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Research Article
Non Proteinaceous Seed Extracts of *Albizia lebbeck* Inhibits Porcine Pancreatic α-amylase

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Abstract

Background and Objective: Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion resulting from one of the complication called hyperglycaemia. α-amylase inhibitors play vital role in managing post-prandial hyperglycemia because it lowers post-prandial blood sugar by inhibiting α-amylase in the digestive organs. In pursue of finding novel α-amylase inhibitors *Albizia lebbeck* (*A. lebbeck*) a traditional Indian medicinal plant was investigated. **Materials and Methods:** The n-hexane and dichloromethane (DCM) extracts of seeds of *A. lebbeck* were prepared and investigated for inhibition towards porcine pancreatic α-amylase (PPA) and human salivary amylase. Gel inhibition of PPA was performed on native polyacrylamide gels. Quantitative estimations were performed using enzyme assays. Means and standard deviations were calculated and compared. **Results:** The n-hexane and DCM extracts were found to inhibit PPA activity on 7.0% native polyacrylamide gel treated with 0.1% starch, whereas, it fails to inhibit salivary α-amylase on the gel. About 25 mg of the n-hexane and DCM extract showed 78.45±3.28 and 61.46±2.05% inhibition of 13 U of PPA with effective IC_{50} value 6.56±0.77 and 8.76±0.98 mg, respectively. **Conclusion:** This study revealed α-amylase inhibitor potential of *Albizia lebbeck* which may helpful to develop medicinal preparations to reduce hyperglycaemia, complications associated to DM.

Key words: Diabetes mellitus, hyperglycaemia, pancreatic α-amylase (PPA), *Albizia lebbeck*, dichloromethane

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus (DM) is a worldwide occurring chronic metabolic disorder that has a substantial influence on the health, quality of life, life expectancy of patients and health care system. Of the DM, about 90% have type 2 diabetes (NIDDM; non-insulin-dependent diabetes mellitus)[1]. Type 2 diabetes is generally associated with the disturbance in carbohydrate, fat and protein metabolism. These disturbances arise due to the defect in insulin secretion, insulin action or both and mainly identified by augmented level of post-prandial blood glucose, hyperglycemia[2]. Hyperglycemia leads to various complications including diabetic retinopathy, loss of vision, nephropathy, amputation and cardiovascular disease[3]. It can also damage body's systems comprising blood vessels and nerves[4].

One of the therapeutic approaches to decrease post-prandial hyperglycemia is by retarding absorption of glucose through inhibition of carbohydrate hydrolyzing enzymes[5]. The α-amylases and α-glucosidase are considered as key carbohydrate hydrolysing enzymes that can be targeted to control post-prandial hyperglycemia. Among two, α-amylases (α, 1, 4-Glucano hydrolases) catalyze the hydrolysis of α-D-(1, 4) glycosidic linkages of starch resulting small oligosaccharides. It is supposed that inhibition of this enzyme can control the post-prandial glucose level and could be an effective strategy for managing hyperglycemia[3].

In post-prandial hyperglycemia activity of these enzymes enhances and degradation of dietary starch proceeds rapidly. Inhibition of α-amylase, as it is responsible for initial degradation of dietary starch leads to minimum accumulation of glucose in the blood. This will reduce the rate of glucose absorption and prevents hyperglycemia and the complications associated with the DM. Currently several amylase inhibitors such as acarbose, miglitol and voglibose are available but resulting side effects such as abdotension, bloating, flatulence and diarrhea limits their utility[6-7]. Many studies are targeted towards searching natural proteinaceous and non-proteinaceous α-amylase inhibitors for drug-design to lower the post-prandial hyperglycemia[8]. Several proteinaceous α-amylase inhibitors are isolated and purified but their proteolytic stability against pepsin and other proteases in the acidic environment of stomach and intestine limit utility[9]. Furthermore, researchers have been established the antidiabetic potential of non proteinaceous extracts of several medicinal plant[10]. These extracts are directly or indirectly used for the treatment of diabetic related complications[11]. Non-proteinaceous extracts of medicinal plants such as pycnogenol, phaseolamin and nephelium lappaceum have been observed to inhibit α-amylase or reduce postprandial hyperglycemia[12-14].

