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Original Article

α-GLUCOSIDASE AND α-AMYLASE INHIBITORY ACTIVITY OF *INDIGOFERA CORDIFOLIA* SEEDS AND LEAVES EXTRACT

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ABSTRACT

Objective: The present study was done to find out the anti-diabetic effect of *Indigofera cordifolia* seeds and leaves extract on intestinal α–glucosidase, α-amylase enzymes *In-vitro*.

Methods: *In-vitro* α–glucosidase and α-amylase assays were performed in order to evaluate anti- diabetic potential of *Indigofera cordifolia* seeds and leaves extract. The dose depended inhibitory effect of aqueous and methanol extract of seeds and leaves was compared with standard acarbose.

Results: α-Amylase inhibitory activity of aqueous extract of seeds and leaves was found to be 65.12% and 55.02% while methanol extract showed 85.59% and 83.40 % inhibition at 4 mg/ml, respectively. In α–glucosidase inhibitory assay the methanol extract of seeds and leaves show more maltase inhibition (IC50 = 2.57±0.29 and 3.47±0.87 mg/mL, respectively) than sucrase (IC = 50 2.73±0.11 and 3.71± 0.46 mg/mL, respectively), at the same time aqueous extract of seeds and leaves showed more maltase inhibition (IC50 = 3.30±0.94 and 5.97± 0.22 mg/mL, respectively) than sucrase inhibition (IC50 = 4.30±0.16 and 6.56±0.62 mg/mL, respectively). Acarbose (standard) showed more maltase inhibition (IC50 = 9.86±0.12 µg/mL) than sucrase (IC50**=** 46.46±1.5 µg/mL) and had 93.40% α-amylase inhibition at 50 µg/ml.

Conclusion: Both the methanol and aqueous extract of *I. cordifolia* seeds and leaves showed strong inhibition against animal α-amylase and α-glucosidase enzyme.

Keywords: *Indigofera cordifolia*, α–glucosidase, α-amylase, Diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM) is a common disease caused by the sugar dysmetabolism characterized as high plasma glucose concentration [1-2]. The burden of diabetes is increasing globally and 346 million people worldwide have diabetes according to World Health Organization [3]. Diabetes has become a common disease due to aged population in the world, bad food habit and environmental pollution [4]. The disease is mainly classified into insulin dependent diabetes mellitus (type I diabetes) and non-insulin dependent diabetes mellitus (type II diabetes). The incidence of type II diabetes is increasing worldwide [5] with hyperglycemia, abnormalities in serum lipids associated with micro- and macro-vascular complications, which are the major causes of morbidity and death in diabetic subjects [6-7].

In the development of type II diabetes postprandial hyperglycemia plays an important role and recent reports revealed that high postprandial plasma glucose level is more harmful than fasting blood glucose. It cannot only cause serious complications but also increase the mortality, so it is important to control postprandial blood glucose level to reduce complications and mortality. One of the therapeutic approaches is to decrease the postprandial hyperglycaemia by insulin, lispro, amylin analogues[8], and retarding an absorption of glucose by inhibition of carbohydrate-hydrolysing enzymes, such as α amylase and α –glucosidase (acarbose and voglibose). Currently α-glucosidase inhibitors are used as first line drugs for reducing postprandial blood glucose level [9-17]. There are many articles related to antidiabetic compounds from plants. However, normalising blood glucose level is a formidable challenge in clinical practice [18].

In diabetes mellitus the insulin-sensitive peripheral tissues, glucose uptake and metabolism in response to insulin get reduced. In the pathogenesis of peripheral insulin resistance defective glucose transport system may perform the significant role. Balancing glucose homeostasis and clearing postprandial glucose burden is a very important step in target tissues [29].

One of the available glucose-lowering treatments is α-Glucosidase inhibitors (AGIs). The α-glucosidase enzyme is necessary for the breakdown of carbohydrates to absorbable monosaccharides which are located in the brush border of the small intestine. The AGIs help in delaying, but do not inhibit the absorption of consumed carbohydrates, reducing the postprandial glucose and insulin peaks [19].

