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MECHANISTIC *IN VITRO* ASSESSMENT OF GLUCOSE-LOWERING AND INSULIN-SENSITIZING ACTIVITIES OF JAMUN NEEM KARELA KWATH JUICE: A POLYHERBAL FORMULATION FOR SUPPORTING HEALTHY BLOOD SUGAR BALANCE

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ABSTRACT

Diabetes mellitus is marked by chronic hyperglycemia due to defective insulin secretion or action. This study evaluated the *in vitro* glycemic control potential of Jamun Neem Karela Kwath Juice, a novel polyherbal liquid formulation manufactured by Multani Pharmaceuticals Ltd., comprising traditional herbs: *Syzygium cumini, Momordica charantia* and *Azadirachta indica*. Glycemic control potential was evaluated through α-amylase and α-glucosidase inhibition assays, using acarbose as the standard, alongside glucose uptake in differentiated L6 myotubes. Cytotoxicity was assessed via MTT assay, with IC_{50} values derived from nonlinear regression in GraphPad Prism. Jamun Neem Karela Kwath Juice exhibited dose-dependent enzyme inhibition. It achieved significant α-amylase inhibition (maximum % inhibition: 31.24%) and potent α-glucosidase inhibition (maximum % inhibition: 47.27%). In L6 myotubes, the formulation markedly boosted glucose uptake, peaking at 2% concentration with a 3.48-fold increase over basal levels. MTT assay indicated concentration-dependent cytotoxicity (IC_{50}: 11.93% in L6 cells), confirming viability at lower doses suitable for functional assays. These results demonstrated Multani Jamun Neem Karela Kwath Juice's insulin-sensitizing properties and potential to promote peripheral glucose uptake, positioning it as a promising therapeutic agent to support healthy blood sugar balance.

Keywords: α-amylase inhibition, α-glucosidase inhibition, Acarbose, Doxorubicin, Glucose uptake assay, MTT assay

INTRODUCTION

Diabetes mellitus represents a complex metabolic disorder marked by persistent hyperglycemia due to impairments in insulin secretion, insulin action, or a combination of both.[1] This condition significantly disrupts the metabolism of carbohydrates, lipids, and proteins, resulting in long-term systemic complications.[1] In 2022, the World Health Organization (WHO) introduced its first-ever global targets for managing diabetes, setting a deadline for 2030. These benchmarks dictate that 80% of people living with diabetes mellitus should be formally diagnosed, and 80% of those diagnosed must maintain proper control over their blood pressure and glycaemic levels. Additionally, the guidelines call for 60% of diabetic individuals over the age of 40 to receive statin therapy, alongside ensuring total (100%) affordable access to insulin and blood glucose self-monitoring tools for everyone with type 1 diabetes mellitus. This paper compiles insights from international specialists on the best approaches to reach these benchmarks. Currently impacting more than 10% of adults globally, diabetes mellitus poses an escalating threat to both public health and the global economy.[1,2] Utilizing a health-augmented macroeconomic model evaluated across 204 countries and territories, we measured the financial impact of the condition from 2020 to 2050.[2] This evaluation focused on three main cost drivers: decreased effective labour supply resulting from diabetes-induced mortality and disability, the direct financial resources required for medical treatment, and the economic burden of informal caregiving.[2] This escalating challenge links to serious microvascular issues such as retinopathy, nephropathy, and neuropathy, alongside macrovascular problems including cardiovascular and cerebrovascular disorders, all of which drive high rates of morbidity, mortality, and economic pressure on health services.[1]

In 2022, the World Health Organization (WHO) launched its inaugural global targets for diabetes management, establishing specific benchmarks to be realized by 2030: the formal diagnosis of 80% of individuals living with diabetes mellitus; target-level glycemic and blood pressure management in 80% of those diagnosed; statin therapy for 60% of diabetic individuals aged 40 years and older; and universal (100%) affordable access to insulin and blood glucose self-monitoring technologies for all patients with type 1 diabetes mellitus. This paper synthesizes international expert perspectives on actionable pathways toward fulfilling these milestones, addressing a disease that currently impacts more than 10% of the global adult demographic and constitutes an escalating epidemiological and economic threat.[1,2] Employing a health-augmented macroeconomic framework encompassing 204 countries and territories, we evaluated the financial ramifications of diabetes from 2020 through 2050 based on three distinct economic drivers: contractions in the effective workforce stemming from diabetes-associated morbidity and mortality, healthcare expenditure allocated to medical interventions, and financial costs linked to informal care provision.[2] Excluding

informal caregiving costs, the forecasted global economic liability is projected at INT$10.2 trillion (in 2017 international dollars), which equates to roughly 0.22% of the annual global gross domestic product (GDP). However, when informal care expenditures are integrated into the model, the projected economic burden increases markedly to INT$78.8 trillion, with a sensitivity range spanning from a baseline of INT$5.5 trillion to an upper limit of INT$152.1 trillion depending on the specific assumptions applied to caregiving variables.

