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Dolichos biflorus Linn attenuate progression of renal damage in alloxan-induced diabetic rats

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SUMMARY

Dolichos biflorus Linn. (*Fabaceae*), commonly known as Horse gram is a medicinal plant, used in folk medicine for treating kidney stones and diabetes mellitus. The purpose of the present study was to investigate the effects of daily oral feeding of various doses of methanolic extract of *Dolichos biflorus* seeds (DB) for 42 days on blood glucose concentrations and kidney functions in Alloxan-diabetic rats. Plasma glucose levels, body weight, serum creatinine, and urinary albumin levels were monitored on 15th, 29th, 43rd day. Renal hypertrophy was assessed as the ratio between the kidney weight and body weight of the rats. Plasma glucose concentrations in Alloxan-diabetic rats were significantly reduced by the administration of DB (350 mg/kg) and DB (700 mg/kg) on day 15 and onwards ($P < 0.01$). After 15 days of Alloxan administration urinary albumin levels (UAE) were over 5 fold higher in diabetic controls as compared to normal controls. Treatment with DB significantly prevented the rise in UAE levels from day 15 to 43 in comparison to diabetic controls ($P < 0.01$). Renal hypertrophy was significantly higher in diabetic controls as compared to non-diabetic controls. Treatments with DB (350 mg/kg) and DB (700 mg/kg) significantly prevented renal hypertrophy ($P < 0.01$) as compared to diabetic controls. DB (175 mg/kg) failed to modify renal hypertrophy. Thus the present study indicates that methanolic extract of *Dolichos biflorus* may be useful in management of hyperglycemia and kidney functions in Alloxan-diabetic rats.

Key words: *Dolichos biflorus*; Kidney stones; Diabetes mellitus; Alloxan-induced diabetes; Renal hypertrophy

INTRODUCTION

Diabetic nephropathy (DNP) is a major cause of illness and premature death in people with diabetes, largely through accompanying cardiovascular diseases and end-stage renal failure. It is a progressive disease ending in chronic renal insufficiency. Indeed, diabetic patients are several times as prone to kidney disease as non-diabetic people and the cumulative risk of diabetic nephropathy in type I and type II diabetes mellitus is about 30% to 50% after 25 years of the disease. Proteinuria heralds the onset of Diabetic nephropathy and the worsening of proteinuria parallels progression of renal disease (Bretzel, 1997; Di Landro D *et al*., 1998; Marcantoni, 1998).

For various reasons in the recent years, the popularity of complimentary medicine has increased. Dietary measures and traditional plant therapies as

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prescribed by Ayurvedic and other indigenous systems of medicine have been used commonly in India. Surveys conducted in Australia and US indicate that almost 48.5 and 34% of respondents had used at least one form of unconventional therapy including herbal medicine (Eisenberg *et al*., 1993; Maclennan *et al*., 1996). Indian figures are not available. The World Health Organization (1980) has also recommended the evaluation of the effectiveness of plants in conditions where we lack safe modern drugs (Upadhayay and Pandey, 1984).

The primary objective of this study was to assess the efficacy of traditional anti-diabetic plant in diabetic nephropathy. Research on medicinal plants has increased recently all over the world. Medicinal plants have been used in various systems, as they have immune potential against numerous diseases. One such plant *Dolichos biflorus* (DB) Linn. (*Fabaceae*) is chosen in this investigation. DB Linn. is a genus of twining herb found in India and is distributed throughout the tropical regions of the Old World. Branches of DB are suberect or twining, glabrescent, leaflets are 2.5 to 5 cm, broardly lanceolate, stipules subulate, seeds 5-6, compressed reniform, grey or redish brown in colour (Basu and Kirtikar, 1984). *D. biflorus* has antitumor activity (Kurtan and Unnikrishan, 1990), the root of the plant is used as expectorant in China (Ayensd and Duke, 1985), the entire dried plant is used in abortion in India (Hemadri and Sasibhushana, 1985), it is also used in menstrual problems (Quisumbing, 1951), the entire dried plant is reported to possess antioxidant effect (Muthu *et al*, 2006), Hypolipidemic effect (Muthu *et al*., 2005), the seeds of the plant are reported to possess an acid stable trypsin-chemotrypsin inhibitor (Mehta and Simlot, 1982), the polyherbal formulation (NR-AG-1and NR-AG-2) Containing seeds of the plant is reported to possess Nephroprotective effect (Harish and Samiulla, 2001), traditionally the seeds are used in heart troubles, liver troubles, eye diseases, diseases of the brain, kidney stones (Basu and Kirtikar, 1984). The antilithiatic activity of seeds of DB Linn. and rhizomes of *Bergenia ligulata* Wall. were reported (Garimella, 2001). The seed diets in normal fasted rats for one week are reported to possess Blood sugar and Total cholesterol lowering effect (Pant *et al*., 1968). Also the seeds are used as indigenous food in the treatment of Diabetes mellitus.

