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In-Vitro Evaluation of Anti-hyperglycemic Property of Methanolic Extract of *Gliricidia Sepium* Leaves

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ABSTRACT

Diabetes mellitus has emerged rapidly due to the societal factors which influence the large population by their lifestyle. Initially, it was known as 'a disease of a rich man' but in recent years it affects all class of people, As we know the medical therapy in started through ancient and traditional medicine from plant origin and there is extensive ancient history if embedded in the area of plant medicine. *Gliricidia sepium* belongs to the family Fabeacae. Firstly this particular plant was found in Central America after that many of the tropical countries employed these plants as aremedy for different disorders as a folore medicine. α-Amylase Inhibition Assay: It is the common method to estimate the amount of reducing sugars in different types of test samples. In this essay, we can measure the amount of reducing sugar generated after-treatment of the test solution with the α-amylase enzyme. α-glucosidase Inhibition Assay: This method involves the reaction of the sample with glucosidase enzyme and p-nitrophenyl glucopyranoside in this assay as concentration is high sample solution or standard solution the lower the absorbance. The findings of the current project indicated that selected plant exerted moderate inhibition in both methods. Maybe they isolate fractions may give clear and productive results.

Key Words: Gliricida Sepium, Glucosidase, Amylase, Hyperglycemia

INTRODUCTION

Diabetes is a heterogeneous metabolic disorder and it is the primary cause of death in recent years worldwide. The complications of diabetes mellitus are quite unbearable, which makes life miserable. This particular disease affects the population of developing countries and also had a marginal effect on the global population. Diabetes mellitus has emerged rapidly due to the societal factors which influence the large population by their lifestyle. Initially, it was known as 'a disease of a rich man' but in recent years it affects all class of people.[1] Diabetes caused due to the insulin resistance as a major cause due to obesity in Indian population in this era. Hyperlipidemic conditions may arise due to high fat intake, lack of physical activities, sedentary lifestyle.[2] There are various classes of drugs are available for therapeutic strategies to manage diabetes selectively type 2 diabetes. The current drugs represent the class of inhibitors of α-glucosidase and α-amylase. These enzymes are known to exhibit their mechanism in the process of breakdown of an oligosaccharide to monosaccharides. This particular endpoint inhibition leads to downstream inhibition of elevated blood glucose levels since the monosaccharide's the other form representing carbohydrates and they can be absorbed by the mucosal barrier by which they show the anti-hyperglycaemic effect. In another case, there is another enzyme inhibition also responsible for controlling diabetes by inhibiting α-amylase by a breakdown of a compound starch molecule to smaller molecules like maltose, triose, dextrin etc. Through this effect, alpha-amylase inhibitors are also employed to control type-2 diabetes. Currently, we have the class of drugs for the management of type 2 diabetes are acarbose and miglitol and they can act by inhibiting glycosidases such as above said enzymes and as far other drugs like voglibose inhibit α-glucosidase. However, most of these drugs from synthetic origin have some limitations; they are nonspecific, which may lead to serious side effects and cause diabetic complication in the body.[3] As we know the medical therapy in started through ancient and traditional medicine

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from plant origin and there is extensive ancient history if embedded in the area of plant medicine. According to the report of the World Health Organisation (WHO), the major population rely only on plant medicines rather than the medicines from the synthetic origin. Currently, the microorganisms are resistant against recently developed antibiotics, and it is because of the extensive and uncontrolled use of antimicrobial drugs. These antibiotics are associated with various side effects since the synthesis involves many harmful chemical substances plant medicines and the antibiotics produced from the plant origin has got a lot of advantages, limited side effects, long history and natural antioxidant principles, patient tolerance and acceptance towards the plant medicine are more. Based on the data and their history in recent years the herbal medicines are gaining more importance in the management of diabetes mellitus as the adverse effects are less and less expensive compared to the drugs from the synthetic origin.[4] The major role of medicinal plants created the overall importance, and it lies not only in their conventional chemotherapeutic potency in traditional medicine but also in their pivotal role as sources of new scaffolds for drug discovery. Gliricidia sepium belongs to the family Fabaceae. Firstly this particular plant was found in Central America after that many of the tropical countries employed these plants as a remedy for different disorders as a folore medicine. It was the Philippines in the 1600s introduced this plant and also Srilanka also introduced this plant in 1800s for the purpose to protect the tea plants a to them to provide shade. Plant Research Center of Coastal Research Station, Karachi started cultivating this plant for a different purpose than to provide manure for coconut trees and to provide shade for beetle leaves and tea plantations. After the Extensive research in this plant revealed that it could be cultivated in the plains of Sindh and Punjab and also in some areas of to the coastal region since the irrigation facility was abundant.

