Indian Journal of Experimental Biology
Vol. 38, August 2000, pp. 781-784

Hypoglycemic effect of aqueous extract of *Enicostemma littorale* Blume (chhota chirayata) on alloxan induced diabetes mellitus in rats

Ravi Vijayvargia**, Monika Kumar & Sarita Gupta*

Department of Biochemistry, Faculty of Science, M.S. University of Baroda, Baroda 390 002, India

Received 21 April 1999; revised 9 March 2000

The whole plant aqueous extract of *E. littorale* was tested for its hypoglycemic activity on normoglycemic, hyperglycemic and alloxan induced diabetic rats. Blood sugar lowering activity was not observed in normoglycemic and glucose loaded hyperglycemic rats in the short time experiment. But in case of diabetic rats, the fall of blood sugar after 30 days treatment with the aqueous extract was found to be significant ($P<0.001$). The decrease in the plasma glucose level was accompanied with decrease in the level of glycosylated haemoglobin and glucose-6-phosphatase activity in liver. The potent anti-diabetic properties of *E. littorale* has been reported for the first time.

In recent times many traditionally used medicinally important plants were tested for their anti-diabetic potential by various investigators in the experimental animals[1-10]. These properties were attributed to different formulations, extracts and active principles. Working on the same line, we have undertaken a study on a traditional medicinal herb chhota chirayata or *E.littorale* Blume for its hypoglycemic activity. It is used as an Ayurvedic medicine in diabetes. Different formulations of the plant are used as a remedy in malaria, abdominal ulcer, rheumatism, hernia, swellings and insect poisoning[11]. *E. littorale* Blume (Family-Gentianaceae) is a 2-5 inches tall herb which grows throughout India up to 1500 feet from Punjab and Gangetic plains to Sri Lanka . It is more common in the plains and near the sea. A preliminary study suggests that the aqueous extract of the herb showed a significant hypoglycemic activity in the alloxan induced diabetic rabbits[12] but no further work was carried out in this direction. Hence the present study was undertaken to investigate the anti-diabetic potential of the medicinally important herb with respect to certain biochemical parameters. Also short term and long term experiments were carried out to elucidate mechanism of action of the herbal extract.

Materials and Methods

Chemicals—Alloxan monohydrate was purchased from Loba chemicals, Mumbai.

Plasma glucose was estimated by glucose oxidase-peroxidase kit purchased from Reckon diagnostics, Baroda. Thiobarbituric acid and glucose-6-phosphate was purchased from SRL Mumbai. All other chemicals used were of the analytical grade.

Animals—Charles Foster male albino rats, approximately of the same age, weighing 150-250 g. were used. Animals were housed under standard conditions and maintained on stock diet and water adlibitum.

Preparation of the aqueous extract—Dry *E. littorale* whole plant was procured locally and got identified in the Botany Department of the M.S. University of Baroda. Fine powder of the plant was made in the electric grinder. The powder was soaked in thrice the amount of water for 2 hr and then boiled for 20 min. Then it was passed through a fine cotton cloth to get the filtrate .The filtrate was concentrated to a final concentration of 1 g/ml by boiling. This was used as the aqueous extract in the study.

Induction of diabetes—120 mg/kg body weight of alloxan in saline (0.9% NaCl) was administered intraperitonially in male Charles Foster rats to induce diabetes. Control rats were injected with saline only. Prior to this the rats were fasted for 12 hr. After a fortnight urine sugar estimation was done by Benedict method[13]. Plasma glucose levels were estimated by GOD-POD kit method[14]. The rats with high urine sugar and > 200 mg/dl plasma glucose were selected for the diabetic group and were stabilized for 4 weeks before starting the aqueous extract treatment.

*Correspondent author
**Present Address: Lab. # 6, National Center for Cell Science, Pune University Campus, Ganeshkhind, Pune 411 007. India
E-mail-sarita_gupta_25@hotmail.com -vijayravi@hotmail.com

Experimental design

(a) Short term experiment

In case of the short term experiment, normoglycemic and glucose loaded hyperglycemic rats were used. In case of the normoglycemic experiment, 12 rats were fasted overnight and divided equally into two groups, viz. control and the test. 0 hr blood withdrawal was done from the orbital sinus and then the controls were fed with 5 ml of the tap water while the tests were given 5ml aqueous extract of the herb. Following this, the blood was withdrawn at 1 and 2 hr and the plasma glucose was estimated by the GOD-POD method for all the samples .For hyperglycemic experiment an additional step was carried out. After 1hr of feeding water to control rats and 5 ml of aqueous extract to the test rats, animals in both groups were given a glucose load of 1g/kg body weight. Thereafter glucose was estimated at 1 and 2 hr respectively.

