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Original Article

Glucose uptake potential in L6 Myotubes by Ficus Racemosa

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ABSTRACT

Introduction: Ficus racemose, mostly as fruits and bark decoction to treat uncontrolled diabetes has been extensively applied in ayurvedic medicine in India. The objective of this study was to assess the uptake of glucose in L6 myotubes by Ficus racemosa. Background: Diabetes is a common metabolic disease characterized by abnormally high plasma glucose levels, leading to major complications, such as diabetic neuropathy, retinopathy and cardiovascular diseases. Presently available oral hypoglycaemic agents have exhibited several side effects. Therefore, more effective oral antihyperglycemic agents, particularly those that normalize both insulin and glucose levels are needed to be found. Method: Cell culture: L6, a mono layer myoblast culture (obtained from NCCS, Pune-Passageno-19) was cultured in the DMEM. In vitro glucose uptake activity: Glucose uptake assay was followed by the methodology of (Gupta et al, 2009). Result: It was observed from the results that Ficus racemosa extract at different concentrations exhibited substantial degree of glucose uptake in skeletal muscle cells, which was compared with that of Standard Metformin. A maximum glucose uptake of 53% was observed for ficus 30mg/ml, whereas metformin exhibited 61% of glucose uptake. The IC50 of ficus extract and metformin was found to be 2.57mg/ml and 1.79mg/ml, respectively. Conclusion: From the study that was conducted, it could be concluded that Ficus racemosa had a better glucose uptake compared to that of Standard Metformin used by diabetic patients.

Keywords: Ficus racemose, glucose, l6 myotube, uptake, myoblast.

Introduction

Diabetes mellitus is a type of metabolic disorders - defects in insulin secretion, or insulin action [1]. The chronic diabetes mellitus (DM) and uncontrolled diabetes lead to many diabetic complications such as diabetic cardiomyopathy, nephropathy, neuropathy, etc [1]. The number of people with diagnosed diabetes is expected to increase by 55% between 2015 and 2040 reaching to 642 million people, with the majority of cases in low- and middle-income countries [2]. In India, diabetes has been quickly increasing, leading to the point of being potentially epidemic with more than 62 million people presently diagnosed with the disease [3]. In 2000, India (31.7 million) had the highest number of people with diabetes mellitus in the world, China (20.8 million) had the second place, and the United States (17.7 million) was the third country regarding the number of people with the disease [3]. Rough estimates have demonstrated that the number of people diagnosed with diabetes in rural areas is one-quarter of that in urban regions in India and other Indian sub-continent countries such as Bangladesh, Nepal, Bhutan, and Sri Lanka [4, 5]. Insulin resistance has been defined as a reduced responsiveness of insulin on a target cell or a whole organ [6], it is not only the major pathophysiological condition of type 2 diabetes (non-insulin-dependent diabetes mellitus) [6], but also exists in type 1 diabetes (insulin-dependent diabetes mellitus) [7]. Impaired glucose uptake in skeletal muscle is present in insulin resistance diabetes [8]. The transmembrane transport of glucose mediated by glucose transporter 4(GLUT4), limits the rate of muscle glucose uptake. GLUT4 which is a protein stored in intracellular vesicles, mainly contributes in regulating insulin-stimulated glucose transport into skeletal muscles and adipose tissues [9, 10]. The insulin-signalling pathway is one of the key pathways responsible for blood glucose regulation. When binding to its receptor, insulin triggers the autophosphorylation of the insulin receptor and insulin receptor substrates (IRS). The insulin resistance and chronic hyperglycaemia are caused by the conditional depletion of GLUT4 [11]. This triggers a lot of signalling cascades, inducing biological responses like glucose uptake into the cell, and glycogen synthesis [2]. The plant

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kingdom demonstrates a large reservoir of biologically active compounds which has not been explored yet. Growing epidemiological studies have suggested that the consumption of fruits, vegetables and few medicinal herbs decrease the incidence of diabetes.

Materials and Methods

Cell culture

L6, a mono layer myoblsst culture (obtained from NCCS, Pune-Passageno-19) was cultured in the DMEM with 10% foetal bovine serum (FBS) and supplemented with penicillin (120 units/ml), streptomycin (75 microgram/ml), gentamicin (160 microgram/ml), and amphotericin B (3 microgram/ml) in 5% CO2 environment. From differentiation, the L6 cells were transferred to DMEM with 2% FBS for 4 days, post confluence.

In vitro glucose uptake activity

This invitro study was conducted by using ethanolic extract of Ficus racemose which was obtained from Green Chem Herbal Extracts and Formulations, Bengaluru.

Glucose uptake assay was performed according to the article published by Gupta et al,2009 [12].

L6 myoblasts grown in 48 -well plate was subjected to glucose uptake as reported. When semi confluent monolayer was formed, the culture was renewed with the respective differentiation media. After attaining complete differentiation, the differentiated cells were incubated with KRP + 0.2% BSA for 18 h at 37^{0}C in the CO2 incubator. After 18h, the media was discarded and the cells were washed with KRP buffer once. The cells were treated with metformin and ficus extract of different concentrations (0.01,0.03,0.1,0.3,1,3,10 and 30 mg/ml), then 2-deoxy glucose (1mM) was added, and after that, they were incubated for half an hour. The supernatant was collected for glucose estimation, and glucose uptake was terminated by washing the cells thrice with 1ml ice-cold KRP buffer. By freezing and thawing three times, the cells were eventually lysed. Cell 1ysate was collected for glucose estimation. Glucose uptake was assessed considering the difference between the initial and final glucose content in the incubated medium by GODPOD method, in which 10µl of the sample and 1ml of the reagent were mixed and incubated for 25 min at 15-25^{0}C or `10 min at 37^{0}C. The absorbance of the standard (A standard) and the sample (A sample) against the reagent blank within 60 min was measured, the time interval from sample addition to the media was discarded, and the cells were washed with KRP buffer once. The cells were treated with Metformin and ficus extract of different concentrations (0.01,0.03,0.1,0.3,1,3,10 and 30 mg/ml) and then 2 – deoxy glucose (1mM) was added and incubated for half an hour. The supernatant was collected for estimating glucose, and the difference between the initial and final glucose content in the incubated medium by GODPOD method was considered to measure glucose uptake.10µl of the sample and 1ml of the reagent were mixed, then incubated for 25 min at 15-25^{0}C or `10 min at 37^{0}C in this method. The absorbance of the standard (A standard) and the sample (A sample) against the reagent blank within 60 min was measured, the time interval from sample addition to reading time must be exactly the same for the standard control and the sample. All the treatments were performed in triplicate with two replicates.

