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In Vitro Anti-Diabetic Activity of Glycyrrhizaglabraethanolic Extract

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ABSTRACT

Objective: Plants offer a variety of medicinal benefits with less side effects and increased efficacy. *Glycyrrhizaglabra*is a medicinal plant used in traditional medicine like ayurveda and siddha. The roots of this plant provides the main source of medicine commonly used in treatment of ulcers, pulmonary and skin diseases. Diabetes is one of the most common metabolic disorder affecting multiple organs. Many studies are being out on medicinal plants to provide treatment for diabetes which is more easily available and has increased efficacy. Thus, the aim of this study is to find the antidiabetic property of Glycyrrhizaglabraethanolic extract.

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Materials and Method: Preparation of glycyrrhizaglabra extract was done by mixing 25 g of glycyrrhizaglabra powder with 250 mL of ethanol and and was extracted using soxhlet apparatus. The antidiabetic properties were tested using alpha amylase and glucosidase inhibitory assay. Different concentrations of glycyrrhiza and standard(acarbose) were used to test inhibition percentage of amylase and glucosidase.

Results: Glycyrrhiza is found to be more effective in inhibition of alpha amylase than standard (acarbose) in lower concentrations like 10 and 20microlitres.However, from 30-50 microlitres, the inhibition percentage of glycyrrhiza is 70-80% while that of standard is 75-85%. The inhibitory action of glycyrrhiza on alpha glucosidase is less compared to standard acarbose in all concentrations however the difference being only 5%.

Conclusion: It can be concluded that glycyrrhizaglabra has good antidiabetic properties.

Keywords: amylase, antidiabetic, glycyrrhizaglabra, glucosidase, inhibition

Introduction

Plants have been a great source of medicine in all cultures from ancient times. Various indigenous plants are being used in the diagnosis, prevention and elimination of diseases. Demand for herbal medicines, health products and pharmaceuticals across the world is increasing as they have less side effects and more efficacy.(1)

Glycyrrhizaglabra Linn commonly known as licorice belongs to the family of Fabaceae. It is a well-known medicinal plant used in traditional medicine like ayurveda and siddha across India for its ethnopharmacological value to cure varieties of ailments(2). The roots and rhizomes are the main medicinal parts of licorice. Licorice root is used mainly for the treatment of peptic ulcer, hepatitis C, and pulmonary and skin diseases, although clinical and experimental studies suggest that it has several other properties useful pharmacological such as anti-inflammatory, antiviral, antimicrobial, anti-oxidative, anticancer activities, immune modulator, hepatic protective and cardio protective effects(3). Licorice oil is also widely used as a natural sweetener and flavoring agent. Glycyrrhizin is the main bioactive component in licorice that imparts the sweet flavor in addition to which some volatile compounds such as flavonoids and saponins are also responsible for the sweetness. Therefore, due to its sweetness and flavoring characteristics, licorice extract is mostly used in the confectionery industry.(4)

Diabetes is a collective metabolic disorder affecting various organs in the body(5). In diabetic patients, glucose utilisation in the body is severely affected because of improper insulin secretion from β-cell of the pancreas(6). Major organs like the pancreas, kidney and liver are damaged due to diabetes(7). The increase in the glycogen catabolism results in low hepatic glycogen level and ultimately hepatic damage(8). Food habits and genetic factors are the main factors responsible for diabetes(9).A study revealed that urbanization of rural India has doubled the rate of diabetes.(10)

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Various modern medicines such as biguanides, sulfonylureas, and thiazolidinediones are available for the treatment of diabetes. However, these medicines exhibit many undesired side effects.(11) The preference for herbal drugs in treatment of diabetes is increasing(12,13,14,15,16). Medicinal plants or natural products involve retarding the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes, such as pancreatic amylase. The inhibition of this enzyme delays carbohydrate digestion resulting in the reduction in glucose absorption rate and consequently decreasing the postprandial plasma glucose rise(17). Researchers are focussed on indigenous medicinal plants that have a high potential in inhibiting α-amylase enzyme activity. Thus, this present study aims to find the anti-diabetic activity of Glycyrrhizaglabra.