Effective management of type 2 diabetes mellitus (T2DM) demands control of postprandial hyperglycemia, a key contributor to advancing diabetic complications.[3,4] A proven therapeutic approach centres on blocking major carbohydrate-digesting enzymes, specifically α-amylase and α-glucosidase.[3,5] α-Amylase breaks down complex polysaccharides into oligosaccharides, whereas α-glucosidase converts disaccharides into absorbable monosaccharides like glucose within the small intestine.[3,5] Suppressing these enzymes delays carbohydrate breakdown and glucose uptake, thereby blunting postprandial blood glucose spikes.[4,5]

Existing synthetic inhibitors of α-amylase and α-glucosidase effectively manage glycemia, yet their use faces constraints from side effects like flatulence, abdominal pain, and diarrhoea.[3,6] These drawbacks have spurred exploration of safer, more tolerable options, especially from natural origins.[4,7] In this scenario, plant-based bioactive polyherbal formulations have gained prominence as a vital therapeutic strategy, drawing broad pharmacological attention for their multitargeted antidiabetic benefits and reduced adverse effects.[8,9,10] The newly developed polyherbal liquid formulation, Jamun Neem Karela Kwath Juice, has potent glycemic control potential, manufactured by Multani Pharmaceuticals Ltd.

MATERIALS AND METHODS

Chemicals/Reagents

For Glucose uptake and MTT Assay
Glucose Uptake-Glo™ Assay Kit (Promega), PBS (Phosphate Buffered Saline), 96-well white plate, CO2 incubator, Luminometer, Insulin (standard), T25 flask (Falcon), DMEM (Dulbecco's Modified Eagle Medium) (Gibco), Trypsin EDTA 0.05% (Gibco), FBS (Fetal Bovine Serum) (Gibco), MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma), DMSO (Dimethyl sulfoxide) (Sigma-Aldrich)

For α-Amylase activity
α-Amylase (EC. 3.2.1.1) Type VI-B: From porcine pancreas, 500,000 units [15.8 units/mg solid at pH 6.9], CNPG3 reagent (2-chloro-4-nitrophenyl-α-D-maltotrioside), Acarbose, Sodium dihydrogen orthophosphate ($NaH_2PO_4 \cdot 2H_2O$), Disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$)

For α-Glucosidase activity
α-Glucosidase isolated from small intestine of rat, Sucrose, Acarbose, Phosphate Buffer, Glucose reagent kit: Glucose oxidase method.

Test Item
Jamun Neem Karela Kwath Juice is a Proprietary Ayurvedic Formulation manufactured by Multani Pharmaceuticals Ltd. The composition of the test item is as below:

Jamun Neem Karela Kwath Juice (Batch No- JNK2512A(R)), Mfg.: 03/26, Exp.: 03/28)

Each 100 ml is prepared from: *Syzygium cumini* (Fr.)*, Momordica charantia* (Fr.), *Azadirachta indica* (Lf.*),* along with permitted: Excipients, Colours and Preservatives**.**

Glucose Uptake Assay in L6 Cells

Standard Curve of 2DG6P
A standard calibration curve was generated using serial concentrations of 2DG6P ranging from 0.625 to 20 µM. The luminescence intensity increased proportionally with increasing concentrations of 2DG6P, demonstrating a strong linear relationship between RLU (Relative Light Units) and 2DG6P (2-deoxyglucose-6-phosphate) concentration. The generated standard curve showed reliable linearity with an R^2 value of approximately 0.998.

Cell Culture and Differentiation
L6 myoblast cells were seeded in 96-well white-walled clear-bottom plates and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were cultured under humidified conditions at 37°C of 5% CO2 until approximately 80–90% confluence was achieved. Differentiation was induced by replacing the growth medium with DMEM containing 2% horse serum. The differentiation medium was renewed every 48 h for 5–7 days until multinucleated myotubes were observed microscopically.

Preparation of Reagents
The Glucose Uptake-Glo™ Assay Kit was used to estimate glucose uptake. The 2DG6P detection reagent was prepared according to the manufacturer's instructions by combining luciferase reagent, $NADP^+$, G6PDH, reductase, and reductase substrate. Insulin was used as the positive control at a final concentration of 100 nM prepared from a 240 µM stock solution. A 0.1 mM solution of 2-deoxyglucose (2DG) was prepared in phosphate-buffered saline (PBS). Serial dilutions of 2DG6P standard ranging from 20 µM to 0.625 µM were prepared in PBS to generate the standard calibration curve.

