ORIGINAL ARTICLE

Hemidesmus indicus root extract ameliorates diabetes-mediated metabolic changes in rats

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India has more than 40 million diabetic people that represent nearly 20% of total diabetes population worldwide. Allopathic medicines are currently used for control of diabetes but often they are overprescribed or found to be dangerous on long-term use due to its toxicity and side effects. Plant-based remedies remain to be one of the most popular complementary treatments for diabetes mellitus and are considered to be natural and comparatively safe. Traditionally, many plants are used in Ayurveda, Sidha and folklore systems of medicines to treat diabetes mellitus, but the pharmacognostic and pharmacological studies on many of these plants are yet to be performed. Hence, the present study aims to explore the anti-diabetic activity of *Hemidesmus indicus* roots in streptozotocin-induced diabetic rats. The oral administration of aqueous extract at doses of 500 mg/kg significantly reduced the blood glucose within 5 h and 12-week treatment reverted the altered levels of insulin, glycosylated hemoglobin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyl transferase (γ—GT) and creatine kinase (CK) to near normal levels in diabetic rats. The results of the present study suggest that *H. indicus* administration not only reduces blood glucose but also offers protection to diabetes-induced metabolic alterations in rats.

Key words: Diabetes mellitus, *Hemidesmus indicus*, metabolic changes, streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease that is growing in prevalence worldwide.[1] It is the most common endocrine disorder affecting more than 100 million people worldwide (6% of the population) and more than 40 million people in India alone. In the next 10 years, it may affect more than five times of people than it does now.[2] Moreover, diabetes is the fastest growing metabolic disease and happens to be the third most common disease in the world after cardiovascular and oncological disorders.

At present, the increasing prevalence of obesity and more frequent food intake lead to childhood and adult type-2 diabetes.[3] Hence, aggressive control is very important to decrease microvascular and macrovascular complications, which are considered to be the major cause of morbidity and mortality in DM.[4] The microvascular complications of diabetes encompass long-term complications of diabetes affecting small blood vessels and macrovascular complications including hypertension and dyslipidemia, which affects large blood vessels and causes heart attack and stroke. Generally, treatment options are either pharmacologic or non-pharmacologic therapy that includes dietary control and physical exercise to reduce the excess weight.

Among the therapies, non-pharmacologic therapy (e.g. diet, exercise and weight loss) remains to be a critical component in diabetes treatment. Dietary management includes the use of traditional medicines mainly derived from plants.[5] Traditional medicine practices include incorporation of plant-, animal- and mineral-based medicines either applied singularly or in combination to treat, diagnose and to prevent the illnesses or to maintain well-being. Even now, approximately 80% of the third world populations are almost dependent on traditional medicines.[6] The ethno-botanical information containing the details of the plants and its use for the treatment of various conditions including DM are available for several plants to date,[4] but there is little information about plants possessing both hypoglycemic and hypolipidemic effects.[7]

H. indicus R.Br. (Indian Sarsaparilla) is used in traditional medicine as one of the Rasayana plants of Ayurveda, for its anabolic effect. Rasayana plants are characteristically anabolic in nature because they stimulate protein synthesis and other metabolic activities. They stimulate the flow of bile and also remove toxins from the body. They are good diuretic agent, increase the flow of urine upto three to four times and serve as an alternative tonic, demulcent (capable of forming a soothing film on the surface of the membrane), diaphoretic and are used to treat venereal diseases, skin diseases, urinary infections, negative emotions (bad moods) and impotence.[8] It also prevents abdominal distention,

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arthritis, rheumatism, gout and epilepsy.[9] The roots of the plant are woody, sweet in taste and possess cooling effect. Roots are one of the well-known drugs in the Ayurvedic system of medicine.[10] *H. indicus* root extract has been reported to protect DNA from radiation-induced strand breaks[11] and also used in traditional medicine for gastric ailments.[12] *H. indicus* mainly consists of essential oils and phytosterols such as hemidesmol, hemidesterol and sponins. 2-hydroxy-4-methoxybenzoic acid (HMBA) isolated from the roots has been shown to possess potent anti-inflammatory, antipyretic and antioxidant properties[13] and also protects ethanol,[14] CCL_4 and paracytomol-induced[15] hepatic damage. The oil from the roots has been shown to contain over 40 minor constituents. Among them, nerolidol, borneol, linalyl acetate, dihydrocarvyl acetate, salicylaldehyde, isocaryophyllene, α-terpinyl acetate and 1, 8-cineol were reported to be important aromatic and bio-active principles.[16]

The hypoglycemic activity of this plant extract was already reported by us,[17] but the ameliorative potential has not been explored so far. The main objective of this study was to assess the ameliorative effect of the *H. indicus* aqueous extract on diabetes-mediated metabolic alterations.

