

In Vitro Cytotoxicity Analysis of Chloroform Extract of Novel Poly Herbal Formulation

S Selvakumar*, Barnali Sarkar

Department of Industrial BioTechnology, Bharath University, Chennai-600073, India.

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ABSTRACT

Objective: It is of interest to investigate the *in vitro* cyto toxicity effects of chloroformic extract of Novel polyherbal formulation were studied. **Materials and Methods:** Cytotoxicity of the crude extract of polyherbal formulation was evaluated on LLCMK2 monkey kidney epithelial cells and Cell viability was determined by using MTT assay. **Results and Discussion:** Our results indicate that the non toxic nature of a poly herbal formulation of Novel polyherbal formulation on control and experimental cell lines. **Conclusion:** The current mode of treatment for various diseases including cancer is based on synthetic drugs. These drugs are effective but they show serious adverse effects and also alter the genetic and metabolic activity of the patient. Furthermore, *in vivo* activity of the active compounds of a poly herbal formulation Novel polyherbal formulation needs to be determined in animal models and human subjects, so as to determine their efficacy in a metabolic environment.

Keywords: Chemotherapy, Cytotoxicity, *Nalla marunthu*, MTT assay, Monkey kidney epithelial cells, Vero cell lines.

INTRODUCTION

Plants have been a source of medicine for thousands of years, and phytochemicals continue to play an essential role in medicine. The use of medicinal plant extracts for the treatment of human diseases is an ancient practice; this has greatly increased in recent years. For a long time, plants are being used in the treatment of cancer¹.

Natural products possess immense pharmacological significance in the development of drugs and were discovered through plant bioprospecting. The majority of drug candidates, such as paclitaxel, etoposide, camptothecin, vinca alkaloids, indole alkaloids, podophyllotoxin derivatives, etoposide and teniposide, currently used in clinical cancer chemotherapy, were originally derived from plants². The efficacy of chemotherapy, radiotherapy, hormonal therapy, or surgery, which are mainly used for the treatment of cancer, are well-known for side effects hence, the identification of novel natural products that possess better effectiveness

against cancer, but less harmful effects have become desirable and therefore, natural products are continuously being explored worldwide. The floral elements of unique arid plant biodiversity of Saudi Arabia are being practiced in folk medicine since ancient times³. As part of our efforts to study traditional Indian medicinal plants for pharmacological activities, our study provides data on the cytotoxic efficacy of a poly herbal formulation.

MATERIALS AND METHODS

Collection of samples

The chloroformic extract of a poly herbal formulation is used for the experiment. The herbal formulation was prepared by the available literature.

Preparation of Herbal medicine

The herbal formulation was prepared in the department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Bharath University, Chennai, India. The equal volume of shade dried leaves of *I. tinctoria*, *A. polygonoides*, *T. portulacostrum* and *C. bonplandianus* were taken in to marter and pistle, The plant material was coarsely powdered, then filtered by muslin cloth and the filtrate was used for further extraction.

Preparation of extracts

1000 grams of polyherbal formulation was packed in three separate round bottom flask for sample extraction using solvents namely Aqueous, Chloroform and Methanol. The extraction was conducted by 250 ml of each solvent mixture for a period of 24 hours. At the end of the extraction the respective solvents were concentrated under

Table 1: *In vitro* Cytotoxicity analysis of chloroformic extract of *Nalla Marunthu*.

