantly when Dolichos biflorus was given to diabetic rats from mean±SD values 362±63.36 to 118±38.55 with p value < 001. S cholesterol and S. triglyceride levels were also decreased significantly with P value < 001. It was concluded that Dolichos biflorus has anti-diabetic and anti-lipidemic effect at daily oral dose and can be used as an adjuvant for management of diabetes mellitus and its associated complications.

Key words: dolichos biflorus antidiabetic lipid profile

INTRODUCTION

Diabetes mellitus is a chronic disease characterized by hyperglycemia and dyslipidemia, which leads to severe complications like nephropathy, retinopathy,
vasculopathy, neuropathy and cardiovascular diseases. Since its prevalence in India is expected to increase from 31.7 million (2000) to 79.4 million in 2030 (1), it is being referred as diabetic capital of world (2).

The conventional treatment of diabetes mellitus includes insulin therapy, diet, exercise, and oral hypoglycemic agents, which either increases insulin sensitivity or stimulate insulin production by active pancreas (3). Though insulin therapy affords a tight and effective glycemic control, its parental mode, frequent dose adjustment, and need for proper refrigeration, favors its non-compliance and episodes of fatal hypoglycemia. The continuous use of oral hypoglycemic agents causes several side effects and toxicity even with physiological doses (4).

Before the introduction of insulin and other hypoglycemic agent, dietary measures and traditional plant therapies by ayurveda and other indigenous system of medicine were used commonly in India. Several Indian medicinal plants have been found to be useful in successfully managing the diabetes. Though no definitive statistics on the use of herbal medicine are available in India, surveys conducted in Australia and USA indicate that almost 48.5% and 34% respondents respectively had used at least one form of unconventional therapy including herbal medicine. WHO has suggested the evaluation of the potential of plants as effective agents, especially in areas, which lack safe modern drugs? Herbal drugs are widely used in the management of diabetes due to their effectiveness, low cost, and less side effects even on long term treatment. Unfortunately active biological components of most of them are not still known (5).

Since diabetes mellitus requires, a long-term treatment the herbal products can be used along with conventional treatment or alone. One such medicinal plant is “Dolichos biflorus”. Commonly known as “Kulthi” in hindi & “Horse Gram” in English, it is commonly used legume in hills of north and south India. It is being used in Ayurvedic medicines for the treatment of piles, pain, constipation enlargement of liver and spleen and urinary stones (6).

In view of the effectiveness of conventional herbal adjuvant, as well paucity of published scientific data on this medicinal plant; the present study has been taken to assess the anti-diabetic and anti-lipidemic effect of dolichos biflorus.

MATERIALS AND METHODS

The study was conducted in department of Physiology at HIHT University, Dehradun during a period of 2 months. All experiments in rats were carried out in accordance with the recommendation and guidelines for care and use of laboratory animals approved by Institutional Animal Ethics Committee. Adult Wistar stain rats weighting 120-150 grams (30-45 days old) were used as experimental diabetic model.

Materials

Streptozotocin (STZ) was used to produce experimental diabetes. Streptozotocin was supplied by Sigma Chemicals Co., St. Louis, MO, and USA. The freshly prepared buffered solution was used to produce experimental diabetes. Dolichos biflorus was procured as seed powder from Gem Granites Chennai (US patent no 5916567) in the amount of 1 kg (7).
Experimental animals

32 Albino rats of wistar strain weighing 125–175 gm of either sex were obtained from the Central Animal House, HIMS, Dehradun, and were maintained under standard laboratory condition. Each animal was housed in plastic cages (43 cm × 29 cm × 15 cm) with normal day night cycle and had free (ad libitum) access to water and food. Streptozotocin (STZ) was used to induce the diabetes in rats. It was obtained from Sigma Aldrich Company. It is available as a dry powder of 50 mg per vial. For induction of diabetes streptozotocin was prepared by dissolving it in 3 ml of 0.1M cold sodium citrate buffer of pH 4.5.

Dosage

Dolichos biflorus was administered in the dose of 300-mg/kg body weight/day for each rat. Calculated dosage of seed powder of Dolichos biflorus was dissolved in the 2 ml of distilled water. The suspension was administered orally by intragastric tube reaching up to lower 1/3rd of oesophagus.