MATERIALS AND METHODS

Plant Material

The roots of *H. indicus* were collected from the Morappur forest area, Dharmapuri District, Tamil Nadu, during the month of April 2005. The plants were authenticated in the Forest Department, Dharmapuri District, Tamil Nadu, where a voucher specimen (FDSC 201) was also submitted. The roots were washed with distilled water, shade dried, powdered and stored in air-tight containers until further use.

Preparation of Root Extract

The powdered roots of *H. indicus* (100 g) were used to obtain their juice using a Turmix electric extractor with 500 ml of sterile distilled water. The extract was filtered and the residue was removed. The extract was concentrated under vacuum to obtain a solid residue and freeze dried and the yield was calculated (3-7% w/v).

Animals

Male albino rats (Wistar strain, 150-200 g) were purchased from Tamil Nadu Veterinary and Animal Sciences University, Madhawaram, Chennai, and housed under standard husbandry conditions (30° C ± 2°, 60 -70 % relative humidity and 12 h: 12 h day-night cycle) and allowed standard pellet rat feed and water ad libitum (free access to water). Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee (IAEC - VIT University).

Induction of Diabetes Mellitus

Diabetes was induced experimentally in rats by single intraperitoneal injection of a freshly prepared solution of streptozotocin (STZ) (Sigma, USA) at a dose of 35 mg/kg bodyweight in 0.1 M cold citrate buffer, pH 4.5. STZ induces DM by destroying the pancreatic beta cells. After 72 h, blood was collected from the tail vein under ether anesthesia with aseptic procedure and blood glucose levels were determined using Autoanalyser, Microlab 2000. Animals were considered to be diabetic if the blood glucose values were above 250 mg/dL and STZ-induced diabetic rats were stabilized in diabetic condition over a period of 7 days[18] and those rats survived alone were selected for this study. Control rats were given citrate buffer (pH 4.5).

Experimental Design

Animals were divided into four groups of six animals each. Group I served as control rats; group II had STZ- treated surviving diabetic rats; group III served as a positive control and received a standard hypoglycemic drug, tolbutamide (100 mg/kg bw/day); group IV rats were treated with aqueous extract at 500 mg/kg/day for 12 weeks by the oral intubation method. Animals were sacrificed at the end of 12 weeks after collecting blood from retro-orbital plexus under ether anesthesia for biochemical estimations.

Biochemical Estimations

Plasma insulin level was determined by using radioimmunoassay kit (Pharmacia, Uppsala, Sweden) with a betamatic counter (Cronex, Dupont, France). The kit included human insulin as standard and I^{125}-labelled human insulin as antibody, which cross-reacts with rat insulin. Determination of total hemoglobin was estimated by the cyanomethemoglobin method,[19] and glycosylated hemoglobin (HbA_1C) was estimated by the modified method.[20] Measurement of serum total cholesterol, triglycerides and serum HDL-cholesterol was determined by using commercial kits (Dialab, Austria). The activities of plasma enzymes, alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1), alkaline phosphatase (ALP; EC 3.1.3.1), gamma glutamyl transferase (γ-GT; EC 2.3.2.2) and creatine kinase (CK; EC 2.7.3.2) were measured by using Ecoline kits (E. Merck) in an Autoanalyser (Microlab 2000).

Toxicity Studies

To assess the toxic effect of the *H. indicus* extract, a median lethal dose (LD 50) study was carried out on different groups of rats. The aqueous extract was administered orally at doses ranging from 100 mg- 1 g/kg/day to 2-5 g/kg/day to different groups of rats (n = 6) for 7 days. The rats were observed for any lethal effect of the test drug and dosage. The median effective dose (ED 50) was calculated for the test drug with respect to its effect on reduction of blood glucose.

Statistical Analysis

Statistical analysis was performed using the SPSS software package, version 9.05. The values were analyzed by one- way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). All the results were expressed in mean ± SD for six rats in each group. P values < 0.05 were considered to be statistically significant.