(b) Long term experiment

For long term experiment 24 rats were taken, 18 of which were diabetic. They formed 4 groups, viz.

1. Control (5 ml of tap water to be fed daily)
2. Aqueous extract treated control (5 ml of the extract to be fed daily)
3. Untreated diabetics (5 ml of the tap water to be fed daily)
4. Aqueous extract treated diabetics. This group was further divided into

(a) Moderate diabetics (plasma glucose levels 200-300 mg/dl).

(b) Severe diabetics (plasma glucose levels > 300mg/dl).

The aqueous extract treatment was carried out for 30 days and blood was collected from the orbital sinus on 0, 10, 20 and 30 day respectively to estimate plasma glucose levels. Glycosylated haemoglobin[15] was estimated for all the groups on the 30 day of the experiment while the rats were sacrificed on 36 day and glucose-6-phosphatase enzyme activity[16] in the liver homogenate was estimated.

Results

The results of present investigation are summarized in Figs 1-3 and the Tables 1and 2. The aqueous extract of *Enicostemma littorale* was tested on laboratory animals for preliminary confirmation of its hypoglycemic potential and possible mode of action i.e. short term or the long term .The results of the short term normoglycemic experiment are shown in the Fig. 1 and that of short term hyperglycemic experiment are shown in Fig. 2.

Long term experiments were done with the diabetic rats along with the controls. Plasma glucose levels of rats on different days of the experiment are shown in Table 1 and the liver glucose-6-phosphatase activity in Table 2. Glycosylated haemoglobin levels are depicted in Fig. 3.

Discussion

It was seen that the extract has no hypoglycemic effect on normoglycemic as well as hyperglycemic rats at short term. But since the experiment was carried out only up to 2 hr and studies[17] have shown that

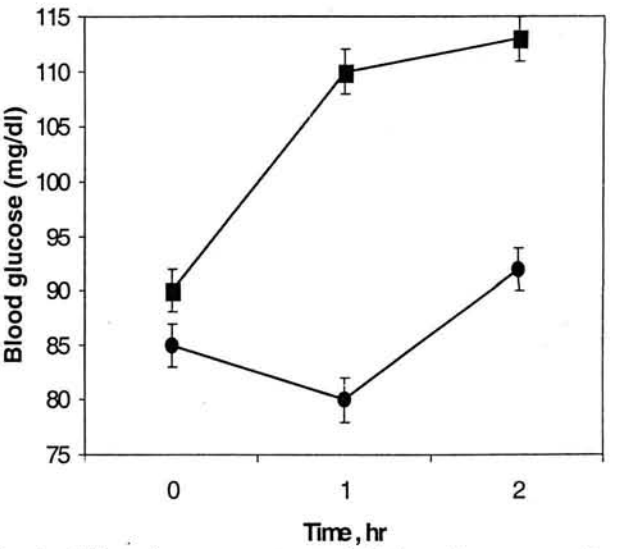

Fig. 1—Effect of aqueous extract of *E. littorale* on normoglycemic, control (-●-) and test (-■-) rats

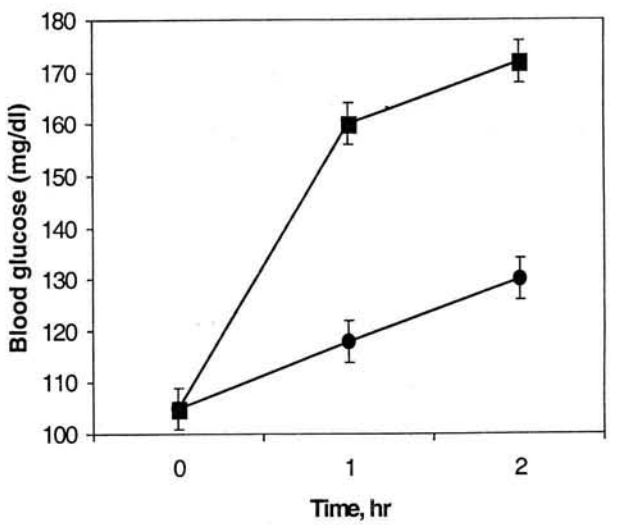

Fig. 2—Effect of aqueous extract of *E.littorale* on hyperglycemic, control (-●-) and test (-■-) rats