Standard Curve Preparation
For standard curve generation, 50 µL of each 2DG6P standard concentration was added into designated wells of a white 96-well plate. Subsequently, 25 µL stop buffer and 25 µL neutralization buffer were added to each well, followed by 100 µL of equilibrated detection reagent. Plates were incubated at room temperature for 60 min in the dark. Luminescence was recorded using the Spectra Max system with an integration time of 1 s. The standard curve was plotted using relative light units (RLU) against 2DG6P concentration.

Treatment and Glucose Uptake Measurement
Differentiated L6 myotubes were washed with PBS and incubated overnight in serum-free medium to reduce basal glucose transporter activity. Cells were treated with insulin (100 nM) or test samples at concentrations of 2%, 1%, and 0.5% for 1 h. Basal control wells received PBS alone. Following stimulation, 0.1 mM 2DG was added to each well and incubated for 30 min at 37°C.

After incubation, the uptake reaction was terminated by removing the medium and washing the cells three times with ice-cold PBS. Stop buffer (25 µL) and neutralization buffer (25 µL) were sequentially added to each well. Thereafter, 100 µL of 2DG6P detection reagent was added and plates were incubated for 1 h at room temperature in the dark. Luminescence was measured using the Spectra Max system.

Calculation of Glucose Uptake
Net RLU (Relative Light Units) values were obtained by subtracting blank readings from sample readings. The concentration of 2DG6P produced in each sample was determined from the standard curve equation. Stimulation activity was expressed as fold increase relative to the basal control.

Cytotoxicity Evaluation in L6 Cells
L6 cells (ATCC- CRL-1458, mouse myoblast cell line) were cultured to assess the cytotoxic effects of the test sample using an MTT assay.

Cell Culture
L6 Cell line was procured from ATCC. Stock cells were cultured in DMEM media supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), and streptomycin (100 µg/mL) in a humidified atmosphere of 5% CO2 at 37 °C until confluent. The cells were dissociated using 0.25% trypsin-EDTA and centrifuged at 1000 rpm for 5 minutes. The culture media was discarded, and the cell pellet was gently re-suspended using 2 mL of complete DMEM medium. Cell viability was checked, and a single-cell suspension of 5.0x10 ^5 cells/ml was prepared.

Table 1: Preparation of Doxorubicin Working Solutions

Step	Preparation Procedure	Final Concentration
Stock Dilution	8.1 µL of 3.7 mM Doxorubicin stock + 291.8 µL DMEM plain media	100 µM
1	150 µL of 100 µM solution + 150 µL DMEM plain media	50 µM
2	150 µL of 50 µM solution + 150 µL DMEM plain media	25 µM
3	150 µL of 25 µM solution + 150 µL DMEM plain media	12.5 µM
4	150 µL of 12.5 µM solution + 150 µL DMEM plain media	6.25 µM
5	150 µL of 6.25 µM solution + 150 µL DMEM plain media	3.125 µM
6	150 µL of 3.125 µM solution + 150 µL DMEM plain media	1.56 µM

Table 2: Preparation of Test Sample Dilutions

Step	Preparation Procedure	Final Concentration
Initial dilution	150 µL of 100% stock solution + 150 µL DMEM plain media	50%
1	150 µL of 50% solution + 150 µL DMEM plain media	25%
2	150 µL of 25% solution + 150 µL DMEM plain media	12.5%
3	150 µL of 12.5% solution + 150 µL DMEM plain media	6.25%
4	150 µL of 6.25% solution + 150 µL DMEM plain media	3.125%
5	150 µL of 3.125% solution + 150 µL DMEM plain media	1.56%
6	150 µL of 1.56% solution + 150 µL DMEM plain media	0.78%

Preparation of Standard and Working Solutions

Preparation of Doxorubicin Standard
A 3.7 mM stock solution of Doxorubicin was prepared and utilized for generating working concentrations. An initial working solution of 100 µM was prepared using plain DMEM medium. Subsequent serial two-fold dilutions were performed to obtain test concentrations ranging from 50 µM to 1.56 µM. (Table 1)

Preparation of Test Sample
For cytotoxicity assessments, the test sample was prepared as 100% stock solutions using plain DMEM medium. Serial two-fold dilutions were subsequently performed to generate treatment concentrations ranging from 50% to 0.78%. (Table 2)

Control Treatment
Control cells were treated with plain DMEM media containing 1% DMSO.

