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Identification of Hyperactivity Glucose Uptake Inhibitor from Artificial Drugs and Plant Extracts with Reference to Diabetes Mellitus

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ABSTRACT

Living cell uses glucose as an energy source and intermediary metabolite. Glucose is transported across the apical membrane of the enterocytes by SLC_5A_1 and later across their basal membrane by SLC_2A_2. Molecule movement by glucose transporter proteins works by facilitating diffusion. There are various kinds of materials were found to inhibit the uptake of glucose by the cell both in vivo and in vitro. The in vitro glucose uptake inhibition study was carried out in the blood cells of diabetic patients and liver cells of alloxan-induced diabetic mice using natural and artificial glucose uptake inhibitors. The present study included 5 diabetic patients in the age group of 54, 63, 65, 67 and 72 years and 5 alloxan-induced diabetic albino mice. All patients had glucose level greater than 150mg/dl. The patients were monitor during a period of one month. In the present study, the effect of various natural and synthetic compounds on glucose uptake by cells was studied and the results are reported in this paper.

Keywords: **A**lloxan-induced diabetic mice, SLC_5A_1, SLC_2A_2, glucose transporter proteins, dodecyl trimethyl ammonium bromide, sodium nitroprusside.

INTRODUCTION

Glucose is a polar molecule; transport through biological membranes requires specific transport proteins. Transport of glucose through the apical membrane of intestinal, choroid plexus and kidney epithelial cells depends on the presence of secondary active Na+/glucose symporters,

SGLT-1 and SGLT-2, which concentrate glucose inside the cells, using the energy provided by cotransport of Na+ ions down their electrochemical gradient[1]. Facilitated diffusion of glucose through the cellular membrane is otherwise catalyzed by glucose carriers (protein symbol GLUT, gene symbol SLC2 for Solute Carrier Family 2) that belong to a superfamily of transport facilitators including organic anion and cation transporters, yeast hexose transporter, plant hexose/proton symporters, and bacterial sugar/proton symporters[2]. GLUTs are integral membrane proteins which contain 12 membrane spanning helices with both the amino and carboxyl termini exposed on the cytoplasmic side of the plasma membrane. GLUT proteins transport glucose and related hexoses according to a model of alternate conformation[3,4,5], which predicts that the transporter exposes a single substrate binding site toward either the outside or the inside of the cell. Binding of glucose to one site provokes a conformational change associated with transport, and releases glucose to the other side of the membrane. The study was carried out in three different methods. In the first method, effect of dodecyl trimethyl ammonium bromide on glucose uptake by red blood cells of diabetic patients was studied. In the second method, effect of *Gynura procumbens* extract on glucose uptake by liver cells of diabetic mice was studied. In the third method, effect of sodium nitroprusside on glucose uptake by red blood cells of diabetic patients was studied. The liver cells of alloxan induced diabetic mice were treated with natural glucose uptake inhibitor obtained from the extract of *Gynura procumbens*. The result shows that the uptake of glucose by mice liver cells were marked decreased by the action of the plant extract. The amount of glucose uptake was decreased in respect to the concentration of inhibitor increased. The artificial glucose uptake inhibitors such as sodium nitroprusside and dodecyl trimethyl ammonium bromide markedly reduce the glucose uptake by the blood cells of diabetic patients in *in vitro* condition. The extract of *Gynura procumbens* contains a natural glucose uptake inhibitor, which has the capacity to inhibit the glucose uptake by liver cells of alloxan-induced diabetic mice in *in vitro* condition.

EXPERIMENTAL SECTION

The present study included 5 diabetic patients in the age group of 54, 63, 65, 67 and 72 years and 5 alloxan-induced diabetic albino mice. All patients had glucose level greater than 150mg/dl. The patients were monitor during a period of one month. All subjects gave informed consent to participate in this study. In the present study the effect of various natural and synthetic compounds on glucose uptake by cells were studied. The study was carried out in three different methods. In the first method, effect of dodecyl trimethyl ammonium bromide on glucose uptake by red blood cells of diabetic patients was studied. In the second method, effect of *Gynura procumbens* extract on glucose uptake by liver cells of diabetic mice was studied. In the third method, effect of sodium nitroprusside on glucose uptake by red blood cells of diabetic patients was studied.