Amylase inhibitors have elements that prevent the absorption of dietary starch by body hence they are also called as starch blockers. Digestive enzyme amylase and other secondary enzymes are necessary for the breakdown of complex carbohydrates such as starch to be absorbed [20-21].

Traditional Indian and Chinese medicines have long used of plant and herbal extracts as anti-diabetic agents [7]. Therefore, investigation on such agents from traditional medicinal plants has become more important and researches are competing to find the new effective and safe therapeutic agent for the treatment of diabetes. The Indian herb *Indigofera. cordifolia* (IC) *is* wildly growing plant in Rajasthan, India and whole plant is used as a tonic and given to cattle as fodder [22]. In Western Rajasthan IC is one of the traditional remedies used for the treatment of hepatitis and diabetes in folk medicine for the times unknown. IC has good amount of phenolic and flavanoid content with strong antioxidant power [23]. In this study, we examined the anti-diabetic activity using different *In-vitro* models.

METHODS AND MATERIALS

Plant material and chemicals

Seeds and leaves of *Indigofera cordifolia* (Fabaceae) were collected during the month of November 2012 from farm at Barmer District, Rajasthaan state of India. The plant material was authenticated by Dr. Ganesh Iyer, botanist at Ruia College, Matunga, Mumbai. Starch and p-nitrophenyl-alpha-d-glucopyranoside (pNPG) were purchased from Himedia, India. Porcine pancreatic α-amylase was procured from Sigma Aldrich Inc., (St Louis, MO). Dinitrosalicylic acid (DNS) and Tris base was obtained from Himedia Laboratory, Mumbai. A

glucose estimation kit was procured from Accurex Biomedical Pvt. Ltd., Thane, Mumbai. Starch, maltose and sucrose were purchased from Sisco Research Laboratories, (Mumbai, India). Acarbose was obtained from Bayer Medical Co. (Germany). All other chemicals and solvents used are of analytical grade.

Plant extraction

Freshly collected plant materials were cleaned to remove adhering dust and then dried under shade. The dried samples were powdered in mixer grinder and used for solvent extraction. The air dried powdered plant material was extracted in soxhlet extractor successively with petroleum ether followed by methanol and water. Each time before extracting with the next solvent, the material was dried in hot air oven at 40 °C. The extracts were concentrated by rotary vacuum evaporator and then dried. The extracts were stored in a refrigerator at 2–8 ˚C for use in subsequent experiments. The aqueous and methanolic extract of *I. cordifolia* was marked as ICA and ICM respectively.

α-Amylase inhibition assay

The α-amylase inhibitory assay was performed according to the method previously described with slight modifications [5, 24]. Briefly 120 µL of IC extracts (20 mg/mL in DMSO) were mixed with 480 µL of distilled water and 1.2 mL of 0.5% w/v soluble potato starch in 20 mM phosphate buffer pH 6.9 containing 6.7 mM sodium chloride in a test tube. The reaction was initiated (0 min) by addition of 600 µL of enzyme solution (4 units/mL in distilled water), 600 µL of the mixture was withdrawn after 3 min into separate test tubes containing 300 µL DNSA color reagent (1g of 3, 5-dinitrosalicylic acid, 30g of sodium potassium tartarate and 20 mL of 2 N sodium hydroxide to a final volume of 100 mL in distilled water) and transferred to a hot water bath maintained at 85-90 °C for 15 min. Afterwards the reaction mixture in each tube was diluted with 2.7 mL distilled waters and the absorbance measured at 540 nm by using a spectrophotometer (UV-1650, Shimadzu, Kyoto, Japan). Test incubations were also prepared for 2.5, 5 10 and 20 mg/mL of *I. cordifolia* to study the concentration dependant inhibition. For each concentration blank incubations were prepared by replacing the enzyme solution with 600 µL in distilled water at the start of the reaction. Control incubations representing 100% enzyme activity which was conducted in a similar manner by replacing IC with 120 µL DMSO. All the tests were run in triplicate. Net absorbance (A) due to the maltose generated was calculated as:

A540 nm*IC = A540 nm*Test – A540 nm*Blank

From the value obtained the percentage (w/v) of maltose generated was calculated from the equation obtained from the maltose standard calibration curve (0-0.1% w/v maltose). The level of inhibition (%) was calculated as:

100-% reaction (at t=3 min)

Where, % reaction = Mean maltose in sample × 100/ Mean maltose in control

Isolation of α-glucosidase from rat small intestine

This assay was carried out to investigate the in vitro inhibitory activity of *Indigofera Cordifolia* on sucrase and maltase (α-glucosidases). Although α-glucosidase isolated from yeast is extensively used as a screening material for α-glucosidase inhibitors but the results did not always agree with those obtained in mammals. Therefore, we used a small intestine homogenate of a rat as α-glucosidase solution because we speculated that it would better reflect the in vivo state [25].

After 20 hours of fasting, small intestine of male Wistar rats (180 g) was collected after sacrificing the animal under anesthesia. The small intestine between the part immediately below duodenum and the part immediately above the cecum was cut and was thoroughly cleaned with saline and an epithelial layer of mucosal tissue was collected by scraping the luminal surface firmly with a spatula. The mucosal scraping was homogenized in phosphate buffered saline (PBS) pH 7.4 containing 1 % triton x 10, and then centrifuged at 12000 rpm for 15 min. The supernatant fraction contained rat small intestinal α-glucosidase. Butanol was added to the supernatant fraction 1:1 proportion and centrifuged at 15000 rpm for 15 min. The aqueous layer was dialyzed overnight against the same buffer. After dialysis, the concentrated enzyme was used as crude α-glucosidase enzyme in the study to observe inhibition by different extracts of *I. cordifolia*. All the preparations were carried out at 4 °C. The protein content of enzyme preparation was estimated [26].

α-glucosidase inhibition assay

The effect of extracts of seeds and leaves of *I. cordifolia* on rat intestinal α-glucosidase activity was assayed according to the method of Matsui et al., with slight modifications [27]. Briefly 0.5 mg protein equivalent of crude α-glucosidase enzyme was incubated with different concentrations of IC for 5 min before initiating the reaction with substrates maltose (6 mM) and sucrose (45 mM) in a final reaction mixture of 1 mL of 0.1 M phosphate buffer pH 7.2. The reaction mixture was incubated for 20 and 30 min at 37°C for substrates maltose and sucrose respectively. The reaction was stopped by adding 1.0 mL of Tris base and α-glucosidase activity was determined by monitoring the glucose released from maltose and sucrose by the glucose oxidase method. Enzyme inhibition data were expressed as IC50 value (the concentration of IC required to inhibit 50% of α-glucosidase activity).

Statistical analysis

All data are expressed as mean ± S. D. for each experiment. Linear regression analysis was used to calculate IC50 values.

RESULTS

α-Amylase inhibitory assay

First, the ability of *I. cordifolia* seeds and leaf extracts to inhibit α-amylase activity in vitro was investigated and the result is presented in Figure 1. The results revealed that *I. cordifolia* leaf extracts inhibited α-amylase in a dose dependent manner (1-4 mg/mL). Aqueous and methanol extract of *I. cordifolia* were studied for their inhibitory effect on α-amylase enzyme involved in starch hydrolysis which is responsible for the increase in postprandial glucose levels in diabetes mellitus. The maximum inhibition of aqueous extract of seeds was 65.12% and of leaves was 52.02% whereas methanolic extract of seed was shown 85.59 % and of leaves was 65.59% at the concentration of 4 mg/mL, while the acarbose showed 93.40% inhibition (data not shown) of α-amylase enzyme.