Table 1—Effect of aqueous extract of the *E.littorale* on plasma glucose levels of the experimental rats

	Control (mg/dl)	Drug treated Control (mg/dl)	Non-drug treated diabetics (mg/dl)	Drug treated diabetics	
				Moderate (mg/dl)	Severe (mg/dl)
0 day	102.05±3.85	97.93±3.27	400.4±45.7	291.2±24.6	577.3±2.84
10 days	105.5±2.03	108.8±2.78	459.8±58.3	108.9±7.59*•	485.3±35.1°
20 days	112.8±3.63	99.3±1.26	451.4±57.8	108.2±5.77*•	419.7±16.6*
30 days	105.8±5.83	109.7±1.66	492.4±58	111.3±3.84*•	404.9±17.4*

* $P > 0.001$ (compared to values on 0 day of the same group)
° $P > 0.05$ (compared to values on 0 day of the same group)
• $P > 0.001$ (compared to non-drug treated diabetic group)

the action may initiate after longer interval, it would be preferable to extend the time of the experiment up to 10 or more hours. Then only something can be said conclusively regarding the mode of action of herbal extract.

The plasma glucose levels in the extract treated group are shown to be higher than that of control animals at the end of two hours. Further studies are required to find out the possible mechanism responsible for this. In case of long term experiment on the diabetic rats, there is a significant hypoglycemic effect evident within 10 days of the aqueous extract administration in case of moderately diabetic rats. The plasma glucose levels changed from 291±24.6 to 108±7.59 mg/dl which is almost the normal level. In case of the severe diabetics, the effect was delayed but by the end of 20 days of the aqueous extract administration, the plasma glucose levels decreased from 577±2.84 to 419±16.6 mg/dl. Thus there was a 61% decrease in the plasma glucose levels of the moderately diabetic rats while in case of the severe diabetics the fall was 30%. The plasma glucose levels of the untreated diabetic group were increasing throughout the entire period. After 30 days of treatment with the aqueous extract, glycosylated haemoglobin levels were in the normal range for controls, extract treated controls and the extract treated moderately diabetic group .On the other hand in case of extract treated severely diabetics the levels were higher than normal but still less elevated than the untreated diabetic group. This observation is consistent with the plasma glucose levels as glycosylated haemoglobin levels corresponds with the plasma glucose levels. Thus the extract has a significant role in preventing the glycosylation of haemoglobin in case of moderate diabetes. The activity of the liver enzyme glucose-6-phosphatase was brought down to almost normal in case of moderately diabetic rats and decreased to a significant extent in a case of severe diabetic rats. Diabetic controls showed a very high activity. Thus the aqueous extract shows its action at the enzyme

Table 2—Effect of aqueous extract of *E. littorale* on glucose-6-phosphatase enzyme activity in experimental rats

Group	Specific activity (units/mg protein)
Control	7.81±0.457
Drug treated control	8.41±0.457
Non-drug treated diabetic	12.43±1.29*
Drug treated diabetic (moderate)	8.79±0.425•
Drug treated diabetic (severe)	10.26±0.775•

• $P<0.02$ (Compared to control group)
* $P<0.002$ (Compared to control group)

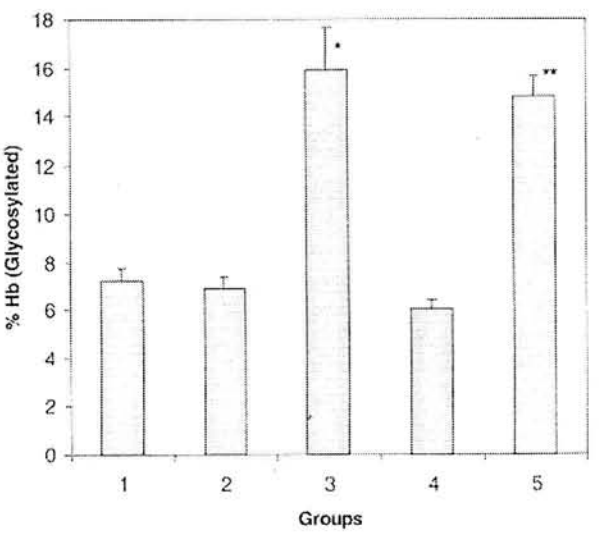

Fig. 3—Effect of aqueous extract of *E. littorale* on the glycosylated Hb levels in (1) control, (2) extract treated control, (3) untreated diabetic*, (4) extract treated diabetic (moderate), (5) extract treated diabetic rats**(severe). *$P > 0.002$. **$P > 0.001$

