



Trade Science Inc.

ISSN : 0974 - 7508

Volume 6 Issue 4

Natural Products

An Indian Journal

Full Paper

NPJ, 6(4), 2010 [201-204]

Antidiabetic activity of bark extract of *Pithecellobium dulce* Benth in alloxan-induced diabetic rats

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Received: 22nd November, 2010 ; Accepted: 2nd December, 2010

ABSTRACT

The present study was undertaken to evaluate the Antidiabetic activity of bark of *Pithecellobium dulce* Benth. Hydro alcoholic bark extract of *Pithecellobium dulce* Benth was subjected to qualitative phytochemical analysis which showed the presence of sterols, alkaloids, glycosides, saponins, tannins, carbohydrates, proteins, flavonoids and phenolic compounds. The extract at dose levels of 200mg/kg.b.wt and 400mg/kg.b.wt were screened for antidiabetic activity in alloxan induced rats. The extract at dose level of 400mg/kg.b.wt showed a significant antidiabetic activity ($p < 0.05$) when compared to standard Glibenclamide and also showed significant decrease in cholesterol and triglyceride levels ($p < 0.05$) when compared to control diabetic rats. © 2010 Trade Science Inc. - INDIA

KEYWORDS

Pithecellobium dulce
Benth;
Antidiabetic activity;
Hydro alcoholic extract;
Alloxan.

INTRODUCTION

Diabetes mellitus a chronic metabolic disorder has become an epidemic, with a worldwide incidence of 5% in the general population^[1]. Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in this increasing prevalence in the past two decades. Ancient Indian Sanskrit literature that deals with the health care system describes the diabetes mellitus as 'madhumeha'. Current studies conducted estimates that diabetes mellitus is most popular and commonly appearing disorder in human population all over the world. A recent development in modern science helps to understand the pathophysiology of diabetes mellitus and rely on the ayurvedic and modern medicine to prevent and treat this disorder. These developments help to identify the potential constituents and develop novel thera-

pies from traditional medicinal plants to combat the diabetes with lesser side effects.

Pithecellobium dulce Benth (Leguminosea) is a small to medium size, evergreen, spiny tree up to 18 m height, and native of tropical America and cultivated throughout the plains of India and in the Andaman's. It is known as 'Vilayati Babul' in Hindi, 'Seema chinta' in Telugu and 'kodukkappulli' in Tamil. The various extracts of leaf, root, and seed of the plant have been reported to be antidiabetic activity, and also bark extract of the plant exhibits astringent activity in dysentery, febrifuge and treatment of dermatitis. The presence of steroids, saponins, lipids, phospholipids, glycosides, glycolipids and polysaccharides has been reported in the seeds^[2-5]. The bark contains 37% of tannins of catechol type. Quercetin, kaempferol, dulcitol and afezilin have been reported from the leaves^[6,7]. Roots have been reported to possess estrogenic activity^[8].

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Studies on alkylated resins from seed oil have been reported recently^[9]. So, an attempt was made to evaluate the antidiabetic potential of hydro alcoholic bark extract of *Pithecellobium dulce* Benth.

MATERIALS AND METHODS

Plant collection

The barks of the plant were collected during March 2010 from Eluru, India. The plant species was identified and authenticated by Department of Botany, Acharya Nagarjuna University, Guntur.

Extraction procedure

The fresh bark of *Pithecellobium dulce* Benth was washed with water, air dried at room temperature, the dried bark material was powdered mechanically. Around 750gm of finely powdered bark was extracted with aqueous ethanol (30:70) by using soxhlet extraction apparatus for about 18 hr. After extraction the solvent was distilled off and extract was concentrated on water bath to a dry residue and dried in a dessicator.

Phytochemical screening

The hydro alcoholic extract was subjected to qualitative phytochemical investigation for the identification of the phytoconstituents viz., sterols, alkaloids, glycosides, saponins, tannins, carbohydrates, proteins, phenolic compounds and flavonoids^[10].

Experimental animals

Healthy adult Wistar rats weighing 150-220 g were used for the antidiabetic activity. The animals were housed in clean polypropylene cages and maintained in a well-ventilated temperature controlled animal house with a constant 12h light/dark schedule. The animals were fed with standard rat pelleted diet and clean drinking water was made available *ad libitum*.

Acute toxicity studies

The acute toxicity test of the extracts was determined according to the OECD (Organization for Economic Co-operation and development) guidelines No. 420. Wistar rats (150-220 g) were used for this study. After the sighting study, starting dose of 2000 mg/kg (p.o.) of the test samples was given to various groups

containing five animals in each group. The treated animals were monitored for 14 days, for mortality and general behavior. No death was observed till the end of the study. The test samples were found to be safe up to the dose of 2000 mg/kg, and, from the results, 200 mg/kg and 400 mg/kg dose were chosen for further experimentation.

