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NEGATIVE REGULATION OF GLUCOSE UPTAKE BY COSTUS PICTUS LEAVES EXTRACT IN 3T3- L1 CELL LINE

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ABSTRACT:
The leaf of Costus pictus is considered as an antidibetic in folklore medicine and is known to reduce the blood sugar, similar as insulin. The aim of the study is to investigate the effect of ethanolic extract of C. pictus leaf on glucose uptake by 3T3- L1 cell line (skeletal muscle cells) involved in glucose utilization. Ethanolic extract of C. pictus leaf was analyzed to study GLUT4 translocation and glucose uptake activity, but it has no direct peripheral action at 300 µg/ml dose comparable with insulin and metformin. The glucose uptake was not enhanced by the extract of C. pictus leaf.

Introduction:

Diabetes is the metabolic disorder of the metabolism of carbohydrates, protein and fat and caused due to absolute or relative degree of insulin resistance. [1] It has became an epidemic with a worldwide incidence of 5% the number likely to increase from 135 million in 1995 to 300 million in 2025. [2] There are more than 30 milion people with dubieties mellitus in India and the incidence is increasing.[3] Insulin and various type of hypoglycemic agents such as biguanids and sulfonylureas, old and new, are available for the treatment of diabetes. However, none of these medications is ideal due to toxic side effects and, in some cases diminution of response after prolong use. [4] So, with increasing incidence of diabetes mellitus in rural population throughout the world and due to adverse effect of synthetic medicine, there is a clear need for development of indigenous, inexpensive botanical source of antidiabetic crude of purified drug.[5] Many plants reported useful for the treatment of diabetes mellitus in ayurveda system of medicine have been tested on experimental animals.[6] Costus pictus (Zingiberaceae) syn. Costus mexicanus (DC) Greene, commonly known as spiral ginger, stepladder or insulin plants, originated in Mexico. In India, it is grown in gardens as an ornamental plant, especially in Kerala, at every household. The species is similar to Costus pictus (Koenig), which is commonly known as "Channakkoova" in Kerala, and leaves of both these species are being used by many people in Kerala for diabetes mellitus. [7,8]

Powder of the leaves of the medicinal plant C.pictus , known to posses therapeutic effect when administered to steptozotocin- induced diabetic rats, is found to reduce blood glucose level by 21% after 15 days of administration.[9] The methanol extract of C.pictus leaf is used to lower blood glucose level in

alloxan- induced diabetic rats.[10] The anti hyperglycemic and insulin secretory activity of an aqueous extract of C.pictus leaf was investigated in streptozotocine- induced diabetic rats.[11] Antioxidant[12,13] and diuretic activity[14] have been evaluated. The antidiabetic property of C.pictus might be due to one or many mechanisms, viz.., promotion of insulin, decrease in absorption of intestinal glucose, inhibition of gluconeogenesis, promotion of peripheral utilization of glucose.

Although antidiabetic activity of C.pictus extract has been reported, the extract mechanism of this effect is yet to be elucidated. The 3T3- L1 cell line is the best characterized cellular model origin to study glucose uptake and GLUT4 translocation. [15, 16] Therefore, we evaluated the effect of ethanolic extract of C.pictus leaf on glucose uptake through glucose transport in 3T3- L1 cell line.

MATERIALS AND METHODS

Plant material

The leaves of C.pictus were collected from Indian Institute of Integrative Medicine (RRL), Medicinal Garden, Jammu. This Plant is identified and authenticated by botanist Dr. S. N. Sharma Department of Taxonomy, I.I.I.M, Jammu, India and a voucher specimen was deposited in the Herbarium of Department of Botany, IIIM Jammu. The voucher specimen was deposited at our department for future reference.

Chemicals and cells

Fetal bovine serum (FBS) was purched from Invitrogen (Carlsbad, CA, USA). Dulbecco's modified Eagle's medium (DMEM) and other culture products were purchased from GIBCO B (San Diego, CA, USA). Trypsine, versene, glucose in PBS solution (TPVG) solution, bovine serum albumin (BSA), insulin, metformin and glucose kit were obtained from Randox Laboratory Ltd. (Ardmore, United Kingdom); dibasic sodium hydrogen phosphate, sodium bicarbonate, magnesium chloride were from Randox Laboratory Ltd. (Mohali India) and SD Fine Chem. (Mumbai, India). All chemicals and solvents used were of analytical grade. The 3T3-L1 cell line (RAT) was obtained from National Centre for Cell Science (Pune, India).

Preparation of plants extract

The leaves of the C.pictus were washed thoroughly with tap water, shade- dried, cut into small pieces and crushed to moderately coarse powder. Extraction was done using 95% ethanol in a soxhlet apparatus for 8 hours. The extract was concentrated using rotary evaporator at 40-45°C under reduced pressure.