Albizia lebbeck (L.) is a medicinal plant belonging to Mimosoid legume and family *Fabaceae*. It is distributed throughout Indian subcontinent with worldwide occurrence. It has a significant role in traditional Ayurvedic medicine for treating various diseases including cataract, asthma, ophthalmopathy, leprosy, diarrhea, poisoning[15]. It also possesses various biological activities including anti-allergic, anti-inflammatory, anti-convulsant, anti-fertility, anti- microbial, anti-arthritic and anti-oxidative activities[16]. This study was carried out to evaluate whether nonproteinaceous seed extracts (n-hexane and DCM extracts) of *A. lebbeck* inhibit porcine pancreatic α-amylase (PPA) activity or not. Gel inhibition of PPA performed on substrate containing native polyacrylamide gels. Quantitative estimations were performed using enzyme assays.

MATERIALS AND METHODS

Sample collection: This study was done at Department of Biochemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India. Dry seeds of *A. lebbeck* were collected in the University campus.

Procurement of instruments and chemicals: Electrophoresis system was obtained from Broviga, India. Porcine pancreas α-amylase, Type I-A (2x crystallized suspension in 2.9 M NaCl containing 3 mM $CaCl_2$) was procured from Sigma Aldrich. Soluble starch, PPA (porcine pancreatic α-amylase), n-hexane, dichloromethane (DCM) and DMSO (dimethyl sulfoxide) were procured from SRL Pvt. Ltd, Mumbai, India. DNSA (3, 5-Dinitrosalicylic acid) was obtained from HiMedia Laboratories, Mumbai, India. All other chemical and reagents were of analytical grade.

Preparation of non-proteinaceous seed extracts: Dried seeds of *A. lebbeck* were crushed to fine powder with the help of mixer grinder. The powder (40 g) was extracted with n-hexane and dichloromethane (DCM) in the ratio of 1:10 w/v separately through maceration (48 h × three times). The crude extracts were filtered and concentrated under controlled temperature 40-50°C. The extracts collected were stored at -20°C. Stock solutions for inhibition assay were prepared by dissolving upto 10 mg of each extract in 1 mL of DMSO and appropriately diluting it in 0.1 M phosphate buffer of pH 6.9 before use.

α-amylase inhibition assay: α-amylase inhibitor activity was assayed based on Bernfeld's method[17]. Increasing concentration of n-hexane and DCM extracts were mixed with PPA in different test-tubes and incubated for 10 min at 37°C. The reaction was started by adding extract-enzyme mixture to test tubes containing 1% starch in 0.1 M phosphate buffer of pH 6.9. These tubes were incubated for 10 min at 37°C and reactions were terminated by adding DNSA (1% 3, 5-Dinitrosalisylic acid, 30% sodium potassium tartarate, 0.2 M NaOH) reagent to the assay mixture. The assay tubes were kept in a boiling water bath for 5 min, cooled under tap water and the colour of maltose liberated was measured at 540 nm. Controls without inhibitor were run simultaneously. One α-amylase activity unit is defined as the amount of enzyme that will liberate 1 mmol of maltose in 1min under the assay conditions (pH 6.9, 37°C). Inhibitory activity is expressed as the percentage of inhibited enzyme activity out of the total enzyme activity.

