Antidiabetic and antihypercholesterolemic effect of *Hemidesmus indicus* Linn.R. root in Alloxan induced diabetic rats.

Received : 19.12.2006
Accepted : 10.01.2007

**Abstract**

Antidiabetic and antihyperlipidemic effect of *Hemidesmus indicus* Linn.R.root. (HIR) was investigated in rats. Administration of HIR (40 mg/g body weight/day) for four weeks significantly decreased the serum cholesterol, triglyceride, free fatty acids and phospholipid. Four weeks treatment of diabetic rats with HIR (40 mg/g body weight/day) showed significant hypoglycemic effect. Results of the present study show that HIR has hypocholesterolemic and antidiabetic effects.

**Keywords** : Antidiabetic activity, hypolipidimic agents, *Hemidesmus indicus*, root and Alloxan.

**Introduction**

Diabetes mellitus is characterized by hyperglycemia together with biochemical alterations of glucose and lipid metabolism (1). Liver is an insulin dependent tissue, which plays a vital role in glucose and lipid homeostasis and is severely affected during diabetes (2). Liver participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. During diabetes a profound alteration in the concentration and composition of lipid. (3). Decreased glycolysis, impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver (4). Many traditional plant treatments for diabetes mellitus are used throughout the world (5). Few of the traditional
plant treatments for diabetes have been shifted scientific scrutiny, and the World Health Organisation has recommended that this area warrants attention (6).

*Hemidesmus indicus* (HI, Family – Asclepiadaceae) known as Nannari in Tamil, and Indian sarsaparilla in English has been extensively used in Ayurvedic system of medicine. Each part of the plant has medicinal value. The root gives cooling effect and used in fever, diabetes, cough, cures blood disorders, and has got diuretic effect (7, 8). This study was thus initiated with the aim of evaluating the effects of an aqueous extract of *Hemidesmus indicus* L. on the blood glucose level and serum lipids in alloxan induced diabetic rats.

**Materials and Methods :**

**Plant material :**

*Hemidesmus indicus* L. roots were collected from Maruthamalai hills, Coimbatore District, Tamil nadu, India. The plant was identified and authenticated at the Department of Botany, Kongu nadu Arts and Science College, Coimbatore. 500g of the plant was soaked overnight in 1.5 litres of 95% ethanol. This suspension was filtered and the residue was resuspended in an equal volume of 95% ethanol for 48 h and filtered again. The two filtrates were pooled and the solvents were evaporated at 40° - 50° c under reduced pressure and lyophilized (9).

**Animals :**

All the experiments were carried out with male Wister rats aged seven to eight weeks (180 – 200g), obtained from the Veterinary Hospital, Thrissur, Kerala, India. The animals were housed in polypropylene cages and provided with water and standard pellet diet (Karnataka Agro Food Corporation Limited, Bangalore, India) ad Libitum. The animals used in the present study were approved by the ethical committee. The care of the animals was as per the “Guidelines for the care and use of Animals in Scientific Research” prepared by the Indian National Science Academy, New Delhi (10).

**Induction of experimental diabetes :**

The rats were injected intraperitonially with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body weight (11). After 2 weeks, rats with moderate diabetes having glycosuria (indicated by Benedict’s qualitative test) and hyperglycemia (i.e. with a blood glucose of 200 – 300 mg/dl) were used for the experiment.

**Experimental procedure :**

In the experiment, a total of 25 rats (15 diabetic surviving rats, 10 normal rats) were used. The rats were divided into 5 groups or five rats each.

**Group 1 :** Normal untreated rats.

**Group 2 :** Diabetic control rats given 1 ml of aqueous solution HIR extract daily using Intragastric tube for 30 days.

**Group 3 :** Diabetic rats given
glibenclamide (600 μg / kg body weight) (12) in 1 ml of aqueous solution daily using an intragastric tube for 30 days.

Group 4: Diabetic rats given HIR extract (400 mg/kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 30 days.

