International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 3 | JUL-SEPT | 2018 | 636-640
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

IN VITRO STUDIES ON ALPHA AMYLASE INHIBITORY ACTIVITY OF INDIAN MEDICINAL PLANTS

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ABSTRACT

Pancreatic alpha amylase inhibitory activity of aqueous extracts of medicinal plants was evaluated in vitro to search new anti-diabetic agents as alternatives to synthetic medicines. Rhizome and leaves of C. longa and leaves of Moringa oleifera L., Azadirachta indica L., Psidium guajava L. and Murraya koenigii L were extracted with hot water and six extracts were tested for presence of PPA inhibitory activity quantitatively and their modes of inhibition were determined. Presence of alpha amylase inhibitors were identified in all extracts in quantitative assay. Aqueous extract of leaves and rhizome of C. longa showed highest anti-amylase potential with IC_{50} values of 0.53±0.10 and 0.96±0.29 mg/ml respectively. IC_{50} values of other extracts ranged between 1.24±0.49 to 4.50±0.38 mg/ml. Three highest inhibition potential showing extracts of C. longa leaves and rhizome and aqueous extract of Moringa oleifera displayed non-competitive, mixed and non-competitive mode of inhibition respectively as determined in terms of changes in Vmax and km values. In conclusion, active constituents of these three extracts possess anti-diabetic properties and can be used in management of diabetes mediated complications.

KEY WORDS

Alpha amylase, hyperglycemia, inhibition potential, medicinal plants, turmeric

INTRODUCTION

Diabetes mellitus is a multifactorial non-communicable disease characterized by elevated levels of blood glucose [1]. Due to modern lifestyle and increased consumption of carbohydrate rich food, the disease continues to be a global health challenge in addition to an economic burden in developing countries. Among the various strategies implemented for management of diabetes related complications, retardation of glucose absorption via inhibition of alpha amylase and alpha glucosidase enzymes is considered as one of the important therapeutic targets [2]. The alpha amylase is an important secretary product of pancreas and salivary gland responsible for the initial step in hydrolysis of complex polysaccharides to a mixture of oligosaccharides and disaccharides in the intestinal mucosa.

The well-known anti-amylase drug, acarbose is an important member of antidiabetic drug families and suitable for managing insulin independent diabetes mellitus complications. However, this drug is associated with gastrointestinal side effects as flatulence, bowel bloating or hypoglycemia, which limit its use in the treatment of diabetes mellitus [3]. Accordingly, investigation and development of alternative strategies for perfect management of postprandial hyperglycemia without any side effects are needed [4].

Herbal medicines used for therapeutic management of diabetes related complications are increasingly becoming popular in modern society as alternatives to synthetic drugs. These are cheaper, accessible and culturally more acceptable holistic medicines with lower negative side effects as compared to synthetic drugs [5, 6]. In India, indigenous herbal remedies have been used since ancient times in reducing the problems associated

with diabetes [7]. The studies on traditional herbal remedies used for diabetes found more than 1,200 species of plants with hypoglycemic activity [8, 9] and a large number of medicinal plants and their formulations are used for treating diabetes. In addition to this, over 200 million people in India with limited access to primary healthcare centers, depend on traditional system of medicine to cater to their healthcare needs [10]. In order to support this traditional knowledge with scientific testing, World Health Organization (1980) has recommended the evaluation of mechanistic properties of the plants effective in such systems [11, 12]. The search for new pharmacologically active agents obtained by screening natural sources such as medicinal plants or their extracts can lead to potent and specific inhibitors for α-amylase [13]. This study was carried out to investigate the potential of aqueous extracts of some commonly used medicinal plants in India to inhibit the activity of Porcine Pancreatic Alpha Amylase enzyme which is structurally and mechanistically similar to the Human Pancreatic Alpha Amylase [14] and to find out the possible mode of inhibition in presence of these extracts.

MATERIALS AND METHODS

The fresh leaves of *Moringa oleifera L.* (MOL), *Azadirachta indica L.* (AIL), *Psidium guajava L.*(PGL), *Murraya koenigii L.* (MKL) and leaves (CLL) and rhizome (CLR) of *Curcuma longa L* were collected from local agricultural fields of Nanded, Maharashtra, India. Porcine pancreatic α-amylase (PPA) and Acarbose were procured from Sigma Aldrich (Co.,St .Louis, USA), while soluble starch and DNSA were obtained from Hi Media (Mumbai, India). Other chemicals were of analytical grade.

