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Antihyperglycemic and antihyperlipidaemic effects of extracts of *Ipomoea reniformis* Chios on Alloxan Induced Diabetic Rats

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Abstract

The ethanol and aqueous extracts of stem of *Ipomoea reniformis* Chois (Family: Convolvulus) was investigated for its antihyperglycemic and antihyperlipidaemic effects of alloxan induced diabetic rats. Diabetes was induced by administration of single dose of alloxan monohydrate (150 mg/kg b. wt). Ethanol and aqueous extracts of *I. reniformis* at doses of 300 and 600 mg/kg b. wt were administered as a single dose per day to diabetes rats for the period of 12 days, respectively. The effect of extracts of *I. reniformis* on blood glucose levels, serum lipid profile (Total cholesterol, triglycerides, phospholipids, low density, very low density and high density lipoprotein) and serum enzymes (ALT, ASP, ALP) were measured in the diabetic rats. The extracts of *I. reniformis* exhibited significant antihyperglycemic and antihyperlipidaemic effects on alloxan induced diabetic rats when compared to the standard drug (Metformin 250 mg/kg). An oral glucose tolerance test (OGTT) was also performed, in which there was a significant improvement in glucose tolerance in rats treated with extracts. A comparison was made between the extracts and antidiabetic drug- glibenclamide (500µg/kg). The present investigation of this plant established pharmacological evidence to support the folklore claim that it is an anti-diabetic agent.

Key words: Alloxan; antihyperglycemic; antihyperlipidaemic; extracts; *Ipomoea reniformis*; rats.

Introduction

Diabetes Mellitus is a heterogeneous disorder characterized by microvascular pathology leading to chronic complications clinically manifested principally in the kidney and retina [1]. A therapy that normalizes the metabolic dysfunctions in diabetes should be expected to prevent, delay or substantially reduce the severity of these long term microvascular complications improving the

quality of life [2]. The use of herbal medicine is widespread, which are used by the people for the treatment of disparate diseases even at this modern era. There are diverse medicinal plants in the world, which are invariable single plant extract or functions or mixture of extracts/ fractions from different plant, which have been carefully standardized for their safety and efficacy [3]. Changes in lipid levels and consequent disorders of lipid metabolism and stress have been observed in diabetic mellitus [4]. Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes [5]. Low density lipoprotein in diabetic patient leads to abnormal metabolism and is associated with increase in very low density lipoprotein (VLDL) secretion and impaired VLDL catabolism. Ultimately this leads to atherosclerotic plaque [6]. The present work was therefore undertaken to substantiate the folklore claims in a scientific manner using animal models.

Ipomoea reniformis Chois (Convolvulus) is commonly known as musakani. It is a small plant found all over India. Leaf juice of this plant is used to treat snake bite [7]. Root is used as a diuretic, laxative, anti-inflammatory, antipyretic [8] and leaf extracts used as an antihyperglycemic and antihyperlipidaemic [9].

Material and methods

Plant material

Stem of *Ipomoea reniformis* was collected from foot hill of Yercaud, Salem, Tamil Nadu, India and authenticated by Dr P. Jayaraman, Plant anatomy Research centre, Chennai, Tamil Nadu, India. Voucher specimens (IRC/065/07) were deposited at our college Museum for future reference.

Preparation of the extract

The powdered material (600g) of stem of *I. reniformis* was extracted separately using ethanol (1000ml) by Soxhlet technique and aqueous extract by cold maceration. The extracts were dried under reduced pressure. The dried extracts were stored in a desiccator and were subjected to further studies.

Preliminary phytochemical screening

The ethanol and aqueous extracts were subjected to preliminary screening for various active phytochemical constituents [10].

Animals

Wistar Albino rats of either sex, weighing 180-200 g, were purchased from M/S Venkateshwara Enterprises (P) Ltd, Bangalore, India and housed under standard environmental conditions (temperature: 24 ± 1^0 C, light/ dark cycle: 12 h). The rats were fed with standard Pellet diet (Amrut (P) Ltd, Bangalore) and water *ad libitum*. Before commencement of the experiment the animals were deprived of food for 10 h but had free access to water. Experimental protocols were reviewed and approved by the Institutional animal ethics Committee (P.Cog/10/2007).