Results

Postprandial blood glucose level has been known to be regulated by glucose uptake, which is a rate limiting step for glucose metabolism. In the present study, differentiated L6 myotubes were used because it was previously established that glucose uptake was higher in differentiated cells than in undifferentiated ones, which is probably due to the presence of glucose transporter-4 (GLUT4) in their expression. The positive control chosen for glucose uptake due to their anti - diabetic activity was Metformin, as it is known specifically for the affirmative effect on the translocation of GLUT4 to the cell surface, thereby promoting glucose uptake. The concentrations of the tested extracts that enhanced glucose uptake in cells by 50% (IC50) were determined by a linear regression analysis between the percentage of glucose uptake against the extract concentrations by using the Graph pad prism.

It was observed from the results that ficus racemosa_extract at different concentrations exhibited substantial degree of glucose uptake in skeletal muscle cells, which was compared with that of Standard Metformin. A maximum glucose uptake of 53% was observed for ficus 30mg/ml, whereas metformin exhibited 61% of glucose uptake as depicted in fig:1. The IC50 of ficus extract and metformin was found to be 2.57mg/ml and 1.79mg/ml, respectively.

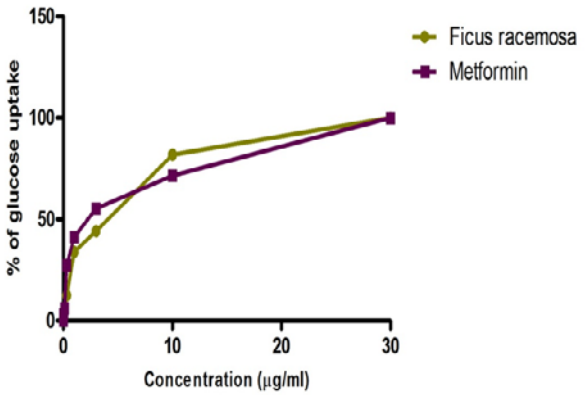

Figure 1: IC50 of ficus extract and metformin

Discussion

Although, diabetes mellitus is not curable, it is controllable. Frequent measurement of glycaemic control in people with diabetes, and suitable adjustment of therapeutic regimens are needed to be done because of progressive nature of the disease [13, 14]. α-amylase catalyses the hydrolysis of α-1,4-glucosidic

linkages of starch, glycogen, and various oligosaccharides, and simplifies the availability of sugars for the intestinal absorption. Inhibition of this enzyme activity in the digestive tract of humans has been considered to be effective to control diabetes by diminishing the absorption of glucose decomposed from starch by this enzyme [15, 16]. The insufficient insulin secretion or response makes it impossible for diabetic patients to control postprandial blood glucose properly, that leads to postprandial hyperglycaemia, therefore it is a major contributing factor for diabetic complications [17].

L6 myotubes is a well-established skeletal muscle model for studying glucose uptake process, and it is one of the key insulin targeted tissues in maintaining the whole body glucose homeostasis, through the stimulation of glucose uptake mediated by GLUT4 translocation [18]. Skeletal muscle is one of the key insulin targeted tissue in maintaining the whole body glucose homeostasis, through the stimulation of glucose uptake mediated by GLUT4 translocation [19]. Plant derived natural compounds have established a platform for developing new drug synthesis with fewer side effects [20]. Plants have been used by men from prehistoric times to get rid of suffering ailments. The folk medicines of almost around the world have relied chiefly on herbal medicine even today [21]. The studies conducted by [22] and [23] suggested that through an intracellular cascade reaction, insulin stimulates translocation of glucose transporters (GLUTs) into the cell membrane of the cells responsive to this hormone with subsequent internalization of glucose molecules. The results of their studies can be compared with the present study as well. In fact, the regulation of GLUT4 trafficking and eventually glucose uptake is the most significant effect of insulin on glucose metabolism [24].

In addition, in a study conducted by [25], it was declared that glucose transport is the key step in insulin-regulated glucose metabolism, including glycolysis, glycogen synthesis and lipogenesis, and it is obvious that the insulin action is clearly affected by a dysfunction in the process in muscles and adipose tissues [25]. Natural products play a major role in the development of drugs for the treatment of human disease [26]. The results of the study that was conducted above demonstrated that Ficus racemosa extract at different concentrations exhibited substantial degrees of glucose uptake in skeletal muscle cells, which were compared with that of Standard Metformin. A maximum glucose uptake of 53% was observed for Ficus 30mg/ml, whereas metformin exhibited 61% of glucose uptake as depicted in fig:1. The IC50 of Ficus extract and metformin was found to be 2.57mg/ml and 1.79mg/ml respectively

Conclusion

From the study that was conducted above it could be concluded that Ficus racemosa had a better glucose uptake compared to that of Standard Metformin used by diabetic patients.

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