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Evaluation of Antidiabetic Activity Mechanism of Ethanol Extract of *Alangium salvifolium* Leaves Employing Cell-based Assays

Mayank Singh Lodhi, Balkrishna Dubey, Ashish Manigauha*
Department of Pharmacology, Technocrats Institute of Technology-Pharmacy, Anand Nagar, Bhopal – 462021 (MP), India
***Corresponding author: E-Mail: ashish.manigaunha@gmail.com**

ABSTRACT

Alangium salvifolium is a restorative plant and rich in phytoconstituents including flavonoids, polyphenol, alkaloids, tannins and so on. The antidiabetic movement of *Alangium salvifolium* definitely detailed however its component activity isn't depicted. Thus in the present investigation, it was wanted to investigate the antidiabetic activity component of ethanol extract of *Alangium salvifolium* leaves by utilizing cell-based examines. The leaves of *Alangium salvifolium* were handled for oil ether, ethanol and aqueous extract. The ethanol extract was screened for its cytotoxicity utilizing MTT examine against 3T3-L1 and RINm5F at various fixations. The glucose take-up in 3T3-L1 cells was essentially upgraded by ethanol remove. Further, insulin discharge from RINm5F cells was adjusted. The results of the examination affirmed that the ethanol extract improves the glucose take-up under *in vitro* conditions.

KEY WORDS: *Alangium salvifolium*, RINm5F, 3T3-L1, Cell-based Assays.

1. INTRODUCTION

Globally the people of different countries develop their own medical system from their ancestors. The Ayurveda is developed by Indian people. The Ayurveda describe the preparation of drugs from the plant materials (Vogel, 1991). Apart from this the Siddha and Unani have been developed by India (Ramarao, 1990; Handa, 1991).

Diabetes mellitus shows the expansion level sugar in blood because of metabolic issue. Diabetes mellitus is an unending ailment that requires long haul medicinal consideration both to confine the advancement of its overwhelming complexities and to oversee them when they do happen (Kothari, 2005; Das, 2005).

The major challenges for researchers are to find out new potent antidiabetic drug with fewer side effects, self-administrable, less expensive and completely reversible. The synthetic drugs is fail to fulfill these challenges. Further the herbal medicines could be used by minimizing above contests. The researchers reported various medicinal plants for their antidiabetic activity but their mechanism of action is not documented.

Alangium salvifolium is used as medicinal plant and documented various pharmacological activity namely antidiabetic, antimicrobial, anticancer, anti-inflammatory, laxative, diuretic, astringent, anthelmintic, emollient and antiepileptic activities. The plant was also demonstrated it therapeutic uses as anti-fungal, anti-microbial, cardiac and anti-fertility (Shetty, 2003; Xavier, 2005; Jain, 2010). The leaves, roots and barks are used for the herbal medicines for the healing of various diseases (Kumar, 2010).

The antidiabetic action of *Alangium salvifolium* has been logically detailed yet its instrument activity isn't portrayed. So looking on above matter we planned to explore the antidiabetic mechanism of *Alangium salvifolium* leaves extracts by employing cell-based assays.

2 MATERIAL AND METHODS

Preparation of extracts: 500 gram of *Alangium salvifolium* leaves were used for the preparation of petroleum ether, ethanol and aqueous extract. The ethanol extract were designated for further study.

***In vitro* cell line study:**

Preparation of buffer solution: The composition of PBS is listed in table.1. The solution were prepared for I liter by mixing different ratio of ingredients presented in table.1.

Cell culture: A rat insulinoma cell line (RINm5F) and 3T3-L1 pre-adipocyte were maintained at 37°C, 100% relative humidity, 5% CO_2, 95% air and the culture medium was changed twice a week.

Table.1. Common composition of PBS

Salt	Concentration (mmol/L)	Concentration (g/L)
NaCl	137	8.0
KCl	2.7	0.2
Na_2HPO_4	10	1.42
KH_2PO_4	1.8	0.24

Determination of cytotoxicity by Micro culture tetrazolium (MTT) assay:

- To each well 100 µl of the weakened cell suspension was included. Following 24 hours, the Different grouping of test extricate and Standard was included the focus rang is 12.5 to 800 ug/ml.
- The last volume of the well was made up to 200µl with medium (Serum Free) to the cells.