Preparation of extracts

The leaves and rhizome materials were washed repeatedly with distilled water to remove any adhered debris and dirt particles. The plant parts were completely dried in shade at room temperature. The dried plant materials were then ground to obtain fine powder. The powdered plant materials were subjected to hot water extraction at 50^{o}C for 12 hrs in 1:10 ratio. The resulting blends were collected, filtered and concentrated in a rotary evaporator. Dried extracts were weighed and dissolved in dimethyl sulfoxide to yield stock solution of extracts.

Alpha amylase inhibition assay

Quantitative α-amylase inhibition assay was performed as per the method described by Stephen and Oboh [15]. Briefly, 100 µl of each extract (1 mg/ml) was mixed with 100µl of α-amylase (1mg/ml) and 100µl of 0.1M phosphate buffer (pH 7.0). This mixture was pre-incubated at room temperature for 10 minutes. After incubation, 100µl of starch (0.1%) solution was added and incubated at room temperature for further 30min. This was followed by addition of 1ml dinitro salicylic acid (DNSA) reagent to stop the reaction and incubation in boiling water bath for 5 minutes. The reaction mixture was removed from water bath, cooled at room temperature and diluted to 10 ml with distilled water. Absorbance of the mixture was taken at 540nm. The blank with 100% enzyme activity was prepared by replacing the plant extract with 100 µl of buffer. A blank reaction was similarly prepared using the plant extract in the absence of the enzyme solution. A positive control sample was prepared using Acarbose (100 µg/ml) and the reaction was performed similarly to the reaction with plant extract as mentioned above. The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below:

$$\% \text{ inhibition} = \frac{\text{O.D of control-O.D Of extract}}{\text{O.D of control}} \times 100$$

Mode of α-amylase inhibition

The method for determining the mode of inhibition of α-amylase in presence of selected plant extracts involved pre-incubation of 100µl of α-amylase solution with extracts at room temperature for 10 minutes. Control was prepared by pre-incubating α-amylase with 100µl of phosphate buffer (pH 7.0) in place of extract. Reaction was started with addition of starch solution at increasing concentration (0.1 – 1.0%). The reaction mixture was incubated for 10 mins at room temperature and terminated by addition of 1 ml of DNSA. The contents were placed in boiling water bath for 5 minutes and diluted 10 ml with distilled water. The amount of reducing sugars released during reaction was determined spectrophotometrically at 540 nm. The concentration of released sugar was determined using standard curve of maltose. The concentrations of sugar obtained such were converted to reaction velocities. The mode of α-amylase inhibition by the extract was determined by plotting a double reciprocal (Line weaver- Burk) plot between reaction velocity (1/V) and

starch concentration (1/[S]) using Michaelis Menten kinetics. The changes in the values of Vmax and Km over control were considered to reveal inhibition mode of extract as explained earlier [16].

RESULTS AND DISCUSSION

PPA inhibitors that reduce postprandial hyperglycemia have been found helpful in controlling insulin independent diabetes mellitus [17,18]. The knowledge about the role of herbal extracts in management of type II diabetes is known since ancient times and currently being used in Ayurveda for the treatment of diabetes. However, due to lack of sustained scientific evidence, these medicinal plants have not gained much importance [19]. In the present study, five indigenous medicinal plants from India were screened for their anti-amylase activity potential. The results on inhibitory activity of aqueous extracts of CLL, CLR, AIL, PGL, MOL and MKL are shown in Table 1. At 1 mg/ml, all the plant extracts showed considerable inhibitory activity in the assay. The PPA activity was completely inhibited in the presence of CLL extract and reduced by 52.66% in presence of CLR extract. In presence of other extracts, the detected range of inhibition was 11.11 to 40.12%. Balaji et al., [20] also studied antidiabetic activity of eight Indian medicinal plants as alpha amylase inhibitors and found that methanolic extracts of *W. somnifera, O. sanctum* and *A. paniculata* were effective inhibitors of amylase. The use of *C. longa* (turmeric), a golden spice in Ayurveda is not new to human being. It has been reported to be used as preservative, coloring agent, in treatment of biliary disorders, anorexia, cough, diabetic wounds and hepatic disorders. Rhizome of *C. longa* is known to possess therapeutic activities and used as an anti-diabetic [21-24], hypolipidaemic [21-23], anti-inflammatory [23,24], anti-diarrheal [22], hepatoprotective [21,22], anti-asthmatic [23] and anti-cancerous drug. Isopropanol and acetone extracts of *C. longa* were found to inhibit starch hydrolytic activity of human pancreatic amylase in the studies made by Ponnusamy et al., [25]. Similarly, Prabhakar et al., [26] noted anti-amylase activity of methanolic and aqueous extracts of *C. longa* rhizome, *A. indica* leaf and bark, *O. santum, W. somnifera, T. cardifolia* and *B. oleracea*. The study indicated that methanol extract of *O. santum* and aqueous extract of *W. somnifera* possesses potent anti-amylase activity among other plants.

