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Pharmacological Study

Dolichos biflorus Linn. ameliorates diabetic complications in streptozotocin induced diabetic rats

Yogesh Saxena, Brijesh Purwar, Harsh Meena[1], Parth Sarthi[2]

Department of Physiology, [1]Department of Pathology, Himalayan Institute of Medical Sciences, SRH University, Dehradun, Uttarakhand, [2]Department of Physiology, Integral Institute of Medical Sciences and Research, Integral University, Lucknow, Uttar Pradesh, India

Abstract

Background: Horsegram (*Dolichos biflorus* Linn.) is a known antilithiatic, hypolipedemic and has free radical scavenging activity and increased production of reactive oxygen species play a role in pathophysiological mechanisms that trigger diabetic complications. **Aim:** To see the effect of daily oral feeding of *D.biflorous* on nephropathy and retinopathy in streptozotocin (STZ) induced-diabetic rats. **Materials and Methods:** A total of 24 healthy rats were randomly grouped into controls, diabetic and diabetic on *Dolichos*. Diabetes was induced by a single dose of STZ (55 mg/kg) and animals were given prepared food and water *ad libitum*. *Dolichos* was orally given at 300 mg/kg/day to rats in diabetic on *Dolichos* group for next 30 days. Fasting blood glucose levels was monitored at beginning and at the end of the experiment while assessment of serum creatinine levels and histopathological study of kidney and retina was carried only at the end of the experiment. Statistical differences between groups were analyzed using analysis of variance followed by, Bonferroni test as *posthoc* test. **Results:** Results indicated improvement in serum creatinine levels and reduced glomerular sclerosing and Bowman's space with interstitial alterations and significantly reduced renal hypertrophy in diabetic rat son *Dolichos* diabetic rats ($P < 0.001$). Retinal layers showed inconsistent improvement in the width of the neuronal layers and decreased vacuolization of plexiform layers and retinal vessel density. **Conclusion:** *D. biflorus* at doses of 300 mg/kg/day for 30 days resulted in gradual but significant decreased diabetic nephropathy.

Key words: Diabetic nephropathy, diabetic retinopathy, *Dolichos biflorus*, experimental diabetes

Introduction

Diabetes mellitusis a global epidemic with ~20% of the diabetics residing in South-East Asia[1] and is associated with vascular and non-vascular complications.[2,3] Progressive complications like retinopathy leading to blindness[4,5] and nephropathy leading to renal failure[5,6] enhances the outlook of this metabolic disorder. Obvious shared features include their dependence on duration, accumulation of metabolites independent of insulin involving vessels and basement membrane of tissues.

Putative pathogenic mechanisms involves the increased production of reactive oxygen species[7,8] and impaired sorbitol pathway[9] leading to deposition of advanced glycosylated products and hemodynamic changes.[10]

Although insulin therapy is effective in prevention of complications, it has its demerits of daily parental administration, variability of effectiveness and high incurred cost.

Dolichos biflorous Linn. known as "*Kulthi*" is widely grown and consumed in hills of Uttrakhand. It is a common ingredient of several of Ayurvedic preparations for bowel disorders and renal stones.[11] Evidence suggest that it has antilithotic,[12] antihepatotoxic,[13] hypolipedemic,[14] sugar lowering effect[15,16] and has potential antioxidant and free radical scavenging property.[17]

In the above context study was conducted to assess the effect of *D. biflorous* on diabetic complications of retinopathy and nephropathyin experimental diabetic rat model.

Address for correspondence: Dr. Yogesh Saxena,
Asso. Prof., Department of Physiology, Himalayan Institute of Medical Sciences, SRH University, Dehradun - 248 140, Uttarakhand, India.
E-mail: drysaxena@rediffmail.com

Materials and Methods

Study design

The interventional study was conducted at the Department of

Physiology of HIHT University during a period of 2 months. Clearance for this study was taken from the Institutional Animal Ethics Committee (IAEC) of the university (No. HIHTPHARMA/I-1/2010/1954; dt. 18.02.10). Adult Wistar stain rats weighting 120-150 g (30-45 days old) were used for experimental diabetes models.

Materials

Streptozotocin (STZ) was used to produce experimental diabetes. It was supplied by Sigma Chemicals Co., St. Louis, MO, USA. A single dose of freshly prepared buffered solution of STZ (Inj. streptozotocin was prepared by dissolving it in 3 ml of 0.1 Mcold sodium citrate buffer of pH 4.5) was used to produce experimental diabetes.

