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Study of Glucose Uptake Activity of *Solanum xantohocarpum* in L-6 Cell Lines

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Abstract: To evaluate the glucose uptake potential of ethanolic extract of different parts such as aerial part, ripe fruit and root of *Solanum xanthocarpum* using L-6 cell lines.This study also evaluated cytotoxicity of extracts by MTT assay.The results showed the extracts did not show any cytotoxicity and possessed effective glucose uptake potential. The ethanolic extract of fruit of *Solanum xanthocarpum* showed better glucose uptake potential when compared to ethanolic extract of aerial part and root.

Key words: Glucose Uptake · Mttassay · Cytotoxicity · *Solanum xanthocarpum*

INTRODUCTION

There is sufficient number of drugs in different systems of medicine for the management of diabetes but the incidence of type-II diabetes is too high. The mainstay of the treatment includes oral hypoglycaemic and insulin [1,2]. They have many side effects with their long term usage [3,4]. Herbal drugs may be preferred as alternatives or adjuncts' because of their relative safety, efficacy and affordability. Herbal drugs are widely prescribed and used, in many forms, in different systems of medicine. They are used though we act data substantiating their usefulness and efficacy. World Health Organization approves of the management of the diabetes mellitus [5].*Solanum xanthocarpum* is used in medicine in various forms, such as decoction, electuary, ghrita. Its roots are one of the constituents of well known Ayurvedic preparation "Dasmula Ashva.In traditional systems of medicine, different parts like leaves, stem, flower, root, seeds and the plant as a whole are used. The drug is used as antiasthmatic, hypoglycaemic, anti-inflammatory,anti-tussive, antipyretic, antispasmodic, antihistaminic and hypotensive. The whole plant is useful in vitiated conditions of vata and kapha, helminthiasis, dental caries, inflammations, flatulence, constipation, dyspepsia, anorexia, leprosy, skin diseases, hypertension, fever, cough, asthma, bronchitis, hiccough, lumbago, haemorrhoids and epilepsy[6]. Phytochemical studies on the genus Solanum showed the presence of alkaloids, flavonoids, steroidal glycoside and steroidal saponins. The flavanoids quercetin and apigenin glycosides are the major chemical constituents which are present in the fruits of *Solanum xanthocarpum* [7].Although the hypoglycemic effect of *Solanum xanthocarpum* has been reported the extract mechanism of this effect has yet to be elucidated. Therefore we evaluated the effect of ethanolic extract of different parts such as aerial part, fruit, root of *Solanum xanthocarpum* on glucose uptake activity through glucose transport in skeletal muscle cells.

MATERIALS AND METHODS

Materials: 3-(4, 5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Bovine Serum Albumin (BSA), 2Deoxy- glucose, Dulbecco's Modified Eagle's Medium (DMEM), Metformin and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Antibiotics from Hi-Media Laboratories Ltd., Mumbai. Insulin (Torrent Pharmaceuticals, 40IU/ml) was purchased from a drug store. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

Cell Lines and Culture Medium: L-6 (Rat, Skeletal muscle) cell culture was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells of L-6 were

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cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Method

Preparation of Extract: The authenticated dried materials was used for extraction, initially the dried materials were made into fine powder; the powder was packed in filter paper and loaded into the thimble. The solvent methanol of 2.5 liter was used for extraction was poured into flask (distilling pot). The soxhlet extraction was performed for 10 hours. Later the extracted solvent was evaporated under reduced pressure to get waxy material. The extractive value of the extraction was obtained by using the relation [8,9].

$$\% \text{ of extraction} = \frac{\text{Weight of dried extract}}{\text{Weight of fresh dried material}} \times 100$$

Preliminary Phytochemical Analysis: Preliminary phytochemical analysis was carried on ethanolic extracts of *Solanum xanthocarpum* as method described [10,11].