Table 1 Inhibition of amylase activity by aqueous extracts of selected medicinal plants at 1.0 mg/ml.

Sr. No.	Extracts	% Inhibition
1	CLL	100
2	CLR	52.66
3	AIL	34.25
4	MOL	40.12
5	PGL	11.11
6	MKL	31.65

IC_{50} values of all extracts were determined by analyzing the dose response curves plotted by testing amylase activity in presence of varying concentrations (0.5-5.0 mg/ml) of extracts (data not shown here). The aqueous extracts of CLL was found to contain potent PPA inhibitor with an IC_{50} value of 0.53±0.10 mg/ml followed by an effective inhibition determined in presence of aqueous extract of CLR with an IC_{50} value of 0.96±0.29 mg/ml. IC_{50} values of all other extracts were ranged between 1.24 ±0.49 to 4.50±0.38 mg/ml. Acarbose which was used as a standard PPA inhibitor showed highly potent anti-amylase activity with an IC_{50} value of 0.15±0.11 mg/ml (Table 2).

Of these six, three extracts including CLL, CLR and MOL showing higher inhibition potential were selected for determining their modes of PPA inhibition using Line weaver Burk plots. Table 3 shows the values of kinetic parameters related to these three selected extracts. As compared to the control set, the rate of amylase catalyzed reaction was found to be lowered in presence of aqueous extracts of CLL, CLR and MOL. The value of affinity constant km was constant to control value in presence of CLL and MOL extracts whereas it was noted higher than control set in presence of CLR extract. Based on these values, it can be noted that the mode of PPA inhibition in presence of CLL and MOL extracts was non-competitive. That means, the active components in these extracts binds to a site other than active site of PPA with the same affinity for free and substrate bound enzyme and interfering with the process of enzyme catalysis [27]. In presence of CLR extract, the mode of inhibition was found to be of mixed type in which active constituents in extract is expected to bind with either free or bound form of enzyme. The increase in the value of km in presence of CLR extract indicated that the active constituent binds with free form of enzyme.

Table 2. Inhibitory concentration (IC_{50}) value of plant extract on alpha-amylase.

Sr.No.	Extract	IC_{50} (mg/ml)
1	CLL	0.53 ± 0.10
2	CLR	0.96 ± 0.29
3	AIL	1.54 ± 0.59
4	MOL	1.24 ± 0.49
5	PGL	4.50 ± 0.38
6	MKL	1.57 ± 0.76
7	Acarbose	0.15 ± 0.11

Table 3. km and Vmax values of extract of medicinal plants and their mode of Inhibition

Parameters	Control	CLL	CLR	MOL
V_{max}	19.29	13.88	18.90	16.12
K_m	0.02	0.02	0.0384	0.02
V_{max}/K_m	876.81	527.55	492.18	644.8
Inhibition mode	--	Non-competitive	Mixed	Non-competitive

CONCLUSION

The results of this study suggest that although, all selected extracts are commonly known for their medicinal properties, very few studies reported their PPA inhibitory potential. In the present study we have not ascertained the chemical nature of the phytoconstituents responsible for PPA inhibitory activity and the active lead molecules needs to be isolated and characterized through *in vitro* and *in vivo* studies. Three extracts including CLL, CLR and MOL exhibiting good enzyme inhibitory potential can be used to lower the postprandial high glucose levels by inhibiting PPA activity.

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Received:04.05.18, Accepted: 07.06.18, Published:01.07.2018

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