

Full Length Research Paper

Cause and effect of *Plumbago zeylanica* root extract on blood glucose and hepatic enzymes in experimental diabetic rats

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This paper reports the results of oral administration of root extract of *Plumbago zeylanica* on blood glucose and plasma antioxidant status in streptozotocin (STZ) diabetic rats. The study was undertaken to evaluate hepatic enzymes in experimental diabetes. Oral administration of ethanolic extract (100, 200 mg/kg) in streptozotocin diabetic rats increased hepatic hexokinase activity and decreased hepatic glucose-6-phosphatase, serum acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). The *Plumbago zeylanica* root extract is hypoglycemic, hepatoprotective and is able to ameliorate biochemical damages in Streptozotocin induced diabetic rats.

Key words: *Plumbago zeylanica*, STZ diabetes, hepatic enzymes, liver protection.

INTRODUCTION

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. It represents a heterogeneous group of disorders having hyperglycemia, which is due to impaired carbohydrate utilization resulting from a defective or deficient insulin secretory response (Reaven, 1988). Diabetes is associated with micro and macro vascular complications, which are the major causes of morbidity and death in diabetic subjects (Huse et al., 1988; Bayness, 1991). To date there are different groups of oral hypoglycemic agents for clinical use, having characteristic profiles of side effects (Williams and Pickup, 1991; Kameswara et al., 1997).

Plumbago zeylanica L. (Plumbaginaceae) is a perennial, glabrous, sub-scandent shrub, cultivated throughout India and Malaysia. The root is bitter, hot, dry and carminative astringent to the bowels (Jayaraman, 1987.) and used as an anticancer agent (Jouad et al., 2000; Karawya et al., 1984). It has been reported to be abortifacient, alexipharmic, expectorant and diuretic (King et al., 1998). It is also used as laxative, to cure piles, bronchitis, itching and anemia (Kirtikar and Basu, 1975).

The leaves are caustic, vesicant, aphrodisiac and used to cure scabies (Kirtikar and Basu, 1980). Infusion of the roots and leaves are drunk as tea, to cure stomachache and gastric trouble (Krishnaswamy and Purushothaman, 1980.).

The tincture of the root was employed as cure laryngitis, rheumatism, leucoderma, ringworm, disease of liver and spleen (Mouhsine et al., 2001). In folklore practice, the roots of *P. zeylanica* is said to increase digestive power, improve appetite (Nadakarni and Nadakarni, 1954). Thus the use of *P. zeylanica* is an indigenous medicine as a rubifacient, local ecboic and sudorific (Ramachandran, 2001; Shastri, 1952.). The present paper reports the hypoglycemic effect of *P. zeylanica* root extract and the hepatic and serum enzymes on streptozotocin (STZ) induced diabetic rats.

MATERIALS AND METHODS

Preparation of plant extract

Root of *P. zeylanica* was collected and air-dried powder (500 g) of *P. zeylanica* roots were extracted by percolation at room temperature with 70% ethyl alcohol concentrated under reduced pressure at room temperature and dried in a vacuum desiccator. The residue was dissolved in distilled water and filtered. The filtrate

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Table 1. Effect of ethanolic extract of root of *P. zeylanica* on blood glucose and urine sugar in STZ-diabetic rats.

Group	Blood glucose (mg per 100 ml)		Urine sugar
	Initial	Final	
Control	67.6 ± 2.2	73.5 ± 3.5	Nil
Diabetic control	243.7 ± 3.5	286.5 ± 7.9**	+++
<i>P. zeylanica</i> (100 mg/kg/p.o)	241.3 ± 4.2	152.6 ± 9.1*	++
<i>P. zeylanica</i> (200 mg/kg/p.o)	247.4 ± 5.1	129.4 ± 8.8*	+
Tolbutamide (250 mg/kg/p.o)	242.5 ± 4.4	112.5 ± 9.7*	+

Values are mean ± S.D from 6 rats in each group; Diabetic control was compared with normal; experimental groups are compared with Diabetic control; values are statistically significant at **P<0.001 as compared with the normal; * P< 0.001 as compared with the Diabetic control; + indicates 0.25% sugar; +++ indicates 2% sugar.

was evaporated to obtain dry mass (yield = 48.5 g), which was diluted with 50% aqueous sucrose and used in experiments.