Preparation of Test Solutions: For *in vitro* studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of Cytotoxicity of Test Extracts in L-6 by MTT Assay: The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line [12].

$$\% \text{ Growth Inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

***In vitro* Glucose Uptake Assay:** Glucose uptake activity of test drugs were determined in differentiated L6 cells. In brief, the 24 hr cell cultures with 70-80% confluency in 40mm petri plates were allowed to differentiate by maintaining in DMEM with 2% FBS for 4-6 days. The extent of differentiation was established by observing multinucleation of cells. The differentiated cells were serum starved over night and at the time of experiment cells were washed with HEPES buffered Krebs Ringer Phosphate solution (KRP buffer) once and incubated with KRP buffer with 0.1% BSA for 30min at 37°C. Cells were treated with different non-toxic concentrations of test and standard drugs for 30 min along with negative controls at 37°C. 20µl of D-glucose solution was added simultaneously to each well and incubated at 37°C for 30 min. After incubation, the uptake of the glucose was terminated by aspiration of solutions from wells and washing thrice with ice-cold KRP buffer solution. Cells were lysed with 0.1M NaOH solution and an aliquot of cell lysates were used to measure the cell-associated glucose. The glucose levels in cell lysates were measured using glucose assay kit (Biovision Inc, USA). Three independent experimental values in duplicates were taken to determine the percentage enhancement of glucose uptake over controls [13,14].

RESULTS

The yield of ethanolic extract of different parts such as aerial part, fruits and root of *Solanum xanthocarpum* are shown in Table 1.

Table 1: yield of ethanolic extract of *Solanum xanthocarpum*

Sl.No	Test material	% yield (w/w)
1	*Solanum xanthocarpum* -Aerial parts	9.44
2	*Solanum xanthocarpum* -Roots	9.64
3	*Solanum xanthocarpum* Fruits	47.20

Table 2: Phytochemical analysis of *Solanum xanthocarpum* ethanolic extract

Sl/No	Test	SXA	SXR	SXF
1	Test for carbohydrates			
	a. Molisch's test	+	+	+
2	Test for Glycosides			
	a. Keller-Killiani test	+	-	-
3	Test for Saponins			
	a. Foam test	-	-	+
4	Test for Alkaloids			
	a. Mayer's test	-	+	-
	b. Dragendrodroff's test	-	+	-
5	Test for Flavonoids			
	a. Alkaline reagent test	+	+	+
6	Test for Phenolics and Tannins			
	a. Ferric chloride test	-	+	+
	b. Test for Tannins	+	+	+
7	Test for Phytosterols and Triterpenoids			
	a. Leiberman-Bucharat test	-	+	+
	b. Salkowaski test	-	+	+
8	Test for fixed oils and fats			
	a. Oily spot test	-	-	-

(+) Present, (-) Absent
SXA: *Solanum xanthocarpum* aerial parts
SXR: *Solanum xanthocarpum* roots
SXF: *Solanum xanthocarpum* fruits

Solanum xanthocarpum aerial parts ethanolic extract showed the presence of carbohydrate, glycoside, flavanoids, tannins. The ethanolic extract of *Solanum xanthocarpum* roots showed the presence of carbohydrate, alkaloids, flavanoids, phenols, tannins phytosterols and triterpenoids. The ethanolic extract of *Solanum xanthocarpum* fruits showed the presence of carbohydrate, saponin, flavanoids, phenols, tannins, phytosterols and triterpenoids The phytochemical constituents present in the ethanolic extarct of different parts such as aerial part, fruit and root of *Solanum xanthocarpum* are shown in Table 2

The cytotoxicity of extracts was evaluated by MTT assay The CTC_{50} value of ethanolic extract of different parts such as aerial part,root and fruit of *Solanum xanthocarpum* are 148.30, 93.70 and 90.00 in L 6 cell lines and results are shown in Table 3

Among the six extracts tested, SXA and SXF were found to have potent activity in enhancing the glucose uptake in L-6 myotubes with 38.43 ± 4.94 and 43.81 ± 3.31 percent uptake over controls. Insulin and metformin showed 131.50 ± 17.62 and 68.35 ± 11.45 percentage glucose uptake over control respectively results are shown in Table 4 and Fig. 1.