Acute toxicity studies

Healthy adult Wistar albino rats of either sex, starved over night, were divided into six groups (in each group 6 animals) and were orally fed with the aqueous and ethanol extracts of *I. reniformis*

in escalating dose levels 100, 200 and 300 mg/kg body weight, respectively. The rats were observed continuously for 2 h for behavioral, neurological and autonomic changes and after a period of 24 and 72 h for any death [11].

Induction of diabetes

Diabetes was induced in the Wister albino rats by a single intraperitoneal (i.p) injection of alloxan (150 mg/kg b. wt) dissolved in normal saline, while control group was injected with buffer only. Stable hyperglycemia was confirmed by estimating the glucose level in blood of rats.

Oral glucose tolerance test

After overnight fasting, a 0 min blood sample (0.2 ml) was taken from the rats in normal control (Group I), diabetic control (Group II), diabetic + Metformin (250 mg/kg) (Group III) diabetic + ethanol extract (300 and 600 mg/kg) (Groups IV and V) and diabetic + aqueous extract (300 and 600 mg/kg) (Groups VI and VII) from tail tip. Glucose solution (2 g/kg) was administered orally immediately. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration [12]. All blood samples were collected in potassium oxalate and sodium fluoride containing tubes and used for the estimation of blood glucose.

Antihyperglycemic activity

The rats were alienated into seven groups (six in each group) and were treated as follows, Group I normal rats received vehicle (1% Carboxy Methyl Cellulose (CMC); group II untreated diabetic control received vehicle; group III, diabetic received Metformin 250 mg/kg; groups IV and V, diabetic rats received ethanol extract 300 and 600 mg/kg, group VI and VII diabetic rats received aqueous extract 300 and 600 mg/kg, respectively, daily by gastric intubations. All the animals were scarified on 12^{th} day in fasting condition by cervical dislocation.

Antihyperlipidaemic activity

Total cholesterol was estimated according to Liebermann Burchard Reaction Method. LDL cholesterol was estimated indirectly by Friedwald's method [13]. Triglycerides (TG) were determined using Hantzsch condensation method. HDL cholesterol was also estimated by Liebermann Burchard Reaction Method.

Determination of biological assay

Rats of all groups were anaesthetized by ether and the blood samples of each were taken by puncturing ratro-orbital pleasure plexus and allowed to clot for 60 min at room temperature. Serum was separated by centrifugation at 3000 rpm at 25^0C for 15 min and analyzed for assorted biochemical parameters. The serum ALT [14], AST [15], ALP levels were measured using the respective spectrophotometric diagnostic kit obtained from Biosino Biotechnology Company Ltd (Beijing, PR China).

Statistical evaluation

All the data are presented as mean ± SEM. The differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnette multiple comparisons test. $P<0.01$ were considered to be significant. [16]

Results

Preliminary chemical test

Our phytochemical studies indicated that ethanol and aqueous extracts of stem of *I. reniformis* contains alkaloidas, flavanoids, glycosides, saponins, terpenes and steroids.

Acute toxicity studies

In performing preliminary test for pharmacological activity in rats, aqueous and ethanol extracts did not produce any significant changes in the behavioral or neurological responses upto 600 mg/kg b. wt. acute toxicity studies revealed the non-toxic nature of the ethanol and aqueous extracts of *I. reniformis.*

Oral glucose tolerance test

Table 1 shows the changes in the levels of blood glucose in normal, diabetic control and experimental groups after oral administration of glucose (2 g/kg). The diabetic rats showed that significant increase in the blood glucose at 1 h and 2 h. In extracts and metformin treated animals blood glucose concentration was significantly decreased after 1 and 2 h. (Table 1)

