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

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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

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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

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glibenclamide (600 ìg / 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 (400mg/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 [A]
Group I	104.64±3.90	9.84±0.65	Nil
Group II	300.80±0.40[a*]	3.38±0.71[a*]	+++
Group III	109.20±1.93[b*]	9.52±0.69[b*]	TRACE
Group IV	111.20±2.05[c*,ens]	9.18±0.71[c*,ens]	Nil
Group V	106.80±2.60[dns]	9.80±0.52[dns]	Nil

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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[a*]	119.90±1.54[a*]	167.54±1.14[a*]	132.80±1.49[a*]
Group III	76.38±0.58[b*]	61.30±0.90[b*]	101.62±0.37[b*]	85.70±0.62[b*]
Group IV	76.80±0.80[c*,ens]	61.38±0.13[c*,ens]	101.73±0.15[c*,ens]	85.79±0.25[c*,ens]
Group V	75.68±0.91[dns]	60.69±0.84[dns]	99.69±0.07[dns]	79.86±0.66[dns]

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

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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]. Intra peritonial administration of alloxan (150mg/kg) effectively induced diabetes in normal rats as reflected by glycosuria, hyperglycaemia, poly phagia 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 antihy perglycemic 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 hypertriacy lglycerolemia 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 hypocholesterolaemic drugs. [25, 26].

Excess of fatty acids in serum produced by the alloxan – induced diabetes

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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. Hyper cholesterolemia and hypertrig-lyceridemia 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.

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