MATERIALS AND METHODS

Plant material

Seeds of *Dolichos biflorus* Linn. (*Fabaceae*) were purchased from local market, Pimpri, Pune, India. Seeds were authenticated by Mr. P.G. Diwakar (Scientist 'D' for Joint Director) Botanical Survey of India; Pune (Voucher no. CAG 1).

Extraction

The seeds were cleared from dust and other foreign materials and initially were shade dried. The seeds were then coarsely powdered in mixer and passed through a 40 mesh sieve. The powdered material was then extracted with methanol by continuous hot percolation method using soxhlet apparatus. The yield of powdered seeds of DB was found to be 5.6% w/w. Phytochemical screening of the methanolic extract of DB revealed the presence of flavonoids, saponins, alkaloids and phytosterols.

Experimental animals

Wistar rats of either sex weighing 150 - 180 g were purchased from National Toxicology Center (NTC), Pune. The rats were housed in standard laboratory conditions with free access to standard pellet diet and water *ad libitum.* Animal Ethics Committee approved the protocol of the study.

Preparation of diabetic nephropathic animals

Alloxan was used for experimental induction of diabetic nephropathy. Diabetes was induced by a single intraperitonial injection of Alloxan monohydrate in citrate buffer (pH 4.5) at a dose of 150 mg/kg body weight of the rat. The diabetic state was confirmed

48 h after alloxan injection by hyperglycemia. Surviving rats with fasting blood glucose level higher than 250 mg/dl were included in the study.

Experimental design

The animals were randomized in the following groups: Rats in group I received saline plus CMC daily and served as normal control. Rats in group II–V received a single intraperitoneal injection of 150 mg/kg Alloxan. Group II received saline daily and served as a diabetic control. Group III received 175 mg/kg of DB, group IV received 350 mg/kg of DB, and group V received 700 mg/kg of DB. The Methanolic extract of DB was dissolved in CMC and given orally everyday for 42 days by force-feeding using a 5-ml syringe. After randomization into various groups, the rats were acclimatized for a period of 2 – 3 days before initiation of experiment. Animals described as fasting had been deprived of food for at least 16 h but had been allowed free access to drinking water.

Sample collection

Fasting blood samples were collected on day 15, 29 and 43 of the treatment from retro-orbital plexus under light ether anaesthesia in two epindorff tubes one containing anticoagulant for separation of plasma and another without anticoagulant for separation of serum. Plasma and serum were separated in a high speed C-24 electric centrifuger (Remi Udyog, New Delhi) at 2000 rpm for 20 min by centrifugation.

Biochemical analysis

Plasma glucose

Plasma Glucose levels were estimated by standard procedure based on glucose oxidase method using commercially available glucose kits (Nirmal laboratories Ltd, India) (Trinder, 1969).

Serum creatinine levels

Serum creatinine levels were measured by standard procedure based on quantitative colorimetric assay on day 15 and 43 using commercially available kits (Nirmal laboratories Ltd, India)

Urinary albumin levels

Urinary Albumin levels were measured by standard procedure based on quantitative colorimetric assay on day 15, 29 and 43 using commercially available kits (Nirmal laboratories Ltd, India).

Body weight

Weight of individual animal was recorded on day 1, 15, 29 and 43.

Renal hypertrophy

On the day 43, animals were sacrificed and the kidney weight was recorded and the degree of renal hypertrophy was expressed as the ratio of the weight of the two kidneys to total body weight.