In-gel inhibition of porcine pancreatic α-amylase: In gel inhibition of porcine pancreatic α-amylase was carried out following 7.0% non-denaturing polyacrylamide gel electrophoresis, native PAGE[18]. Porcine pancreatic α-amylase was loaded in lane without or with n-hexane and DCM extract separately. The constant current (100 V) was supplied to the gel till the tracking dye "bromophenol blue" (BPB) reaches the bottom of the gel. After electrophoresis gel was placed in a 1% soluble starch solution in 0.1 M phosphate buffer, pH 6.9, for 1 h at 4°C. After 1 h, the gel was carefully rinsed with Milli-Q water. α-amylase activity was observed on the polyacrylamide-starch matrix as clear bands on a blue-colored background after staining with a Lugol's reagent (1 g iodine dissolved in 100 mL of 1 M potassium iodide) for 10 min. The absence of clear bands in lane containing inhibitor with enzyme confirms inhibitory efficacy. The gel was washed, in order to remove the excess iodine solution and then photographed.

Statistical analysis: All experiments were conducted in triplicate. Means and standard deviations were calculated and compared. The analysis was performed using Microsoft Excel 2010.

RESULTS AND DISCUSSION

In the present investigation the inhibition potential of n-hexane and DCM extracts of *A. lebbeck* was studied against α-amylase. The inhibition of PPA by n-hexane extracts is shown in Fig. 1.

About 25 mg of the n-hexane extracts inhibited PPA by 78.45±3.28% in solution assay under standard condition. The crude DCM extract with concentration of 25 mg mL^{-1} was found to inhibit 61.46±2.05% activity of PPA as shown in Fig. 2.

The IC_{50} is the concentration of an inhibitor where the activity of PPA reduced by half. The IC_{50} values were found to be 6.56±0.77 mg mL^{-1} for crude n-hexane and 8.76±0.98 mg mL^{-1} for DCM extract (Fig. 3).

Recently different solvent extracts of bark of *A. lebbeck* were screened for inhibition against salivary amylase and

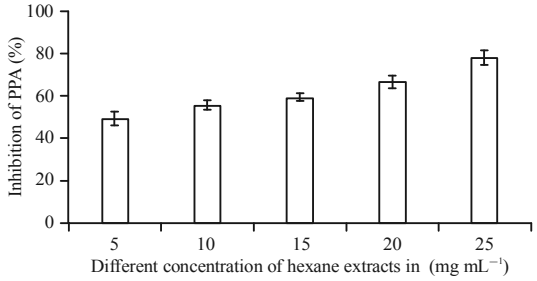

Fig. 1: Inhibition of PPA by n-hexane extract. Increasing concentration was pre-incubated with PPA at 37°C for 10 min
Results are presented as Mean±SD

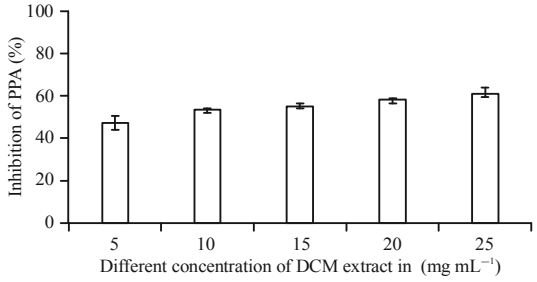

Fig. 2: Inhibition of PPA by DCM extract. Increasing concentration was pre-incubated with PPA at 37°C for 10 min
Results are presented as Mean±SD

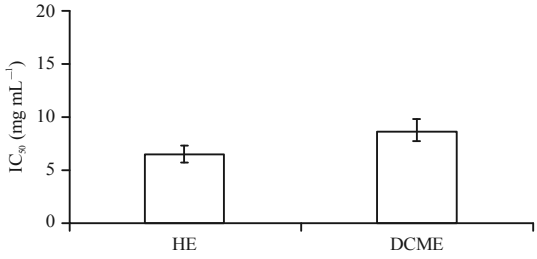

Fig. 3: IC_{50} values of n-hexane (HE) and dichloromethane (DCME) extracts
Results are presented as Mean±SD