MATERIAL AND METHODS

Plant Material: The *Gliricidia Sepium* was available in the summer season late May-June period in the selected areas of Harapanahalli town. Professor K. Prabhu, Department of Pharmacognosy, S.C.S College of Pharmacy, authenticated and certified the plant. Specimen no: SCSCOP.Ph.Col.Herb. no.002/2011-12

Extraction: The plant was shade dried at room temperature for a long time. The dried plant material grounded into coarse powder and tightly accumulated in soxhlet apparatus column successive extraction has been done with desired solvents. The extracts were concentrated under reduced pressure and stored in an airtight container at below 10°C.

Chemicals and reagents: Acarbose the standard drug, Porcine pancreatic amylase, α-glucosidase are procured from Sigma-Aldrich. Starch is procured from Loba Chemie, DNSA from SRL Chemicals other chemicals are from high media.

Instrumentation: Merck UV-visible spectrophotometer (model prove 600), Saurtorius Weighing balance, Rotaflash evaporator .

Experimental:

α-amylase enzyme inhibition assay: Porcine pancreatic α-amylase (0.5 mg/ml) concentration is prepared by dissolving it in 20 mM soum phosphate buffer solution of 6.9 pH then it was mixed with the sample at various concentrations (20-100 µg/ml) to which 1% of starch solution and 100 µl buffer were added. The reaction was allowed to be carried out at the temperature of 37°C for 5 min and after that, it can be terminated by adding 2 ml of 3,5-dinitro salicylic acid a colouring reagent. The reaction mixture was heated to the temperature of 100°C up to 20min and then dilute the mixture with 10ml purified water in an ice bath. inhibitory activity was determined by measuring the intensity of the colour at 540 nm in Merck prove 600 spectrophotometers. [6,7]

α-glucosidases Inhibition assay:

The α-glucosidase inhibitory activity was done by to the standard method were the extract (50µL) and α-glucosidase solution(100µL) were incubated at 25°C for 10 min followed by the addition of 50µL of 5mM/L solution of ρ-nitrophenyl-α-D-glucopyranoside in 0.1m/L phosphate buffer (pH 6.9). The reaction mixture turns yellow was then incubated at 25-30°C for 5 min and the absorbance was measured at 405nm using Merch prove 600 UV –VIS spectrophotometer.[8-9]

$$\text{Percentage Inhibition} = \frac{\text{Abs c} - \text{Abs t x 100}}{\text{Abs c}}$$

Ac= Absorbance of the control and At = Absorbance of the test

The inhibitory concentration (IC_{50}) value is nothing but the concentration which is required to inhibit 50% of its inhibitory property under the assayed conditions. The IC50 values were determined by plotting the % inhibition

vs log inhibitor concentration and calculated the mean inhibitory values.

Statistical Analysis: This study is analyzed by using Dunnets test in graph pad prism version 5.0

RESULTS

In the present investigation, the Ethanolic extract of *Gliricidia Sepium* was evaluated for in-vitro anti-hyperglycemic activity by α-amylase and α-glucosidase inhibitory assay. In the α-amylase method, the anti-hyperglycemic activity for acarbose, at 100µg/ml was found to be 65.75 and the extract

showed 48.46 at. The results clearly state that EEGS significant ability to show inhibition of α-amylase with **IC_{50} 101.42**. The results are depicted in table no.01 and fig.01

In α-glucosidase inhibitory assay the Ethanolic extract of *Gliricidia sepium* extract showed dose-dependent activity, the anti-hyperglycemic activity for acarbose, at 100µg/ml, was found to be 71.27 and the extract showed 55.47. The results clearly state that EEGS significant ability to show inhibition of α-glucosidase still better than the α-amylase with **IC_{50} 86.81**. The results are depicted in table no.02 and fig.02