Group 5: Normal rats given HIR extract (400 mg/kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 30 days.

At the end of 30 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in two different tubes that is one with anticoagulant – potassium oxalate and sodium fluoride for plasma and another without anticoagulant for serum separation. Plasma and serum were separated by centrifugation.

Analytical procedure:

Blood glucose was estimated by O – Toluidine method (Sasaki et al) (1972) (13). Lipids were extracted from serum by the method of Folch et al (1957) (14). Total cholesterol and triglycerides were estimated by the method of Zlatkis et al (1953) (15) and Foster and Dunn (1973) (16). Free fatty acid and phospholipids were analyzed by the method of Falholt et al (1973) (17) and Zilversmit et al (1950) (18).

Statistical analysis:

All values were expressed as the mean ± SD obtained from a number of experiments (n). Data from all the tables of normal animals, diabetic control animals, reference drug treated and HIR extract treated animals were compared by ONE WAY ANOVA followed by Duncan’s Multiple Range Test (DMRT) (19).

Results:

Blood Glucose:

Table 1. Blood glucose and urine sugar of normal and experimental rats

| Groups | Blood glucose (mg/dl) | Plasma insulin (µ U/ml) | Urine sugar ^ |
|--------|-----------------------|-------------------------|--------------|
| Group I | 104.64±3.90 | 9.84±0.65 | Nil |
| Group II | 300.80±0.40a* | 3.38±0.71a* | +++ |
| Group III | 109.20±1.93b* | 9.52±0.69b* | TRACE |
| Group IV | 111.20±2.05c,ens | 9.18±0.71c,ens | Nil |
| Group V | 106.80±2.60dns | 9.80±0.52dns | Nil |
Values are given as Mean ± SD (n = 5 rats).

Statistical comparison: a: Group I and II, b: Group II and III, c: Group II and IV
d: Group I and V, e: Group III and IV
* P < 0.05, ns – not significant.
A – Indicates 0.25% sugar and (+++) indicates more 1% sugar.

### Table 2.

Changes in levels of Cholesterol, Free fatty acid, triglycerides and phospholipids in serum of normal and experimental animals.

| Groups  | Cholesterol (mg/dl) | Free fatty acid (mg/dl) | Triglycerides (mg/dl) | Phospholipids (mg/dl) |
|---------|---------------------|-------------------------|-----------------------|-----------------------|
| Group I | 75.25±0.91          | 59.73±0.68              | 99.64±0.20            | 79.19±0.24            |
| Group II| 174.20±0.58<sup>a</sup> | 119.90±1.54<sup>a</sup> | 167.54±1.14<sup>a</sup> | 132.80±1.49<sup>a</sup> |
| Group III| 76.38±0.58<sup>b</sup> | 61.30±0.90<sup>b</sup> | 101.62±0.37<sup>b</sup> | 85.70±0.62<sup>b</sup> |
| Group IV| 76.80±0.80<sup>c,ens</sup> | 61.38±0.13<sup>c,ens</sup> | 101.73±0.15<sup>c,ens</sup> | 85.79±0.25<sup>c,ens</sup> |
| Group V | 75.68±0.91<sup>dns</sup> | 60.69±0.84<sup>dns</sup> | 99.69±0.07<sup>dns</sup> | 79.86±0.66<sup>dns</sup> |

Values are given as Mean ± SD (n = 5 rats).

Statistical comparison: a: Group I and II, b: Group II and III, c: Group II and IV
d: Group I and V, e: Group III and IV
* P < 0.05, ns – not significant.

Results:

Blood glucose:

Table 1 shows the levels of blood glucose and urine sugar of normal and experimental rats. There was a significant elevation in blood glucose in alloxan diabetic rats which decreased significantly to normal level in experimental rats when compared with normal rats. Administration of HIR extract and glibenclamide separately tends to bring the parameters significantly towards the normal.

In diabetic rats the urine sugar was (+++) but in the case of HIR extract treated rats showed no urine sugar as seen in normal rats. These effects were compared with glibenclamide.