Statistical analysis

Values are expressed as mean ± S.E.M. Statistical analysis was performed using one way ANOVA. Dennett's test was used for multiple comparisons. The values were considered to be statistically different when $P < 0.05$.

RESULTS

Plasma glucose

The effect of Alloxan and the plant extract on plasma glucose levels is shown in Table 1. The plasma glucose levels were markedly raised (up to 5 times) in the diabetic controls as compared with non-diabetic controls on the 15^{th} and 43^{rd} day of the experiment ($P < 0.01$). Treatment with DB for 42 days significantly reduced the plasma glucose levels. DB 350 mg/kg and DB 700 mg/kg treatment groups showed significant protection against Alloxan hyperglycemia ($P < 0.01$) from day 15 and onwards. While DB (175 mg/kg) treatment group showed significant reduction in plasma glucose levels only on day 29 ($P < 0.05$) and day 43 ($P < 0.01$) of the experiment.

Table 1. Effect of treatment of methanolic extract of *Dolichos biflorus* on Plasma glucose (mg/dl) levels in alloxan induced diabetic rats

Gr. no.	Treatment	Plasma glucose (mg/dl)		
		Day 15	Day 29	Day 43
1	Normal control	64.39 ± 3.38	61.96 ± 2.96	63.98 ± 1.18
2	Diabetic control	296.26 ± 12.78$^{\#\#}$	289.77 ± 11.39$^{\#\#}$	287.83 ± 14.46$^{\#\#}$
3	Diabetic + DB (175 mg/kg/day)	263.37 ± 9.87	258.24 ± 10.50*	237.42 ± 9.14**
4	Diabetic + DB (350 mg/kg/day)	233.49 ± 13.05**	182.78 ± 6.61**	149.90 ± 5.17**
5	Diabetic + DB (700 mg/kg/day)	201.13 ± 12.33**	154.26 ± 6.51**	137.25 ± 3.22**

Results are presented as mean ± SEM. (n = 5), ANOVA followed by Dunnett test. $^{\#\#}P < 0.01$ when compared with Normal control; $^{*}P < 0.05$, $^{**}P < 0.01$ when compared with Diabetic control.

Table 2. Effect of treatment of methanolic extract of *Dolichos biflorus* on Body weight (g) in alloxan induced diabetic rats

Gr. no.	Treatment	Body weight (g)			
		Day 1	Day 15	Day 29	Day 43
1	Normal control	163.47 ± 3.54	181.43 ± 4.37	196.28 ± 4.61	218.92 ± 5.05
2	Diabetic control	172.93 ± 3.023	125.67 ± 2.13$^{\#\#}$	118.33 ± 2.16$^{\#\#}$	104.80 ± 2.78$^{\#\#}$
3	Diabetic + DB (175 mg/kg/day)	169.88 ± 2.74	134.26 ± 1.26	139.40 ± 5.98*	163.2 ± 5.33**
4	Diabetic + DB (350 mg/kg/day)	173.17 ± 5.41	138.4 ± 2.24	150.40 ± 3.12**	171.2 ± 4.93**
5	Diabetic + DB (700 mg/kg/day)	166.79 ± 4.53	161.22 ± 3.01**	169.4 ± 3.64**	178.8 ± 5.54**

Results are presented as mean ± SEM. (n = 5), ANOVA followed by Dunnett test. $^{\#\#}P < 0.01$ when compared with Normal control; $^{*}P < 0.05$, $^{**}P < 0.01$ when compared with Diabetic control.

Body weight

The effect of Alloxan and different plant extracts on the body weight of rats is summarized in Table 2. The basal values in the controls and treated groups were not significantly different from each other. The increase in the body weight in the non-diabetic control was significantly higher as compared to diabetic controls. Treatment with DB (700 mg/kg) showed significant protection against loss in weight on day 15 and onwards ($P < 0.01$) whereas treatment with DB (350 mg/kg) showed significant protection against loss in weight on day 29 and on day 43 ($P < 0.01$), While DB (175 mg/kg) treatment group showed significant activity only on day 29 ($P < 0.05$) and day 43 ($P < 0.01$) of the experiment.