Acute toxic study

8 healthy randomly selected rats were taken for the study of acute toxic effect of dolichos biflorus. The rats were fasted overnight and the Dolichos biflorus was administered in the dose of 2-gm/kg-body weight intra-gastrically. Rats were observed continuously for first 3 hr and were monitored for three days for mortality and general behavior, signs of discomfort and neurological manifestations. No mortality and adverse effects were observed at this dose.

Induction of diabetes

Overnight fasted rats were tested for basal values of FBS (fasting blood sugar) and given single dose of STZ intravenously in tail vein at the dose of 55 mg/kg body weight. Following which the rats were kept for the next 24 hours on 5% glucose solution in their cage to prevent early hypoglycemia following immediate oxidative degeneration of beta cells. Fasting blood glucose levels were measured after 72 hours of administration of STZ and were considered as 1st day for the study. Animals with blood glucose greater than 250 mg/dl were included in the study. The control animals were injected with equal volume of vehicle.

Experimental procedure

Thirty-two rats were randomly divided into 4 groups of 8 animals each (Group I–Group IV). Group I and Group II were labeled as normal rats, whereas Group III & Group IV were induced diabetes and included in study as experimental rats. Group II (normal rats) and Group IV (diabetic rats) were administered Dolichos biflorus in dose of 300 mg/kg/day intragastrically for a period of next 30 days. Group I (normal) and Group III (diabetic) rats were administered same volume of vehicular fluid up to a period of 30 days.

Group I : Normal
Group II : Normal rats on Dolichos
Group III : Diabetic
Group IV : Diabetic rats on Dolichos

All the rats were given normal prepared
food and water ad libitum. In all the groups the fasting blood sugar levels were estimated on 1\textsuperscript{st}, 8\textsuperscript{th}, 15\textsuperscript{th}, 22\textsuperscript{nd} and 30\textsuperscript{th} day. On 30\textsuperscript{th} day overnight fasted rats were sacrificed for drawing sample for lipid profile from beating heart and tissue of pancreas for histology.

**RESULTS**

Intravenous injection of streptozotocin (STZ) produced cardinal signs of type 1 diabetes i.e. polydipsia and polyphagia in group III & IV rats. As expected fasting blood sugar levels on the day 1 of experimental period was raised among the same groups and were statically significant when compared to their basal levels. Following administration of Dolichos in-group IV rats the levels of FBS decreased significantly along the days from 8\textsuperscript{th} to 30\textsuperscript{th} experimental day (P<0.001). The FBS levels in diabetic rats on Dolichos (Group IV) rats were significantly lower than the corresponding FBS levels in diabetic rats (Group III) from 8\textsuperscript{th} day to the 30\textsuperscript{th} Day of experimental period (P<0.001). Surprisingly there was no effect on the blood sugar levels in the normal rats on 4 weeks of administration of Dolichos (Group II). Levels of FBS remained nearly the same as day 1 in both normal (Group I) and diabetic rats (Group III) on all the days of the experiment.

Serum triglyceride and total cholesterol levels were found to be increased significantly among (Group III) diabetic rats as compared to group I rats at the end of 4 weeks. Administration of Dolichos biflorus produced a decline in serum triglyceride and total cholesterol levels in diabetic rats on Dolichos (Group IV) which were statistically significant when compared with diabetic rats (group III) at end of experimental period, however administration of Dolichos biflorus in Group II rats did not produce any significant effect on blood glucose and lipid profile (Table II).

**Estimation of biochemical parameters and histology:**

Fasting Blood Glucose: Using all aseptic precautions rat-tail vein was pricked and fasting blood glucose level was estimated by Glucometer (Abbotts Diabetes Care Inc Alameda USA).

Serum Cholesterol and Triglycerides: Overnight fasted rats were sacrificed by cervical dislocation and the abdomen was opened by mid line incision. Thorax was cut open to reach the heart and blood was collected from beating heart for estimation of serum cholesterol and serum triglycerides.

Histology: The pancreas was identified, isolated and was preserved in 10% formalin in saline for histology.