2.1 Effect of Dodecyl Trimethyl Ammonium Bromide on Glucose Uptake by Red Blood Cells of Diabetic Patients

To determine the effect of dodecyl trimethyl ammonium bromide on glucose uptake by red blood cells of diabetic patients. Twelve-hour fasting blood samples were collected from three diabetic patients at three different times. The determination of RBC glucose uptake was studied by following method. Each 1 ml of the blood samples of each patient was taken in 5 separate test tubes labeled C1 (initial), C2 (final), T1, T2 and T3. The glucose present in the C1 test tube was

immediately measured by GOD-POD method. Then add 0.1M, 0.2M and 0.3M of dodecyl trimethyl ammonium bromide solution to the test tubes T1, T2 and T3 respectively. Then the tubes (including C2) were incubated at 37°C for 30 minutes. After 30 minutes the glucose content of each sample was estimated using GOD-POD method. From the amount of glucose present in the blood, the effect of dodecyl trimethyl ammonium bromide on glucose uptake was determined.

2.2 Effect of *Gynura procumbens* Extract on Glucose Uptake by Liver Cells of Diabetic Mice

To determine the effect of *Gynura procumbens* extract on glucose uptake by liver cells of diabetic mice. The extract was further subjected to solvent-solvent partitioning starting from hexane as the least polar solvent, followed by solvents of increasing polarities, which are ethyl acetate and butanol. The crude fractions were then subjected for evaluation of glucose uptake assay described below. Alloxan induced diabetic mice liver cells were separated from the liver tissue using mortar and pestle and then placed in phosphate buffer (pH 7.4, 0.1M), stored in 4°C before the experiment. During glucose uptake study, 1 ml of the solution containing liver cells were taken in 5 different test tubes (C, T1, T2, T3, T4 and T5) and pre-incubated with phosphate buffer (pH 7.4; 0.1M) at 37˚C for 10 min. To all the test tubes except control add 1 ml of the plant extracts at varying dilution. The glucose uptake reaction was initiated by adding glucose (100mg/100ml) to the buffer solution that contains liver cells. After incubation at 37˚C for 30 min, cells were washed three times with phosphate buffer (pH 7.4; 0.1M) and solubilized with SDS. The glucose content of the cells was estimated by GOD-POD method and ortho-toluidine method.

2.3 Effect of Sodium Nitroprusside on Glucose Uptake by Red Blood Cells of Diabetic Patients

To determine the effect of sodium nitroprusside on glucose uptake by red blood cells of diabetic patients. Each 1ml of the blood samples of each patient was taken in 5 separate test tubes labeled C1(initial), C2(final), T1, T2 and T3. The glucose present in the C1 test tube was immediately measured by GOD-POD method. Then add 0.1M, 0.2M and 0.3M of sodium nitroprusside solution to the test tubes T1, T2 and T3 respectively. Then the tubes (including C2) were incubated at 37°C for 30 minutes. After 30 minutes the glucose content of each sample was estimated using GOD-POD method. From the amount of glucose present in the blood, the effect of sodium nitroprusside on glucose uptake was determined.

2.4 Estimation of Blood Glucose

Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. In a subsequent peroxidase catalyzed reaction, the oxygen liberated is accepted by the chromogen system to give a red colored quinoemine compound. The red color so developed is measured at 510nm and is directly proportional to glucose concentration. Taken 3 set of test tubes and marked as blank, standard and test. 20µl of serum sample was taken the test tube, and then added 20 µl of glucose standard reagent was added to the blank, Test and standard tubes. These solutions are mixed well and incubated at 37°C for 10 minutes or room temperature for 30 minutes. Then added 1.5 ml of deionized water to all the tubes and mixed well. Read the absorbance at 490–550nm against a reagent blank.

RESULTS AND DISCUSSION

The aim of the present study was to investigate the influence of various natural and artificial compounds on the uptake of glucose by the cells of diabetic patients in *in vitro* condition. Subjects were selected according to the degree of blood glucose level, age and sex. A female at the age of 54 whose body weight is 65 kg has the glucose level of 202 mg/dl. A male at the age of 63 whose body weight is 70 kg has the glucose level of 212mg/dl. As such experiment has been made in 5 patients and the result was also the same i.e all of them have increased fasting glucose level. The results show that the uptake of glucose by the blood samples after treatment with sodium nitroprusside - the artificial glucose uptake inhibitor was marked decreased.