RESULTS

Effect on Blood Parameters

The effect of the *H. indicus* extract on plasma insulin, total hemoglobin, glycosylated hemoglobin and liver glycogen at the end of 12 weeks of study period is given in Table 1. In diabetic rats, the levels of liver glycogen, plasma insulin and total hemoglobin were significantly ($F > 0.05$; $P < 0.001$) decreased with elevated levels of glycosylated hemoglobin. Oral administration of *H. indicus* extract significantly ($F > 0.05$; $P < 0.001$) increased the levels of liver glycogen, plasma insulin, total hemoglobin to near normal level and also restored the glycosylated hemoglobin level.

Effect on Lipids

Table 2 presents the levels of serum lipids in control and in diabetic rats at the end of 12 weeks of treatment period. Total cholesterol, triglycerides and LDL cholesterol levels were significantly ($F > 0.05$; $P < 0.001$) elevated in diabetic rats with decreased HDL cholesterol level. Oral administration of *H. indicus* aqueous extract brought back the levels of serum lipids to near normal.

Effect on Serum Marker Enzymes

The effect of the *H. indicus* aqueous extract on the activities of serum enzymes on 12 weeks of treatment is given in Table 3. The significantly ($F > 0.05$; $P < 0.001$) elevated levels of AST, ALT, ALP, γ-GT and CK in diabetic rats were normalized on treatment with *H. indicus* extract.

Toxicity Studies

H. indicus extract-treated rats appeared normal and there was no toxic effect on rats up to 20-50 times of the effective dose (500 mg/kg). There were no deaths in any of these groups.

Table 1: Effect of *H. indicus* (500 mg/kg/day) on plasma insulin, hemoglobin, glycosylated hemoglobin and hepatic glycogen in normal and streptozotocin-induced diabetic rats at the end of 12 weeks of treatment period

Groups	Dose (mg/kg/day)	Plasma insulin (μU/mL)	Total hemoglobin (g/dL)	Glycosylated hemoglobin (%)	Liver glycogen (mg/g of wet tissue)
Control	-	15.62 ± 1.3	15.50 ± 0.5	5.5 ± 0.4	9.75 ± 0.74
Diabetic control	-	6.89 ± 1.0*	12.95 ± 1.0*	7.2 ± 0.5*	5.69 ± 0.69*
Diabetic + Tolbutamide	100	13.90 ± 1.4#	14.10 ± 1.1#	4.5 ± 0.3#	8.23 ± 0.12#
Diabetic + *H. indicus* extract	500	13.98 ± 1.8#	15.95 ± 0.6#	4.9 ± 0.5#	9.55 ± 1.32#

Each value is expressed as mean ± SD for six rats in each group (n = 6). *Different from normal control, $F > 0.05$ (ANOVA) and $P < 0.05$ (DMRT). # Different from diabetic control, $F > 0.05$ (ANOVA) and $P < 0.05$

Table 2: Effect of *H.indicus* (500 mg/kg/day) on serum total cholesterol, triglycerides, HDL and LDL levels in normal and streptozotocin-induced diabetic rats at the end of 12 weeks of treatment period

Groups	Dose (mg/kg/day)	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL cholesterol (mg/dL)	LDL cholesterol (mg/dL)
Control	-	80 ± 2.13	84 ± 1.0	27.7 ± 1.1	32.2 ± 1.5
Diabetic control	-	126 ± 2.9*	125 ± 2.5*	26.0 ± 1.5	46.1 ± 2.1*
Diabetic + Tolbutamide	100	80 ± 1.5#	87 ± 1.9#	29.5 ± 1.8#	33.2 ± 2.5#
Diabetic + *H. indicus* extract	500	77 ± 1.8#	83 ± 1.6#	28.5 ± 1.4#	32.0 ± 2.1#

Each value is expressed as mean ± SD for six rats in each group (n = 6). *Different from normal control, $F > 0.05$ (ANOVA) and $P < 0.05$ (DMRT). # Different from diabetic control, $F > 0.05$ (ANOVA) and $P < 0.05$

Table 3: Effect of *H.indicus* (500 mg/kg/day) on serum marker enzymes aspartate transaminase, alkaline phosphatase, alkaline phosphatase, γ- glutamyl transferase and creatine kinase in normal and streptozotocin-induced diabetic rats at the end of 12 weeks of treatment period

