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Invitro alpha amylase and alpha glucosidase inhibition activity of crude ethanol extract of *Cissus arnottiana*

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ABSTRACT

Diabetes mellitus is one of the most common endocrine diseases characterized by hyperglycemia due to absolute or relative deficiency of insulin. One anti diabetic therapeutic approach is to reduce gastrointestinal glucose production and absorption through the inhibition of alpha amylase enzyme plays a major role in preventing rise in postprandial glucose level in diabetics. The main aim of the current study was to screen the ethanol extract of Cissus arnottiana fruits for its in vitro alpha amylase and alpha glucosidase activity. The preliminary phytochemical screening of this plant crude ethanol extract showed presence of maximum compounds, hence the ethanol extract have under taken for its alpha amylase and alpha glucosidase inhibition activity. All the tested concentrations of extract of C. arnottiana showed significant inhibitory activity. At the concentration of 10mg/ml the plant showed appreciable alpha amylase and alpha glucosidase inhibitory activity (78.91%, 81.25%) with IC_{50} value 2.95mg, 2.81mg respectively.

Key words: Diabetes mellitus, hyperglycemia, amylase, inhibition, *Cissus arnottiana.*

INTRODUCTION

Diabetes mellitus is an endocrine disorder characterized by hyperglycemia is associated with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. A therapeutic approach for treating diabetes is to decrease postprandial hyperglycemia. This can be achieved through the inhibition of carbohydrate hydrolyzing enzymes such as alpha glucosidase and alpha amylase [2, 3]. Alpha amylase and glucosidase inhibitors are drug-design targets in the development of compounds for the treatment of diabetes, obesity and hyperlipaemia [4]. Plants have long been used for the treatment of diabetes, particularly in developing countries where most people have limited resources and do not have access to modern treatment. Because of the possible importance of these inhibitors in plant physiology and animal and human nutrition, extensive research has been conducted on their properties and biological effects [5]. Ethno botanical information indicates that more than 800 plants are used for the treatment of diabetes throughout the world [6] but still there is an insufficient scientific proof of their antidiabetic activity [7].

Cissus arnottiana is an erect woody tree which belongs to Vitaceae family. *Cissus* is a genus of about 350 species of tropical and subtropical, chiefly woody vines of the grape family (Vitaceae). They are often used as medicinal plants because they contain some bioactive compounds such as vitamins, proteins, carbohdrates and polyphenols among others. The bioactive compounds are contained in their leaves, stems and roots or bark, which makes these plants to be used medicinally in indigenous system of medicine [8]. *Cissus sicyoides* reported significantly reduced the levels of blood glucose, urinary glucose and urinary urea, as well as both the food and fluid intake and the volume of urine

excreted, in streptozotocin-diabetic rats [9]. The *Cissus quadrangularis* exhibit anti-diabetic property in alloxan induced diabetic rats by Stimulation of Surviving ß-cell to release more Insulin [10].

The scientific knowledge and biological activity of the plant *Cissus arnottiana* is least explored. Hence the present study was evaluated for its in vitro α-glucosidase and α-amylase enzyme inhibition activity.

MATERIALS AND METHODS

Fruit collection and extraction

The plant fruits were collected in surrounding area of Ranga Reddy District of Andhra Pradesh, India and identified by Botanical Survey of India, Coimbatore, Tamilnadu, India. The shade dried fruit powder was exhaustively extracted with ethanol by soxhlet apparatus and the extract was concentrated by vacuum drying.

