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Cellular Level Catalysation of Glucose by Organ Level Cells as a Possible Therapeutic Scope for Diabetes Mellitus

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ABSTRACT

A polyherbal concoction was tested on various organs derived cell lines to understand the glucose uptake and metabolism at laboratory level. The findings clearly show that the tested concoction has positively catalysed the glucose uptake and metabolism suggesting its limited value in protecting the organ damage due to hyperglycemia. Although the study was done in lab simulated cell culture model, how the concoction may act in complex system is not easy to predict based on our lab study. Nevertheless the concoction possess some positive value for the management of diabetic comorbidities but further intense research and double blind randomized clinical trial is required to establish the conclusive merit of the same. Details of the study are presented in the paper.

Keywords: Hyperglycemia, polyherbal concoction, diabetes mellitus, glucose utilization assay

INTRODUCTION

Polyherbal concoctions are gaining importance especially in the arena of health care- wellness and fitness. The metabolic disorder like diabetes mellitus can be managed well if the necessary lifestyle changes and botanical based traditional recipes are imbibed and integrated into the daily life. Through such disciplined approach the treatment requirement for diabetes mellitus can be minimized significantly.[1,2,3]

The polyherbal formulation tested contains the following herbs such as *Andrographis paniculata, Syzygium cumini, Tinospora cordifolia, Momordica charantia, Cyperus rotundus, Zingiber officinale, Piper nigrum, Adhatoda vasica* each and every herb used in the formulation besides having strong mention in the ancient literature also have invaluable scientific proof for the management of diabetes mellitus.[4,5,6]

Glucose being the primary energy source of all cells in human body irrespective of either the segregation or the role of different organs absorbs and metabolizes glucose.

Certain cells in the liver do synthesize glucose and in strict medical parlance synthesized glucose by the liver is sufficient to meet the daily demand of the glucose by human being.

The increased glucose burden may cause severe stress and distortion to several organs and progressively the organs may wane of from their functions.[7] Therefore the remedy that is likely to be integrated into the daily lifestyle must catalyse the organ level glucose absorption and glucose metabolism and thereby the possible shock and its ramification radiating out of high blood glucose can be avoided.

We in the present paper have studied a polyherbal concoction (DCOD) in the uptake and metabolism of glucose by several cell lines representative of various organs such as HepG2, INS-1, L-6 myoblast

and 3T3L-1. The details are presented in the paper.

MATERIALS AND METHODS

The polyherbal concoction used in the present study contains the following herbs *Andrographis paniculata, Syzygium cumini, Tinospora cordifolia, Momordica charantia, Cyperus rotundus, Zingiber officinale, Piper nigrum, Adhatoda vasica*.

Similarly the following cell lines to elaborately represent various critical organs we have used in the study and the details are given below

HepG2: HepG2 is a human hepatoma cell (liver) which is non-tumorogenic immortalized cell line used extensively in diabetic research.

INS-1: Insulin secreting cell which is considered to be the gold standard cell line that represents fairly the pancreatic islet beta cells used for diabetic research.

L 6 myoblast: This cell line is the best model to study the likely glucose uptake by muscle cells.

3T3L-1: The mouse derived cell line which is used to study the adipose tissue response to glucose challenge.

Cytotoxicity assay

The cytotoxicity assay was determined as per the standard procedure.

In brief cells were seeded into 96-well plates at a density of 8000 cells per well, within a volume of 100 μl. The cells were allowed to attach on the plate and then treated with polyherbal concoction at the following concentrations such as 50 μg/ml, 100 μg/ml, and 200 μg/ml.

