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Research Article

IN VITRO α-AMYLASE AND α-GLUCOSIDASE INHIBITORY EFFECTS OF ETHANOLIC EXTRACT OF *EVOLVULUS ALSINOIDES (L.)*

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ABSTRACT
Diabetes mellitus is a disorder in carbohydrate metabolism of endocrine system due to an absolute or relative deficiency of insulin secretion, action, or both. There has been an enormous interest in the development of alternative medicines for type 2 diabetes, specifically screening for phytochemicals with the ability to delay or prevent glucose absorption. Pancreatic *α*-amylase and glucosidase inhibitors offer an effective strategy to lower the levels of post prandial hyperglycemia via control of starch breakdown. The goal of the present study was to provide *in vitro* evidence for potential inhibition of α-glucosidase and α-amylase enzymes by using the ethanolic extract of *Evolvulus alsinoides*. The results showed a significant (more than 70%) reduction in *α* –amylase activity as well as 50% reduction in *α-glucosidase activity*. The present study suggests that the extract of *Evolvulus alsinoides* effectively act as alpha amylase and alpha glucosidase inhibitor leading to a reduction in starch hydrolysis and hence eventually to lowered
glucose levels.
Key words: Anti diabetic, α-glucosidase inhibitor, *Evolvulus alsinoides,* α-amylase

INTRODUCTION

Diabetes mellitus is a chronic disease characterized by elevated blood glucose levels, disturbances in the carbohydrate, fat and protein metabolism[1]. Over several years diabetes mellitus has become a major health problem worldwide; reaching epidemic proportions[2]. Diabetes mellitus is considered to be a serious issue in many countries and traditional methods using medicinal plants to control diabetes is gaining momentum. The synthetic hypoglycemic agent/s does produce serious side effects and whereas drug derived from medicinal plants are frequently consider being safe and cost effective[3].

Herbal medicines with anti-diabetic potential have different modes of action – mimic insulin, act on insulin secreting beta cells or modify glucose utilization. Herbs which modify glucose utilization act by altering the viscosity of gastrointestinal contents, delaying gastric emptying or delaying glucose absorption[4]. Absorption of glucose can be delayed by reducing the rate of digestion of starch. Pancreatic α-amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to a mixture of smaller oligosaccharides consisting of maltose, maltotriose, and a number of a-(l-6) and a-(1 - 4) oligoglucans. These are then acted on by α-glucosidases and further degraded to glucose which on absorption enters the blood-stream. Degradation of this dietary starch proceeds rapidly and leads to elevated PPHG (post-prandial hyperglycemia). It has been shown that activity of HPA (human pancreatic α-amylase) in the small intestine correlates to an increase in post-prandial glucose levels, the control of which is therefore an important aspect in treatment of type 2 diabetes. Hence, retardation of starch digestion by inhibition of enzymes such as α-amylase plays a key role in the control of diabetes. Inhibitors of pancreatic α-amylase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the post-prandial serum glucose levels[5].

Some inhibitors currently in clinical use are acarbose, miglitol, and voglibose are known to inhibit a wide range of glycosidases such as *α*-glucosidase and *α*-amylase. Because of their nonspecificity in targeting different glycosidases, these hypoglycemic agents have their limitations and are known to produce serious side effects. The main side effects of these inhibitors are gastrointestinal viz., bloating, abdominal discomfort, diarrhea and flatulence[6]. Therefore, the search for more safer, specific, and effective hypoglycemic agents has continued to be an important area of investigation with natural extracts from readily available traditional medicinal plants offering great potential for discovery of new antidiabetic drugs[7,8,9]. The aim of the present study is to examine the *in vitro* α-amylase and glucosidase inhibitory activity of ethanolic extract of *Evolvulus alsinoides.*

MATERIALS AND METHODS

Collection of Plant Material

The whole plant of *Evolvulus alsinoides* (L.) used for the investigation was obtained from Coimbatore district, Tamilnadu, India. The plant was authenticated by Dr. P.Satyanarayana, Botanical survey of India, TNAU Campus, Coimbatore. The voucher number is BSI/SRC/5/23/2011-12/Tech.-514. Fresh plant material was washed under running tap water, air dried and powdered.

