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In vitro antidiabetic activity of *Caesalpina digyna* (R.) methanol root extract

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ABSTRACT

Diabetes is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. Recent decades have experienced a sharp increase in the incidence and prevalence of diabetes mellitus. One antidiabetic therapeutic approach is to reduce gastrointestinal glucose production and absorption through the inhibition of carbohydrate digesting enzymes such as α- amylase and α-glucosidase and α-amylase. Inhibition of amylase and glucosidase enzymes involved in digestion of carbohydrates can significantly decrease the post prandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in management of blood glucose. The aim of the current study was to screen the methanol extract of root of Caesalpinia digyna for its in vitro antidiabetic activity. Our assay result suggests that methanol extract of Caesalpinia digyna exhibit dose-dependent increase in percentage inhibitory activity on α-glucosidase enzymes (IC_{50} 402.23±10.14 µg/ml) and α-amylase (IC_5 of 686.94 ±3.98 µg/ml). Acarbose was used as a standard drug.

Key words: *in vitro* antidiabetic; *Caesalpinia digyna*, α-glucosidase, α-amylase enzymes,

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INTRODUCTION

Diabetes is one of the major causes of premature death worldwide. Every ten second a person dies from diabetes related causes mainly from cardiovascular complications. In 2007, diabetes caused 3.5 million deaths globally [1]. Diabetes affects mainly the developing countries like India. Indeed, India presently has the largest number of diabetic patients in the world and has been infamously dubbed as the 'diabetic capital of the world' [2]. Diabetes mellitus is epidemic in India as a result of societal influence and changing lifestyles. Diabetes has been known in India for centuries as 'a disease of rich man' but now spread among all masses [3].

Caesalpinia digyna is a large scandent, prickly shrub or climber, up to 10 m tall growing wild in the scrub forest of the eastern Himalayas in Assam and West Bengal, the eastern ghats in Andhra Pradesh, and in Madhya Pradesh [4], [5]. The ethanol-water extract of roots inhibits the growth

of Mycobacterium tuberculosis [6]. Several members of species of genus *Caesalpinia* like *C. sappan* and *C. bonducella,* etc., are used for wide variety of ethanomedical properties such as anti-inflammatory, antioxidant, antidiabetic and hepatoprotective [7]. *Caesalpinia digyna* is traditionally used for the treatment of diabetes and anti-inflammatory diseases [8]. The genus *Caesalpinia* consists of several member of species like *C. sappan, C. bonducella,* etc., are used traditionally for treatment of inflammation, hepatotoxicity and diabetes. Inhibition of amylase and glucosidase enzymes can be an important strategy in management of post prandial blood glucose level in type 2 diabetes patient [9]. Thus, objective of our study is to investigate the root of *Caesalpinia digyna* for its *in vitro* antidiabetic activity.

MATERIALS AND METHODS

Potato starch, trichloroacetic acid, Folin-Ciocalteau reagents were purchased from SD Fine Pvt. Ltd., Mumbai, 3,5-dinitrosalicylic acid, Tris buffer, linoleic acid, ammonium molybdate, were purchased from Hi-Media Pvt. Ltd., Mumbai, α-amylase, α-glucosidase enzymes, xanthine oxidase, quercetin, hypoxanthine, pyrocatechol were purchased from SRL Pvt. Ltd., Mumbai. Glucose assay kit from Agappe diagnostic Pvt. Ltd., Kerala, Acarbose was obtained from Bicon Pvt. Ltd., Chennai, ferrozine, (2'2-azobis (2-amidino propane) dihydrochloride), butylated hydroxy toluene from Loba Cheme. All other chemicals used in the study were obtained commercially and were of analytical grade.The roots of *Caesalpinia digyna* (CD) were purchased from Abirami Botanicals, Tuticorin, TN India and authenticated. The specimen was preserved in the Herbarium section of the department (Voucher No.22/2009/10).

Extraction of root

The shade dried CD roots were powdered mechanically and sieved through sieve no 20 and stored in an air tight container. The extraction was carried out by hot percolation method using Soxhlet apparatus. The solvent used was methanol. About 100 gm of powder was extracted with 600 ml of methanol. The extract was concentrated to dryness under controlled temperature 40-50°C. The percentage yield was found to be 10.15%. The extract was preserved in refrigerator till further use.

In vitro methods employed in antidiabetic studies [10]

Inhibition of alpha amylase enzyme

A total of 500 µl of test samples and standard drug (100-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle [11], [12].

Inhibition of alpha glucosidases enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract

for 5 min at 37°C. The reaction was initiated by adding 1ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 10 min at 37°C. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method [13-15].

Calculation of 50% Inhibitory Concentration (IC_{50})

The concentration of the plant extracts required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by

I % = (Ac-As)/Ac X 100, [16]

where Ac is the absorbance of the control and As is the absorbance of the sample.

RESULTS

The dried root of *Caesalpinia digyna* Rottler was extracted with methanol. The percentage yield of extract was found to be 10.15. Preliminary phytochemical screening of the extract of CD revealed the presence of carbohydrates, flavonoids, tannins, steroids and triterpenoids and glycosides.