Serum lipids:

The effect of HIR extract on serum lipids of normal and experimental rats is summarized in Table 2. A marked increase in the level of cholesterol, free fatty acids, triglycerides and phospholipids were observed in
diabetic rats. Treatment with HIR extract significantly reduced the lipid levels.

**Discussion:**

Alloxan is well known for its selective pancreatic islet â– cell toxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms (20). Intrapерitoneal administration of alloxan (150mg/kg) effectively induced diabetes in normal rats as reflected by glycosuria, hyperglycaemia, polyphagia and polydipsia when compared with normal rats (21). In our present study we have observed that an aqueous extract of *Hemidesmus indicus* root can reverse these effects. The possible mechanism by which HIR extract brings about its antihyperglycemic action may be by potentiation of pancreatic secretion of insulin from â– cell of islets or due to enhanced transport of blood glucose to peripheral tissue. This was clearly evidenced by the increased level of insulin in diabetic rats treated with HIR extract. In this context a number of other plants have also been reported to have antihyperglycemic and insulin – release stimulatory effect (22,23).

Hypercholesterolemia and hypertriglycerolemia are major risk factors for atherosclerosis and related occlusive vascular disease (24). Clinical complications such as atherosclerosis could be diminished and life prolonged when blood lipids are lowered by hypcholesterolaemic drugs. (25, 26).

Excess of fatty acids in serum produced by the alloxan – induced diabetes promotes conversion of excess fatty acids into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins (27). The abnormal high concentration of serum lipids in the diabetic subject is, mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in alloxan diabetic rats (28,29) and significant increase observed in our experiment was in accordance to these studies. The marked hyperlipidaemia that is characteristic of the diabetic state may therefore, be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (30).

Activities of enzymes suggest that enhanced lipid metabolism during diabetes is shifted towards carbohydrate metabolism and it enhances the utilization of glucose at the peripheral sites. One of the possible actions of HIR extract may be due to its inhibition of endogenous synthesis of lipids.

**Conclusion:**

It can be concluded from the data that HIR extract significantly reduces the levels of serum lipids, which are actively raised in alloxan diabetes rats. HIR extract has beneficial effect on plasma insulin. Moreover its antihyperlipidemic effect could
represent a protective mechanism against the development of atherosclerosis.

References:

1. Arky RA. Clinical correlates of metabolic derangements of diabetes mellitus in: Kozak GP. (Ed), Complications of Diabetes mellitus, Saunders WB. Philadelphia, 1982; 16-20.

2. Seifter S, England S. Energy metabolism, In: Arias I, Popper H, Schacter D, et al (Eds.). The liver: Biology and Pathobiology, Rauen Press, New York, 1982; 219 – 49.

3. Sochor M, BaquerNz, McLean P. Glucose over and under utilization in diabetes: Comparative studies on the change in activities of enzymes of glucose metabolism in rat kidney and liver. Mol. Physiol. 1985; 51:68.

4. Baquer N. Glucose over utilization and under utilization in diabetes and effects of antidiabetic compounds. Ann Real Acad Farm 1998; 64:147-80

5. Swanston Flatt SK, Day C, Bailey CJ and Flatt RR. Traditional plant remedies for diabetes. Studies in the normal and Streptozotocin diabetic mice. Diabetologia 1990; 33:462-4.

6. WHO Expert Committee on diabetes mellitus second report. Technical Report Series 646. World Health Organization. Geneva 1980; 61.

7. Role of Biotechnology in medicinal and aromatic plants: Volume XIII.

8. Deepak Acharya and Rai M.K. Traditional knowledge for curing various ailments among Gonds and Bharias of Patalkot valley, M.P., India 1996 -2000.

9. Pandey VN, Rajagopalan SS, ChowdhorryDP: An effective ayurvedic hypoglycemic formulation. J. Res. Ayur. Sid. 1995, 16: 1-14.