After 48 h of incubation at 37∘C, the medium was removed and replaced with fresh medium containing 10% FCS and 0.5 mg/ml MTT (dissolve 25 mg MTT in 50 ml complete culture medium) and incubated for 3 h at 37∘C. The medium was then removed and the MTT crystal (purple formazan) was dissolved in DMSO (200 μl/well) to solubilize the formazan crystals formed in the cells. The absorbance was read at 540 nm using a microplate reader. The cytotoxic effect of the polyherbal concoction was calculated from the control value and accordingly the concentration to be used in the subsequent experiment was arrived. [8, 9, 10]

$$\% \text{ Cell death} = 1 - (\text{Absorbance of test well} / \text{Average of the untreated}) \times 100$$

Glucose utilization byHepG2

The glucose utilization by HepG2 cells was determined. The HepG2 cells were dislodged by brief exposure to 0.25% Trypsin in phosphate-buffered saline, counted, suspended in fresh growth medium, and then seeded at a density of 6 000 cells per well into a 96-well culture plate and allowed the cells to adhere and grow in a humidified incubator with 5% CO2 at 37∘C for three days.

Two cell-free rows were also included to serve as blanks. On day three after seeding, without changing the medium, the polyherbal concoction was added (up to a concentration of 200 μl) did not exhibit any cytotoxic activity. After 48 h incubation, the medium was removed and replaced with a 25 μl incubation buffer (RPMI medium diluted with PBS, 0.1% BSA and 8 mm glucose) and further incubated for an additional 3 h at 37∘C.

1,1-Dimethylbiguanide hydrochloride (0.1 μg/ml) was used as positive control while the negative control (untreated) contained only the incubation buffer without polyherbal concoction.

After incubation, 10 μl of the incubation medium was removed from each well and transferred into a new 96-well plate and then 200 μl of glucose oxidase reagent was added to determine the concentration of glucose in the medium. After 15min of incubation at 37∘C, the absorbance was measured at 492 nm by microtitre plate reader.

The amount of glucose utilized was calculated as the difference between the cell-free and cell-containing wells. The percentage of glucose utilization was calculated in relation to the untreated

controls. Cell viability in the representative wells was determined using the MTT assay.

Glucose utilization by L6 Myoblasts

The glucose utilization in L6 myoblasts cells was determined. The L6 cells were seeded into 96-well culture plates at a density of 3 000 cells/well and allowed to adhere until 90% confluence was reached. Two cell-free rows were also included to serve as blanks for glucose utilization assay. After 90% confluence, the culture medium was removed and replaced with DMEM containing 2% FBS and cultured for an additional five days.

Fourty-eight hours (48 h) prior to the glucose utilization assay, the culture medium was replaced and the polyherbal concoction was added at the concentrations described above. A column was also treated with insulin (4 μg/ml) instead of polyherbal concoction to serve as a positive control. The cells were then incubated in the presence of the polyherbal concoction for an additional 48 h.

After the incubation period, the medium was removed and replaced with 25 μl incubation buffer containing RPMI medium diluted with PBS, 0.1% BSA, and 8mM glucose and incubated for further 3 h at 37∘C. Five microlitres (5μl) of the incubation medium was removed from each well and then placed into a new 96-well plate into which 200 μl of glucose oxidase reagent was added per well to determine the concentration of glucose in the medium.

After 15 min of incubation at 37∘C, the absorbance was measured at 520 nm using a microtitre plate reader. The amount of glucose utilized was calculated as the difference between the cell-free and cell-containing wells. The percentage of glucose uptake was calculated in relation to the untreated controls. Cell viability in the representative well was determined using the MTT assay. [8, 9, 10]

Lipid Accumulation in 3T3-L1 Preadipocytes

Lipid accumulation in 3T3-L1 preadipocytes cells was determined according to the method described by Oyedemi et al. The 3T3-L1 preadipocytes were seeded at a density of 6 000 cells per well into a 48-well culture plate and were allowed to grow until 100%confluence was reached. Two days after confluence, the preadipocytes were treated for an additional two days with various concentrations of the polyherbal concoction or positive control (Rosiglitazone; 0.4 μg/ml).