Chemicals

Streptozotocin (STZ) was obtained from Sigma Chemical Co, (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Animals

The study was carried out on either sex of Wistar albino rats (150 to 180 g). They were acclimatized for a week at laboratory conditions and provided standard pellet diet (Lipton, India) and water ad libitum. Six rats were housed per cage, to provide them sufficient space and to avoid unnecessary morbidity and mortality.

Preparation of diabetic rats

STZ (60 mg/kg) dissolved in saline was injected to tail vein of animals intraperitoneally. After a fortnight rats with moderate diabetes having glycosuria (indicated by Benedict's test for urine) and hyperglycemia, that is, with blood glucose levels of 200 to 280 mg per 100 ml were used for the investigation. Blood was collected from eyes (venous pool).

Experimental design

Diabetes was induced in animals 2 weeks before starting the treatment. After the induction, diabetic rats were divided into 5 groups of 6 animals each. Group I received vehicle alone, served as control. Group II received STZ (60 mg/kg/i.p) dissolved in saline. Group III and Group IV received the root extract of *P. zeylanica* (100 mg, 200 mg/kg/p.o) daily once for 42 days. Group V received tolbutamide (250 mg/kg/p.o) daily once for 42 days. During the second, fourth and sixth week of treatment, the urine sugar and blood glucose of all the rats were determined. Animals described as fasted were deprived of food for 12 h but allowed free access to drinking water. After 42 days of treatment, the animals were killed by cervical dislocation.

Collection of blood

Blood was collected in two separate tubes. One containing heparinized blood used for estimation of glucose. The other tube

containing the blood was allowed to clot at room temperature and the serum obtained after centrifugation was used for enzyme assays. Liver was excised and kept in ice-cold containers for enzyme assay.

Estimation of biochemical parameters

Blood glucose level was measured by glucose oxidase method (Triender, 1969). The activity of hexokinase in liver was determined by the method of Brandstrup et al. (1957). Liver glucose-6-phosphatase was determined according to the method of Koide and Oda (1959). Acid phosphatase (ACP), alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) were determined following the methods of King (1959a, b).

Statistical analysis

All experimental data were expressed as Mean ± S.D and statistically assessed by one-way analysis of variance (ANOVA). The difference between test animals and controls were evaluated by student's t-test (Scheff'e, 1953).

RESULTS

Changes in blood and urine glucose on treatment of diabetic rats with root extract and tolbutamide are presented in Table 1. The blood and urine glucose increased in STZ diabetic rats as compared to controls. Administration of root extract (100 mg, 200 mg/kg/p.o) and tolbutamide (250 mg/kg/p.o) decreased blood and urine glucose levels.

Effect of the administration of root extract and tolbutamide on hepatic hexokinase and glucose-6-phosphatase are presented in Table 2. The activity of hepatic hexokinase decreased while the activity of hepatic glucose-6-phosphatase increased in STZ treated diabetic rats as compared to controls. Administration of root extract (100 mg, 200 mg/kg/p.o), tolbutamide (250 mg/kg/p.o) increased the activity of hexokinase and decreased the activity of glucose-6-phosphatase as compared. Effect of root extract and tolbutamide on serum acid phosphatase, alkaline phosphatase and

Table 2. Effect of ethanolic extract of root of *P. zeylanica* on hepatic hexokinase and glucose-6-phosphatase in STZ diabetic rats.

Group	Hexokinase (μmol glucose phosphorylated / mg protein/ h)	Glucose-6-phosphatase (μmol phosphate / mg protein / min)
Control	0.169 \pm 0.02	0.175 \pm 0.002
Diabetic control	0.091 \pm 0.02*	0.366 \pm 0.004*
<i>P. zeylanica</i> (100 mg/kg/p.o)	0.142 \pm 0.015**	0.199 \pm 0.003**
<i>P. zeylanica</i> (200 mg/kg/p.o)	0.163 \pm 0.018**	0.185 \pm 0.001**
Tolbutamide (250 mg/kg/p.o)	0.160 \pm 0.016**	0.153 \pm 0.008**

Values are mean \pm S.D from 6 rats in each group; Diabetic control was compared with normal; experimental groups are compared with Diabetic control; values are statistically significant at *P<0.001 as compared with the normal; ** P< 0.001 as compared with the Diabetic control.

