ANTIHYPERGLYCEMIC ACTIVITY OF EDIBLE CACTUS (Carraluma fimbriata)

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ABSTRACT:

As the prevalence of diabetes increases, there is a need to develop new drugs for effective treatment. Plants and herbal bioactive compounds have emerged as an alternative treatment option. The Caralluma fimbriata used in this study is known for its diverse biological effects. Diabetes is a long-lasting condition that will affect your body by not turning the food into energy. The will breakdown most of the food you eat and will release the energy into the blood stream. Obesity is a risk factor that will lead to diabetes. Diabetes is a chronic metabolic disorder which can be well controlled by maintaining a balanced diabetic diet. So, the main goal of therapy in patients with diabetes mellitus is improving metabolic control. The specific goals of therapy are maintaining normal or near-normal blood glucose concentrations by synchronizing hypoglycaemic drugs with food intake and physical activity, optimizing serum lipid and lipoprotein concentrations, helping patients attain desirable body weights, preventing and treating complications of diabetes, and improving overall health. **Objective:** This study was designed to investigate the antihyperglycemic activity of an ethanolic extract of the leaves of C. fimbriata. **Conclusion:** This study concludes that the edible cactus (C. fimbriata) has the hyperglycaemic activity.

KEYWORDS: Alpha amylase, alpha glucosidase, Caralluma fimbriata, diabetes, glucose absorption, 3-2,5-diphenyl tetrazolium bromide assay.

INTRODUCTION:

Diabetes is a chronic metabolic disease that is increasing worldwide. It is characterized by hyperglycaemia with impaired carbohydrate, protein and fat metabolism. This may be due to inactivity or insulin resistance as a direct result of the destruction or dysfunction of pancreatic beta cells. Diabetes is spreading like an epidemic and India is becoming the diabetes capital of the world [1]. It is estimated that there are daily Indians with diabetes worldwide today. Statistics show that up to 79.4 million people will suffer from this disease by 2030 in India alone. Control of postprandial hyperglycaemia is key to the treatment of type 2 diabetes and its complications [2-3]. General approaches include the administration of exogenous insulin and oral hypoglycaemic agents such as alpha- glucosidase inhibitors, sulfonylureas, and biguanides. Caralluma fimbriata (family Apocynaceae) is a succulent wild cactus native to the arid regions of Tamil Nadu. It is known for its hyper lipidemic, hepatoprotective, anti-

obesity and anti-cancer antioxidant activity with no history of side effects. In this study, ethanolic extracts of C. fimbriata leaves were tested for antihyperglycemic activity [4-5].

MATERIALS AND METHODS:

Reagents and extract:

Ethanol extract of C. fimbriata leaves obtained free of charge from Green Chem Herbal Extracts and Formulations. Alpha-amylase and alpha-glucosidase enzymes were purchased from Hi media, Mumbai. L6 myoblast cultures were obtained from the National Center for Cellular Sciences (NCCS). All other reagents and chemicals used, such as ethanol and dimethyl sulfoxide (DMSO), are of analytical grade.

Preparation of cell culture:

The monolayer myoblast (obtained from NCCS) used to be cultured in DMEM with 10% fetal bovine serum (FBS) and supplemented with penicillin (120 units/mL), streptomycin(75µg/mL), gentamicin (160 µg/mL), and amphotericin B (3 µg/mL) in a 5% CO2 environment. For differentiation, the L6 cells had been transferred to DMEM with 2% FBS for 4 days, post confluence. The extent of differentiation was once as quickly as set up through watching the multinucleate of cells.

Cytotoxic study (3-2,5-diphenyl tetrazolium bromide assay):

The cytotoxicity of the test extracts was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Plant extracts were prepared in DMSO for cytotoxicity analysis. Cells were seeded in 48-well plates at a concentration of 5×10 ^4cells/well. After incubation for 24 hours, the cells were washed with 200 µl of 1X phosphate-buffered saline (PBS; pH 7.4) and incubated in serum-free medium at 37°C and a CO 2 incubator for 1 hour. After starvation, the cells were treated with various concentrations of test extracts (1-1000 µl/ml) for 24 hours. At the end of the treatment, the medium of the control cells and cells treated with the extract was removed and 50 µl of MTT containing PBS (5 mg/ml) was added to each well. The cells were then incubated in a CO2 incubator at 37°C for 4 hours. The purple formazan crystals formed were then dissolved by adding 150 litres of DMSO and mixed efficiently by pipetting up and down.

Spectrophotometric measurement of absorption of formazan magenta dye was done by using multimode reader.