The cells were then cultured for an additional ten days in normal culture medium (DMEM with 10% FBS) and the medium replaced every two to three days. After ten days, the culture medium was removed and gently washed with PBS. The cells were then allowed to fix at room temperature for approximately 1 h by adding 500 μl per well of 10% formaldehyde in PBS. The fixative solution was aspirated and later stained by adding 200 μl of pre warmed oil red solution (6 ml of stock solution (0.5 g oil red dye in 100 ml isopropanol) in 4ml of distilled water) for 15 min at 37∘C. After 15 min of incubation, excess dye was extensively washed with water and the plate dried in an oven at 37∘C. The dye was further extracted by adding isopropanol (250 μl per well) after which 200 μl was transferred to a 96-well plate and the absorbance measured at 520 nm using a microtitre plate reader. [10]

Glucose metabolism by insulin secretion

The glucose metabolism assay as a reflection of insulin secretion in INS-1 cells using MTT (tetrazolium) colorimetric assay was determined according to the method described by Janjic and Wollheim. The INS-1 cells were cultured in RPMI containing 5% FCS. The cells were seeded into 96-well plates at a density of 8000 cells per well, with a volume of 100 μl. The cells were left to attach overnight and treated with polyherbal concoction or PBS (which serve as a control) in the presence or absence of glucose (20 mM). After 48 h of incubation at 37∘C, the medium was removed from the cells and 100 μl of DMEM medium containing 10% FCS and 0.5 mg/ml MTT

was added and further incubated for additional 30min at 37∘C. The medium was later aspirated and MTT crystal (purple formazan) dissolved in DMSO (200 μl/well). The absorbance was read at 540 nm using a microplate reader.

Lipase Inhibition Assay

The lipase inhibition assay was determined according to the method described by Lewis and Liu. Briefly, 10 μl of the polyherbal concoction or positive (orlistat; 50μg/ml) or negative control (distilled water) was added to the well of 96-well plates. Thereafter, porcine pancreatic solution (10mg/ml) was (freshly prepared in 50mMTris-HCl buffer-pH 8.0) added and then centrifuged to remove the insoluble material and was then added at 4 times the volume to each of the sample (40 μl). After 15 min of incubation, 170 μl of substrate solution (20 mg pNPP in 2ml isopropanol was added to 18 ml 50 Mm Tris-HCl buffer containing 20 mg gum Arabic, 40 mg sodium deoxycholate, and 100 μl Triton X-100) were then added and incubated for 25 min at 37∘C; the absorbance was then measured at 405 nm using a spectrophotometer and the percentage inhibition was calculated using the following equation:

% Inhibition = (1 − Absorbance of the test well/ Absorbance of the untreated (control)× 100)

RESULTS

Effect of polyherbal concoction on various cell lines

The polyherbal concoction did not exhibit any cytotoxic activity in all the cell lines tested up to a concentration of 200µg/ml. Table- 1

Concentration (µg/ml)	% Cell death vis-à-vis different cell types			
	HepG2	L6 myoblast	3T3L-1	INS-1
50	5	11	7	12
100	22	14	18	21
200	31	35	28	36

Effect of polyherbal concoction on glucose utilization in HepG2 hepatocytes

The polyherbal concoction in a dose dependent manner increased the glucose utilization by HepG2 cells and the rate of glucose utilization due to polyherbal concoction treatment was comparable with the results of 1,1-Dimethylbiguanide hydrochloride. Table-2

Sample Details	Glucose utilization (% of control)
Control	100
1,1-Dimethylbiguanide hydrochloride (0.1 µg/ml)	185
Polyherbal concoction	
a. 50 µg/ml	142
b. 100 µg/ml	154
c. 200 µg/ml	165

Effect of polyherbal concoction on glucose utilization by L6 myoblast

The polyherbal concoction has marginally increased the glucose utilization by L-6 myoblast cells and the effect was not influenced by concentration of polyherbal concoction. Table- 3

Sample Details	Glucose utilization (% of control)
Control	100
Insulin (6 µg/ml)	160
Polyherbal concoction	
a. 50 µg/ml	105
b. 100 µg/ml	109
c. 200 µg/ml	111