3.1 Effect of Dodecyl Trimethyl Ammonium Bromide on Glucose Uptake by Red Blood Cells of Diabetic Patients:

Table 1: Blood glucose levels in patients (initial control) (mg/dl)

S. No	Sex	Age	Body weight	Glucose level in initial control (mg/dl)	Glucose level in final control (mg/dl)
P1	FM	54	65	202	154
P2	M	63	70	212	160
P3	M	65	68	223	156
P4	FM	67	66	240	190
P5	M	72	75	261	178

Table 2: Blood glucose levels in 0.1M dodecyl trimethyl ammonium bromide treated sample (mg/dl)

S. No	Sex	Age	Body weight	Glucose level in final control (mg/dl)	Glucose level in test sample (mg/dl)
P1	FM	54	65	154	166
P2	M	63	70	160	171
P3	M	65	68	156	174
P4	FM	67	66	190	201
P5	M	72	75	178	222

Table 3: Blood glucose levels in 0.2M dodecyl trimethyl ammonium bromide treated sample (mg/dl)

S. No	Sex	Age	Body weight	Glucose level in final control (mg/dl)	Glucose level in test sample (mg/dl)
P1	FM	54	65	154	172
P2	M	63	70	160	175
P3	M	65	68	156	178
P4	FM	67	66	190	208
P5	M	72	75	178	229

The amount of glucose uptake inhibited by the blood sample treated with 01.M solution was found to approximately 6.8 mg/dl. The same results were obtained when treated with 0.2 and 0.3M solutions of sodium nitroprusside. The amount of glucose uptake was decreased in respect to the concentration of inhibitor increased.

Table 4: Blood glucose levels in 0.3M dodecyl trimethyl ammonium bromide treated sample (mg/dl)

S. No	Sex	Age	Body weight	Glucose level final control (mg/dl)	Glucose level in test sample (mg/dl)
P1	FM	54	65	154	180
P2	M	63	70	160	183
P3	M	65	68	156	182
P4	FM	67	66	190	216
P5	M	72	75	178	230

3.2 Effect of *Gynura Procumbens* Extract on Glucose Uptake by Liver Cells of Diabetic Mice:

Table 5: Glucose levels in liver cell solution in initial and final control solution (mg/dl)

S. No.	Glucose levels in initial control (mg/dl)	Glucose levels in final control (mg/dl)
M1	100	68
M2	100	75
M3	100	70
M4	100	59
M5	100	64

Table 6: Glucose levels in liver cells solution treated with 1% *Gynura procumbens* extract (mg/dl)

S. No.	Glucose levels in control sample (mg/dl)	Glucose levels in test sample (mg/dl)
M1	68	73
M2	75	81
M3	70	79
M4	59	68
M5	64	72

Table 7: Glucose levels in liver cells solution treated with 2% *Gynura procumbens* extract (mg/dl)

S. No.	Glucose levels in control sample (mg/dl)	Glucose levels in test sample (mg/dl)
M1	68	77
M2	75	86
M3	70	84
M4	59	73
M5	64	79

The liver cells of alloxan induced diabetic mice were treated with natural glucose uptake inhibitor obtained from the extract of *G. procumbens*. The inner and outer glucose-binding sites are probably located in transmembrane segments 9, 10, 11[6]; also, the QLS motif located in the seventh transmembrane segment could be involved in the selection and affinity of transported substrate[7,8]. Each glucose transporter isoform plays a specific role in glucose metabolism determined by its pattern of tissue expression, substrate specificity, transport kinetics, and regulated expression in different physiological conditions[9]. To date, 13 members of the GLUT/SLC2 have been identified[10]. The ethnomedical use of the *Bauhinia monandra* in the

management of diabetes and stimulating insulin release is one of the modes of action of the butanol fraction and some of its subfractions[11].

Table 8: Glucose levels in liver cells solution treated with 3% *Gynura procumbens* extract (mg/dl)

S. No.	Glucose levels in control sample (mg/dl)	Glucose levels in test sample (mg/dl)
M1	68	82
M2	75	91
M3	70	88
M4	59	79
M5	64	86

3.3 Effect of Sodium Nitroprusside on Glucose Uptake by Red Blood Cells of Diabetic Patients:

Table 9: Blood glucose levels in patients (initial control and final control) (mg/dl)

S. No.	Sex	Age (Yr)	Body weight (Kg)	Glucose level in initial control (mg/dl)	Glucose level in final control (mg/dl)
P1	FM	54	65	202	154
P2	M	63	70	212	160
P3	M	65	68	223	156
P4	FM	67	66	240	190
P5	M	72	75	261	178