- The plates were then brooded at 37°C for ideal time, and minute examination was done and perceptions recorded like clockwork.
- Following 72 hours, the medication arrangements in the wells were disposed of and 20µl of MTT was added to each well. The plates will be delicately shaken and hatched for 3 hours at 37°C.
- The supernatant must be evacuated and 200µl of solubilization arrangement was included and the plates were tenderly shaken to solubilize the shaped formazan. The absorbance was estimated at a wavelength of 570nm. The rate development restraint was determined.

Glucose uptake assay:

- Glucose take-up action of test drugs were resolved in separated, 3T3-L1 cell Line.
- The 3T3L1 (mouse fibroblast) cell lines were refined in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% Fetal Calf Serum (FCS), 4mm Glutamine and 1% anti-toxin/antimitotic, in a 5% CO_2 hatchery at 37°C in brief, the 24 hr cell societies with 70-80% intersection in 40mm petri plates and permitted to separate 4-multi day.
- The degree of separation was set up by watching multinucleation of cells. The separated cells were serum starved medium-term and at the season of trial cells were washed with HEPES cradled Krebs Ringer Phosphate arrangement (KRP cushion) once.
- After then brooded with KRP cushion with 0.1% BSA for 30min at 37°C.
- Cells were treated with various non-lethal convergences of test and standard medications for 30 min alongside negative controls at 37°C.
- Fixations going from 12.5 µg/ml to 800 µg/ml of concentrate.
- D-glucose arrangement was added at the same time to each well and hatched at 37°C for 30 min.
- After hatching, the take-up of the glucose was ended by desire of arrangements from wells and washing thrice with super cold KRP cushion arrangement.
- Cells were lysed with 0.1M NaOH arrangement and an aliquot of cell lysates were utilized to gauge the cell-related glucose.
- The glucose levels in cell lysates were estimated utilizing glucose test unit. Three free trial esteems in copies were taken to decide the rate upgrade of glucose take-up over controls.

Insulin release assay: The assay was carried out in RIN m5F cells.

- Cells are washed with KRB (Krebs-Ringer Bicarbonate) cradle (100 µL) to evacuate serum and afterward the phones (1x105cells/well) are seeded into the 96well microtitre plate and hatched for 24 hours at 37°C.
- Different centralizations of test tests are readied utilizing 100 µL KRB cradle and hatched for a hour at 37°C.
- After brooding, the insulin was estimated by sandwich ELISA techniques. Sandwich ELISA Procedure (MercodiaRat Insulin ELISA) Twenty five µL of the cell satisfies and 50 µL of the compound conjugate are added to hostile to insulin covered 96 well small scale titer plate.
- The entire blend is brooded for 2 hours at room temperature; the hatched arrangement is then washed with KRB cradle. Two hundred µL of the substrate (tetra methyl benzidine) is added to each well and brooded for 15 minutes.
- After the hatching, 50 µL of the stop arrangement (0.5% H_2SO_4) is included and is set a shaker for 5sec to guarantee legitimate blending. The absorbance was estimated at 450 nm (Li, 2016; Fang, 2008; Ashcroft, 1973; Horwitz, 1975).

Statistical analysis: The outcomes were communicated as mean ± SEM of three autonomous investigations. The correlation between treated cells and untreated cells (control) was assessed utilizing single direction ANOVA pursued by Dunnett's various examination test.

3 RESULTS AND DISCUSSIONS

The study was conducted to investigate the antidiabetic mechanism of ethanol extract of *Alangium salvifolium* leaves by employing cell-based assays.

Cell viability study: In the present investigation, the ethanol extract of *Alangium salvifolium* was screened for its cytotoxicity utilizing MTT test against 3T3-L1 and RINm5F at various fixations. The cytotoxicity of the ethanol separate leaves of *Alangium salvifolium* was observed to be portion subordinate. The ethanol extract of *Alangium salvifolium* demonstrated some noteworthy lethality to the 3T3 and RINM5f cell lines with a LC_{50} esteem more noteworthy than 1000 µg/ml affirming the sheltered idea of the concentrate.