MTT Assay Procedure
To each well of the pre-labelled 96-well microtiter plate, 100 µL of the prepared cell suspension (50,000 cells/well) was added and incubated at 37°C with 5% CO2. After 24 h of incubation, the supernatant was removed, and the monolayer was rinsed with media. To each pre-designated well, 100 µL of test drugs at various concentrations was added and incubated for 24 hours. After incubation, the test solutions in the wells were discarded, and 100 µL of MTT reagent (4 mg/10 mL of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37 °C in 5% CO2. The supernatant was removed, and 100 µL of DMSO was added. The plates were then gently shaken to solubilize the formazan crystals. The absorbance was measured using a microplate reader at 590 nm wavelength using a multimode plate reader, Spectra Max i3X, Molecular Devices.

Calculation of Inhibition
Percentage inhibition was determined using the formula:

$$\% \text{ Inhibition} = (\text{OD of Control}) - (\text{OD of Sample}) \times 100 / (\text{OD of Control})$$

Note: IC_{50} is calculated using Graph pad prism 5.

Statistical Analysis

IC_{50} values represent the half maximal inhibitory concentration, a quantitative measure of the concentration required to inhibit a biological process by 50%. These values were determined by constructing dose-response curves and were derived from nonlinear regression analysis (curve fit) based on a variable sigmoid dose-response curve using GraphPad Prism 5.0 software (GraphPad, San Diego, CA, USA). Nonlinear regression modelled the observational data as a nonlinear combination of the model parameters and one or more independent variables, with data fitted by successive approximation.

α-Amylase Inhibition Assay
This assay evaluated the ability to inhibit α-Amylase, an enzyme responsible for breaking down complex carbohydrates into simple sugars. The inhibitory potential of the test item against α-amylase was evaluated using an *in vitro* cell-free enzyme assay, employing acarbose as the reference standard.

Phosphate Buffer Preparation (40 mM, pH 6.9)
Phosphate buffer (40 mM, pH 6.9) was formulated by dissolving sodium dihydrogen orthophosphate (6.24 g/L; solution A) and disodium hydrogen phosphate dihydrate (7.12 g/L; solution B). These solutions were blended at a 45:55 (v/v) ratio, with the final volume brought to 200 mL using deionized water.

Preparation of Enzyme Solution
α-Amylase (3.246 mg) was dissolved in 100 mL of phosphate buffer (40 mM, pH 6.9).

Preparation of Standard Solution
Acarbose (50 mg) was dissolved in 50 mL of phosphate buffer and serially diluted to achieve a working concentration of 100 µg/mL in phosphate buffer (pH 6.9).

Preparation of Test Sample
The test sample was prepared by considering the original stock as 100% and making further dilutions using the same phosphate buffer (pH 6.9).

α-Amylase Inhibition Assay Procedure
Phosphate buffer (120 µL, pH 7.0) was pre-incubated with varying concentrations of the test sample or acarbose standard, along with 60 µL of α-amylase solution, at 37°C for 3 min. Following this, 60 µL of CNPG3 substrate reagent was introduced, and the reaction proceeded at 37°C for an additional 3 min. The mixture was then boiled for 2 min to terminate the reaction, cooled to room temperature, and absorbance was recorded at 405 nm. A control reaction was performed under the same conditions without the test sample.

Calculation of Inhibition

Percentage inhibition was determined using the formula:

% Inhibition = Absorbance (control) - Absorbance (test) X 100 / Absorbance (control)

Note: IC_{50} is calculated using Graph pad prism 6.

α-Glucosidase Inhibition Assay
This assay measured inhibition of alpha-glucosidase, which converts disaccharides into glucose. The inhibitory potential of the test item against α-glucosidase was evaluated using an *in vitro* cell-free enzyme assay, employing acarbose as the reference standard.

Phosphate Buffer Preparation (80 mM, pH 7.0)

Phosphate buffer (80 mM, pH 7.0) was formulated by dissolving sodium dihydrogen phosphate A.R. ($NaH_2PO_4 \cdot 2H_2O$; 12.48 g/L; solution A), and disodium hydrogen phosphate anhydrous (Na_2HPO_4; 11.35 g/L; solution B). These solutions were blended at a 39:61 (v/v) ratio, with the final volume brought to 200 mL using deionized water.

Preparation of 37 mM Sucrose Substrate Solution
Sucrose (1.2665 g) was dissolved in 100 mL of 80 mM phosphate buffer (pH 7.0) with thorough mixing until fully dissolved.