**Statistical analysis**

Interpretation and analysis of the data thus obtained was carried out using standard statistical method of significance. Calculated data was tabulated, analyzed for the significance of variance by ANOVA (Analysis of variance) and results were confirmed by post hoc test Bonferroni. Values of continuous variable (fasting blood sugar) between the groups were analyzed for difference by Mann- Whitney test. Analysis of data was done by using SPSS ver.10 and EXCEL (Win XP). Significance was set at 0.05 with < 0.05 as statistically significant.
In histological study of pancreas, dolichos treated rats (group IV) in Fig. 3, showed restoration of normal architectural pattern of islets of langerhans with appearance of α cell in the center, which was lost in the diabetic rats (Fig. 2) when compared to normal architecture (Fig. 1).

Data obtained showed that FBS levels decreased when Dolichos biflorus was given to diabetic rats from mean±SD values 362±63.4 to near normal (118±38.5) by the end of 4 weeks of treatment by dolichos (Fig. 4).

**TABLE I**: Comparison of Fasting Blood sugar levels (FBS), of different groups (Group I: Control; Group II: Normal rats on Dolichos; Group III: Diabetic rats; Group IV: Diabetic rats on Dolichos) at different experimental days.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FBS (mg/dl)</th>
<th>GR I (n=8)</th>
<th>GR II (n=8)</th>
<th>GR III (n=8)</th>
<th>GR IV (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1\textsuperscript{st} day</td>
<td>86.4±2.2</td>
<td>89.3±4.7</td>
<td>362.8±39.1 YYY,^^^</td>
<td>362.6±63.7 YYY,^^^</td>
<td></td>
</tr>
<tr>
<td>8\textsuperscript{th} day</td>
<td>84.9±3.7</td>
<td>87.5±4.6</td>
<td>368.3±29.1 YYY,^^^,mmm</td>
<td>243.7±65.7 YYY,^^^^,^^^^,mmm</td>
<td></td>
</tr>
<tr>
<td>15\textsuperscript{th} day</td>
<td>86.1±2.0</td>
<td>87±4.5</td>
<td>352.4±43.8 YYY,^^^,mmm</td>
<td>167.0±68.4 YYY,^^^^,^^^^,mmm</td>
<td></td>
</tr>
<tr>
<td>22\textsuperscript{nd} day</td>
<td>84.0±2.5</td>
<td>86.1±3.7</td>
<td>359.6±42.7 YYY,^^^,mmm</td>
<td>146.2±49.9 YYY,^^^^,^^^^,mmm</td>
<td></td>
</tr>
<tr>
<td>30\textsuperscript{th} day</td>
<td>84.2±2.6</td>
<td>86.5±3.4</td>
<td>377.3±32.8 YYY,^^^,mmm</td>
<td>118.0±38.5 YYY,^^^^,^^^^,mmm</td>
<td></td>
</tr>
</tbody>
</table>

The values are mean±SD; Statistical analysis done by Repeated measures ANOVA and post Hoc test Bonferroni comparison test. The mark Y indicates comparison with group I; ^ indicates comparison with group II; Mark ¥ indicate comparison with Group III. “P” Value: Y P<0.05, YY P <0.01, YYY P<0.001; ^P<0.05; ^P<0.01; ^^^P<0.001; ¥P<0.05, ¥¥P<0.01, ¥¥¥P<0.001. The f indicates comparison with 1\textsuperscript{st} day and * indicates comparison with 8\textsuperscript{th} day. fP<0.05, fp<0.01, ffp<0.001; *P<0.05, **P<0.01, ***P<0.001.