Sample Extraction

100g of dried plant powder was extracted in 500ml of ethanol in a water shaker for 72hrs. Repeatedly extraction was done with the same solvent till clear colorless solvent is obtained. Obtained extract was evaporated to dryness by using a rotary vacuum evaporator at 40-50^{o}C and stored at 0-4^{o}C in an air tight container.

In vitro α-amylase inhibition study

The α-amylase inhibitory activity was determined according to the method described by Jyothi *et al.,* 2011[10]. Briefly, the total assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme, and the plant extracts in

the concentration range 10-100µg/ml were incubated for 10 min at room temperature followed by addition of 200 µl of 1% starch in all the test tubes. The reaction was terminated with addition of 400 µl of 3,5 dintrosalycylic acid (DNSA) color reagent, placed in boiling water bath for 5 minutes, cooled at room temperature and diluted with 15 ml of distilled water and the absorbance measured at 540nm. The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing various concentrations of the plant extracts prepared with different solvent prepared with DMSO. The results were expressed as % inhibition calculated using the formula:

$$\text{Inhibition activity (\%)} = \frac{\text{Abs (control)-Abs (extract)}}{\text{Abs (control)}} * 100$$

The IC_{50} values (inhibitor concentration at which 50% inhibition of the enzyme activity occurs) of the plant extracts were determined b y performing the assay as above with varying concentrations of the plant extracts ranging 20 to 100µg. The IC_{50} values were determined from plots of percent inhibition vs log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

α-glucosidase inhibition assays

The yeast α-glucosidase was dissolved in 100mM phosphate buffer, pH 6.8 was used as enzyme source; 10mM *para*nitrophenyl-α-D-glucopyranoside was used as substrate. *Evolvulus alsinoides* extract powder was weighed and mixed with dimethylsulfoxide to get a concentration of 20-100µg/ml. The different concentration of plant extract was mixed with 320µl of 100mM phosphate buffer (pH 6.8) and 50 µl of 10mM PNPG in the buffer and then it was incubated at 30 °C for 5 minutes. After the incubation, 20µl of the buffer containing 0.5 mg/ml of the enzyme was added and further incubated at 30°C for five minutes. Finally, 3.0 ml of 50mM sodium hydroxide was added to the mixture and the absorbance (A) was measured at 410nm on a spectrophotometer. The enzyme without plant extract was used as a control[11].

$$\text{\% Inhibition} = \frac{A_{410}\text{ control} - A_{410}\text{ test}}{A_{410}\text{ control}} * 100$$

The IC_{50} values (inhibitor concentration at which 50% inhibition of the enzyme activity occurs) of the plant extracts were determined by performing the assay as above with varying concentrations of the plant extracts ranging 20 to 100µg. The IC_{50} values were determined from plots of percent inhibition vs log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

RESULTS AND DISCUSSION

Diabetes mellitus (DM) is a common endocrine system disease that causes metabolic disorders and which leads to multiple organ damage syndrome. Clinical admiral diabetes is divided into two types, with more than 90% of patients having Type II diabetes[12]. The number of diabetes cases was 171 million in 2000 and is expected to rise to 366 million in 2030[13]. Inhibition of α-glucosidase (EC 3.2.1.20) and α-amylase (EC 3.2.1.1), enzymes involved in the digestion of carbohydrates, can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in the management of postprandial blood glucose level in type 2 diabetic patients and borderline patients[14,15].

Intestinal α-glucosidase is a glucosidase acting as a key enzyme for carbohydrate digestion, located at the epithelium of the small intestine. α- glucosidase has been recognized as a therapeutic target for the modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality that occurs in Type II DM[16]. Several natural α-glucosidase and α-amylase inhibitors including acarbose, voglibose and miglitol are clinically used as a treatment, but their prices are high and clinical side effects occur[17,18]. Natural products are still the most available source of α- glucosidase inhibitors[19]. Therefore, screening of alpha-amylase and glucosidase inhibitors in medicinal plants has received much attention. Therefore, in the present study we investigated α- amylase and α- glucosidase inhibitory activity by using the ethanolic extract of *Evolvulus alsinoides*.