D. biflorus was procured as seed powder from Gem Energy Industry Ltd, Chennai (US patent no 5916567; authorized dealer) in the amount of 1 Kg.

Healthy adult Wistar rats weighing 120-150 g (30-45 days old) were procured from central animal house of the university. The animals were individually housed in labeled plastic cages (43 cm χ 29 cm χ 15 cm) under standard laboratory conditions. The room temperature was maintained at 25°C ± 3°C and animals were allowed freshly prepared food and water *ad libitum*. They were subjected to the regular cycle of the day and night and observed for gain in weight for 3-4 days.

Experimental design

A total of 24 rats were studied, which were randomly grouped under control, diabetic rats and diabetic rats on *Dolichos*. Each group comprised of 8 rats.

- Group I: Control rats
- Group II: Diabetic rats
- Group III: Diabetic rats on *D. biflorus* (n = 8).

Method of induction of diabetes (Group II and III)

Every time six healthy rats (STZ 50 mg vial sufficient for only 6 rats) were fasted and their fasting blood glucose (FBS) was assessed. Following anesthesia (Ether) each rat were weighed and single dose of freshly prepared STZ (at 55 mg/kg) was given in the tail vein using all aseptic precautions. Those animals whose FBS level exceeded 250 mg/dl at 72 h after injection were considered as diabetic.[18]

Control animals were injected with the equivalent amount of cold citrate buffer (pH 4.5). All the doses were administered at a volume not exceeding 1 ml/100 g body weight of rats. The site was cleaned and smeared with antibiotic ointment to prevent infection.

Following the procedure all the studied rats were fed freshly prepared feed and had access to water *ad libitum*. Rats of Group III were given measured *Dolichos* seed powder at of 300 mg/kg/day in suspension by intra gastric feeding tube (Ramson no. 6) for 30 days. At the 30th day (0 day is the day of onset of diabetes), the rats were measured for weight, FBS, Serum creatinine and sacrificed for harvesting of tissues for histology.

Bio chemical analysis

- Measurement of Glucose: For the determination of blood glucose using Gluco-check (model: Abbot`s Glucometer XCE115-3098), whole blood was taken from the tail vein of all rats[19]
- Plasma creatinine: Blood samples for the measurement of blood chemistry were drawn into pre-chilled Ethylene di-amine tetra acetic acid (EDTA-containing tubes and immediately placed on ice. All tubes were centrifuged within several minutes of collection and the plasma samples were assayed for creatinine using RA-50 semi auto-analyzer.

Histological study

For the study and comparison of pancreatic islets of Langerhans, retina and kidney, rats from all the groups were sacrificed by cervical dislocation and dissected for retrieval of pancreatic tissue, enucleation of eyeballs and resection of both the kidneys. The samples were fixed in 10% formalin, stained with H and E and photographed by microscope with × 20 and × 40 magnifications. In diabetic rats tissue of pancreatic Langerhans and the beta cells degenerated irreversibly.[8] The kidneys were observed for histological changes like glomerular thickening with decreased bowmen space, tubular vacuolization and moderate interstitial fibrosis [Figure 1a-c]. H and E sections of the retina were observed for decreased neuronal layer with thickening of basement membrane, blood vessels invasion and vacuolization of plexiform layers [Figure 2a-c].

Statistical analysis

The data were expressed as mean ± standard error of the mean statistical differences between groups were analyzed using analysis of variance (ANOVA) followed by, Bonferroni test as *posthoc* test. The difference was considered to be statistically significant at $P < 0.05$. Statistical software IBM SPSS (Version -17) was used for analysis.

Results

Rats that had received STZ became diabetic at a frequency of 75% (12/16 × 100). Weight and basal FBS levels of all the groups were not significantly different from each other.