Cytotoxicity assay

The extract was separately dissolved in 1 ml of dimethyl sulfoxide (DMSO) and the volume was made up to 10 ml with maintenance medium to obtain a stock solution of 1 mg/ml concentration, sterilized by filtration and further dilution were made from the stock. The cytotoxicity assays were carried out using 0.1 ml of cell suspension containing 10,000 cells seeded in each well of a 96-well micrititre plate (Tarzans India Pvt. Ltd., Kolkata, India). Fresh medium containg different concentration of the test sample was added after 24 hours of seeding. Control wells were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 72 hours. The morphology of the cells was inspected under the microscope for detectable alterations, i.e., loss of monolayer, granulation and vacuolization in the cytoplasm. The cytopathogenic effect (CPE) was scored. The 50% cytotoxic concentration (CTC_{50}) was determined by the standard Microculture Tetrazolium MTT. [17, 19]

Preparation of cell culture

Monolayers of 3T3- L1 cells were maintained at subconflucent conditions in growth medium (DMEM with 4.5 g/l glucose, 100 U/ml streptomycin and 10% fetal bovine serum). Cells were maintained in a humidified 37°C incubator with ambient oxygen and 5% CO_2. Cells were maintained in a continuous passage by trypisinization of subconfluent culture using TPVG solution.

Glucose uptake by 3T3- L1 cell line

Glucose uptake activity was estimated by the methods described by Pareek et al. cells were cultured on 6- well plates and incubated for 48 hours at 37° CO_2 incubator. When semi-confluent monolayer was formed, the culture was renewed with serum free DMEM containing 0.2% BSA and incubated for 18 hours at 37° in the CO_2 incubator. After 18 hours, the medium was discarded and cells were washed with Krebs ringer phosphate (KRP) buffer once. The cells are treated with insulin, slandered drug and plant extract, and glucose (1M)) was added and incubated for half an hour. The supernatant was collected for glucose estimated and glucose uptake was terminated by washing the cells three times with 1 ml ice-cold KRP buffer. Cells were subsequently lysed by freezing and thawing three times. Cells lysate was collected for glucose estimation. Glucose uptake was calculated as the difference between the initial and final glucose content in the incubated medium by Glucose Oxidase- Peroxidase (GOD-POD) method. Six groups containing five wells of plate (n=5) each were taken as given in Table 1.

Statistical analysis

Data were presented as mean±standard error and analyzed using one way analysis of variance

(ANOVA) with Dunnett test.

Table 1: Effect of C.pictus on glucose uptake in 3T3- L1 cell line

Group no	Incubation medium	Glucose uptake(mg/dl/30 min)
1.	Control	61.4 ± 3.53
2.	Insulin(1 IU/ml)	118 ± 1.92**
3.	Metformin(100 µg/ml)	93.6 ± 2.59**
4.	Insulin(1 IU/ml) + Metformin(100 µg/ml)	108.2 ± 3.24**
5.	C.pictus extract (300 µg/ml)	54.8 ± 2.49
6.	C.pictus extract (300 µg/ml) + Insulin(1 IU/ml)	113 ± 2.14**

RESULTS

The extract of C.pictus was evaluated for its cytotoxic activity by 3-(4, 5 dimethyl thizole-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. C.pictus extract showed CTC_{50} value of 358.3 µg/ml in 3T3- L1 cell line. In vitro study on glucose utilization in 3T3- L1 Cell line was studied and results are given in Table 1.

DISCUSSION

Glucose uptake was not significantly increased by C.pictus extract and the extract was found to be less effective than insulin. The glucose uptake was significantly more in all groups tested except in the C.pictus group, when compared to the control group. Results were compared with those of insulin (Injectable antidiabetic) and metformin (Oral antidiabetic), which were used as a standard anti diabetic drugs. Insulin (1 IU/ml) and metformin (100 µg/ml) enhanced the glucose uptake by 163.73 ± 4.64% and 87.50 ± 6.25%, respectively, over control. The extract was also tested with insulin to confirm any synergistic effect, but results indicated that extract did not have any synergistic effect with insulin. The extract and insulin enhanced the glucose uptake in 3T3- L1 cell by 165.64 ± 5.15% over control, when used together. In India, C.pictus is grown in garden, especially in Kerala, Where the fresh raw leaves are eaten by diabetic people. It is turning out be a munching dietary supplement for diabetes, as it is thought to lower raised blood sugar level considerably.

In conclusion, we found that the extract of C.pictus leaves does not significantly stimulate glucose uptake by 3T3- L1 cells. It appears that C.pictus has no direct peripheral action. Further studies with estimation of insulin and insulin receptor may give more insight into the mechanism of the antidiabetic activity of C.pictus.

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