Original Article

Anti-diabetic activity of cassia fistula (alpha amylase – inhibitory effect)

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ABSTRACT

Aim: To evaluate the anti-diabetic activity of cassia fistula. Materials and methods: Cassia fistula extract used in the study here was obtained from Green Chem Herbal extracts and formulations, Bengaluru, India. DMEM, FBS, and Pioglitazone, were obtained from Sigma Aldridge co India. Acarbose, dinitrosalicylic acid, DMSO is obtained from Sigma Aldrich co. India. Alpha amylase enzymes were procured from Himedia, Mumbai. All the roger chemicals used in the study were up to analytical grade. The study was carried out as an in vitro study by the method of Bernfeld. Results: cassia fistula showed high alpha amylase inhibitory activity. Conclusion: It can be concluded that cassia fistula shows high alpha amylase inhibitory activity and can be used as medication for diabetes.

Keywords: Alpha glycosidase, anti-diabetic, cassia fistula, diabetes mellitus, hypoglycaemia.

Introduction

Diabetes mellitus, a chronic metabolic disorder, has now become epidemic with a worldwide incidence of 5% in the general population. More than 100 million of the world's population has already reached the diabetic mark and the number of people suffering from it is expected to soar up to 366 million [1]. Decreased physical activity, increasing obesity and stress, and changes in the food consumption have been implicated in this increasing prevalence in the past two decades [2]. Overt diabetes affects 2-3% of the total world population. Type I and type 2 diabetes are treated with exogenous insulin and oral hypoglycemic agents; respectively, in conventional therapy [3]. The demand for using natural products with antidiabetic activities by patients has increased, although there are different kinds of oral hypoglycemic agents available along with insulin for the treatment of diabetes [4]. The patients with noninsulin-requiring diabetes have been treated orally in traditional medicine with a variety of plant extracts for many years. In India, in ancient literature (Ayurveda), a number of plants are stated for the treatment of diabetic conditions.

Many traditional healers applied flowers of Cassia fistula Linn (Indian laburnam) belonging to the family Leguminosae widely in the most of the herbal preparations for diabetes [5]. Traditionally, it is used to cure diabetes, constipation, skin diseases, fever, abdominal pain, and leprosy [6]. This plant is reported to contain antibacterial, antioxidant, and antiinflammatory activities [7]. The constituents reported in this plant are alkaloids [8], flavonoids, and anthraquinone glycosides [9].

The traditional use of the flowers of C. fistula for diabetes has not been proven so far. Moreover, the main focus has been put on ethanol and aqueous extracts for diabetes by the researchers, but a considerable number of investigations have declared that the petroleum ether, benzene, and chloroform extracts were also found to have anti-diabetes activities [9-11]. Being aware of the effective extracts and isolating the active fraction from the effective extract would be a helpful method for developing new drugs. The standard fraction of an active extract may demonstrate to be better therapeutically, less toxic and inexpensive compared to the pure isolated compounds. Considering these facts, proving its traditional use, identifying the active antidiabetic extract of C. fistula in diabetes-associated complications, and identifying the active antidiabetic fraction of the active extract are the aims of the present study.

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Patients and Methods

Plant material:

Cassia fistula extract used in the study here was obtained from Green Chem Herbal extracts and formulations, Bengaluru, India.

Chemicals used:

DMEM, FBS, and Pioglitazone, were obtained from Sigma Aldridge co India. Acarbose, dinitrosalicylic acid, DMSO is obtained from Sigma Aldrich co. India. Alpha amylase enzymes were procured from Himedia, Mumbai. All the roger chemicals used in the study were up to analytical grade.

In – vitro α- amylase inhibitory assay (Bernfeld 1955)

In vitro amylase inhibition was studied by the method of Bernfeld [12]. In brief, 100µL of different concentrations of Cassia (1, 3, 10, 30, 100, 300 and 1000µg/ml) were allowed to react with 200µl of α-amylase enzyme (Hi media RM 638) and 100µL of 2mM of phosphate buffer (pH-6.9). After 20-minute incubation, 100µl of 1% starch solution was added. 200µl of the enzyme was replaced by buffer to apply the same process for the controls. The reference standard was Acarbose. After incubation for 5 minutes, 500µl of Dinitrosalicylic acid reagent was added to both the control and the test. They were maintained in boiling water bath for 5 min. The amount of absorbance was recorded at 540 nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was computed using the formula