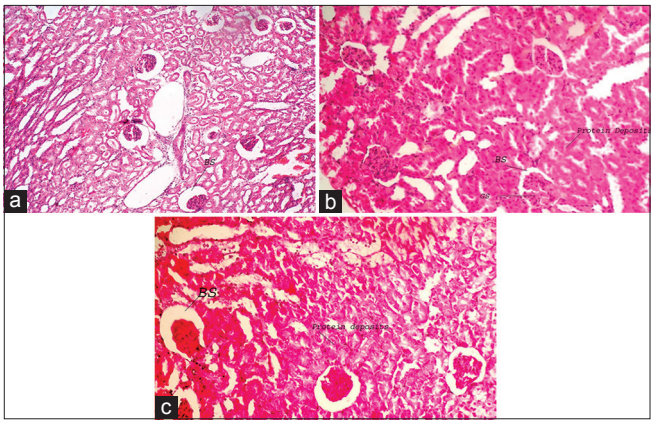

Figure 1: Normal rat: Kidney at ×20 normal Bowman`s space with normal architecture and no evident sclerosis seen: Normal tissue. (b) Diabetic rat ×40: Giant retinal vessels occupying the ganglion layer and inner plexiform layer. Lumen filled with red blood cells; Lucentare as seen in inner plexiform. (c) Diabetic rat on Dolichos: Kidney at ×20: Bowman`s space are not decreased with minimal disruption of architecture; protein deposits are minimal with less tubular obliteration; minimal infilteration

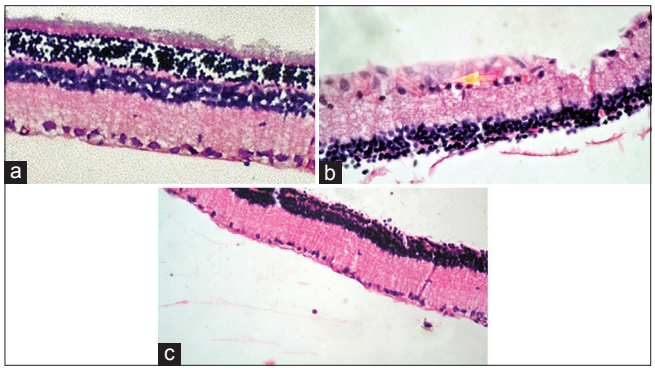

Figure 2: (a) Normal rat: All layer of retina are distinct and regularly arranged. (b) Diabetic rat ×40: Giant retinal vessels occupying the ganglion layer and inner plexiform layer. Lumen filled with red blood cells; Lucentare as seen in inner plexiform. (c) Diabetic rats on Dolichos: ×40: Less disruption of layers including photo receptors layer; few blood vessels in ganglion cell layer; little vacuolization in plexiform layer

Diabetes was associated with reduced gain in body weight when compared with the control rats and the reduction was statistically significant (P = 0.008, 0.02). However, the reduction was less in diabetic rats on *Dolichos*. The data related to the body weight are shown in Table 1.

Biochemical finding

No statistically significant difference was observed between groups in the mean FBS values prior to administration of STZ suggesting that all animals were non-diabetics. Repeated measure ANOVA for the differences in the mean values of the FBS between the groups suggested that FBS values of diabetic rats were higher at both 3rd and 30th day of development of diabetes and the difference was statistically significant ($P < 0.001$) in comparison to controls. Group III showed statistically significant difference to control at 3rd day only ($P < 0.001$). Although higher value of FBS was observed on 30th day in group III but it was not statistically significant when compared with the controls [Table 2].

No significant difference in creatinine mean values was observed between the groups. However there was a definite increase in the plasma creatinine levels in diabetic rats. The diabetic rats showed significant renal hypertrophy (ratio of renal weight/rat weight) which decreased in diabetic rats on *Dolichos* (0.85 ± 0.1; $P < 0.001$) [Table 3].

Histopathological finding

The kidney specimens of the diabetic group showed markedly severe destruction in glomerular and tubule-interstitial lesions such as glomerular sclerosis, atrophy, interstitial expansion and interstitial cellular infiltration [Figure 1b] as compared with those of the control group [Figure 1a]. In the diabetic rats on *D. biflorus*, general morphology of glomerulus and tubule-interstitial lesions were much improved and showed quite normal appearance [Figure 1c].

Retinal histology shows infiltration of blood vessels in the layers of retina (ganglion layer) and disruption of the plexiform layer with vacuolization and thickening of the basement membrane [Figure 2b] when compared with the normal rats [Figure 2a]. The retinal layers of the diabetic rats on

Table 1: Weight changes of the rats

Parameter	Controls (*n*=8)	¥Diabetes (*n*=8)	¥Diabetes on *Dolichos* (*n*=8)
Basal weight	129±6.85	126.86±1.35	124.95±4.78
30th day weight	136.37±6.62	119.22±1.74**	122.15±6.82*

¥Comparison of weight on 30th day with control rats shows decrease in mean values in diabetic rats (P=0.008) and diabetic rats on *Dolichos* (P=0.023), *P<0.05, **P<0.01, ***P<0.001, Values in mean±SEM, SEM: Standard error of themean