Table 10: Blood glucose levels in 0.1M SNP treated sample (mg/dl)

S. No.	Sex	Age	Body weight	Glucose level in final control (mg/dl)	Glucose level in test sample (mg/dl)
P1	FM	54	65	154	160
P2	M	63	70	160	165
P3	M	65	68	156	163
P4	FM	67	66	190	198
P5	M	72	75	178	186

Table 11: Blood glucose levels in 0.2M SNP treated sample (mg/dl)

S. No.	Sex	Age	Body weight	Glucose level in final control (mg/dl)	Glucose level in test sample (mg/dl)
P1	FM	54	65	154	168
P2	M	63	70	160	172
P3	M	65	68	156	173
P4	FM	67	66	190	207
P5	M	72	75	178	194

Fresh juice of *Zingiber officinale* produced a time dependent decrease in blood glucose level significantly compared to both glibenclamide and metformin. *Z. officinale* juice increases insulin sensitivity leads to hypoglycemic action inspite of insulin depletion by alloxan and additional non insulin related hypoglycemic action could be inferred[12].

Table 12: Blood glucose levels in 0.3M SNP treated sample (mg/dl)

S. No.	Sex	Age	Body weight	Glucose level in final control (mg/dl)	Glucose level in test sample (mg/dl)
P1	FM	54	65	154	175
P2	M	63	70	160	178
P3	M	65	68	156	178
P4	FM	67	66	190	216
P5	M	72	75	178	202

On the basis of sequence similarities, the GLUT family has been divided into three subclasses. Acipimox treatment would enhance insulin sensitivity by reducing endogenous glucose production and increasing peripheral glucose uptake. Furthermore, we hypothesized that increased insulin-stimulated peripheral glucose uptake would involve improved distal insulin signaling at the level of AKT, GSK-3, and GS in muscle[13]. The AS160 CBD directly regulates contraction-induced glucose uptake in mouse muscle and that calmodulin provides an additional means of modulating AS160 RAB-GAP function independent of phosphorylation. These findings define a novel AS160 signaling component, unique to contraction and not insulin, leading to glucose uptake in skeletal muscle[14]. Administration of insulin into the lungs does indeed increase nonhepatic glucose uptake, that skeletal muscle is the site of that effect, and that the effect manifests by 1 hour after insulin inhalation[15]. Diabetes mellitus is a metabolic disorder in the body characterized by hyperglycemia altered metabolism of lipids, carbohydrates and proteins with an increased risk of complications of vascular disease[16]. Navneet *et al.* concluded that aqueous extracts of *Momordica balsamina* seeds is having significant antihyperglycemic potential and can be further fractionated in order to get a responsible molecule for this vary action[17]. Acute IL-6 treatment enhances insulin-stimulated glucose disposal in humans *in vivo*, while the effects of IL-6 on glucose and fatty acid metabolism in vitro appear to be mediated by AMPK. IL-6 did not increase whole-body glucose disposal in either healthy subjects or patients with type 2 diabetes, whereas it reduced insulin concentrations in the patients to values comparable with those of the healthy subjects, suggesting that IL-6 might have favorable effects on insulin action[18]. Two synthetic detergents: triton X-100 and dodecyl trimethyl ammonium bromide have been found very strong inhibitors. Kinetic studies showed that these detergents behaved as mixed type inhibitors. The Na^+-dependent transport of amino acids (aspartic acid, lysine, phenylalanine) are only poorly affected by dodecyl trimethyl ammonium bromide, while triton X-100 inhibits unspecifically all the transport studied[19].

The result shows that the uptake of glucose by mice liver cells were marked decreased by the action of the plant extract. The amount of glucose uptake inhibited by the 1% solution of plant extract was approximately 7.4 mg/dl. The same results were obtained when treated with 2 and 3% solutions of plant extract. The amount of glucose uptake was decreased in respect to the concentration of inhibitor increased. The *in vitro* glucose uptake inhibition study was carried out in the blood cells of diabetic patients and liver cells of alloxan-induced diabetic mice using natural and artificial glucose uptake inhibitors. The artificial glucose uptake inhibitors such as sodium nitroprusside and dodecyl trimethyl ammonium bromide markedly reduce the glucose uptake by the blood cells of diabetic patients in *in vitro* condition. The extract of *G. procumbens* contains a natural glucose uptake inhibitor, which has the capacity to inhibit the glucose uptake by liver cells of alloxan-induced diabetic mice in *in vitro* condition.

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