In Vitro Hypoglycemic Activity of Ethnopharmacological Important Plant of Chhattisgarh *Plummbago Zelynica* (Chitrak) against 3T3 – L1 diabetes Cell Lines

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Abstract: Plumbago zeylanica is a native plant of India belonging to the family Pumbago zeylanica. The aim of the present research was to evaluate the cytotoxicity and glucose uptake activity of methanolic extract of roots of Plumbago zeylanica using 3T3 cell lines. The results were found that the extracts showed cytotoxicity and the methanolic extract showed better glucose uptake potential. The results were compared with Plumbago zeylanica extract and rosiglitazone, which was used as the standard anti diabetic drugs. Rosiglitazone (100 µg/ml) and Plumbago zeylanica (500 µg/ml) enhance the glucose uptake over control.

Keywords: Hypoglycemic Activity, Cytotoxicity and Glucose Uptake Activity, Methanolic extract

1. INTRODUCTION

Diabetes mellitus (DM) is described as metabolic disorder of hypoglycaemia which results in a low blood glucose levels and disturbances of carbohydrates, fats and protein metabolisms resulting from defects in insulin secretion, insulin action or both[1]. Diabetes mellitus (DM) affects all body systems and the main brunt is borne by eyes, kidneys, skin and nerves[2].

Herbal medicines have long been used effectively in the treatment of Diabetes Mellitus. Selected Plant has been used for several centuries for the treatment of various ailments. The present work was undertaken to study the effect of isolated bioactive compounds from selected plant on glucose uptake in different cell lines.

2. EXPERIMENTAL METHODS

Table 1: Regional vernacular name of *plumbago zeylanica* linn

Language	Common name
Hindi:	Chitraka / Chitramol
Sanskrit:	Chitra
Guajarati:	Agni / Vahini
Kannada:	Chitramula
Bengali:	Chitra
Punjabi:	Veellakeduveli
Malayalam:	chitrakmula/ chitramoolam
Tamil:	Chita
Telugu:	Kodiveli/ chitramoolam
English:	Lead wory, Ceylon lead wart
Oriya:	Ogni

2.1. Cytotoxicity assay (MTT assay)

Glucose uptake activity of test drugs were determined in differentiated 3T3 and Vero cells. In brief, the 24 hr cell cultures with 70-80% confluences 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 multinucleate of cells. The differentiated cells were serum starved overnight 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. 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. Three independent experimental values in duplicates were taken to determine the percentage enhancement of glucose uptake over controls.

2.2 Assay *In vitro* glucose uptake assay

Glucose uptake activity of test drugs were determined in differentiated 3T3 and Vero cells. In brief, the 24 hr cell cultures with 70-80% confluences 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 multinucleate of cells. The differentiated cells were serum starved overnight 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. 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

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3. RESULTS

In the MTT assay (Fig. 1), the methanolic extract of the roots of *Plumbago zeylanica* was screened using MTT for its cytotoxicity against three cell lines namely 3T3-L1 at concentrations to determine the IC50 value. The cytotoxicity of the methanolic extract roots of *Plumbago zeylanica* was found to be dose dependent. The methanolic extract of roots of *Plumbago zeylanica* did not confer any significant lethality to the healthy 3T3-L1 cell lines with an LC50 value greater than 1000 µg/ml confirming the safe nature of the extract. Among the cell lines studied, less cytotoxicity was seen towards than 3T3-L1 cell line.

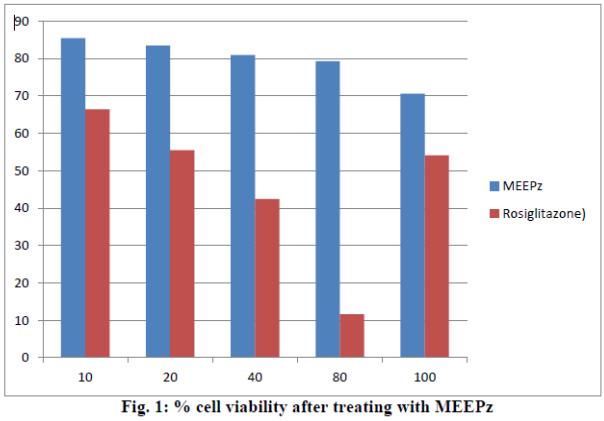

Fig. 1: % cell viability after treating with MEEPz

Medicinal plant (*Plumbago zeylanica*) enhances the glucose uptake by GLUT4 translocation and was proven by *in vitro* glucose model. The 3T3-L1 cell lines are the best characterized cellular model origin to study glucose uptake and GLUT4 translocation. Hence, in this research 3T3-L1 cell lines are used to determine the glucose uptake activity of methanolic extract of roots of *Plumbago zeylanica* and the results are presented in Table 2.

Table 2: *in vitro* glucose uptake studies in *3T3*-L1 cell lines.

Test Sample	Conc" (µg/mL)	% Glucose uptake over control
Plumbago zeylanica	500	28.29 ± 2.64
Rosiglitazone	100	58.59 ± 3.3

4. CONCLUSION

The research clearly shows the importance of *Plumabgo zeylenica* as a useful medicinal plant. *Plumabgo zeylenica* is used throughout the world for therapeutic purposes. It is evident from the review of the research that *plumbago*

zeylanica is used for centuries in Ayurvedic medicine for the treatment of various disease. It is most important medicinal plant extensively used in herbal formulations. *Plumbago zeylanica* L. has great potential to be integrated into conventional medical practice for the treatment and management of various metabolic syndromes, hepatotoxic, diabetes, inflammation, cancer and other disease complications.

The ethanol, methanol, petroleum ether and other solvent extract from the leaves, roots and stems of *Plumbago zylanica* have anti diabetic, anti-microbial, antiviral, antioxidant, antifungal, anti-allergic and other wonderful medicinal properties.

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