Table 2: Change of blood sugar

Parameter	Controls (*n*=8)	Diabetes (*n*=8)	Diabetes on *Dolichos* (*n*=8)
Basal FBS	86.4±2.17	87.4±3.32	88.5±5.17
FBS on 3rd day	84.9±3.65	357.6±39.02***	362.6±55.45***
FBS on 30th day	84.2±2.58	372.6±32.17***	118±30.09###

Values in mean±SEM, Analyzed by repeated measure ANOVA followed by inter-group comparison by *posthoc* test Bonferroni. *Comparison of FBS in control rats, with diabetic rats and diabetic rats on *Dolicho*. *P<0.05; **P<0.01, ***P<0.001, #Comparison of FBS in diabetic rats and diabetic rats on *Dolichos*. #P<0.05; ##P<0.01; ###P<0.001, SEM: Standard error of the mean, FBS: Fasting blood sugar, ANOVA: Analysis of variance

Table 3: Biochemical analysis in blood and renal hypertrophy (kidney weight/rat weight)

Parameter	Controls (*n*=8)	Diabetes (*n*=8)	Diabetes on *Dolichos* (*n*=8)
S. Creatinine	0.66±0.06	0.75±0.07	0.71±0.06
Renal hypertrophy	0.77±0.09	1.4±0.07	0.85±0.1***

*Comparison of renal hypertrophy among diabetic rats on *Dolichos* and diabetic rats; *P<0.05; **P<0.01, ***P<0.001; Values in mean±SEM, SEM: Standard error of the mean, S. Creatinine: Serum creatinine

Dolichos showed lesser development of the features of diabetes following 30 days of *Dolichos* only in five cases; however, rest of the three diabetic rats did show early changes of diabetic retinopathy (DR).

Discussion

Plants are part and parcel of human society from the dawn of civilization and extensive use of *D. biflorus* as food for both animals and human beings is well-known. The seeds are consumed by humans after cooking or frying. They are eaten whole or after grinding into a meal, unlike other seeds, which are consumed after splitting.

The present study provided evidence those 30 days of *D. Biflorus* at 300 mg/kg/day may provide benefit in the diabetic nephropathy, but is less likely to prevent the development of DR. *Dolichos* treated diabetic rats had significantly decreased bowman's space, less sclerosis and inflammatory cells when compared with diabetic rats. In diabetes mellitus, increased blood glucose, lipids, oxidized low-density lipoprotein and oxygen free radicals can induce glomerulo-sclerosis and chronic tubule-interstitial damage in the kidneys leading to DN.[3,20] A progressive decline in the glomerular filtration rate due to loss of functioning nephrons alters the physiological functioning of diabetic kidney.[21] Elevated serum creatinine seen in diabetic rats levels was not elevated in *Dolichos* treated diabetic rats. Decreased blood glucose levels in these *Dolichos* treated rats,

shows that the effect is dependent on the serum glucose levels, which were decreased to near normal in them. The markedly reduced total body weight with elevated kidney weight (renal hypertrophy) of the diabetic group when compared with control group was significantly decreased with amelioration of sclerosis of glomerulus and decreased interstitial infiltration during 30 days of the supplementation of *Dolichos* seed powder at 300 mg/kg in group of diabetic rats on *Dolichos*.

Microvascular changes in DR might be related to hyperglycemia-induced intramural pericyte death and thickening of the basement membrane and may lead to disruption of the blood-retinal barrier.[22] In present study, retinal vessel density is significantly increased, with thickened basement membrane and vacuolization of plexiform layer seen in diabetic animals as compared to normal rats. Intracellular hyperglycemias has been linked to an overproduction of extracellular matrix; hyperglycemia may also decrease production of trophic factors for endothelial and neuronal cells and the intracellular oxidative stress in endothelial cells plays a key role in endothelial dysfunction.[23] *Dolichos* treated rats showed variable response to the *Dolichos* treatment for 30 days. Some of the diabetic rats showed decreased thinness of retinal layers with less disruption of ganglionic and plexiform layers when treated with *D. biflorus* for 30 days while others did show changes in DR even after *Dolichos* intervention.

In diabetes, there is also increased production of free radicals[24] that can initiate per-oxidation of lipids, which in turn stimulates glycation of protein and causes long-term complications.[25] Researchers have shown antioxidant potential of methanolic extract of *D.biflorus*, thus it may reduce long-term complication produced in diabetes.[26] *Dolichos* is known for its antioxidant and free radical scavenging effect[17,26] and may therefore decrease the oxidative damage of the membrane lipids in renal tissues. Earlier studies have shown its hypolipidemic effect,[13] which may also contribute to the lesser degree of damage in the renal tissue.