The *in vitro* α-amylase inhibitory studies demonstrated that *Evolvulus alsinoides* extract has both α- glucosidase and α-amylase inhibitory activity. The percentage inhibition at 100, 80, 60, 40 and 20 µg/ml concentrations of plant extract showed a concentration-dependent reduction in percentage inhibition. Thus the highest concentration of 100 µg/ml tested showed a maximum inhibition of nearly 63% and the standard acarbose showed the inhibitory activity of 74% (Figure 1). The plant extract produced a weak α-glucosidase enzyme inhibition when compared with alpha amylase. The maximum inhibition was found to be 53% at a concentration of 100 µg/ml (Table 1) .

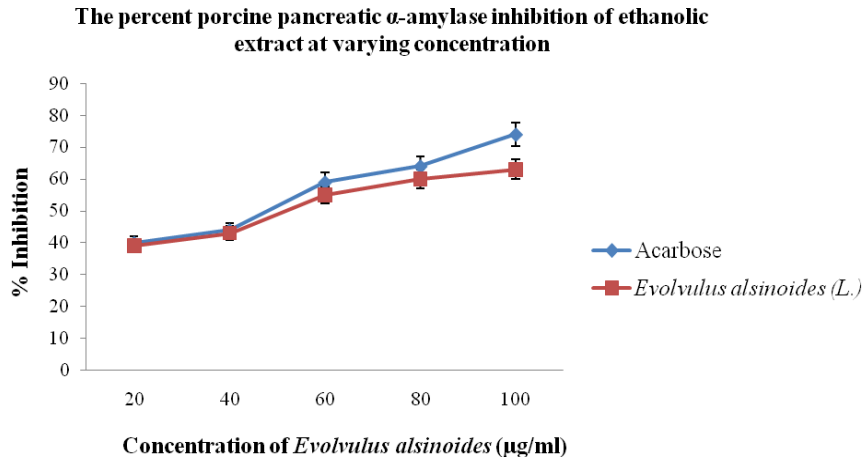

Figure: 1
Values are expressed as mean±SD (n=3)

Table 1: The percentage inhibition of α-glucosidase by ethanolic extract of *Evolvulus alsinoides (L.)*

Plant species	Concentration(µg/ml)	Inhibition(%)	IC_{50} (µg/ml)
Evolvulus alsinoides (L.)	20	36.8	86 ± 0.268
	40	38.0	
	60	39.4	
	80	43.6	
	100	52.9	

Values are expressed as mean±SD (n=3)

Acarbose like drugs, drugs that inhibit α-glucosidase and amylase present in the epithelium of the small intestine, have been demonstrated to decrease post-prandial hyperglycaemia[20] and improve impaired glucose metabolism without promoting insulin secretion in NIDMM patients[21]. These medications are most useful for people who have just been diagnosed with type 2 diabetes and who have blood glucose levels only slightly above the level considered serious for diabetes. They also are useful for people taking sulfonylurea medication or metformin, who need an additional medication to keep their blood glucose levels within a safe range. Therefore, the retardation and delay of carbohydrate absorption with a plant-based α-glucosidase inhibitor offers a prospective therapeutic approach for the management of type 2 diabetes mellitus and borderline patients[22,23]. The results of this study indicate that the administration of some of *Evolvulus alsinoides* an probably manage the postprandial blood glucose levels and confirm the usage of these plants.

CONCLUSION

Our *in vitro* studies indicated that *Evolvulus alsinoides* had a α-amylase and glucosidase inhibitory activity and it might possess therapeutic antidiabetic effects in the type II diabetes mellitus. The result obtained from the present study (*in vitro*) will be confirmed by taking up *in vivo* studies in future.

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