**TABLE II**: Inter group comparison of values (Mean±SD) of S. Cholesterol and S. Triglyceride levels, body weight, and food and water intake after 4 weeks.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (n=8)</th>
<th>Group II (n=8)</th>
<th>Group III (n=8)</th>
<th>Group IV (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Cholesterol (mg/dl)</td>
<td>67.0±12.8</td>
<td>61.1±12.7 ss</td>
<td>86.3±7.7 s</td>
<td>58.2±7.1 ***</td>
</tr>
<tr>
<td>S. Triglyceride (mg/dl)</td>
<td>65.6±17.8</td>
<td>56.2±7.8 ss</td>
<td>106.7±7.2 ***</td>
<td>60.5±3.7 ***</td>
</tr>
<tr>
<td>Food intake (g/animal/day)</td>
<td>19.5±3.4</td>
<td>17.2±1.5</td>
<td>39.2±2.1 s</td>
<td>32.4±4.7 ss</td>
</tr>
<tr>
<td>Water intake (g/animal/day)</td>
<td>21.9±3.3</td>
<td>19±3.72</td>
<td>69.7±4.7 s</td>
<td>60.9±3.8 ss</td>
</tr>
<tr>
<td>Initial Body weight (g)</td>
<td>129±6.9</td>
<td>128.7±6.3 ss</td>
<td>125.4±3.3 ss</td>
<td>124.9±5.8</td>
</tr>
<tr>
<td>Body weight after 4 weeks (g)</td>
<td>180.9±6.0</td>
<td>177.9±5.25 ss</td>
<td>159.5±6.1 **</td>
<td>172.4±7.0 **</td>
</tr>
</tbody>
</table>

The values are mean±SD; *<0.05 (significant); **<0.01 (more significant); ***<0.001 (highly significant), ns (not significant). Data analyzed by one-way ANOVA followed by Post Hoc Test Bonferroni. ^ Comparison between Group II with Group I # Comparison between Group III with Group I ## Comparison between Group III with Group IV.

In histological study of pancreas, dolichos treated rats (group IV) in Fig. 3, showed restoration of normal architectural pattern of islets of langerhans with appearance of α cell in the center, which was lost in the diabetic rats (Fig. 2) when compared to normal architecture (Fig. 1).

Fig. 1: Photomicrograph of normal rats (group I) showing normal acini and normal population in Islets of Langerhans (H&E × 200).
Fig. 2: Photomicrograph of Streptozotocin treated rats (group III) showing marked necrotic changes in Islets of Langerhans and exocrine part. β cells in centre of Islets of Langerhans were appeared in lesser number (H&E × 200).

Fig. 3: Photomicrograph of Dolichos biflorus treated diabetic rats (group IV) showing restorage of normal architectural pattern. β cells appeared to be normal in centre part of Islets of Langerhans (H&E × 200).

Fig. 4: Line Diagram showing trends of the mean values of fasting blood sugar levels of various days among diabetic rats (group III n = 8) and diabetic rats on Dolichos biflorus (group IV n = 8) @ 300 mg/kg/day.

The gain in body weight in the untreated diabetic rats (Group III), from 125.4±3.3 g to 159.5±6.1 (27.1%) was much less than that in normal rats (Group I) from 129±6.9 to 180.9±6.1 (40.2%). However following administration of Dolichos biflorus for 4 weeks the, gain in weight in the diabetic treated animals (Group IV) was almost equal to that in normal (38.03%). There was statistically significant difference between the weight of diabetic rats (Group III) and diabetic rats on dolichos (Group IV) after 4 weeks of administration of the powder (P=0.002) (Table II).
As expected significant increase in the water and food intake was seen in the diabetic groups (Group III) as compared to normal rats (Group I). But after treatment for 4 weeks with Dolichos biflorus, Group IV rats showed a decreased intake (32.45±4.7 g) of the food as compared to the diabetic rats, which still showed polyphagia (39.24±2.1 g). At the end of 4 weeks mean values of water intake also decreased by 12.6% in diabetic rats on Dolichos as compared to diabetic rats and the difference was statistically significant (Table II).

DISCUSSION

Intravenous administration of STZ (dose of 55 mg/kg body weight), induced hyperglycemia, and other cardinal features of diabetes including polyphagia, polydipsia and loss of body weight in albino rats of both group III & group IV. The blood glucose levels remained constantly high during experimental period in-group III rats. Histological examination of pancreas of diabetic rats showed necrosis and loss of normal architecture of islets of langerhans. Similar results were observed by Devi et al (4) and Bhandari et al (8) who also reported high blood sugar level with single injection of streptozotocin in albino rats.