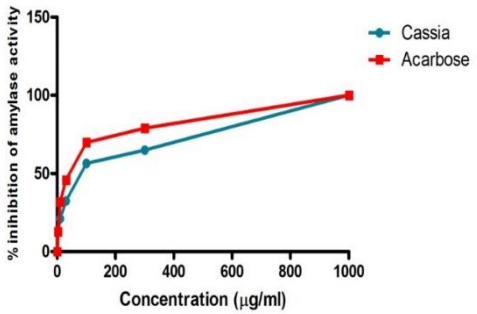
$$\% \text{ inhibition} = [(\text{Control} - \text{Test})/\text{Control}] * 100$$

Suitable reagent blank and inhibitor controls were simultaneously applied.

Results

α – amylase inhibitory activity

The results of the present study exhibited strong α – amylase inhibitory activity of the Cassia extract which is compared with the standard Acarbose. A maximum inhibition of 88.65% was observed at a concentration of 1000µg/ml by Cassia extract, which was compared with that of standard Acarbose that showed inhibition of about 93%. The IC50 of the extract was found to be 73.16µg/ml and for Acarbose 34.83µM.



Discussion

Cassia fistula Linn. also known as golden shower, Indian laburnum, is categorized in the family of Leguminoceae. It is used in the treatment of hematemesis, pruritis, intestinal disorders, leucoderma, diabetes, and as antipyretic, analgesic and laxative in folk medicine. The size of Cassia fistula is moderate. And there are rich sources of flavonoids, anthraquinones and polysaccharides in various species of Cassia. The existence of flavonol and xanthone glycosides has been already reported in the bark of the plant Cassia fistula. So, isolating the phytoconstituent responsible for alpha amylase inhibition has been attempted to be done [13].

Hasenah Ali et al. [14] conducted a research regarding α-Amylase inhibitory activity of some Malaysian plants which are used to treat diabetes, with particular focus on Phyllanthus amarus. Six Malaysian plants' extracts which had a reputation of being useful in treating diabetes, were selected and examined for α-amylase inhibition using an in vitro model. Two different protocols (with and without pre-incubation) were used to study their inhibitory activities, and the results showed that Phyllanthus amarus hexane extract had α-amylase inhibitory properties. In the test without pre-incubation, hexane and dichloromethane extracts of Anacardium occidentale, Lagerstroemia speciosa, Averrhoa bilimbi Pithecellobium jiringa and Parkia speciosa were not active. The isolation of dotriacontanyl docosanoate, triacontanol and a mixture of oleanolic acid and ursolic acid were the results of extraction and fractionation of Phyllanthus amarushexane extract. Dotriacontanyl docosanoate and the mixture of oleanolic acid and ursolic acid are reported from this plant species for the first time. All compounds were tested in the α-amylase inhibition assay and the results demonstrated that the oleanolic acid and ursolic acid (2:1) mixture was a potent α-amylase inhibitor with IC_{50} = 2.01 µg/ml (4.41 µM) which is significantly related to the α-amylase inhibition activity of the extract. Three pure pentacyclic triterpenoids, oleanolic acid, ursolic acid and lupeol demonstrated to inhibit α-amylase.

A research conducted by [15] on in vitro α- amylase and α-glucosidase inhibitory activities of the ethanolic extracts of dioscorea villosa tubers demonstrated that the α – amylase inhibitory activity also showed a dose - dependent increase ranging from 10.71 ± 0.00 to 71.88 ± 3.77 percent with a concentration range of 1.5µg/ml - 1000µg/ml and the IC50 value was computed as 72.44 µg / ml when compared with acarbose, IC50 value 83.23 ± 0.39 µg/mL.