In vitro alpha amylase and α-glucosidase inhibition study

The α-amylase and α-glucosidase inhibitory effect of plant extracts was determined according to the standard method [11]. For alpha glucosidase inhibition, yeast α-glucosidase was dissolved in 100 mM phosphate buffer, pH 7.0, containing bovine serum albumin 2 g/liter and sodium azide 0.2 g/liter which was used as enzyme source. Paranitrophenyl- α-d-glucopyranoside was used as substrate. *Cissus arnottiana* fruit extract was weighed and serial dilutions of 1, 2.5, 5, 10, 15 mg/ml were made up with equal volumes of dimethylsulfoxide and distilled water. 10µl of extract dilutions was incubated for 5 min with 50µl enzyme source. After incubation, 50µl of substrate was added and further incubated for 5 min at room temp. The pre substrate and post substrate addition absorbance was measured at 405 nm on a microplate reader. The increase in absorbance on substrate addition was obtained. Each test was performed three times and the mean absorption was used to calculate percentage α-glucosidase inhibition. Acarbose was used as positive control with various concentrations 1, 2.5, 10 mg/ml. Percentage α-glucosidase inhibition was calculated according to the following formula [12]:

$$\text{Percentage of inhibition} = \frac{[(\text{Control } 405 - \text{Extract } 405)] \text{ X } 100}{\text{Control } 405}$$

The alpha amylase inhibition activity of the plant was assayed by the same method used for alpha glucosidase inhibition activity. For alpha amylase inhibition assay the enzyme porcine pancreatic amylase and substrate Para nitro phenyl alpha D- maltopentoglycoside were used [12].

$$\text{Percentage of inhibition} = \frac{[(\text{Control } 540 - \text{Extract} 540)] \text{ x} 100}{\text{Control} 540}$$

RESULTS

The dried fruit of Cissus arnottiana was extracted with different polarity of solvents. The preliminary phytochemical screening of ethanol extract showed presence of maximum compounds like phenols, flavonoids, tannins, steroids, triterpenoids and glycosides. Hence the ethanolic extract of *Cissus arnottiana* assayed for in vitro alpha amylase and alpha glucosidase inhibitory activity. Four different concentration were tested, the extract showed good inhibitory effect at all the tested concentrations (1, 2.5, and 10 mg/ml**)** at a higher concentration of 10 mg/ml the maximum inhibitory effect of ethanol extract was showed significant alpha amylase and alpha glucosidase inhibitory activity (78.91%, 81.25%) with IC50 value of 2.95mg, 2.81mg respectively (Figure 1and 2). The experiment was repeated for three times and results expressed as mean value of inhibition activity percentage. Acarbose was used as standard of various concentrations.

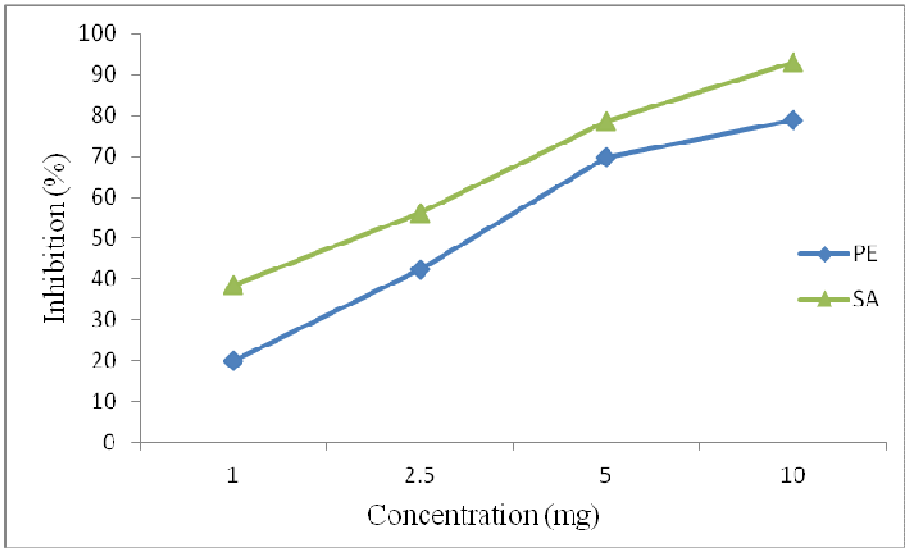

Figure 1: Alpha amylase inhibition activity of ethanol extract of *Cissus arnottiana*