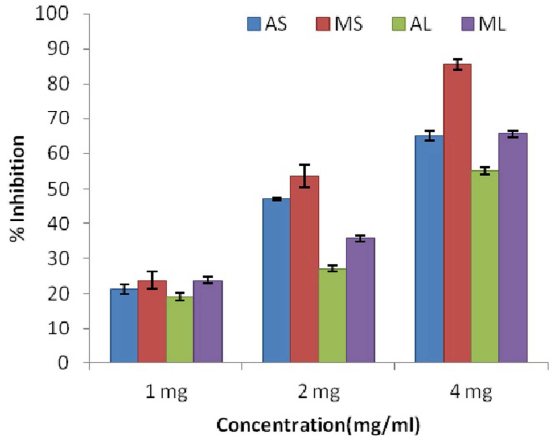

Fig. 1: α-Amylase inhibitory activity of I. cordifolia seeds and leaf extracts

α-Glucosidase inhibitory assay

As can be observed in Fig. 2, the *in vitro* assay of α-glucosidase confirmed that both aqueous and methanol extracts had α-glucosidase inhibitory activity. A dose dependant inhibition of α-glucosidase enzymes such as sucrase and maltase was observed by

aqueous and methanol extracts of seeds and leaves (Figure 2. A, B. Figure 3. C, D). The IC50 values of aqueous extracts of seeds and leaves for sucrase was found to be 4.30±0.83 and 5.76±0.68 mg/mL while methanol extracts 2.14±0.47 and 3.43±0.32 mg/mL respectively. The IC50 values for maltase inhibitory activity was found to be 6.69±0.28 and 6.56±0.35 mg/mL for aqueous extract, and 3.47±0.59 and 3.71±0.64mg/mL for methanol extracts of leaves and seeds of *I. cordifolia* respectively (Figure 2. C and D). The standard drug acarbose showed more sucrase (IC50 = 9.55±1.11 µg/mL) than maltase (IC50 = 46.66 ±0.73 µg/mL) inhibition.

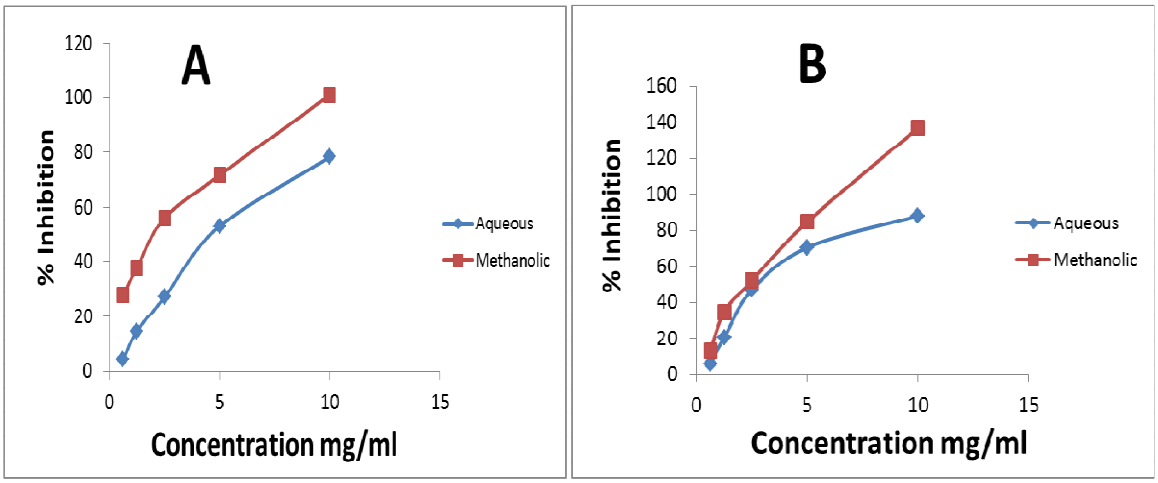

Fig. 2: α-Glucosidase inhibitory activity of *I. cordifolia* on maltase enzyme.

