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Evaluation of in-vitro cytotoxicity of extract/fractions of *Calotropis gigantea* leaves against L-6 cell line

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Calotropis gigantea known commonly as Akda is potent medicinal plant and widely distributed throughout India. The goal of this research work was to evaluate the in vitro cytotoxic potential of the extract of *Calotropis gigantea*. The extract was screened for *in vitro* cytotoxicity by means of SRB assay against L-6 cell line. The results would enable more rational exploitation of the plant in both traditional and orthodox medicine.

Keyword: Cytotoxicity assay, SRB assays, *Calotropis gigantea*, L-6 cell line.

1. Introduction

Calotropis gigantea Linn. (Asclepiadaceae) commonly known as milkweed or swallow-wort, is a common wasteland weed. *Calotropis* belongs to Asclepiadaceae or Milkweed or Ak family which includes 280 genera and 2,000 species of world-wide distribution but most abundant in the sub-tropics and tropics, and rare in cold countries. Different parts of this plant such as the leaves, stem, flowers, and root bark are prescribed by traditional healer in Asia in a variety of disorders of gastrointestinal, cardiovascular, biliary, hepatic, urinary and respiratory origin^{1, 2}. Traditionally *C. gigantea* is used as analgesic³, cures toothache and earache^{4, 5}, sprain⁶, anxiety^{7, 8}, epilepsy⁹ and in mental disorders¹⁰. Various scientific studies reported this plant as contraceptives for human¹¹, sedative, anxiolytic, anticonvulsant¹², analgesic¹³ and wound healer¹⁴.

The plant is considered crude drug of Bangladesh and new oxipregnane- oligoglycosides named *Calotropis A* and *B* have been isolated from the root of *C. gigantea*. Cardenolide glycosides calotropin frugoside and 4-O- Beta- D-glucopyransyl frugoside were also obtained as the cytotoxic principles from the root of *C. gigantea*. This study attempts to determine the in vitro cytotoxic effect of *Calotropis gigantea* R.Br. leaves extract on L-6 cell lines. The results would enable more rational exploitation of the plant in both traditional and orthodox medicine¹⁵.

2. Material and Methods

2.1 Collection and Authentication

The fresh leaves (whole plant) of *Calotropis gigantea* was collected during June 2011, from the ABS Botanical gardens Karipatti Salem district, Tamil Nadu. The plant species was identified and authenticated by taxonomist Dr. A.

Balasubramanian. A voucher specimen was retained in the department for future reference.

2.2 Preparation of the Extract

The collected fresh plant materials of *C. gigantea* were successively extracted with 95% ethanol by continuous hot percolation method using soxhlet apparatus. The solvent was removed under reduced pressure. The extract obtained was kept for drying and stored in vacuum desiccator.

2.3 In vitro Cytotoxic Screening

The laminar airflow bench was swabbed with 70% ethanol. UV lamp and the laminar airflow system were switched on 30 minutes before the initiation of work. All reagents used (DMEM, Serum and TPVG) were brought to 37 °C in a serological water bath prior to use. Discarded the medium from the culture vessel and added sufficient volume of TPVG to wash the monolayer. Discarded the TPVG and fresh TPVG and kept for 2 minutes. At room temperature and then discarded. The culture vessel was transferred to 37 °C and incubated for 3-5 minutes until the cells started detaching. The culture vessel was then gently tapped against the palm and observed for cell detachment. Then a known quantity of medium was added and gently pipette down a couple of times to get a homogenous cell suspension. From the cell suspension a known volume was taken to which equal volume of 0.4% trypan blue was added and the cells were counted using haemocytometer. The dead cells took up the stain whereas the viable cells did not.

Total number of viable cells = $NV \times Df \times Cf$

Where,

NV = No. of viable cells. i.e. Total no. of cells - Dead cells

Df = Dilution factor

Cf = Conversion factor

Percentage Viability =

$$\frac{\text{Total No. of Cells} - \text{Dead Cells}}{\text{Total No. of Cells}} \times 100$$

0.75 to 1 million viable cells were transferred to sterile tissue culture bottles and required amount of medium with 10% serum was added and the bottles were incubated at 37 °C in CO₂ incubator. The passage number, split ratio and data were recorded.

2.3.1 Sulphorhodamine B (SRB) assay

SRB is a bright pink aminoxanthene dye with two sulfonic groups. Under mild acidic conditions, SRB binds to protein basic amino acid residues in Trichloroacetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of at least two orders of magnitude. Colour development in SRB assay is rapid, stable and visible. The developed colour can be measured over a broad range of visible wavelength in either a spectrophotometer or a 96 well plate reader. When TCA-fixed and SRB stained samples are air-dried, they can be stored indefinitely without deterioration^[16].

The percentage growth inhibition was calculated using the formula below:

% Growth inhibition =

$$100 - \frac{\text{Mean OD of Individual test group}}{\text{Mean OD of Cont}} \times 100$$

3. Result and discussion

Determination of Total Cell Protein Content by Sulphorhodamine B (SRB) assay method. The extract/fractions of *Calotropis gigantea* leaves were screened for their cytotoxicity activity by SRB assay method. *Calotropis gigantea* leaves extract/fractions showed higher cytotoxicity against L-6 cell line.

On the basis of cytotoxicity assay we decided the dose for Glucose uptake assay using L-6 cell line and on isolated rat hemi-diaphragm in table 1.

Table 1: *In vitro* cytotoxic screening

Cell Type	Cell line used	Ethanol extract $\mu\text{g/ml}$	Chloroform fraction $\mu\text{g/ml}$	n-Butanol fraction $\mu\text{g/ml}$
Normal	L-6	364	167	376

The extract of *Calotropis gigantea* leaves exhibited a strong cytotoxicity for L-6 cell lines. Chemical constituents reported from the extracts of leaves are alkaloids, flavonoids, tannins, steroids, saponins and glycosides. These biologically active compounds may be responsible for the *in-vitro* cytotoxic activity of extract against the L-6 cell lines. Further isolation and identification of the active compounds as lead in the crude extracts is recommended for the drug development.

4. Conclusion

The results of our study revealed that the extract of *C. gigantea* exhibit potent cytotoxic activity against L-6 cell line. Further *in vivo* and *in vitro* with different human cell lines study is required to demonstrate the antitumor activity of this plant and isolated the lead compound responsible for this activity, might be utilized for the development of novel anticancer drug.

5. Conflict of interest

The authors declare that they have no conflict of interest.

6. Acknowledgement

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