In the present study, the ethanol extract of *Alangium salvifolium* was screened for its cytotoxicity using MTT assay against 3T3-L1 and RINm5F at different concentrations. The cytotoxicity of the ethanol extract leaves of *Alangium salvifolium* was found to be dose dependent. The ethanol extract of *Alangium salvifolium* showed some

significant lethality to the 3T3 and RINM5f cell lines with an LC_{50} value greater than 1000 µg/ml confirming the safe nature of the extract.

3T3-L1 assay: Result of the percentage cell viability of ethanol *Alangium salvifolium* extract in 3T3-L1 cell line with concentration rang 12.5 to 800 µg/ml are presented in table.2.

RINm5F assay: Result of the percentage cell viability of ethanol *Alangium salvifolium* extract in RINm5F cell line with concentration rang 12.5 to 800 µg/ml are presented in table.3.

The findings of study demonstrated that the extract was safe and do not produce adverse effect. It expressed the extract can be administer safely.

The extract was chosen for the cytotoxicity measure in glucose take-up utilizing cell lines. The tissues of skeletal muscle took an interest in insulin acceptance and lead to glucose take-up process. The entire procedure of glucose take-up by skeletal tissue performed by expanding utilitarian glucose transport atoms in the plasma layer. Consequently not reacting insulin invigorated skeletal muscle cause non-insulin-subordinate diabetes mellitus. 3T3-L1 cells assume boss job for utilization of glucose take-up from fat. Further is likewise reflect have a flawless insulin flagging pathway and express the insulin-delicate GLUT4 and furthermore the RINm5F cells are considered to produces insulin and it is one of most extreme widely utilized insulin emitting cell line (Hallsten, 2002; Roy, 2014). The herbal extract increases the glucose uptake by GLUT4 translocation and was proven by *in vitro* glucose model. The glucose utilization in 3T3 cell lines exhibited that the extract were initiate to be prominent over control.

Table.2. Effects of ethanol extract of *Alangium salvifolium* on 3T3-L1

Concentration (µg/ml)	Ethanol extracts
12.5	92.1±0.15
25	81.3± 0.73
50	69.5± 0.59*
100	52.2 ±0.68*
200	39.8±0.42*
400	22.5± 0.37*
800	11.6± 0.54*

Values are expressed as mean ± standard deviation (n=3) per plate for three times' experiments. *P < 0.05 denote significant difference as compared to untreated cell (control).

Table.3. Effects of ethanol extract of *Alangium salvifolium* on RINm5F

Concentration (µg/ml)	Ethanol extracts
12.5	88.6±0.72
25	77.3± 0.49
50	61.9± 0.25*
100	45.1 ±0.37*
200	28.7±0.68*
400	19.4± 0.51*
800	8.2± 0.83*

Values are expressed as mean ± standard deviation (n=3) per plate for three times' experiments. *P < 0.05 denote significant difference as compared to untreated cell (control).

Thus, it tends to be reasoned that the *Alangium salvifolium* was observed to be nontoxic and safe and furthermore might be viable in glucose take-up. The real glucose transporter communicated in skeletal muscle and fat tissue is GLUT-4 is Trans situated from an intracellular film stockpiling site to the plasma layer. The aftereffects of the present examination exhibited that the ethanol extract of *Alangium salvifolium* upgrades the glucose take-up under in vitro conditions. This may because of the nearness of phyto constituents in the plants of or because of its impact on the receptors on the cell film.

However, *in vivo* studies have to be carried out to substantiate the *in vitro* results by employing different *in vivo* models and clinical trials for their effective utilization as therapeutic agents.

4. CONCLUSION

Alangium salvifolium are rich in secondary metabolite such as alkaloid, glycoside, flavonoids, polyphenol etc. In the present examination, it was wanted to investigate the counter diabetic system of *Alangium salvifolium* leaves separates by utilizing cell-based tests. The consequences of the investigation affirmed that leaves extract of *Alangium salvifolium* upgrades the glucose take-up under in vitro conditions. This may because of the nearness of phyto constituents in the plants or because of its impact on the receptors on the cell film. *Alangium salvifolium* leaves might be utilized as a potential contender for antidiabetic and antiobesity tranquilize improvement. Further unthinking in vivo investigations of *Alangium salvifolium* and wellbeing evaluation are suggested.

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