6-2-deoxyglucose assay-glucose uptake test:

The diabetic activity of the test extract was determined by fluorescently labelled 6-(N-(7 nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2 deoxy glucose (6-NBDG). L6 myotubes (10,000

cells/well) were seeded in 96-well plates and allowed to reach approximately 80% confluence. Cells were then differentiated using 2% FBS and various concentrations of extract (1-100 µg/ml) dissolved in DMSO were added. At the end of treatment, 10 µM insulin was added to stimulate glucose uptake and incubated for 15 minutes. Approximately 20 µg/200 ml of 6-NBDG was added and incubated for 10 minutes in the dark. Glucose uptake (%) was measured using a multimode reader (Perkin Elmer) with excitation/emission filters 466/540 nm.

Alpha amylase inhibitory assay:

In vitro amylase inhibition was examined using the Bernfeld method. Plant extracts were dissolved in ethanol. Briefly, 100 µl of test extract was reacted with 200 µl of alpha amylase enzyme (Hi Media RM 638) and 100 µl of 2 mM phosphate buffer (pH 6.9). After 20 minutes of incubation, 100 µl of 1% starch solution was added. The same was done for a control in which 200 µl of enzyme was replaced with buffer. After a 5-minute incubation, 500 µl of 3,5-dinitrosalicylic acid (DNA) reagent was added to both controls and tests. They were kept in a boiling water bath for 5 minutes. Absorbance was recorded on a spectrophotometer at 540 nm and the percent inhibition of the a-amylase enzyme was calculated.

Alpha glucosidase inhibitory activity:

Alpha-glucosidase inhibitory activity of the enzyme is described by Sancheti et al. With minor changes. Plant extracts were dissolved in ethanol. The reaction mixture consisted of 50 L 0.1 M phosphate buffer, pH 7.0, 25 L 0.5 mM 4-nitrophenyl-α-D-glucopyranoside (dissolved in 0.1 M phosphate buffer, pH 7.0), 10 l of the test extract and 25 l of alpha consists of Glucosidase solution (stock solution 1 mg/ml in 0.01 M phosphate buffer, pH 7.0, diluted to 0.1 U/ml in the same buffer, pH 7.0 immediately before analysis). Then the reaction mixture was incubated at 37°C for 30 minutes. The reaction was stopped by adding 100 L of 0.2 M sodium carbonate solution and the enzymatic hydrolysis of the substrate was monitored by the amount of p-nitrophenol released into the reaction mixture at 410 nm using a microplate reader.

RESULTS:

Cytotoxicity assays differ for extracts Concentrations from 1 to 1000 µg/ml at 24-hour intervals. As a result, both the extract and standard material were found to contain metformin. showed a dose-dependent decrease in cell proliferation rate. This was less than 50% even at the highest dose of 1000 µg/ml. IC50 from Extracts and metformin were above 1000 µg/ml and above 1000 µM.

The non-metabolizable fluorescent glucose analogue 6-NBDG is increasingly being used to study cellular glucose transport. Intracellular accumulation of exogenously administered 6-

NBDG is thought to reflect concomitant gradient-dependent glucose uptake by glucose transporters (GLUTS). The glucose uptake capacity of the extract was evaluated at various concentrations from 1 to 100 µg/ml. Plant extracts were dissolved in DMSO. Insulin (10 µM)and standard metformin (10 µg/mL) were used as positive controls. Results showed that theextract dose-dependently enhanced glucose uptake in L6 myotubes compared to standard metformin. The maximum percentage of uptake of the extract was found to be 66.32% ± 0.29%, whereas 10 µg/ml metformin had 74.44% ± 1.72% and insulin had 85.55% ± 1.14% glucose uptake [6-7].

The results showed a potent alpha-amylase inhibitory activity of the test extract comparedto standard acarbose. Typical acarbose inhibition was approximately 93.32% ±1.08%, whilethe test extract achieved a maximum inhibition of 77.37% ± 3.23% at a concentration of 1000 µg/ml. The IC50 was found to be 41.75 µg/mL for the extract and 34.83 for acarbose [8].

The in vitro alpha-glucosidase inhibitory activity of the test extracts was determined. The maximal inhibition of the extract was 83.05% ±1.69% at 1000 µg/ml, comparable to standard acarbose which showed a maximal inhibition of 95.34% ± 0.42% at 1000 µM. The IC50 for the extract and acarbose were 66.71 µg/ml and 45.69 µg/ml.

CONCLUSION

This study has revealed the ethanolic leaf extract of C.fimbriata has the potent inhibitory activity on enzymes of glucose metabolism, alpha glucosidase, alpha amylase, providing its antihyperglycemic activity.

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