Preparation of Standard Solution
Acarbose (50 mg) was dissolved in 50 mL of phosphate buffer and serially diluted to achieve a working concentration of 32 µg/mL in phosphate buffer (pH 7.0).

Preparation of Test Sample
The test sample was used directly in the assay without dilution.

α-Glucosidase Inhibition Assay Procedure
α-Glucosidase solution (50 µL) was combined with 250 µL of phosphate buffer or test sample and incubated at 37°C for 30 min. Sucrose substrate (500 µL) was then added, followed by incubation at 37°C for 20 min. The reaction was terminated by boiling for 2 min, cooled to room temperature, and glucose levels were estimated using the glucose oxidase method.

Glucose estimation (Glucose Oxidase Method)
Sample (100 µL) was mixed with 500 µL of glucose reagent (Glucose reagent kit), incubated at room temperature for 10 min, and absorbance was measured at 510 nm.

Calculation of Inhibition

Percentage inhibition was determined using the formula:

% Inhibition = Absorbance (control) - Absorbance (test) X 100 / Absorbance (control)

Note: IC_{50} is calculated using Graph pad prism 6.

RESULTS

Glucose Uptake Assay results in L6 cells

Estimation of 2DG6P (Standard Curve)
A standard calibration curve was generated using serial concentrations of 2DG6P ranging from 0.625 to 20 µM. The luminescence intensity increased proportionally with increasing concentrations of 2DG6P, demonstrating a linear relationship between relative light units (RLU) and 2DG6P concentration. The generated standard curve showed good linearity with an R^2 value of approximately 0.998, as shown in Table 3.

The graph demonstrated a concentration-dependent increase in luminescence signal with increasing concentrations of 2DG6P as shown in Figure 1.

Effect of Test Item on Glucose Uptake in L6 Cells
The glucose uptake activity of the test item was evaluated in differentiated L6 myotubes using the 2DG uptake assay. Insulin (100 nM) was used as the positive control and produced a 4.28-fold increase in glucose uptake compared to the basal control. Jamun Neem Karela Kwath juice enhanced glucose uptake at all tested concentrations. The highest stimulation activity was observed at 2% concentration with a 3.48-fold increase over the basal control, as shown in Table 4.

Relative production of 2DG6P by test item, Jamun Neem Karela Kwath Juice and Insulin
The graph showed increased glucose uptake activity in Jamun Neem Karela Kwath Juice -treated cells compared to basal control. The stimulatory activity ranged from 3.08-fold to 3.48-fold depending on concentration, as shown in Figure 2.

Cytotoxicity Evaluation in L6 cells
Prior to estimation of Glucose uptake, the cytotoxic effects of the test item on L6 Cells were evaluated by MTT assay following 24 h treatment. The test item exhibited concentration-dependent inhibition of cell viability. Jamun Neem Karela Kwath Juice showed an IC_{50} value of 11.93%. The standard compound Doxorubicin exhibited an IC_{50} value of 12.52 µM under identical experimental conditions as shown in Table 5.

IC_{50} Graph for Cytotoxicity Assessment
The IC_{50} graph demonstrated concentration-dependent cytotoxicity of the test item in L6 cells. Concentrations maintaining approximately 80% cell viability were considered suitable for subsequent glucose uptake studies, as shown in Figure 3.

Note: L6 cells were exposed to the test sample for 24 hours, and the test item, Jamun Neem Karela Kwath Juice showed inhibition at IC_{50} value of 11.93%. Under identical experimental conditions, the reference compound Doxorubicin exhibited an IC_{50} value of 12.52 µM.

The concentration having 80% cell viability will be taken for downstream assay.

Effects on α-Amylase Activity

The evaluated test item, namely Jamun Neem Karela Kwath Juice, demonstrated inhibitory activity against α-amylase in a concentration-dependent manner, indicating its potential role in glycemic control. The test item exhibited a gradual increase in percentage inhibition with increasing concentration. The standard drug, Acarbose, exhibited the highest inhibition among all groups**.** The test item showed significant inhibition of alpha-amylase with maximum % inhibition: 31.24%. The standard drug, Acarbose, showed a % inhibition of 61.94%, as shown in Table 6.

Note: IC_{50} value was not determined as maximum inhibition observed was < 50%

Effects on α-Glucosidase Activity

The evaluated test item, namely Jamun Neem Karela Kwath Juice, demonstrated inhibitory potential against α-glucosidase in a concentration-dependent manner, indicating its potential role in glycemic control. The test item exhibited a gradual increase in percentage inhibition with increasing concentration. The standard drug, Acarbose, exhibited the highest inhibition among all groups. The test item, Jamun Neem Karela Kwath Juice showed inhibition activity of alpha-glucosidase with maximum % inhibition: 47.27%. The standard drug Acarbose showed significant inhibitory activity with maximum % inhibition: 81.93%, respectively, as shown in Table 7.