Table 1: α-amylase enzyme inhibition assay

Concentration ((µg/mL)	Acarbose	IC_{50}	EEGS	IC_{50}
20	33.96±1.07		25.01±0.93	
40	39.21±2.98		28.71±1.85	
60	47.85±1.42	65.85	32.72±2.84	101.42
80	57.72±1.42		38.59±1.42	
100	65.75±0.93		48.46±1.93	

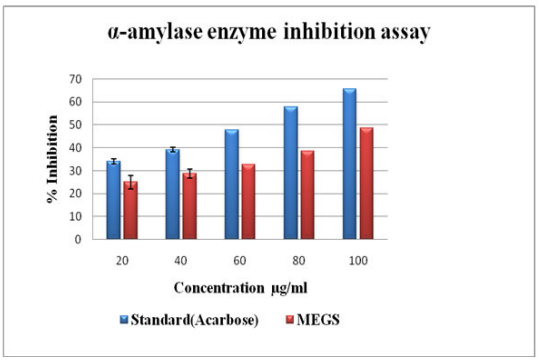

Figure 1: α-amylase enzyme inhibition.

Table 2: α-glucosidase enzyme inhibition assay

Concentration (µg/mL)	Acarbose	IC50	EEGS	IC50
20	36.21±1.49		20.98±3.03	
40	40.53±2.59		30.47±3.27	
60	44.83±3.12	62.82	34.2±4.75	86.81
80	59.78±4.43		46.27±2.17	
100	71.27±2.64		55.47±3.49	

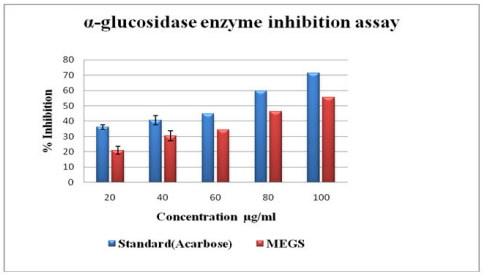

Figure 2: α-glucosidase enzyme inhibition.

DISCUSSION

Diabetes is termed as starvation of plenty and this disease results in decreasing the efficiency to produce insulin. Such condition may lead to hyperglycemia and are managed balanced diet with well fibre content, regular physical activity and medications and insulin supply.[10] The recent literature reveals there are about 45000 plant species are currently used as phytomedicines among them, Among them, many of the plants are proved to possess antihyperglycemic effects.[11]

The bioactive compounds from plants are the main factors which are reported in many kinds of literature that most of the polyphenolics possess an inhibitory effect on α-amylase and α-glucosidase and also on adipocytes[12]. Compounds from plants have also shown the beneficiary effect on glucose uptake in the liver and this is vital as the organ is an important regulator of plasma glucose level and plays a key role in glucose metabolism and regulation.[12]

α-Amylase Inhibition Assay: It is the common method to estimate the amount of reducing sugars in different types of test samples. In this assay, we can measure the amount of reducing sugar generated after-treatment of the test solution with the α-amylase enzyme. It shows that acarbose has exhibited 65.75±0.93at highest concentration. which is the dose-dependent inhibition against α-amylase. However, the effect of the selected plant was acceptable which is exhibited as 48.46±1.93. The inhibition effects exerted by the plant observed with the evidence of enhancement of intensity in colour, which resulted in little higher absorbance values compared to the standard drug.

α-glucosidase Inhibition Assay: This method involves the reaction of the sample with glucosidase enzyme and p-nitrophenyl glucopyranoside in this assay as concentration is high sample solution or standard solution the lower the absorbance reading will be which indicates the low level activity of α - glucosidase towards its substrate and thus it gives higher inhibitory activity. The activity exhibited that acarbose has exhibited 55.47±3.49 at the highest concentration which is the dose-dependent inhibition against α-glucosidase

CONCLUSION

The findings of the current project indicated that selected plant exerted moderate inhibition in both methods. But anyhow the validation of the methods is essential within Vivo screening by using animal models to strengthen the data obtained, so definitely this study us the route map with effective and attractive preliminary results to take it further may be the isolate fractions may give the clear and productive results Hence, a further study with more focus on the isolation of active constituents and in-vitro and in-vivo pharmacological screening.

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