Groups	Dose (mg/kg/day)	AST (U/L)	ALT (U/L)	ALP (U/L)	γ GT (U/L)	CK (U/L)
Control	-	210 ± 8.0	181 ± 1.5	233 ± 1.7	12.54 ± 1.0	352 ± 1.2
Diabetic control	-	230 ± 8.0*	185 ± 1.5*	240 ± 1.2*	13.95 ± 1.2*	630 ± 0.3*
Diabetic + Tolbutamide	100	222 ± 7.9#	182 ± 1.2#	231 ± 1.2#	12.60 ± 1.6#	352 ± 1.2#
Diabetic + *H. indicus* extract	500	222 ± 6.5#	182 ± 1.8#	233 ± 1.8#	12.65 ± 1.5#	356 ± 1.2#

Each value is expressed as mean ± SD for six rats in each group. *Different from normal control, $F > 0.05$ (ANOVA) and $P < 0.05$. #Different from diabetic control, $F > 0.05$ (ANOVA) and $P < 0.05$

DISCUSSION

The present investigation was aimed to evaluate the ameliorative effects of *H. indicus* extract on STZ-induced diabetes-mediated metabolic alterations in rats. Earlier reports in our laboratory showed the hypoglycemic effect of *H. indicus* extract in STZ-induced diabetic rats.[17] The observed effect on insulin, total and glycosylated hemoglobin and glycogen in experimental rats might be due to the stimulatory effect of *H. indicus* extract on the regenerating β-cells and also on the surviving β-cells in diabetic rats. It has been reported that STZ administration produces partial destruction of pancreatic β-cells with permanent diabetes condition.[21] A number other plants have also been shown to exert hypoglycemic activity through stimulation of insulin release.[22,23] The stimulatory activity of *H. indicus* extract was compared with the effect of tolbutamide. It was long been used to treat DM and it stimulates insulin secretion by acting on the pancreatic β-cells. From the results, it appears that *H. indicus* extract stimulates the surviving functional β-cells for insulin release and might also induce the regeneration of β-cells. Two mechanism have been proposed for regeneration of β-cells, which includes budding of the pancreatic ductal epithelium (islet-neogenesis)[24] and replication of existing β-cells.[25]

The decrease in hemoglobin content of diabetic rats might be due to increased formation of glycosylated hemoglobin. Administration of *H. indicus* extract restored the level of total hemoglobin to normal level (15.95 g/dL). It was reported that insulin deficiency increases glycogen breakdown and thereby decreases liver glycogen content.[26] The restoration of glycogen content by *H. indicus* extract in STZ-induced diabetic rats may be due to increased insulin secretion and reactivation of the glycogen synthase enzyme system. Insulin has been shown to activate the glycogen synthase enzyme system for the synthesis of glycogen.[27] Glycogen synthase, a glycosyl transferase enzyme converts excess glucose residues one by one into a polymeric chain for storage as glycogen.

AST is present in the cells of the liver, heart, skeletal muscles, kidneys and pancreas. It is released into serum in larger quantities when any one of these tissues gets damaged. ALT, an enzyme found primarily in the liver, serves as an indicator of liver status and its elevated levels in serum indicates liver damage. These enzymes are directly associated with the conversion of amino acids to ketoacids. Increased levels of ALP indicate bone disease, liver disease or bile tract blockage. γ-glutamyl transferase catalyzes the transfer of the γ-glutamyl peptides to another peptide or L-amino acids or water. Increased activity of γ-GT indicates the liver damage and it was reported to be increased in STZ-induced diabetes rats.[28] CK, also known as creatine phosphokinase, that catalyzes the phosphorylation of creatine to creatine phosphate has been shown to be increased in STZ-induced DM.[29]

The increase of serum AST, ALT ALP has already been reported to be associated with liver dysfunction and leakage of these enzymes to the liver cytosol and into the blood stream under DM.[30] Reduction in the activity of AST, ALT, ALP, γ-GT and CK by *H. indicus* extract treatment indicates the protective role of extract against STZ-induced haptotoxicity and necrotic changes.

Our observations are in agreement with the reports by several researchers that STZ induced DM and insulin deficiency leads to increased blood glucose,[31] increased levels of cholesterol and triglycerides,[7] increased levels of alkaline phosphatase,[32] transaminases,[33] γ-GT[28] and CK[29] enzymes.

To the best of our knowledge, this is the first report that oral administration of *H. indicus* extract (500 mg/kg/day) exhibits significant ameliorative effect on diabetes-mediated metabolic alterations by lowering the levels of total cholesterol, triglycerides, LDL-cholesterol with a mild increase in HDL-cholesterol level in rats. Further, the pharmacological and biochemical investigations are underway to indentify the antidiabetic active principle in the *H. indicus* root extract and to elucidate its mechanism of action.

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