A study was conducted by [16] on a comparative study of alpha amylase inhibitory activities of common anti-diabetic plants at Kharagpur 1 block. Acalypha indica, Allium cepa, Allium sativum, Azadirachta indica, Musa sapientum, Mangifera indica, Murraya, Ocimum sanctum, Phyllanthus amarus and Tinospora cordifolia were examined for their alpha amylase inhibitory activities to establish anti-diabetic potentials. The plant extracts were prepared sequentially with petroleum ether, hexane, chloroform, ethanol and aqueous. The obtained extracts were subjected to in vitro alpha amylase inhibitory assay using starch azure as a substrate and porcine pancreatic amylase as the enzyme. Statistical difference and linear regression analysis were performed by using Graphpad prism 5 statistical software. Ethanol extracts of Mangifera indica, Azadirachta indica and petroleum ether extract of Murraya koenigii (at a concentrations 10-100µg/ml) showed maximum

percentage inhibition on alpha amylase activity with an IC50 value of 37.86 ± 0.32µg/ml, 62.99 ± 1.20µg/ml and 59.0 ± 0.51µg/ml; respectively, when compared with acarbose (IC50 value 83.33 ± 0.75µg/ml). The results showed that Mangifera indica, Azadirachta indica and Murraya koenigii might be effective in lowering post prandial hyperglycemia.

A research done by G. Nivetha etal [17] on Comparative Evaluation of Anti-Diabetic Activity of Lemon Grass Oil and Tulasi Oil demonstrated that the lemon grass oil and Tulasi oil have anti hypoglycaemic properties. It showed that these plants have hypoglycaemic effects. Use of lemon grass oil and Tulasi oil on a regular basis by diabetic patients can be beneficial in lowering the blood glucose and potentiates other therapies used in diabetes treatment.

A study conducted by [18] on the topic in vitro alpha-amylase inhibition and in vivo antioxidant potential of Amaranthus spinosus in alloxan-induced oxidative stress in diabetic rats says that Amaranthus spinosus Linn. (Amaranthaceae), commonly called as ''Mulluharivesoppu'' in Kannada, is applied in traditional system of medicine for the treatment of diabetes in India. The current study deals with the scientific evaluation of alpha amylase and the antioxidant potential of methanol extract of A. spinosus (MEAS). The goal of this study was to examine in vitro alphaamylase enzyme inhibition by CNPG3 (2-chloro-4-nitrophenol a-D-maltotrioside) and in vivo antioxidant potential of malondialdehyde (MDA), glutathione (GSH), catalase (CAT) and total thiols (TT) in alloxan-induced diabetic rats of a methanolic extract of A. spinosus. Blood sugar was also determined in MEAS-treated alloxan-induced diabetic rats. MEAS showed significant inhibition of alpha-amylase activity and IC50 46.02 lg/ml. Oral administration of MEAS (200 and 400 mg/kg) for 15 days showed significant reduction in the elevated blood glucose, MDA and restores GSH, CAT and TT levels as compared with a diabetic control. The present study provides evidence that the methanolic extract of A. spinosus has potent alpha amylase, anti-diabetic and antioxidant activities.

A research accomplished by [19] found that preliminary phytochemical screening revealed the presence of steroids, flavoniods, polyphenols, tannins and triterpenoids in Cassia auriculata and sterols, triterpenoids, flavoniods, naphthalene glycosides in Cassia angustifolia. In the alpha amylase inhibition assay, Acarbose (at concentration 100 µg/ml) showed 50.35% inhibitory effect on alpha amylase activity with IC_{50} values 102.76±0.65 µg/ml. Both plant extracts showed appreciable on alpha amylase inhibition effect when compared with acarbose. In the alpha glucosidase inhibition assay, the ethanolic extract of C. auriculata and C. angustifolia (at concentration 100 µg/ml) exhibited 36.2% and 30.45% on alpha glucosidase inhibitory effect with IC50 values 134.9±0.54 µg/ml.

A study done by [20] found that there was a dose-dependent increase in percentage inhibitory activity against alphaamylase enzyme. A percentage inhibition of 31.10% and 49.14 % were shown in 200µg/ml and 1200µg/ml plant extract, respectively. Glucose diffusion assay was used for the in vitro antidiabetic evaluation. Maximum decrease in glucose diffusion was observed in Cassia Fistula L. ethanolic extract as compared to control values. Glucose diffusion is useful in vitro index to predict the effect of plant fibers on the delay in glucose absorption in GI tract. In addition to glucose adsorption, the delay in glucose diffusion might be attributed to the physical obstacle presented by fiber particles towards glucose molecules and entrapment of glucose within the network formed by fibers [20].

Conclusion

It can be concluded that cassia fistula shows high alpha amylase inhibitory activity and can be used as green medicine for diabetes mellitus.

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