Fibers of the *Dolichos* make a sheath in the intestine to reduce the absorption of carbohydrates and natural insulin into the blood stream. This enables energy to be sustained for a period of at least 3-4 h. Decreased levels of sugar in blood were observed by the authors in their earlier work.[16] Both good control of sugar and presence of antioxidant effect could have decreased the onset of nephropathy, which was observed in this study. In agreement to these findings, researcher reported that antioxidants and good control of diabetes leads to improved renal functions.[21] These findings suggest that *Dolichos* may improve the disturbed metabolism associated with diabetes.

Furthermore, effect of the *Dolichos* powder remains for a longer time when compared with the conventional available medicines as after the intake of normal conventional medicine, the left over insulin in the pancreas is absorbed very quickly in the blood stream when compared with the *Dolichos* powder and hence the frequency of the intake of the powder will be less.

Conclusion

D. biflorus at doses of 300 mg/kg/day for 30 days resulted in gradual but significant decreased diabetic nephropathy and fasting blood sugar. It is therefore suggested that the seed of *Dolichos* may be used as a supplement to the conventional anti-diabetic therapy; however, further studies are required to look for its long-term effect and its possible mechanism of action.

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हिन्दी सारांश

स्ट्रेप्टोझोटोसिन प्रेरित मधुमेही चूहों में मधुमेहजन्य उपद्रवों पर कुलत्थी के प्रभाव का प्रायोगिक अध्ययन

योगेश सक्सेना, ब्रिजेश परवर, हर्ष मीना, पार्थ सारथी

कुलत्थी (हार्स ग्राम), एक हाइपोलिपिडेमिक, अँटिलिथिअटिक और फ्री रेडिकल स्केवेन्जिग गतिविधि के लिये ज्ञात औषधि है। प्रतिक्रियाशील ऑक्सीजन प्रजातियों के उत्पादन में होनेवाली वृद्धि, मधुमेह जटिलताओं को विकसित करने में मुख्य भूमिका निभाती हैं। इस अध्ययन में स्ट्रेप्टोझोटोसिन द्वारा प्रेरित मधुमेही चूहों में नेफ्रोपैथी और रेटिनोपैथी पर कुलत्थी के प्रभाव का प्रायोगिक अध्ययन किया गया है। बेतरतीब ढंग से चुने हुए २४ स्वस्थ चूहों को स्वस्थ चूहों, मधुमेही चूहों और मधुमेही चूहों में परीक्ष औषधि कुलत्थी दी गयी, इन ३ समूह में बांटा गया। समूह २ और ३ के चूहों मे स्ट्रेप्टोझोटोसिन की एक खुराक (५५ मि.ग्रा./कि.ग्रा.) द्वारा मधुमेह प्रेरित किया गया। तृतीय समूह के मधुमेही चूहों मे मौखिक रूप से कुलत्थी चूर्ण अगले ३० दिनों तक ३०० मि.ग्रा./कि.ग्रा. मात्रा में दिया गया। सीरम क्रिएटिनिन का स्तर और वृक्क व नेत्रपटल का हिस्टोपैथोलॉजिक अध्ययन प्रयोग के अंत मे किया गया, जबकि उपवास रक्त ग्लूकोज स्तर का शुरुआत में और प्रयोग के अंत में आंकलन किया गया। कुलत्थी से तृतीय समूह के मधुमेही चूहों मे सीरम क्रिएटिनिन के स्तर में सुधार पाया गया। इस समूह में मधुमेह के द्वारा ग्लोमेरुलर स्क्लेरोसिंग, बोमन स्पेस और साथ में इन्टरस्टिशियल अल्ट्रेशन भी कम पाया गया। तथा वृक्क कि अतिवृद्धि पर भी प्रभाव (P>0.001) पाया गया। नेत्रपटल के परतों में न्यूरोनल परतों की चौड़ाई में असंगत सुधार दिखा और प्लेक्सीफॉर्म परतों के व्हॅक्युओलायझेशन तथा नेत्रपटल सिराओं के घनता में कमी हुई। कुलत्थी चूर्ण की खुराक ३० दिनों के लिए ३०० मिलीग्राम/किग्रा/दिन लेने पर मधुमेही उपद्रव –नेफ्रोपैथी में क्रमिक लेकिन महत्त्वपूर्ण कमी हुई।