Table 3: Cytotoxic properties of test drugs on L6 cell line.

Sl. No	Name of the test extract	Test Concn (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	SXA	1000	71.77 ±2.16	
		500	70.56±3.21	
		250	61.65±1.71	
		125	45.38±4.94	
		62.5	34.62±2.55	148.30 ± 20.20
2	SXR	1000	62.18±5.42	
		500	31.21±3.90	
		250	30.31±3.81	
		125	28.62±2.12	
		62.5	25.96±0.12	93.70 ± 7.80
3	SXF	1000	69.84 ± 5.61	
		500	68.23 ± 3.78	
		250	68.15 ± 3.71	
		125	64.54 ± 2.31	
		62.5	30.24 ± 3.19	90.00 ± 0.00

Table 4: *In vitro* glucose uptake studies in L-6 cell line

Sl.No	Name of the extract	Test concentration (µg/ml)	% glucose uptake over control
1.	SXA	100	38.43 ± 4.94
2.	SXR	100	2.10 ± 1.00
3.	SXF	100	43.81 ± 3.31
4.	Insulin	1 IU/ml	131.50 ± 17.62
5.	Metformin	100	68.35 ± 11.45

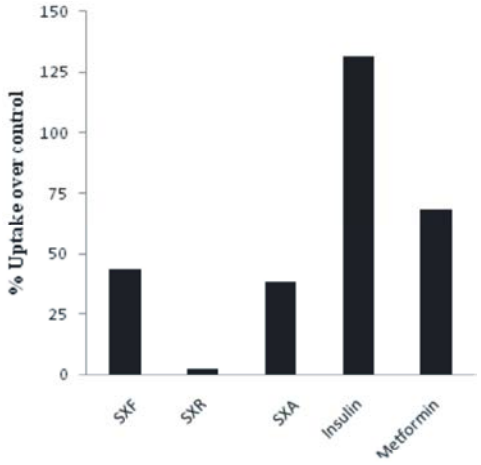

Fig. 1: Enhancement glucose uptake by test extracts

DISCUSSION

The cytotoxicity of extracts was evaluated by MTT assay. The results show the extracts did not show any cytotoxicity. Glucose utilization in L-6 cell lines was studied by *in vitro* methods. The results show the extracts has effective glucose uptake potential GLUT-4 is an important component of the insulin signal transduction network that regulates glucose transport.It is an intracellularly sequestered insulin responsive glucose transporter.GLUT-4 might be involved in the increased uptake of glucose by L-6 cell lines in the presence of ethanolic extract of *Solanum xanthocarpum*. Results were compared with insulin (injectable anti diabetic) and metformin (oral anti diabetic), which were used as the standard anti diabetic drugs. Insulin (1IU/ml) and metformin (100 ìg/ml) enhance the glucose uptake by 131.50 % and 68.35% over control. Skeletal muscle is the tissue comprising the bulk of the body's musculature and is the major site of glucose uptake and utilisation. Type-2 diabetes is inability of the body to utilise insulin, which may result in defective skeletal muscle uptake of glucose [15]. Defects in insulin stimulated skeletal muscle glucose uptake are common pathological states in non-insulin-dependent diabetes mellitus (NIDDM) [16]. The major glucose transporter expressed in skeletal muscle and adipose tissue is GLUT4. In acute deficiency of insulin, GLUT4 is rapidly translocated from an intracellular membrane storage site to the plasma membrane [17]. The results obtained in the present study demonstrate that the ethanolic extract of the fruit of *Solanum xanthocarpum* shows better glucose uptake when compared to the extracts of other parts of the plant under *in-vitro* condition. This maybe due phytochemical constituents present in fruit extract and also due to its effect on the receptors on the cell membrane in L-6cell lines. *In -vivo* studies are to be carried out to substantiate the *in-vitro* results on of *Solanum xanthocarpum*.

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