level also. Thus it can be concluded that the aqueous extract of the herb do not show any hypoglycemic activity at short term on normoglycemic as well as hyperglycemic rats but shows a significant plasma glucose lowering on long term treatment of the diabetic rats, especially in case of the moderately diabetic group (61%). The fall in plasma glucose is manifested in decreased levels of the glycosylated haemoglobin. The liver glucose-6-phosphatase activity is also regulated by the extract. Thus the aqueous extract of the *Enicostemma littorale* has a very potent anti-diabetic effect especially in case of moderate diabetes.

References

1 Pugazhenthi S & Murthy, P S, Partial purification of a hypoglycemic fraction from the unripe fruits of *Mormodica charantia* . *Indian J of Clinical Biochem*, 10 (1995) 19.

2 Sheela C G & Augusti K T, Anti-diabetic effects of S-allyl cysteine sulphoxide (SACs) isolated from garlic *Allium sativum* Linn. *Indian J Exp Biol*, 30 (1992) 523.

3 Sharma S R, Dwivedi S K, Swarup D, Hypoglycemic and hypolipidemic effects of *Cinnamomum tamala* Nees leaves. *Indian J Exp Biol*, 34 (1996) 372.

4 Chandrasekar B, Bajpai M B & Mukherjee S K, Hypoglycemic activity of *Swertia chirayita* (Roxb ex Flem) Karst. *Indian J Exp Biol*, 28 (1990) 616.

5 Srivastava Y, Bhat H V, Verma Y & Venkaiah K, Antidiabetic and adaptogenic properties of *Momordica charantia* extract : An experimental and clinical evaluation. *Phytotherapy Res*, 7 (1993) 285.

6 Mitra S K, Gopumadhavan S, Murlidhar T S, Anturlikar S D & Sujatha M B, Effect of D-400, a herbomineral preparation on lipid profile, glycated haemoglobin and glucose tolerance in streptozotocin induced diabetes in rats. *Indian J Exp Biol*, 33 (1995) 798.

7 Kalia A N, Agrawal D K, Saini A S & Lal H, Hypoglycemic study of some umbelliferae seeds in albino rats. *Indian J Clin Biochem*, 7 (1992) 49.

8 Chattopadhayay R R, Hypoglycemic effect of *Ocimum sanctum* leaf extract in normal and streptozotocin diabetic rats. *Indian J Exp Biol*, 31 (1993) 891.

9 Hossain M Z, Shibib B A & Rahman R, Hypoglycemic effect of *Coccinia indica*: inhibition of key gluconeogenic enzyme glucose-6-phosphatase. *Indian J Exp Biol*, 30 (1992) 418.

10 Puri D, Prabhu K M & Murthy P S, Hypocholesterolemic effect of hypoglycemic principle of fenugreek(*Trigonella foenum Gracecum*) seeds. *Indian J Clin Biochem*, 9 (1994) 13.

11 Chopra R N & Chopra I C, *Medicinal plants of India*,(Indian Council of Medical Research, N. Delhi), 1 (1954) 382.

12 Vyas D S, Sharma V N, Sharma H K & Khanna N K, Preliminary antidiabetic activity of *Aegle marmelos* and *Enicostemma littorale*. *J Res Ind Red Yoga and Homeop*, 14 (1979) 3.

13 Brandt R, Guyer K E & Banks W L Jr., Urinary glucose and Vitamin C. *Am. J. Clin. Pathol*, 68 (1977) 592.

14 Trinder P, Glucose oxidase method. *Annals. Clin. Biochem*, 6 (1969) 24.

15 Parker K M, England J D, Da Costa J, Hess R L & Goldstein D E, Improved colorimetric assay for glycosylated haemoglobin. *Clin Chem*, 27 (1981) 669.

16 Baginski E S, Foa P P & Zak B, *Methods in enzymatic analysis*, 2, 2nd Edition (Academic Press Inc., NewYork), 876.

17 Saxena A M, Bajpai M B, Murthy P S & Mukherjee S K, Mechanism of blood sugar lowering by Swerchirin containing hexane fraction (SWI) of *Swertia chirayita*. *Indian J Exp Biol*, 31 (1993) 178.