Evaluation of *in vitro* α-amylase inhibitory activity using CD Root extract

There was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme. At a concentration 100µg/ml of extract showed a percentage inhibition 23.62 ± 0.2454 and for 1000 µg/ml it was 61.31 ± 0.3729. The extract gave an IC_{50} value of 686.94 ± 3.98 µg/ml. The IC_{50} value of standard drug acarbose was found to be 325.50 ± 4.7 µg/ml (Table 1).

Evaluation of *in vitro* α-glucosidase inhibitory activity using CD Root extract

The CD methanol extract revealed a significant inhibitory action on α-glucosidase enzyme. The percentage inhibition at 100-1000 µg/ml concentrations of CD extract showed a concentration-dependent increase in percentage inhibition. The percentage inhibition varied from 85.71 ± 0.918 to 30.78 ± 0.4855 for highest concentration to the lowest concentration of 100 µg/ml. The concentration required for 50% inhibition (IC_{50}) was found to be 402.23±10.14 µg/ml whereas the α -glucosidase inhibitory activity of positive control acarbose produced percentage of 40.73 ± 1.39 for 100 µg/ml and 91.58 ± 1.39 for 1000 µg/ml. The IC_{50} value of standard drug acarbose against α-glucosidase was found to be 325.50 ± 4.7 µg/ml (Table 2).

DISCUSSION

Lack of insulin affects the metabolism of carbohydrates, proteins, fat and causes significance disturbance of water and electrolyte homeostasis [17]. Recent advances in understanding the activity of intestinal enzymes (α-amylase and α-glucosidase both are important in carbohydrate digestion and glucose absorption) have lead to the development of newer pharmacological agents. A high postprandial blood glucose response is associated with micro- and macro-vascular complications in diabetes and is more strongly associated with the risk for cardiovascular

diseases than are fasting blood glucose. α-Glucosidase enzymes in the intestinal lumen and in the brush border membrane play main roles in carbohydrate digestion to degrade starch and oligosaccharides to monosaccharides before they can be absorbed. It was proposed that suppression of the activity of such digestive enzymes would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation [18]. Alpha-glucosidase inhibitor retards the digestion of carbohydrates and slows down the absorption. Acarbose and miglitol are competitive inhibitor of α-glucosidases and reduces absorption of starch and disaccharides [19]. Hence one of the therapeutic approaches for reducing postprandial (PP) blood glucose levels in patient with diabetes mellitus is to prevent absorption of carbohydrate after food intake. Inhibition of these enzymes (α-amylase and α-glucosidases) reduced the high postprandial (PP) blood glucose peaks in diabetes [20]. Acarbose and miglitol are competitive inhibitor of α glucosidases and reduces absorption of starch and disaccharides [19]. The α-amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates. Acarbose is complex oligosaccharides that delay the digestion of carbohydrates. It inhibits the action of pancreatic amylase in breakdown of starch. Synthetic inhibitor causes side effect such as abdominal pain, diarrhoea and soft faeces in the colon. Our finding reveals that *Caesalpinia digyna* efficiently inhibits α-amylase enzyme *in vitro*. The reaction mechanisms involved in inhibition of α-amylase enzymes by plant protein inhibitors are not clearly understood. But there are some suggestions that the plant protein (flavanols) might cause conformational changes in structure [21]. Chemical investigations of the plant have shown the presence of caesalpinine A, cellallocinnine, ellagic acid, gallic acid, bergenin, bonducellin, intricatinol and tannins [22-25].

The results suggest that methanol extract of *Caesalpinia digyna* root efficiently inhibits α-glucosidase enzymes *in vitro*. The antidiabetic action of *Caesalpinia digyna* can also be attributed to the intestinal α-glucosidases inhibitory activity.

Table 1: α-amylase inhibition by *Caesalpinia digyna* (Rotter.) methanol extract

Sample	Concentration (µg/ml)	% inhibition	IC $_{50}$ µg/ml
CD	100	23.62 ± 0.2454	686.94 ± 3.98
	200	33.89 ± 0.1400	
	400	39.66 ± 0.2454	
	800	55.63 ± 0.3704	
	1000	61.31 ± 0.3729	
ACARBOSE (standard)	100	33.75 ± 0.2425	325.50 ± 4.7
	200	49.22 ± 0.3704	
	400	59.20 ± 0.2833	
	800	67.22 ± 0.1433	
	1000	73.97 ± 0.3736	

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM. The IC50 value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions.

Table 2: α-glucosidase inhibition by *Caesalpinia digyna* (Rotter.) methanol extract

Sample	Concentration (µg/ml)	% inhibition	IC $_{50}$ µg/ml
CD	100 200 400 800 1000	30.78 ± 0.4855 39.67 ± 0.9151 56.08 ± 0.5067 64.01 ± 1.908 85.71 ± 0.918	402.23 ± 10.14
ACARBOSE (standard)	100 200 400 800 1000	40.73 ± 1.39 49.34 ± 1.04 63.48 ± 0.91 72.56 ± 1.22 91.58 ± 1.39	230.71 ± 7.89

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM. The IC50 value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions.

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