Administration of Dolichos biflorus to diabetic rats (Group III), decreased fasting blood sugar levels from mean value of 362.63±63.36 on first day to a mean value of 118.00±38.55 on 30th day. Neelakantan et.al (1999) also reported the hypoglycemic effect of Dolichos biflorus in diabetic rats. The effect of decrease in FBS levels was attributed to absorption of carbohydrate, which could be due to the formation of a slimy sheath in intestine. However they also reported an increase in insulin levels after the administration of Dolichos biflorus in diabetic rats (7). Slimy nature of the gastro intestine lumen was also seen in diabetic animals (Group IV) on Dolichos biflorus in the present study. The restoration to near normal architecture of islets of langerhans in pancreas of diabetic rats on Dolichos biflorus (group IV) could correlate with increase production of insulin from rejuvenating α cells (Fig. 3). Diabetic rats on Dolichos biflorus showed significant decrease in values of FBS up to the 30th experimental day but did not reach up to normal level. Since the mean survivals of diabetic rats were less, an increase in the number of treatment days could have brought the level toward normal. The possible mechanism of the action could be a decreased absorption of the carbohydrate from the gut following dolichos intake, as it form some barrier (mucoid collection). Study needs to be done to ascertain the exact mechanism of action. The decrease levels of FBS in diabetic rats and not in normal rats following ingestion of dolichos suggests failure of the counter regulatory effect (Alpha cells of islets) in diabetic rats as compared to normal rats.

Following decrease in level of FBS in dolichos fed diabetic rats the food intake improved and polydipsia decreased (less decrease to basal values) as compared to the diabetic rats after 4 weeks of experimental period. Also a near normal weight gain was seen in these rats which substantiate the normalization of FBS levels to pre diabetic levels in this group.
The lipid levels are usually elevated in diabetes mellitus and persistent rise in lipid levels is a risk factor for coronary heart disease. Decreased insulin activates lipoprotein lipase, which leads to hypertriglyceridemia (9). Insulin deficiency also leads to hypercholesterolemia due to metabolic abnormalities (10), and had been reported in STZ induced diabetic rats (11). In the present study diabetic rats showed hypercholesterolemia and hyper triglyceridemia, which is in agreement with the findings of the aforementioned authors. Administration of Dolichos biflorus to diabetic rats for 4 weeks caused decreased rise in the serum cholesterol and triglyceride levels (P<0.001) and these values showed a reduction towards values of normal rats. This implies that Dolichos biflorus can prevent or may be helpful in reducing the chronic complications due to abnormalities of lipid metabolism seen in diabetes mellitus.

Muthu et al (2005) in their study also reported a profound decrease in serum cholesterol and triglyceride levels in high fed rats when they were given mentholic extract of Dolichos biflorus in dose of 200-400 mg/kg/day. The possible mechanisms suggested for cholesterol lowering effect were inhibition of hepatic cholesterol genesis or due to increased excretion of fecal sterol (12). Like many species Dolichos biflorus may stimulate hepatic microsomal cytochrome P 450 dependent aryl hydrolase activities which is believed to be involved in the hydroxylation of endogenous steroid such as cholesterol (13) and thereby increases the catabolic conversion of cholesterol to bile acid in liver (14).

The triglyceride level in the diabetic rats on giving Dolichos biflorus decreased significantly and possible mechanism of its lowering effect described by other author. According to Muthu A.K et al supplementation of Dolichos biflorus extract lowered the concentration of triglyceride level significantly in high fat fed diet rats (12). High fat fed rat showed decrease activity of lipoprotein lipase in adipose tissue (15). Stimulation of the activities of skeletal muscle lipoprotein lipase and adipose tissue hormone sensitive lipase may be responsible for the increased uptake of triglycerides from plasma by skeletal muscle and adipose tissue (16).

The study concludes that Dolichos biflorus can be used as an adjuvant in the anti diabetic regime for the control of hyperglycemia. The hypoglycemic and hypolipidemic action as seen by the authors in dolichos fed diabetic rats will be studied further to elaborate the pharmacokinetic and mode of action of the powder of Dolichos biflorus.

Since the mean survival age for diabetic rats was not more than one month, the study was restricted for a period of one month, which is the limitation of the study. Non-estimation of plasma insulin levels among the groups of rats is also a limitation of the study.

Conclusion

Dolichos biflorus has anti-diabetic and anti lipidemic effect therefore it can be used as an adjuvant for management of diabetes mellitus and its associated complication. Because of paucity of work on hypoglycemic effect of Dolichos biflorus, exact mechanism
involved in decrease of blood sugar levels is not known.

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REFERENCES