Note: IC_{50} value was not determined as maximum inhibition observed was < 50%.

Table 3: Estimation of 2DG6P (standard curve)

Conc. of 2DG6P (µM)	OD		Mean OD	Normalization
blank	3599	3994	3796.5	0
0.625	10157	10153	10155	6358.5
1.25	17730	17728	17727.5	13931
2.5	31672	31941	31806.5	28010
5	72039	67212	69625.5	65829
10	137755	139618	138686.5	134890
20	249668	236287	242977.5	239181

Table 4: Glucose uptake activity of Test Sample

Samples	Conc.	OD	Production of 2DG6P	Stimulation activity (in fold)
Control	0	110500	8.78	1.00
Insulin	100nM	453203	37.61	4.28
Jamun Neem Karela Kwath Juice	2%	369370	30.56	3.48
	1%	335299	27.69	3.15
	0.50%	327903	27.07	3.08

Table 5: Cytotoxicity of Test Item in L6 Cells

Cytotoxicity of Test Item in L6 Cells								
Compound Name	**Conc. In %**	**n = 1**		**n = 2**		**Mean % Inhibition**	**Standard Deviation**	**IC_{50}**
		OD at 590nm	**% Inhibition**	**OD at 590nm**	**% Inhibition**			
Control	0	1.031	0.00	1.022	0.00	0.00	0.000	
Jamun Neem Karela Kwath Juice	0.78	0.887	13.97	0.878	14.09	14.03	0.087	
	1.56	0.799	22.50	0.762	25.44	23.97	2.077	**11.93%**
	3.125	0.662	35.79	0.658	35.62	35.70	0.123	
	6.25	0.548	46.85	0.578	43.44	45.15	2.407	
	12.5	0.474	54.03	0.443	56.65	55.34	1.859	
	25	0.344	66.63	0.351	65.66	66.14	0.692	
	50	0.113	89.04	0.145	85.81	87.43	2.282	
Standard (Doxorubicin) (µM)	1.5	0.881	14.55	0.894	12.52	13.54	1.432	**12.52 µM**
	3.125	0.786	23.76	0.778	23.87	23.82	0.079	
	6.25	0.658	36.18	0.668	34.64	35.41	1.089	
	12.5	0.555	46.17	0.568	44.42	45.30	1.235	
	25	0.442	57.13	0.464	54.60	55.86	1.789	
	50	0.343	66.73	0.381	62.72	64.73	2.836	
	100	0.215	79.15	0.243	76.22	77.68	2.067	

Table 6: Summary of Alpha-amylase Inhibitory Activity

Sample Name	Conc. (µg/ml)	Absorbance (405nm)	% Inhibition
Control	0	1.44	0
Acarbose (Standard)	0.19	1.34	7.26
	0.38	1.20	16.95
	0.76	1.08	25.25
	1.56	0.95	34.25
	3.12	0.84	41.86
	6.25	0.55	61.94
Jamun Neem Karela Kwath Juice	0.3906	1.01	5.65
	0.78125	0.98	8.25
	1.525	0.92	13.94
	3.125	0.90	15.81
	6.25	0.84	21.14
	12.5	0.85	20.77
	25	0.74	31.24

Note: IC_{50} value was not determined as maximum inhibition observed was < 50%

Table 7: Summary of Alpha-glucosidase Inhibitory Activity

Sample Name	Conc. (µg/ml)	Absorbance (405nm)	% Inhibition
Control	0	1.024	0
Acarbose (Standard)	1	0.819	20.02
	2	0.561	45.21
	4	0.412	59.77
	8	0.312	69.53
	16	0.254	75.20
	32	0.185	81.93
Jamun Neem Karela Kwath Juice	0.78125	0.880	14.06
	1.5625	0.810	20.90
	3.125	0.760	25.78
	6.25	0.690	32.62
	12.5	0.610	40.43
	25	0.540	47.27