Materials and methods

Preparation of *Glycyrrhizaglabra*extract

The*Glycyrrhizaglabra*plant powder was collectedand used for ethanolic extraction. 25 g of *Glycyrrhizaglabra* powder was mixed with 250 mL of ethanol and extracted using soxhlet apparatus.

Antidiabetic activity

α-Amylase Inhibitory Assay

To a test tube, 250 µL of pancreatic porcine α-amylase (1 U/mL, dissolved in the buffer (pH 6.9) and 100 µL of test extract at a concentration ranging from 15.6 to 250 mg/L were added. The mixture was pre-incubated at 37°C for 15 min, before the addition of 250 µL of 0.5% starch. The mixture was then vortexed and incubated again at 37°C for 15 min followed by the reaction termination using 1 mL of dinitrosalicylic acid color reagent. The tubes were placed in a boiling water bath for 5 min, cooled to room temperature and diluted. Two hundred microliters of the reaction mixture were taken into a 96-well clear plate, and the absorbance was read at 540 nm using Elisa plate reader. The control α-amylase at 1 U/mL without any inhibitor represented 100% enzyme activity. Appropriate test extract controls containing the reaction mixture except the enzyme were used to correct for the color interference. A known α-amylase inhibitor, acarbose was used for comparison studies. The percentage inhibition of the test sample on α-amylase was calculated

α-Glucosidase Inhibitory Assay

The *Glycyrrhizaglabra* powder, various concentrations of each extract were prepared in 10 mM potassium phosphate buffer (pH 6.8). To a 96-well clear plate, a reaction mixture containing 20 µL extract at different concentrations, 20 µL α-glucosidase (0.5 U/mL) and 60 µL of 10 mM potassium phosphate buffer (pH 6.8) were pre-incubated at 37°C for 15 min before adding 20 µL of 5 mM p-nitrophenol-α-D-glucopyranoside substrate. The mixture was then incubated at 37°C for the reaction to take place. After 15 min, 80 µL of stop solution containing 200 mM sodium carbonate was added. Then the absorbance at 405 nm was recorded using the microplate reader. The positive control sample was the mixture of the enzyme and substrate without inhibitors. The sample controls and blanks were the mixtures of sample and control, respectively, except α-glucosidase was instead with buffer,

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respectively. The inhibition (%) of the test sample on α-glucosidase was calculated same way as with α-amylase assay. Acarbose, a prescribed drug for α-glucosidase inhibition, was also used for comparison purpose.

Results and Discussion

α-Amylase Inhibition

From the results of this study, it can be observed that glycyrrhiza inhibits the function of the enzyme alpha-amylase(Figure1). Glycyrrhiza is found to be more effective in inhibition of alpha amylase than standard (acarbose) in lower concentrations like 10 and 20microlitres. The inhibition percentage of glycyrrhiza is 65% while that of acarbose is 45% in 10 microlitres concentration. In 20microlitres concentration, the percentage of inhibition of glycyrrhiza and standard is 65% and 60% respectively. However, from 30-50 microlitres, the inhibition percentage of glycyrrhiza is 70-80% while that of standard is 75-85%.

Alpha amylase is an enzyme that catalyses the hydrolysis of starch into sugars(19,20). The pancreas and salivary gland secrete amylase to hydrolyse dietary starch into disaccharides and trisaccharides which are converted by other enzymes to glucose to supply the body with energy.Therefore, by preventing the action of this enzyme, we are indirectly inducing a therapeutic effect on diabetes by controlling the level of glucose in the blood(21).