Urinary albumin levels

Urinary albumin levels in the Alloxan treated and

Table 3. Effect of treatment of methanolic extract of *Dolichos biflorus* on Urinary albumin (g/l) levels in alloxan induced diabetic rats

Gr. no.	Treatment	Urinary albumin (g/l)		
		Day 15	Day 29	Day 43
1	Normal control	0.22 ± 0.03	0.24 ± 0.05	0.24 ± 0.05
2	Diabetic control	0.91 ± 0.09$^{\#\#}$	1.07 ± 0.09$^{\#\#}$	1.80 ± 0.09$^{\#\#}$
3	Diabetic + DB (175 mg/kg/day)	0.85 ± 0.06	0.78 ± 0.16**	0.64 ± 0.06**
4	Diabetic + DB (350 mg/kg/day)	0.79 ± 0.05	0.64 ± 0.15**	0.47 ± 0.05**
5	Diabetic + DB (700 mg/kg/day)	0.74 ± 0.02*	0.67 ± 0.12**	0.41 ± 0.02**

Results are presented as mean ± SEM. (n = 5), ANOVA followed by Dunnett test. $^{\#\#}P < 0.01$ when compared with Normal control; $^{*}P < 0.05$, $^{**}P < 0.01$ when compared with Diabetic control.

Table 4. Effect of treatment of methanolic extract of *Dolichos biflorus* on Serum creatinine (mg/dl) levels in alloxan induced diabetic rats

Gr. no.	Treatment	Serum creatinine (mg/dl)		
		Day 15	Day 29	Day 43
1	Normal control	0.67 ± 0.03	0.65 ± 0.04	0.68 ± 0.04
2	Diabetic control	1.24 ± 0.09$^{\#\#}$	1.56 ± 0.13$^{\#\#}$	1.83 ± 0.11$^{\#\#}$
3	Diabetic + DB (175 mg/kg/day)	1.21 ± 0.04	1.33 ± 0.02	1.58 ± 0.05
4	Diabetic + DB (350 mg/kg/day)	1.12 ± 0.03	1.28 ± 0.03*	0.98 ± 0.04**
5	Diabetic + DB (700 mg/kg/day)	1.03 ± 0.03	0.94 ± 0.02**	0.88 ± 0.02**

Results are presented as mean ± SEM. (n = 5), ANOVA followed by Dunnett test. $^{\#\#}P < 0.01$ when compared with Normal control; $^{*}P < 0.05$, $^{**}P < 0.01$ when compared with Diabetic control.

nondiabetic control is shown in Table 3. The mean rise in Urinary albumin levels in diabetic controls over the period of 43 days was significantly high as compared to normal control ($P < 0.01$). Treatment with DB (700 mg/kg) showed significant reduction in urinary albumin levels on day 15 ($P < 0.05$) and on day 29 and day 43 ($P < 0.01$), While DB (350 mg/kg) and DB (175 mg/kg) treatment groups, showed significant reduction in urinary albumin on day 29 and day 43 ($P < 0.01$).

Serum creatinine

The effect of Alloxan and treatment with plant extract on the creatinine levels is shown in Table 4. Diabetic control group showed significant increase in Serum creatinine compared to the normal control group throughout the duration of the experiment. Treatment with DB (700 mg/kg) showed significant reduction in serum creatinine level on day 29 and day 43 ($P < 0.01$) whereas treatment with DB (350 mg/kg) showed significant reduction in serum creatinine level on day 29 ($P < 0.05$) and on day 43 ($P < 0.01$), while treatment with DB (175 mg/kg) did not show any activity on this parameter.

Renal hypertrophy

Table 5 summarizes the effect of Alloxan and treatment with different doses of plant extracts on renal hypertrophy. The weight of kidneys was significantly higher in diabetic controls as compared to controls ($P < 0.01$). Treatment with DB (700 mg/kg) and DB (350 mg/kg) significantly prevented renal hypertrophy ($P < 0.01$ and $P < 0.05$ respectively) as compared to diabetic controls along with reduction in kidney weight. Treatment with DB (175 mg/kg) failed to modify renal hypertrophy. Renal hypertrophy measured as the ratio of kidney weight to total body weight in, Normal control, Diabetic controls, DB (175 mg/kg), DB (350 mg/kg), DB (700 mg/kg) treated group.