10. Anonymous, Guidelines for care and use of animals in scientific research, Revised edition, Indian National Science Academy, New Delhi (2000).

11. Katsumata K, Karsumata y, Ozawa T and Katsumata K: Potentiating effects of combined usage of three sulfonylurea drugs on the occurrence of alloxan diabetic rats. Horm. Metab. Res. 1999, 25: 125-126.

12. Pari L and Uma Maheswari J. Anti hyperglycemic activity of Musa Sapentium flower: Effect on lipid peroxidation in alloxan diabetic rats Phytother Res 2000; 14:1-3.

13. Sasaki T, Rinsho Kagaku, Matzy S and Sonal A. Effect of acetic acid concentration on he color reaction in the O- toluidine boric acid method for blood glucose estimation. 1972; 1: 346-53.

14. Folch J, Less M and Solane SGH. A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem. 1957; 26; 497-509.

15. Zlatkis A, Zak B and Bogle GJ. A method for the determination of serum cholesterol J Clin Med 1953; 41:486-92.
16. Foster LB, Dunn RT. Stable reagents for determination of serum triglycerides by colorimetric hantzsch condensation method. Clin. Chem. 1973; 19: 338-40.

17. Folholt K, Falholt W and Lund B. An easy colorimetric method for routine determination of free fatty acids in plasma. Chem. Acta. 1973; 46: 105-11.

18. Zilversmit DB and Davis AK. Micro determination of phospholipids by TCA precipitation. J. Lab. Clin. Med. 1950; 55: 155-61.

19. Bennet P and Franklin NH. Statistical analysis in chemistry and chemical industry. New York: John Wiley and Sons, USA. 208-27.

20. Papaccio G, Pisanti FA, Latronico MV, Ammendola E and Galdieri M. Multiple low dose and single high dose treatments with streptozotocin do not generate nitric oxide J. Cell Biochem 2000; 77 (1) : 82-91.

21. Calabresi P and Chabner BA. Antineoplastic agents. In Goodman A, Rall JW (Eds). The pharmacological basis of therapeutics. 8th Edition Pergmann Press, New York. 1209-63.

22. Prince PSM, Menon VP and Pari L Hypoglycemic activity of Syzigium cumini seeds: Effect on lipid peroxidation in Alloxan diabetic rats. J. Ethanopharmacol 1998; 61: 1-7.

23. Pari L and Uma Maheswari J. hypoglycemic effect of Musa sapreitum L. in Alloxan induced diabetic rats. J. Ethanopharmacal 1999;

24. Castelli WP, Garison RJ, Wilson PW, Abbot RD, Kalousdian S and Kaund WB, Incidence of coronary artery disease and lipoprotein cholesterol levels, J. Am. Med. Assoc. 256 (1986) 2835.

25. Lipid Research Clinics Program, The lipids research clinics coronary primary prevention trial results. 1. Reduction in incidence of coronary heart disease, J.Am. Med. Assoc. 251 (1984a) 251.

26. Lipid Research Clinical Program, The lipid research clinics coronary primary prevention trial results. 11. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering, J. Am. Med. Assoc. 251 (1984b) 365.

27. Bopanna KN, Kannan J, Sushma G, Balaraman R, Rathod SP. Antidiabetic and antihyperlipaemic effects of neem seed kernel powder on alloxan diabetic rabbits. Indian J. Pharmacol 1997; 29: 162-7.

28. Sharma SR, Dwivedi SK and Swarup D. Hypoglycemic and hypolipidaemic effects of Cinnamomum tomala nees leaves. Ind.J. Exp. Biol.1996; 34: 372-4.

29. Pushparaj P, Tan CH and Tan BKH. Effects of Averrhoa bilimili leaf extract on blood glucose and lipids in Streptozotocin diabetic rats. J. Ethanol Pharmacol 2000; 72: 69- 76.

30. Goodman LS and Gilman A. The pharmacological basis of therapeutics, 7th Edition. Mac Millan, New York, 1985; 1490-510.