Note: IC_{50} value was not determined as maximum inhibition observed was < 50%

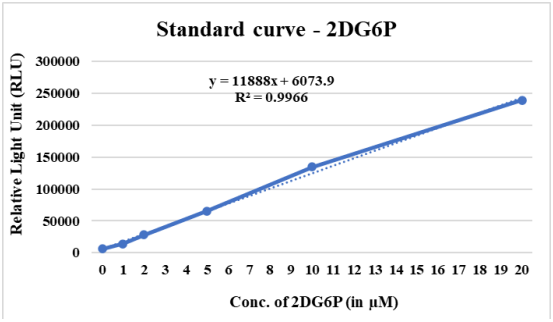

Figure 1: Standard curve for 2DG6P

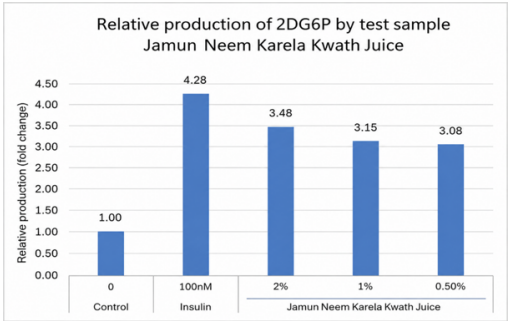

Figure 2: Relative production of 2DG6P by test item, Jamun Neem Karela Kwath Juice and Insulin

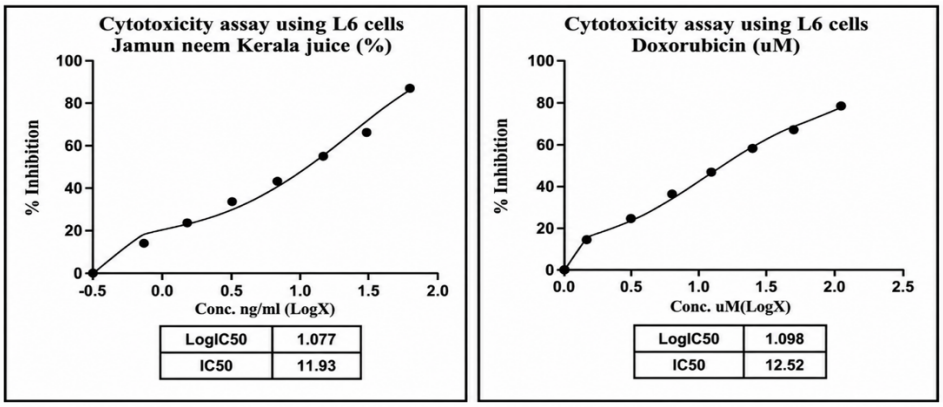

Figure 3: IC_{50} Graph for Cytotoxicity Assessment

DISCUSSION

The rising global incidence of Type 2 Diabetes Mellitus has emphasized the need for safer and more effective therapeutic interventions capable of controlling hyperglycemia and minimizing diabetes-associated complications.[1] Postprandial hyperglycemia is a major contributor to the development and progression of complications such as nephropathy, neuropathy, retinopathy, and cardiovascular disorders.[1,4] Consequently, therapeutic strategies aimed at delaying carbohydrate digestion and improving peripheral glucose utilization are considered beneficial in diabetes management.[5] In this regard, polyherbal formulations have attracted considerable scientific interest due to their multitargeted mechanisms, synergistic therapeutic actions, and relatively lower adverse effects.[8,11,12] The present study evaluated the glycemic control potential of Jamun Neem Karela Kwath Juice using multiple *in vitro* models, including α-amylase inhibition, α-glucosidase inhibition, glucose uptake stimulation in L6 myotubes, and cytotoxicity assessment.

Inhibition of α-amylase and α-glucosidase is a well-established strategy for reducing postprandial blood glucose levels.[13] α-Amylase catalyzes the hydrolysis of complex polysaccharides into oligosaccharides, whereas α-glucosidase further converts disaccharides into absorbable glucose molecules in the intestine.[5] In the present study, Jamun Neem Karela Kwath Juice demonstrated concentration-dependent inhibition of both enzymes, suggesting its potent role in managing healthy blood sugar balance. The α-amylase inhibition assay revealed significant inhibitory activity with a maximum inhibition of 31.24%. The progressive increase in inhibition with increasing concentration indicated dose-dependent enzyme suppression.[14,15] Although acarbose exhibited greater potency at lower concentrations, the substantial inhibitory effect of the formulation highlights its ability to interfere with carbohydrate digestion. This activity may be attributed to the combined action of phytoconstituents present in the herbal ingredients of the formulation.[16] Similarly, Jamun Neem Karela Kwath Juice exhibited potent α-glucosidase inhibitory activity with maximum inhibition of 47.27%. The inhibitory effect increased consistently with concentration, indicating strong suppression of glucose-generating enzymatic pathways.[4] Under the tested conditions, the formulation demonstrated greater maximum inhibition than acarbose. Since α-glucosidase is involved in the terminal stage of carbohydrate digestion, inhibition of this enzyme is particularly important for reducing intestinal glucose absorption and limiting postprandial glucose excursions.[5] Therefore, the strong α-glucosidase inhibitory activity observed in this study suggested that Jamun Neem Karela Kwath Juice may contribute effectively to glycemic regulation following carbohydrate intake.