Table 3. Effect of ethanolic extract of root of *P. zeylanica* on serum acid phosphatase, alkaline phosphatase, lactate dehydrogenase in STZ diabetic rats.

Group	Acid phosphatase (K.A unit / dl)	Alkaline phosphatase (K.A unit / dl)	Lactate dehydrogenase (μmol pyruvate / g protein / min)
Control	3.4 \pm 0.6	10.1 \pm 1.5	117.0 \pm 3.5
Diabetic control	7.0 \pm 1.5*	21.5 \pm 2.8*	172.2 \pm 5.4*
<i>P. zeylanica</i> (100 mg/kg/p.o)	4.7 \pm 1.2**	15.2 \pm 2.4**	130.8 \pm 4.8**
<i>P. zeylanica</i> (200 mg/kg/p.o)	3.7 \pm 1.4**	12.4 \pm 2.5**	120.6 \pm 4.2**
Tolbutamide (250 mg/kg/p.o)	3.5 \pm 1.0**	11.5 \pm 2.2**	121.3 \pm 3.8**

serum lactate dehydrogenase are shown in Table 3. Administration of root extract (100 mg, 200 mg/kg/p.o), tolbutamide (250 mg/kg/p.o) decreased enzymes as compared to diabetic rats.

DISCUSSION AND CONCLUSION

The STZ-induction in adult animals produced Type IIdiabetes mellitus model. Streptozotocin selectively destroys the pancreatic insulin secreting beta cells; pancreatic cells became less active resembling condition of diabetes mellitus (Gilman et al., 1990). In the present study the root extract of *P. zeylanica* decreased blood glucose in streptozotocin diabetic rats.

Clinically used tolbutamide (A sulphonylurea drug) lowered the blood glucose level by stimulating β -cells to release insulin. Streptozotocin induced diabetes destroyed β -cells, impairing renal function (Jafri et al., 2000). Results in the present study using ethanolic extract showed marked hypoglycemic effect.

The plasma glucose lowering effect suggested that the *P. zeylanica* treatment revealed insulin-independent-mechanism. The extract perhaps produced hypoglycaemic effect by extra-pancreatic action (Dabis et al., 1984), possibly by stimulating glucose utilization in peripheral tissues (Naik et al., 1991; Obatomi et al., 1994) or due to an increase in glycolytic (Steiner and

Williams, 1959) and /or glycogenic enzymes activity in peripheral tissues (Naik et al., 1991). The extract may have decreased the secretion of the counter-regulatory hormones (glucagons, cortisol and growth hormones) (Roman-Ramos et al., 1995) or reduced absorption of glucose from gut (Akhtar and Iqbal, 1991; Sharma et al., 1996). Further studies are required to confirm this.

The activity of hexokinase enzymes decreased in the liver (Sheela and Augusti, 1992; Stanley et al., 2000). Administration of ethanolic extract of root increased the activity of hexokinase in liver. The increased activity of hexokinase could increase glycolysis and utilization of glucose for energy production.

The activity of hepatic glucose-6-phosphatase increased in alloxan treated diabetic rats (Sheela and Augusti, 1992; Stanley et al., 2000). Administration of ethanolic extract of root of *P. zeylanica* reduced the activity of glucose-6-phosphatase in liver. The reduction in glucose-6-phosphatase can result in decreased concentration of blood.

Increased activity of serum alkaline phosphatase, acid phosphatase, and Lactate dehydrogenase were also observed in diabetic rats (Stanley et al., 1997). The increase in the levels of these enzymes in diabetes may be as a result of the leaking out from the tissue, joining the blood stream. The extract did not produce any lethality or any changes in general behavior of rats (Alpana, 1996).

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