



The Study of glucose uptake activity in *Alli chooranam* (*Nymphaea nouchali burm.f*) in L6 cell lines

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Abstract

The Alli (Nymphaea nouchali burm.f) is a well-known medicinal plant used in Siddha system of medicine for the treatment of Diabetes mellitus. The effectiveness of glucose lowering herbs is by stimulating glucose uptake by adipose and muscle tissues, hindering the glucose absorption from intestine and prohibiting glucose production from hepatocytes. This study was carried out to determine the glucose uptake activity using in vitro cell culturing methods on L6 cell lines. Treatment of the cells with plant extract significantly increased glucose uptake, the highest concentration (100 µg/ml) giving 46.2%. (P<0.05). The study concluded that aqueous extract increases glucose uptake confirms the anti-diabetic activity of Alli chooranam (N.nouchali).

Keywords: *Alli, Diabetes, Glucose uptake, L6Cell line study, Nymphaea nouchali, Siddha system.*

Introduction

The diabetes is a progressive disease and with disease progression life style management may not be adequate, though still an integral part management, pharmacotherapy then become inevitable. World Health Organization reported (2009) that about 422 million people worldwide have suffered in diabetes & 1.6 million deaths occurred in attributed to Diabetes in every year. The Glucose uptake by GLUTs is the first step of glucose metabolism. In skeletal muscles, both insulin and contractile activity stimulate translocation of glucose transporter GLUT -4 protein from an intracellular membrane pool to the plasma membrane. Resistance to this stimulatory effect of Insulin is a major pathological feature of

Diabetes. The L6 cells are represent a good model for glucose uptake because they have been used extensively to elucidate the mechanism of glucose uptake in muscle, have an intact insulin signaling pathway and express the insulin-sensitive GLUT-4^[6]

The Preparation and standardization of medicinal herbs are urgently needed for future studies and therapies. The *Alli (Nymphaea nouchali Bum,f)* large aquatic herb of the family Nymphaeaceae, commonly known as Water lily (*Alli* in Tamil) is a medicinal plant widely used in Siddha which is known for its anti-diabetic activity mentioned in classical literatures. Plant distributed throughout India, Kashmir, Siberia & Europe. An infusion of

the flower and fruit is given in diarrhea and diaphoretic. Flowers are reputed to be anti-aphrodisiac.

Although anti-diabetic activity of Alli have been reported, lack of sufficient literature on rhizome of Alli (*Nymphaea nouchali* Burm.f) in diabetes. This study was focused on evaluating the glucose uptake activity of rhizome and flowers of Alli in L6 cell lines.

Materials and Methods

2.1. Collection and Authentication of Plant

The flower & rhizome of Alli (*Nymphaea nouchali* Burm.f) freshly collected from various places of Kerala. Identified and authenticated by the Medicinal Botanists at Government Siddha Medical College and Hospital, Palayamkottai. This herbal formulations purified according to the suitable procedure methods described in Siddha classical literature. The drug is dried and subjected to size reduction to get uniform coarse powder.

2.2. Preparation of cell culture

The L6 (rat myoblast cell line) cells was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium. The cell line was cultured in 25 cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO₂ incubator.

Results

Glucose uptake in L6 cell lines was studied in invitro.

Table.1. Shows results of glucose uptake of rhizome and flowers of Alli (*Nymphaea nouchali* Burm.f)

Concentration of sample(µg/mL)	Absorbance	Glucose (mg/dl)	% glucose uptake
Control	0.1077	36.12882	0.00
Sample code: AC-50			
25	0.1202	40.32204	10.39933
50	0.1757	58.93995	38.70233
100	0.2013	67.52768	46.49776

Average OD of Standard = 0.2981

2.3. Glucose uptake assay

The cells were trypsinized (500µl of 0.025% Trypsin in PBS/ 0.5mm EDTA solution for 2 minutes and passaged to T flasks in complete aseptic conditions. The cells were then sub cultured to a 24 well plate. After attaining 80% confluency cells were kept in DMEM without glucose for 24 hours. Samples were added to grown cells at a final concentration of 25 µg/mL, 50 µg/mL and 100 µg/mL from a stock solution of 1mg/mL and incubated for 24 hours in DMEM containing 300mM glucose. An untreated control with high glucose was also maintained. After incubation cells were isolated by spinning at 6000 rpm for 10 minutes. Supernatant was discarded and 200µl of cell lysis buffer (1mTris Hcl, 0.25M EDTA, 2M Nacl, 0.5% Triton) was added.

2.4. Calculation

The incubation was done for 30 minutes at 4°C and the glucose uptake was estimated using high sensitivity glucose oxidase kit method (Coral Clinical Systems: Lot No; RGLU1091). All experiments were repeated in triplicates and mean average was used for calculations.

$$\text{Total Glucose in mg/dL} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 100$$

$$\% \text{ Glucose uptake} = \frac{\text{OD of Test} - \text{OD of Control}}{\text{OD of Test}} \times 100$$

2.5. Statistical analysis

Statistical analysis were carried out with one way analysis of variance and the differences between the samples determined by Dunnett's multiple comparison test. The data were expressed as the mean ± standard deviation and values were considered significant at p < 0.05.