Table 1 Oral glucose tolerance test in experimental animals

Groups / Treatment/mg/kg	Blood glucose level (mg/dL)				
	0 min	**30 min**	**60 min**	**90 min**	**120 min**
Normal control	80.33 ± 5.54	82.46±4.22	80.22±2.64	80.68±4.64	82.62±4.88
Diabetic control	225.6 ± 4.88	298.22± 2.2*	298.22± 2.2*	320.00±8.42*	332.26±8.60*
Metformin 250	108.14 ± 6.6**	188.12 ± 1.9**	172.44±4.62**	148.42±2.22**	120.00±8.84**
Ethanol extract 300	82.48± 2.6***	184.00 ± 4.4***	156.6 ± 3.8***	128.66±8.89***	98.6±7.28***
Ethanol extract 600	80.42±2.2***	172.00±4.2***	154.26±2.4***	122.22±9.62***	94.8±8.86***
Aqueous extract 300	84.28±4.6***	186.22±2.4***	158.24±4.6***	130.48±4.62**	99.8±6.22***
Aqueous extract 600	84.62±2.8***	170.20±4.6***	149.42±1.2***	119.28±6.68***	90.48±8.44***

Values are mean ±SEM, n= 6, (One way ANOVA Followed by Dunnette multiple comparison test). **, *** denotes statistically significance of P<0.05, P<0.01, P<0.001, when compared with respective normal control

Antihyperglycemic activity

The effect of extracts of *I. reniformis* on blood glucose levels in alloxan induced diabetic rats is shown in Table 2. The initial blood glucose levels of the diabetic rats selected for the study were in the range of above 200 mg/100dL.

In alloxan (150 mg/kg) induced rats, the BGL significantly increased blood glucose level from 289.33 ± 4.43 to 348.5 ± 3.42 mg/dl. Ethanol extract of *I. reniformis* (300 and 600 mg/kg) given upto 12th day after alloxan treatment, showed decreased blood glucose levels significantly from 301.83 ± 6.62 to 172.00 ± 5.75 and 292.82 ± 6.6 to 152.66 ± 7.29 mg/dl and aqueous extract (300 and 600 mg/kg) of *I. reniformis* showed from 294.00± 3.63 to 156.50± 5.55 and 294.83± 5.52 to 150.17± 4.36, respectively.

Table 2 Antihyperglycemic effect of extracts of *I. reniformis* Chois on alloxan induced diabetic rats

Groups / Treatment/mg/kg	Blood glucose level (mg/dL)				
	Initial	**3rd day**	**6th day**	**9th day**	**12 day**
Normal control	86.33 ± 1.91	86.00 ± 1.90	87.83 ± 1.90	88.83 ± 2.37	88.83 ± 1.56
Diabetic control	289.33 ± 4.43	304.0 ± 4.32	319.0 ± 4.43	334.0 ± 4.08	348.5 ± 3.42
Metformin 250	287.5 ± 4.73**	264.66 ± 4.67**	222.16 ± 5.84**	174.33 ± 6.97**	152.0 ± 7.75**
Ethanol extract 300	301.83 ± 6.62**	282.00 ± 4.41***	256.83 ± 5.21***	213.5 ± 6.72***	172.00±5.75***
Ethanol extract 600	292.82 ± 6.6**	270.5 ± 7.7***	233.16 ± 8.6***	203.33 ± 9.52***	152.66 ± 7.29***
Aqueous extract 300	294.00± 3.63**	270.66± 3.73***	243.64± 5.34***	202.68± 8.25***	156.50± 5.55***
Aqueous extract 600	294.83± 5.52**	272.32± 5.09***	237.33± 4.2***	194.50± 5.41***	150.17± 4.36***

Values are mean ±SEM, n= 6 (One way ANOVA Followed by Dunnette multiple comparison test). **, *** denotes statistically significance of P<0.05, P<0.01, P<0.001, when compared with respective normal control

Antihyperlipidaemic activity

The lipid profiles in control and experimental rats are depicted in Table 3 in alloxan induced diabetic rats, there was a significant (P<0.001) increase of total cholesterol, triglycerides, phospholipids, and low density lipoproteins (LDL) and very low density lipoprotein (VLDL) cholesterol and significant (p<0.001) decreases in high density lipoprotein (HDL) cholesterol in serum compared with normal control. The extracts treated rats were significantly (p<0.001) decreased the total cholesterol, triglycerides, phospholipids and LDL and VLDL cholesterol and significantly (p<0.001) increased HDL cholesterol.

