INTRODUCTION

Diabetes mellitus is a complex and a diverse group of disorders that disturbs the metabolism of carbohydrate, fat and protein. The number of diabetes mellitus cases has been increasing worldwide in recent years. In 2000, the world health organization estimated a total of 171 million of people with diabetes mellitus from the global population, and this report projected to increase to 366 million by 2030 [1]. With a long course and serious complications often resulting in high death rate, the treatment of diabetes spent vast amount of resources including medicines, diets, physical training and so on in all countries. Thus searching for a new class of compounds is essential to overcome diabetic problems. There is continuous search for alternative drugs [2]. Inhibition of alpha amylase and alpha-glucosidase enzymes can be an important strategy in management of post prandial blood glucose level in type 2 diabetes patient [3]. The chosen medicinal plant namely as Physalis minima leaf L belongs to the Solanaceae family. Thus, objective of the present study is to investigate the in vitro antidiabetic activity of methanolic extract of Physalis minima leaves.

MATERIALS AND METHODS

Study materials

The mature Physalis minima L. leaf (Sodakku thakkaali) was collected in May 2013 from Vaduvur, Thiruvurur District, Tamil Nadu, India. The leaves were washed several times with distilled water to remove the traces of impurities from the leaf. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The Physalis minima L. leaf extract (PMLE) was stored in refrigerator until used. Doses such as 10, 20, 30, 40 and 50µg/ml were chosen for in vitro antidiabetic activity.

METHODS

Physalis minima leaves. Various concentrations from 10 μg ml⁻¹ to 50 µg ml⁻¹ were prepared in methanol along with the standard antidiabetic drug, acarbose. There was a dose dependent percent inhibition by the extract against α-amylase (16% - 84%) α-glucosidase (18% - 76%) and glucose uptake by yeast cells (16% - 78%). Logarithmic regression analysis revealed the IC₅₀ of 28.72 µg ml⁻¹ (α-amylase), 30.82 µg ml⁻¹ (α-glucosidase) and 31.45 µg ml⁻¹ (glucose uptake by yeast cells) with a potency and preference for α-amylase over α-glucosidase inhibition and glucose uptake by yeast cells by the Physalis minima leaf extract.

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Acarbose as standard was used as a control.

**IN VITRO ANTIDIABETIC ACTIVITY**

**In vitro α-amylase inhibition study**

In vitro α-amylase inhibition assay was carried out by the method of Apostolidis [4].

**In vitro α-glucosidase inhibition study**

The α-glucosidase inhibitory activity was determined according to the method described by Apostolidis [4].

**Glucose uptake in Yeast cells**

Yeast cells were prepared according to the method of Gupta daksha et al. [5].

**RESULTS AND DISCUSSION**

In this present study, in vitro α-glucosidase inhibitor activity of ethanolic extract of *Physalis minima* L. leaves were evaluated. The retardation and delay of carbohydrate absorption with a plant-based α-glucosidase inhibitor (Table 2 and fig 2) offers a prospective therapeutic approach for the management of type 2 diabetes mellitus. The IC$_{50}$ values show that *Physalis minima* L has 31.54% and standard 21.45%.

The in-vitro α-amylase inhibitory studies demonstrated that *Physalis minima* L has an efficient anti diabetic activity (Table 1 and Fig 1). The percentage inhibition at 10, 20, 40, 60, 80 μg/ml concentration of crude plant extracts shown concentration dependent reduction in percentage inhibition. At a concentration of 10μ/ml of *Physalis minima* L extract showed 16 % of inhibition and 54% for 80 μg/ml extracts and standard showed a inhibition of 92.84 %.

The rate of glucose transport across cell membrane in yeast cells system is presented in Table 3 and Fig 3. The increase in glucose uptake by the yeast cell at different glucose concentrations i.e. 10, 20, 40, 60, 80 μg/ml respectively. The ethanolic extract of *Physalis minima* L exhibited significantly higher activity at all glucose concentrations showing the maximum increase in 80 μg/ml Glucose concentration. Results also indicated that *Physalis minima* L. had greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug metformin.

The in vitro assays of the present study concluded that Ethanolic extract of *Physalis minima* L. leaves possess hypoglycemic activity and can be used in the management of diabetes. The active principles of *Physalis minima* L. leaves are responsible for inhibitory action of α-amylase, α-glucosidase and increased glucose uptake in Yeast cells.

A study of ancient literature indicates that diabetes (Madhumeha/Prameha) was fairly well known and well conceived as an entity in India. Regulation of glucose level in the blood of the diabetic patient can prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species [6].

α-glucosidase catalyzes the final step in carbohydrate digestion which leads to postprandial hyperglycemia. Inhibitors of α-glucosidase are useful in the control of hyperglycemia as they delay carbohydrate absorption.
digestion and causing reduced glucose absorption rate which consequently reduce the postprandial plasma glucose rise [7]. These inhibitors have been found useful in the control of diabetes mellitus over many years [8, 9]. Many scientists have investigated the plants containing various phytochemicals that exhibit additive and synergistic interaction in antidiabetic properties which exert positive health-promoting effects [10].

The intestinal digestive enzymes alpha-amylase plays a vital role in the carbohydrate digestion. One antidiabetic therapeutic approach reduces the post prandial glucose level in blood by the inhibition of alpha-amylase enzyme. These can be an important strategy in management of blood glucose [11].

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide in to mono and disaccharide. In our experimental study it was observed that ethanolic and aqueous extract of Physalis minima L.demonstrated significant Alpha amylase inhibition activity as compared to standard drug acarbose.

In Yeast (Saccharomyces cerevisiae), glucose transport takes place through facilitated diffusion. Type 2 Diabetes is characterized by the deficiency of insulin causing increased amount of glucose in blood. After the treatment of the yeast cells with these plant extracts, the glucose uptake was found to increase in a dose dependent manner. The increase in glucose uptake by the yeast cell at different glucose concentrations i.e. 10, 20, 40, 60, 80 µg/ml respectively. The ethanolic extract of Physalis minima L. exhibited significantly higher activity at all glucose concentrations showing the maximum increase in 80 µg/ml Glucose concentration. Results also indicated that Physalis minima L. had greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug metformin.

Sucharitha and Estari [12] investigated the hypoglycemic effects of extracts from P. minima in alloxan-induced diabetic rats. The powdered plant parts were successfully extracted with boiling water using soxhlet extractor. The Wister strains of male albino rats were used for the present study. The antihyperglycemic activity of the crude aqueous extracts of P. minima different parts were studied in alloxan-induced diabetic rats. The toxicity study results showed that the medium lethal dose (LD50 ) of the extracts is higher than 1 g/kg body weight and hence, in a single dose administration, the plant extracts had no adverse effect. There is no significant level of reduction in fasting blood glucose level was noticed for the aqueous extracts of root and stem of P. minima. On chronic administration, the effect of P. minima flower and leaf causes a fall in fasting blood sugar of rats. These findings clearly established that the antidiabetic efficacy of the flower and leaf extract of P. minima are almost equal and both exhibited more potent antidiabetic activity by reducing the blood glucose level significantly than all other root and stem extracts.

The in vitro assays of the present study concluded that ethanolic extract of Physalis minima L. leaves possess hypoglycemic activity and can be used in the management of diabetes. The active principles of Physalis minima L. leaves are responsible for inhibitory action of α-amylase, α-glucosidase and increased glucose uptake in Yeast cells.

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