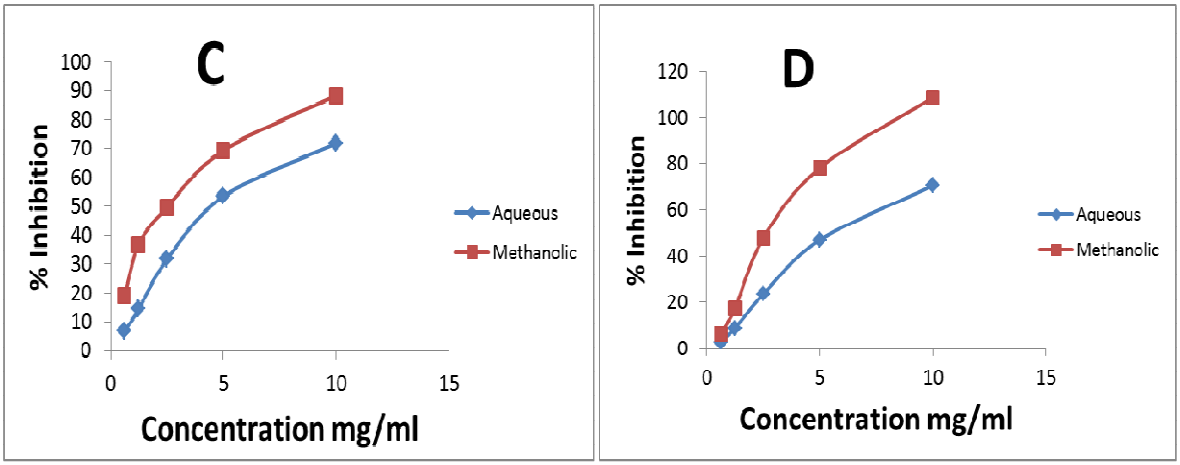

Fig. 3: α-Glucosidase inhibitory activity of *I. cordifolia* on sucrase enzyme.

DISCUSSION

Diabetes is characterized by high blood sugar levels which can cause serious complications such as organ failures and/or destruction of the kidneys, eyes, and various cardiovascular diseases. Therefore, the treatment methods mainly focus on reducing fluctuations in blood sugar levels and their related complications. One of the therapeutic approaches is to decrease the postprandial hyperglycemia by retarding the absorption of glucose through the inhibition of carbohydrate-hydrolyzing enzymes, such as α-amylase and α-glucosidase [25]. α-amylase catalyses the hydrolysis of α-1,4-glucosidic linkages of starch, glycogen and various oligosaccharides and α-glucosidase further breaks down the disaccharides into simpler sugars, readily available for the intestinal absorption. The inhibition of their activity in the digestive tract of humans is considered to be effective to control diabetes by diminishing the absorption of glucose decomposed from starch by these enzymes. Therefore, effective and nontoxic inhibitors of α-amylase and α-glucosidase have long been sought [9]. Agents with α-glucosidase and α-Amylase inhibitory activity have been useful as oral hypoglycemic agents for the control of hyperglycemia in patients with diabetes. There are many natural sources with α- glucosidase, α-amylase inhibitory activity and preventing an excessive postprandial rise of blood glucose level by α-glucosidase and α-amylase inhibition from natural resources is effective in real life as well.

Present study highlighted the inhibitory effect of the plant extract on the activity of intestinal α- glucosidase and α-Amylase in a dose dependent fashion which may be the another way for the management of postprandial hyperglycemia in diabetes. The results give scientific support for the proper use of *I. cordifolia* in folk medicine for the treatment of diabetes and this work could help to develop medicinal preparations or nutraceutical for diabetes and related conditions. Further, *I. cordifolia* may interfere with or delay absorption of dietary carbohydrates in the small intestine, leading to suppression of an increase in plasma glucose after a meal. α-glucosidase inhibitor has been reported to decrease symptoms of diabetes patients [28].

CONCLUSION

This study suggested that the methanol and aqueous extract of *I. cordifolia* seeds and leaves are an effective inhibitor of animal α-amylase and has strong inhibitory activity against microbial α-

glucosidase that may provide a way to regulate the carbon source such as starch in the fermentation processing. Thus, we suggest that *I. cordifolia* can be used to suppress postprandial hyperglycemia in diabetic patients. To understand the inhibitory mechanisms more clearly, we are currently working on isolation and purification of the active constituent from the extract of *I. cordifolia*.

CONFLICT OF INTERESTS

Declared None

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