Table 5. Effect of treatment of methanolic extract of *Dolichos biflorus* on Kidney weight (g) and Kidney weight / Body weight ratio in alloxan induced diabetic rats

Gr. no.	Treatment	Kidney weight (g)	Kidney weight / Body weight
1	Normal control	0.702 ± 0.03	0.423 ± 0.02
2	Diabetic control	0.949 ± 0.04$^{\#\#}$	0.587 ± 0.03$^{\#\#}$
3	Diabetic + DB (175 mg/kg/day)	0.847 ± 0.03*	0.519 ± 0.08
4	Diabetic + DB (350 mg/kg/day)	0.779 ± 0.04**	0.474 ± 0.06*
5	Diabetic + DB (700 mg/kg/day)	0.717 ± 0.03**	0.445 ± 0.04**

Results are presented as mean ± SEM. (n = 5), ANOVA followed by Dunnett test. $^{\#\#}P < 0.01$ when compared with Normal control; $^{*}P < 0.05$, $^{**}P < 0.01$ when compared with Diabetic control.

DISCUSSION

Alloxan induced diabetes in rodents results in development of nephropathy similar to early stage clinical diabetic nephropathy (Mogensen and Rasch, 1980; Sassy-Prigent *et al.*, 1995). Urinary albumin levels are a selective marker of glomerular injury and elevated rates of albumin excretion are a harbinger of progressive nephropathy (Viberti *et al.*, 1982). Rodents such as rats and mice exhibit albuminuria normally (Mauer, 1978; Yotsumoto *et al.*, 1997) and during a period of 42 days, normal controls showed a rise in UAE by over 3 folds. UAE levels in the diabetic group, however, were many folds higher than normal controls. Treatment with DB prevented the rise in UAE levels with varying degree as seen in diabetic controls. The effect was more with DB (700 mg/kg) and less with DB (175 mg/kg) and DB (350 mg/kg). The observations of the present study indicate that the extracts of DB protect glomerulus from the injurious effects of diabetes. The histopathological examination of the kidneys from different groups supports this point as DB (700 mg/kg) and DB (350 mg/kg) treated kidneys were within normal limits. Diabetes induction by Alloxan has been known to produce increase in kidney weight relative to body weight (Kang, 1982). In the present study, the average net weight of kidney of diabetic controls was significantly higher than non-diabetic controls. This is consistent with the previous finding of Yotsumoto *et al.*, 1997. Treatment with DB (350 mg/kg) and DB (700 mg/kg) significantly ($P < 0.05$ and $P < 0.01$ respectively) prevented renal enlargement while DB (175 mg/kg) failed to bring any effect on this parameter. Previous studies on this respect have correlated the degree of renal enlargement with the degree of glycemic control (Rasch, 1979) while others have contradicted the glycemic theory, in the present study DB (350 mg/kg) ($P < 0.01$) and DB (700 mg/kg) ($P < 0.05$) exerted anti-hyperglycemic effect and prevented renal enlargement, anti-hyperglycemic properties may have played a role in preventing renal enlargement. Rasch (1980) reported that the rise in body weight was far less in the poorly controlled diabetic rats as compared to well-controlled diabetic rats. Similar observations were made in this study. Body weight was significantly affected by DB extract. In present study statistically significant increase was seen in serum creatinine levels in the Diabetic control group. Treatment with DB (350 mg/kg) and DB (700 mg/kg) significantly prevented the rise in serum creatinine level while in case of DB (175 mg/kg) treatment group there was not much effect on this parameter.

In conclusion, treatment with methanolic extract of DB [notably DB (350 mg/kg) and DB (700 mg/kg)] to diabetic rats prevented the increase in Serum creatinine, UAE excretion, and renal hypertrophy as well as caused reduction in plasma glucose levels. Treatment also halted the progression of weight loss. The present study showed that the DB exhibits potent nephroprotective effect (prevented renal enlargement in animals and attenuated the rate of increase in microalbuminuria along with reduction in renal hypertrophy) in diabetic rats, prevention of renal enlargement may be due to the anti-hyperglycemic effect of DB. Hence the traditional claim of this plant is proved. However, further work is warranted to pinpoint the exact mechanism of nephroprotective effect of DB and to identify the active constituent (s) responsible for such an effect.

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