In addition to enzyme inhibition, enhancement of peripheral glucose utilization is a critical aspect of managing healthy blood sugar balance.[17] Skeletal muscle is a major site of insulin-mediated glucose disposal, and impaired glucose uptake in muscle cells is a characteristic feature of insulin resistance in T2DM.[17,18] Therefore, the glucose uptake assay performed in differentiated L6 myotubes provides important insight into the insulin-sensitizing potential of the formulation.[17] The glucose uptake assay demonstrated that Jamun Neem Karela Kwath Juice enhanced glucose uptake in L6 cells at all tested concentrations. The highest activity was observed at 2% concentration, producing a 3.48-fold increase in glucose uptake compared with basal control, whereas insulin treatment resulted in a 4.28-fold increase. These findings demonstrated that the formulation promotes glucose utilization in skeletal muscle cells. The enhancement of glucose uptake suggests improved glucose transport and intracellular glucose utilization, which is particularly relevant in insulin-resistant conditions where impaired glucose uptake contributes to hyperglycemia.[17,18] The observed stimulatory activity therefore supports the insulin-sensitizing potential of the formulation and complements the enzyme inhibitory effects demonstrated in earlier assays.[17,19] The standard curve of 2DG6P generated during the glucose uptake assay showed strong linearity with an R^2 value of approximately 0.998, confirming the reliability and accuracy of the assay system. The concentration-dependent increase in luminescence further validated the sensitivity of the experimental model used for glucose uptake quantification. These observations supported the validity of the glucose uptake enhancement produced by Jamun Neem Karela Kwath Juice.

Assessment of cytotoxicity is essential for determining the biological safety of a formulation prior to mechanistic cellular studies.[20] The MTT assay performed in L6 cells demonstrated concentration-dependent reduction in cell viability following treatment with Jamun Neem Karela Kwath Juice. The formulation exhibited an IC_{50} value of 11.93%, whereas the standard cytotoxic agent doxorubicin showed an IC_{50} value of 12.52 µM under identical conditions. Importantly, lower concentrations of the formulation maintained approximately 80% cell viability and were therefore considered appropriate for glucose uptake studies. These findings demonstrated that the concentrations used for evaluating glucose uptake were within an acceptable range and did not produce excessive cytotoxicity. Consequently, the cytotoxicity results support the reliability of the observed biological activity. Overall, the findings of the present study demonstrated that Jamun Neem Karela Kwath Juice exerts a potent role in managing healthy blood sugar balance through multiple complementary mechanisms. The formulation effectively inhibited α-amylase and α-glucosidase enzymes involved in carbohydrate digestion while simultaneously enhancing glucose uptake in skeletal muscle cells. Such multitargeted activity is advantageous for maintaining healthy blood sugar balance.[8,11] The polyherbal composition of the formulation may further provide synergistic therapeutic benefits through the combined action of diverse phytoconstituents.[11,21] Collectively, the present study provides experimental evidence supporting the potential of Jamun Neem Karela Kwath Juice to support healthy blood sugar balance. The findings established a scientific basis for its traditional therapeutic use and suggested that the formulation may aid in regulating healthy blood sugar balance and improving peripheral glucose utilization.[22]

CONCLUSION

The present study demonstrated that Multani Jamun Neem Karela Kwath Juice exhibits robust glycemic control potential through complementary mechanistic pathways. The formulation demonstrated concentration-dependent inhibition of α-amylase and α-glucosidase, underscoring its capacity to retard carbohydrate digestion and attenuate postprandial glucose absorption. Concurrently, Multani Jamun Neem Karela Kwath Juice enhanced glucose uptake in differentiated L6 skeletal muscle cells, indicative of improved peripheral glucose disposal and insulin-sensitizing effects. Cytotoxicity assessment via MTT assay further confirmed that biologically active concentrations of the formulation maintained acceptable cellular viability for downstream functional assays.

The combined enzyme-inhibitory and glucose-uptake-enhancing activities observed in this study support the therapeutic potential of Multani Jamun Neem Karela Kwath Juice in promoting healthy blood sugar balance. The findings sync with scientific validation for the traditional use of its constituent in the management of metabolic conditions and demonstrate the test item, Multani

Jamun Neem Karela Kwath Juice, as a potent candidate for optimal glycemic control**.**

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