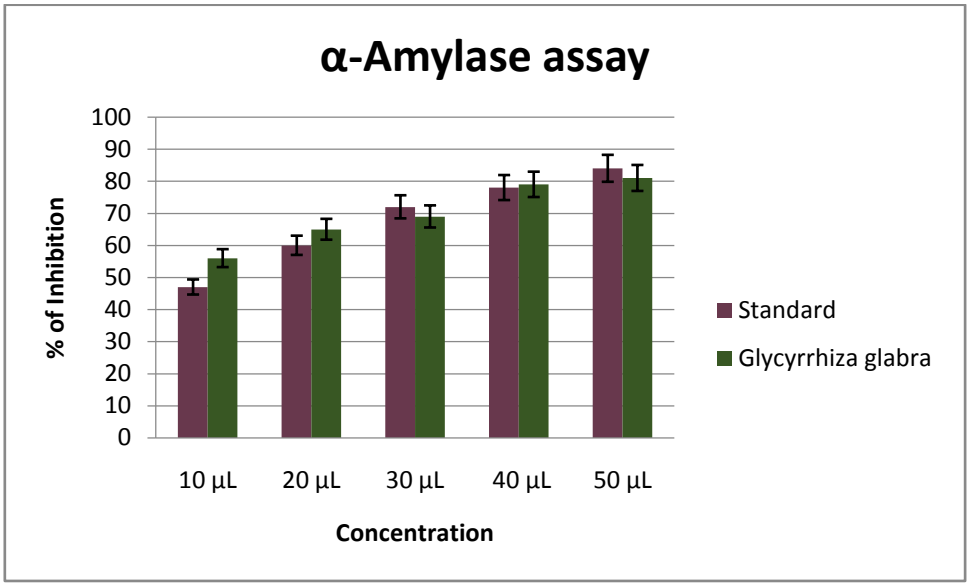

Figure 1: Effect of Glycyrrhizaglabra and acarbose (standard) on α-amylase inhibition

α-Glucosidase Inhibition

It can be observed from Figure2, that the inhibitory action of glycyrrhiza on alpha glucosidase is less compared to standard acarbose in various concentrations however the difference is only 5%. Increase in concentration of glycyrrhiza and standard shows increased inhibition percentage of 70% and 75% respectively on alpha glucosidase.

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Glucosidase is an enzyme that catalyzes hydrolysis of starch to simple sugars. It is located in the intestine of humans and aids in digestion of dietary carbohydrates and starches to produce glucose for intestinal absorption, which in turn, leads to increase in blood glucose levels(22,23). Thus, drugs which have an inhibitory effect on glucoside are used in treatment of diabetes.

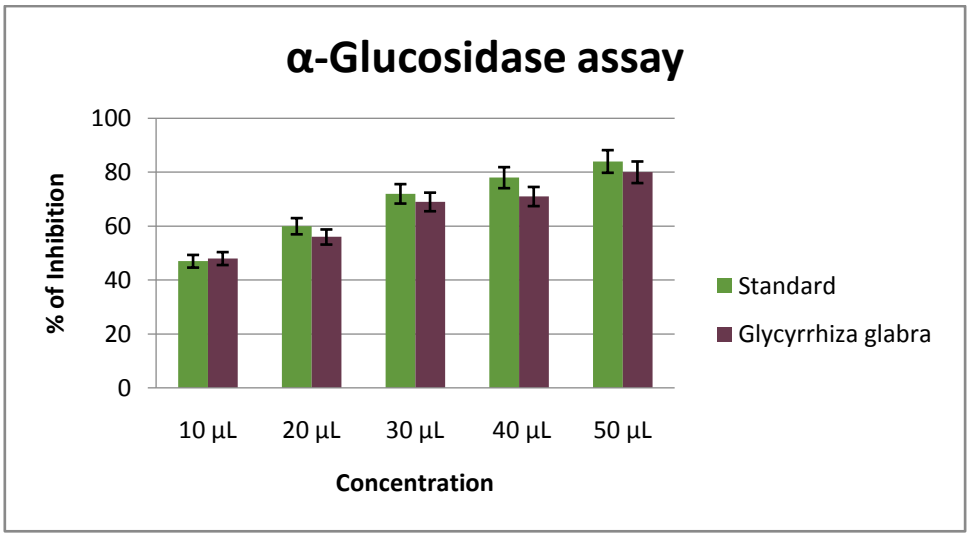

Figure 2: Effect of Glycyrrhizaglabra and acarbose (standard) on α-glucosidase inhibition

Conclusion

From this study it can be concluded that glycyrrhizaglabra's increased inhibitory effect on alpha amylase and glucosidase can be used in synthesizing antidiabetic medicines. Inhibition of α-glucosidase and α-amylase enzyme activity leads to a reduction inglycemic index in diabetic patients and can reduce the incidence of post prandial hyperglycemia.

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