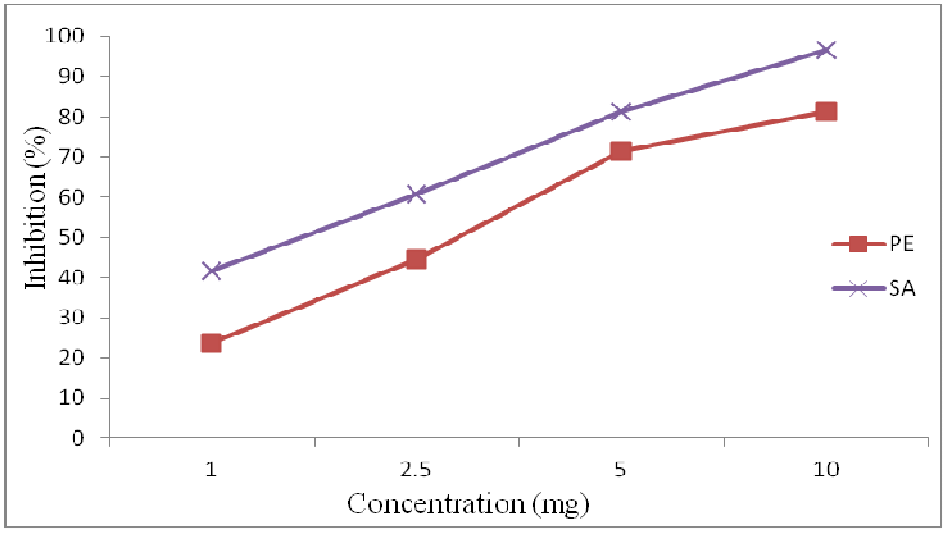

Figure 2: Alpha glucosidase inhibition activity of ethanol extract of *Cissus arnottiana*
PE: Plant extract, SA: Standard acarbose

DISCUSSION

Diabetes mellitus cases were 171 million in 2000 and expected to raise 366 million on 2030 [13]. Two types of DM are currently known [14] and antioxidants used to reduce the risk of chronic diseases [15]. α – glucosidase (EC 3.2.1.20) and α –amylase (EC 3.2.1.1) enzymes play a major role in type 2 diabetic patients and borderline patients [16, 17]. Commonly used synthetic anti oxidants like BHT and BHA [18] possessed side effects [19]. Many herbal extracts has been reported for their anti-diabetic activities and being used in ayurveda for the treatment of diabetes. Therefore, screening of these two enzymes in plant has received more attention. In this study, the preliminary phytochemical screening of ethanolic fruit extract showed presence of compounds that are reported as anti diabetic principles [20, 21, 22]. The medicinal plants or natural products involve retarding the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes. Several α- amylase inhibitors including acarbose, voglibose and miglitol are clinically used for treatment but their prices are high and clinical side effects occur [23]. Hence screening of α-glucosidase inhibitors from plants and synthetic sources is increasing and inhibitors of these enzymes

have been recently developed from natural sources [24]. In this study, *in vitro* effect of different concentrations of ethanolic extract of *Cissus arnottiana* fruit was evaluated. At the concentration the 20mg/ml of plant extract showed significant inhibitory activity.The present study indicated that *Cissus arnottiana* could be useful in management of postprandial hyperglycemia.

The results indicate that ethanol extract of the plant fruit of *Cissus arnottiana* showed appreciable inhibition activity. But the *in vitro* inhibitory activity does not always relate to the corresponding *in vivo* activity. Thus proof of concept needs to be demonstrated in preclinical animal studies [25]. For safety and efficacy to be established, it was essential to confirm the *in vivo* study.

CONCLUSION

In this present study we evaluated in vitro alpha amylase and alpha glucosidase activity of crude ethanol extract of *Cissus arnottiana* fruit. The plant showed significant inhibition activity, so further the compound isolation, purification and characterization which is responsible for inhibiting activity, has to be done for the usage of anti diabetic agent.

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