Table.2.Average glucose in varying concentration of plant extract

Concentration	OD1	OD2	OD3	Glucose (mg/dL)	Glucose (mg/dL)	Glucose (mg/dL)	Average (Glucose (mg/dL))	stdev	Error
Control	0.1114	0.1027	0.109	37.37	34.4515	36.56491	36.1288	1.507333	0.502444
25	0.1161	0.1254	0.1191	38.9466	42.0664	39.953	40.322	1.592297	0.530766
50	0.1798	0.1646	0.1827	60.3153	55.2163	61.2881	58.9399	3.261209	1.08707
100	0.2077	0.211	0.1852	69.6746	70.7816	62.1268	67.52767	4.709924	1.569975

Figure.1 Average glucose in varying concentration of plant extract

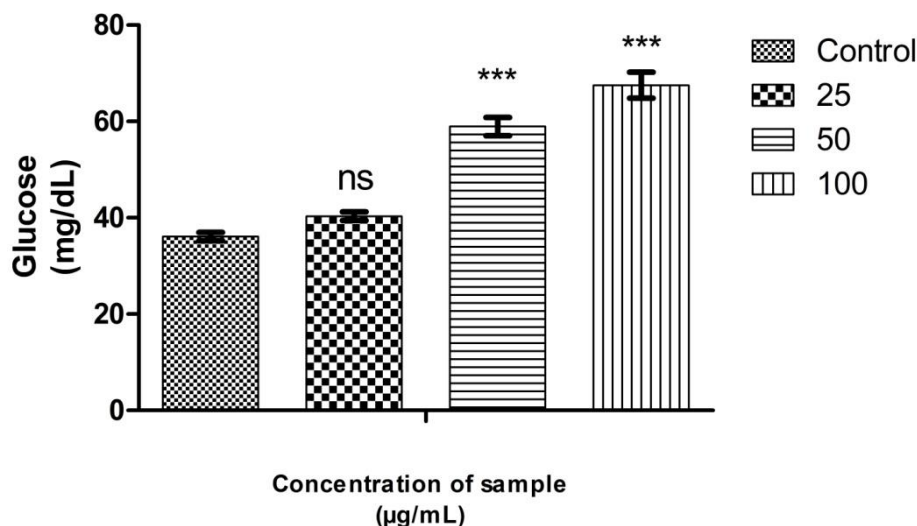
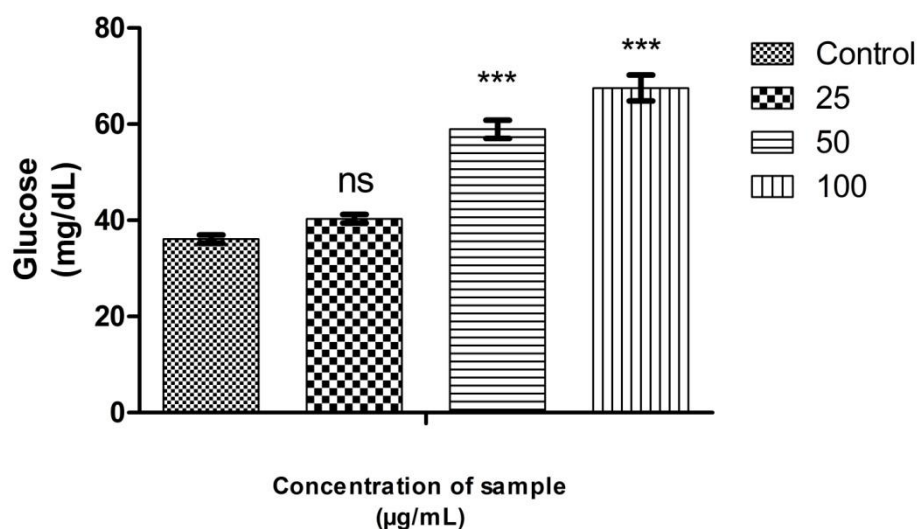


Table 3 Percentage of glucose uptake in varying concentration of plant extract

Concentration	Percentage of glucose uptake 1	Percentage of glucose uptake 2	Percentage of glucose uptake 3	Average	St dev	Std error
Control	0	0	0	0	0	0
25	4.048234	18.10207	8.480269	10.21019	7.18485	2.39495
50	38.04227	37.60632	40.33935	38.66265	1.46834	0.489447
100	46.36495	51.32701	41.14471	46.27889	5.091698	1.697233

Figure.2 Percentage of glucose uptake in varying concentration of plant extract



Discussion

In the present study significant glucose uptake was recorded for L6 cells at different concentrations 50 µg/ml and 100µg/ml. With the highest concentration (100 µg/ml) giving increased uptake 46.2% (P <0.0001). The result obtained in this study on glucose uptake using L6 cells demonstrated that Alli (*Nymphaea nouchali* Burm.f) increased glucose uptake in L6 cell lines.

Conclusion

Findings suggests that Alli (*Nymphaea nouchali* Burm.f) exhibits in anti-diabetic activity might be due its phytochemical constituents. This supports the usage of this plant as an alternative in the treatment of Diabetes. More studies are required to confirm its mechanism of action thereby providing alternative therapy to contribute normal blood sugar and prevention of diabetic complications.

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Conflict of interest

The authors declare no conflict of interest in the present work.

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