Antidiabetic screening

For experiment overnight fasted Wistar rats was induced by a single intraperitoneal administration of alloxan monohydrate (150 mg/kg.b.wt) in 0.2 ml saline [154 mM NaCl] just prior to injection. Those animals with fasting blood glucose level more than 300 mg/dl at 72 h after alloxan administration were divided into four groups of six animals each. Group I served as diabetic control and received 0.3% CMC, Group II served as positive control and received glibenclamide (10 mg/kg.b.wt), orally. Groups III and IV received the hydro alcoholic extract, orally at a dose of 200 mg/kg.b.wt and 400 mg/kg.b.wt respectively. The treatment was continued for fourteen days by administering the extract or drug or 0.3% CMC, once daily. After the treatment period the blood glucose level, cholesterol and triglyceride were estimated.

Estimation of blood glucose

Blood samples were collected by orbital sinus puncture under mild ether anaesthesia and blood glucose was estimated by electronic glucometer (Accu-Check active).

Estimation of cholesterol and triglyceride

For the estimation of cholesterol and triglyceride, the blood samples were collected in Eppendroff's tubes (1 ml) containing 50 µl of anticoagulant (10% trisodium citrate) and plasma was separated by centrifuging at 6000 rpm for 15 min and estimated in UV/Vis Spectrophotometer (Shimadzu). The absorbance of the sample and of the standard was measured against the reagent blank at 500 nm.

The sample solution was prepared by adding 10 µl of the plasma and 1000 µl of the reagent blank, and the standard solution was prepared by adding 10 µl of the standard (Cholesterol and Triglyceride) with 1000 µl of the reagent blank.

The values are expressed as mg/dl. Concentration

TABLE 1 : Effect of hydro alcoholic extract of *Pithecellobium dulce* bark on blood glucose level

Group	Dose	Glucose level		
		Normal	After alloxan	After treatment
Control	0.3% CMC	84±3.49	325±14.21	350±18.34
Glibenclamide	10 mg/kg.b.wt	88±6.06	300±20.06	98±6.24 ^a
Hydro alcoholic extract of <i>Pithecellobium dulce</i>	200mg/kg.b.wt	95±8.19	355±16.15	130±12.16
Hydro alcoholic extract of <i>Pithecellobium dulce</i>	400mg/kg.b.wt	78±5.25	360±15.02	102±5.08 ^a

Values are Mean ± SEM of six animals. Statistical significance: a=p<0.05 as compared to vehicle control

TABLE 2 : Effect of hydro alcoholic extract of *Pithecellobium dulce* bark on cholesterol and triglyceride levels

Group	Dose	Cholesterol level		Triglyceride level	
		Normal	After treatment	Normal	After treatment
Control	0.3% CMC	65±6.07	170±19.11	75±15.12	168±16.24
Glibenclamide	10 mg/kg.b.wt	63±8.01	82±3.08 ^a	61±4.32	110±6.12 ^a
Hydroalcoholic extract of <i>Pithecellobium dulce</i>	200 mg/kg.b.wt	78±7.12	135±6.22	68±8.10	144±8.01
Hydroalcoholic extract of <i>Pithecellobium dulce</i>	400 mg/kg.b.wt	59±5.14	92±5.21 ^a	82±6.21	114±5.32 ^a

Values are Mean ± SEM of six animals. Statistical significance: a=p<0.05 as compared to vehicle control

of the sample = Absorbance of sample x concentration of standard/absorbance of standard^[11].

RESULTS AND DISCUSSION

The qualitative phytochemical evaluation of hydro alcoholic extract showed the presence of sterols, alkaloids, glycosides, saponins, tannins, carbohydrates, proteins, phenolic compounds and flavonoids.

In alloxan treated rats, there was significant increase in blood glucose, cholesterol and triglyceride levels. Oral treatment with 200 mg/kg.b.wt and 400 mg/kg.b.wt of hydro alcoholic extract of bark of *Pithecellobium dulce* significantly reduced the blood glucose, cholesterol and triglyceride. Hydro alcoholic extract at 400 mg/kg.b.wt showed significant decrease in blood glucose level (p<0.05) when compared to the standard glibenclamide (TABLE 1). In diabetic control there was a significant increase in cholesterol and Triglyceride levels. The standard glibenclamide and hydro alcoholic extract at 400 mg/kg.b.wt showed significant decrease in cholesterol and Triglyceride levels (TABLE 2).

CONCLUSION

Alloxan monohydrate as the diabetes-inducing agent was that it is known to produce diabetes mellitus irreversibly with a single dose administration by selective necrotic action on the beta cells of pancreas leading to insulin deficiency^[12]. Insulin deficiency leads to increased blood glucose, cholesterol and triglyceride levels^[13]. Hydro alcoholic extract showed significant decrease in blood glucose level, cholesterol and triglyceride levels. Flavonoids, proteins and saponins have been reported to possess significant anti-diabetic activity and antilipidemic activity^[14,15]. *Pithecellobium dulce* Benth bark extract showed the presence of flavonoids, proteins and saponins; hence the activity of the plant may be due to this phytoconstituents. Investigations are in progress to explore the possible mechanism of action.

ACKNOWLEDGEMENT

We are thankful to the management of Sir C. R. Reddy College of Pharmaceutical sciences, Eluru, A.P, India for providing necessary facilities.

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