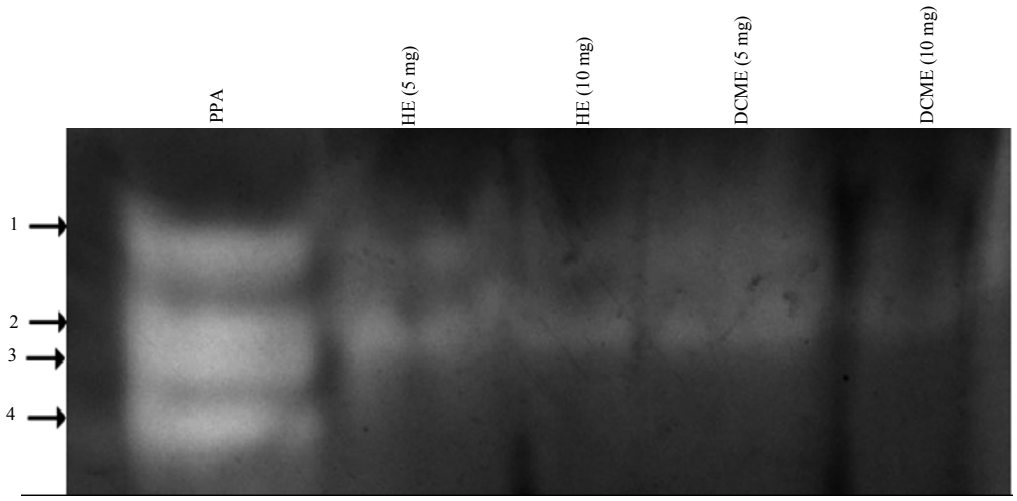

Fig. 4: Zymogram of PPA activity in absence and presence of n-hexane and DCM extracts on 7.0% native-PAGE

found to have substantial inhibition potential[19]. Zymography is an electrophoretic technique for detection and visualization of enzyme activity. This is important tool to assess the enzyme activity of complex biological samples on polyacrylamide gel containing a specific substrate. Amylase zymography was an electrophoretic technique which explores amylase activity directly on a polyacrylamide gel as discrete bands of starch hydrolysis[20]. The PPA showed 4 activity bands in absence of n-hexane and DCM extracts (Fig. 4).

The fast migrating isoforms of PPA (band 3 and 4) were failed to produce activity bands with all tested concentrations of extracts of n-hexane and DCM (5 and 10 mg) suggesting complete inhibition. The slow migrating isoforms (band 1 and 2) of PPA were found to produce little activity bands with all tested concentrations of n-hexane and DCM extracts. Maintenance of normal levels of glycemic control in the blood is proved to be effective strategy to treat patient suffering with DM[10]. This is done by targeting the carbohydrate-hydrolyzing enzymes such as α-amylase in the digestive tract. The α-amylase inhibitors prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and subsequently lessening the postprandial plasma glucose rise[21].

Many studies targeted α-amylase inhibitors to design drugs for controlling hyperglycemia in DM and many plants in different countries are screened for possible candidates[22,23]. Considerable inhibition potential towards PPA was observed in both selected crude extracts. The PPA has frequently been used to simulate the human α-amylase because it is structurally and chemically analogous to human α-amylase[24]. Substantial inhibition to PPA by n-hexane and DCM extracts of *A. lebbeck* could be useful in targeting α-amylase to design drugs for controlling hyperglycemia.

CONCLUSION

Our results suggest that both extracts are good source of amylase inhibitory activity but required high crude phenolic concentration for inhibition. To explore more about interactions between target enzymes and inhibitor constituent from the phenolics, the purification and characterizations of these constituents are necessary.

SIGNIFICANCE STATEMENTS

This study discovers the anti-diabetic potential of non proteinaceous extracts of seeds of *Albizia lebbeck* that can be helpful to develop medicinal preparations to reduce hyperglycaemia, one of the complications associated to DM. This study focused further to explore more about interactions between target enzymes and inhibitor constituent from these crude phenolics. The purification and characterizations of these constituents are necessary for future prospective of this study.

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