Table 3 Antihyperlipidemic effect of extracts of *I. reniformis* Chois on alloxan induced diabetic rats

Groups / Treatment/mg/kg	Total cholesterol	Triglycerides	HDL	LDL	VLDL
Normal	78.16 ± 2.39	64.30 ± 2.14	45.50 ± 2.52	45.50 ± 2.52	16.83 ± 1.83
Diabetic	96.33 ± 2.38	98.16 ± 2.82	32.30 ± 1.96*	23.50 ±	23.16 ± 3.42*
Metformin 250	80.33 ± 2.59**	72.00 ± 2.59**	50.60 ± 1.45**	17.00 ± 1.56**	14.83 ± 2.16**
Ethanol extract 300	81.05 ± 2.95**	68.83 ± 2.59***	53.00 ± 3.32***	19.60 ± 2.73***	23.50 ± 1.34***
Ethanol extract 600	80.00 ± 1.92**	67.66 ± 3.42***	52.66 ± 1.77***	18.50 ± 2.94***	24.66 ± 2.21***
Aqueous extract 300	82.16± 3.86**	69.80± 3.26***	52.66±2.25***	19.66±2.05***	21.66± 3.42***
Aqueous extract 600	79.03± 4.08**	66.5± 2.52***	44.89±3.82***	17.66± 2.33****	23.16± 1.56***

Determination biochemical parameters

Serum enzymes (ALP, AST and ASP) total protein levels are shown in Table 4. ALP, ASP levels were increased significantly ($p<0.001$) in alloxan treated diabetic rats in comparison with normal animals. The extracts significantly ($p<0.001$) decreased the elevated ALP, AST and ASP levels in treated rats. A significant ($p<0.05$) decrease in total protein level was observed with control rats. Administration of extracts of *I. reniformis* stem restored the protein levels to near normal.

Table 4 Effect of extracts of *I. reniformis* on serum profile in normal and alloxan induced diabetic rats

Groups/Treatment/mg/kg	Biochemical parameters		
	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal control	36.42 ± 0.8	136.14 ± 2.6	118.20 ± 4.8
Diabetic control	64.26 ± 1.2*	170.20 ± 4.8*	134.28 ± 2.2*
Metformin 250	42.46 ± 1.4**	141.80 ± 2.6**	128.12 ± 1.9**
Ethanol extract 300	47.15 ± 1.1***	144.00 ± 4.4***	126.6 ± 3.8***
Ethanol extract 600	44.26±2.2***	142.00±4.2***	120.26±2.4***
Aqueous extract 300	46.28±4.6***	144.22±2.4***	124.24±4.6***
Aqueous extract 600	43.62±2.8***	143.20±4.6***	121.42±1.2***

Discussion

Diabetic mellitus is a metabolic disease associated with impaired glucose metabolism which in effect adversely alters intermediary metabolism of lipids and proteins. Most of the complications of the diabetic state are initiated by the generation of free radicals: for instance LDH oxidative modification, leading to atherosclerosis [17, 18] occurs only in the presence of free radicals.

The presence study was conducted to study the anti-diabetic activity of extracts of *I. reniformis* in rats as well as to provide on introductory approach for the evaluation of its traditional preparation in order to scientifically validate the therapeutic preparation of this plant in the control of diabetes. This study shows that the extracts of *I. reniformis* produces a marked decrease in blood glucose at 300 and 600 mg/kg body weight in alloxan diabetic rats after 12 days treatment. Our findings are in agreement with our previous report [9]. The anti-diabetic effect of extracts of *I. reniformis* may be due to increased release of insulin from the existing β cells of pancreas similar to that observed after sulphonylurea administration.

Lipid profile, which is altered in diabetes state, is one of the significant factors in development of cardiovascular diseases. Studies have shown that increased plasma triglycerides and cholesterol levels may be a risk factor for vascular disease [19, 20]. Hyperlipidemia is a recognized complication of diabetic mellitus characterized by elevated levels of cholesterol, triglycerides and phospholipids and changes in lipoprotein composition. In the alloxan induced diabetes in rats, the rise in blood glucose is accompanied by an increase in the serum cholesterol, TG, LDL, VLDL and decrease in HDL. The treatment with extract reduced cholesterol, LDL, VLDL and improved HDL in diabetic rats.

The enzyme ALP is located in the cytoplasm and will be released into circulation after cellular damage. In addition, the soluble enzymes ALT/AST are released when injury involves organelles such as mitochondria [21].

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