Effect of polyherbal concoction on triglyceride accumulation in 3T3-L1 preadipocytes

The polyherbal concoction has only marginally increased the glucose utilization by 3T3- L-1 preadipocytes cells and the effect was not influenced by concentration of polyherbal concoction. Table- 4

Sample Details	Triglyceride accumulation (% of control)
Control	100
Rosiglitazone (0.4 µg/ml)	159
Polyherbal concoction	
a. 50 µg/ml	112
b. 100 µg/ml	121
c. 200 µg/ml	132

The effect of polyherbal concoction on MTT reduction by INS-1 cells

Sample Details	MTT reduction in % after 30min
Control (Without glucose)	18
Control (with glucose)	34
Polyherbal concoction	
a. 50 µg/ml	17
b. 100 µg/ml	22
c. 200 µg/ml	28
Polyherbal concoction (With glucose)	
a. 50 µg/ml	18
b. 100 µg/ml	24
c. 200 µg/ml	32

The glucose thrust could not influence the effect of polyherbal concoction on INS-1 cells. Table- 5

The effect of polyherbal concoction on pancreatic lipase activity (%)

The polyherbal concoction has significantly decreased the lipase accumulation; however the activity was comparable with Orlistat at concentration level 50 µg/ml (Orlistat is a pure compound whereas the test product is a polyherbal preparation) Table- 6

Sample DSetails	% Lipase inhibition
Enzyme	0
Orlistat (50 µg/ml)	71
Polyherbal concoction	
a. 50 µg/ml	15
b. 100 µg/ml	23
c. 200 µg/ml	34

DISCUSSION

The present study has revealed that the tested concoction has promising therapeutic value for the management of diabetes mellitus possibly to manage various comorbidities accompany diabetes mellitus.

The management strategy of diabetes mellitus must go beyond the paradigm of hyperglycaemia reduction in the blood. The strategy also must include enhancing the ability of glucose uptake and glucose metabolism by the cells of several key organs such as liver, islets of pancreas, kidney, musculo skeletal tissue, adipocyte etc.

Glucose being the principle source of energy for all living cells, hyperglycemia condition may challenge the threshold limit of the organs due to high glucose burden and progressively such situation may cause severe fatigue, wilting and weaning the functional ability of the cells in many organs and in due course, the cellular fatigue in collective expression is likely to impair the total function of the organs and which are described as diabetic comorbidities such as nephropathy, neuropathy, retinopathy, myocardial infarction, stroke (ischemic/hemorrhagic), nonfatal congestive heart failure, stable angina, transient ischemic attack, peripheral vascular disease, gastrointestinal event, urinary tract infection, genital infections (non-UTI), genital disorders, ulcer of the skin etc.

Therefore the treatment approach should enable and empower the cells in every critical organs to assimilate and metabolize the glucose in order to not to suffer from hyperglycemia.

The concoction we have studied on the following cell lines such as HepG2, L-6 myoblast, INS-1 and 3T3-L1 showed that the concoction exhibited concentration linear effect in glucose uptake and glucose metabolism by all the above cell lines at the laboratory level.

The findings suggest that the concoction may be empowering the cellular absorption of glucose possibly at several organ(s) level and thereby the concoction may retard the progressive erosion of the organ function and the post descent organ failures which are quite common due to prolonged hyperglycemia. However more intense scientific scrutiny and randomized double blind active control based clinical evaluation may be required to establish our early preliminary observation.

The real challenge of the medical world in dealing with diabetes mellitus is not just the management or correction of hyperglycemic condition alone but the challenge also must be on limiting the organ damage which is otherwise called as comorbidity condition.

Comorbidity conditions account for about 80% of mortality and morbidity among diabetic patients.

The comorbidity burden singularly and collectively favours also an increased infection rate, prolonged suffering and illness, prolonged carriage rate of the pathogens, high endurance and exorbitant treatment cost.

Our study has given a small hope at least directly and indicatively and only further intense study shall validate the real scope of the concoction for managing the global killer- Diabetes mellitus.

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