S.No	Concentration µg/ml	Absorbance 540nm	% cell Viability
1	100	0.07	6.3
2	50	0.18	16.3
3	25	0.25	22.7
4	12.5	0.43	39.0
5	6.25	0.67	60.9
6	3.12	0.89	80.9
7	Control cells	1.10	100

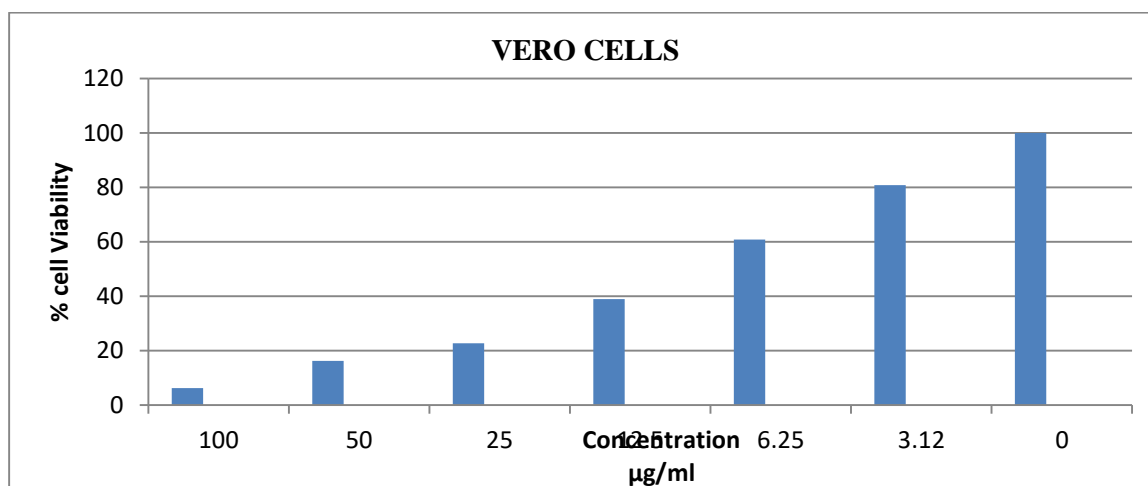


Figure 1: The Percentage of Cell viability on *Nalla marunthu*.

reduced pressure and keep it in water bath (at 50°C). Now the extracted experimental solutions were stored in refrigerator.

Preparation of stock solutions

Stock solutions were prepared by dissolving 4 mg of crude extracts in 100 µL dimethyl sulfoxide and then diluted with RPMI-1640 cell culture medium to make 400 µg/mL. All solutions were sterilized by passing through 0.22 µm syringe-adapted filters and stored at -20°C until use.

Cytotoxicity analysis

Cytotoxicity of the crude extracts was evaluated on LLCMK2 monkey kidney epithelial cells. Cells were grown in RPMI- 1640 culture medium with L-glutamine and 25 mM HEPES (Steinheim, Germany). The medium was supplemented with 2 mg/mL NaHCO₃ (sigma), 10 µg/mL hypoxanthine (Sigma), 11.1 mM glucose (sigma), 10% FBS (BioWhittaker®, Verviers, Belgium) and 5µg/mL gentamicin. The cells were incubated at 5% O₂, 5% CO₂, and 90% N₂ in humidified incubator (SHEL LAB™, Sheldon MfgInc, OR, USA) at 37°C until confluent before used for cytotoxicity assay. Trypsinated cells were distributed in 96 well plates at 10,000 cells in 100 µL per well and incubated for 48 h to allow them to attach before adding the extract. After 48 h the medium was removed completely from each well, and 100 µL of fresh culture medium was then added. Thereafter 100 µL of crude extracts (400 µg/mL) were added in row H and then serially diluted to row B to give concentrations ranging from 200 – 3.125 µg/mL. Cells in row A served as controls without drug (100% growth). The cells with or without extracts were incubated at 37°C for 72 h before determining their viability. Each concentration level was tested in triplicate⁴.

MTT Assay

Cell viability was determined using MTT assay⁵. After 72 h of incubation, the culture medium in each well with or without extract was removed completely from the assay plates and replaced by 100 µL of fresh culture medium. Then 10µL of 5 mg/mL Thiazolyl Blue Tetrazolium Bromide, MTT (Sigma) were added into each well to achieve a final concentration of 0.45mg/mL before incubated for 3 h at 37°C. After 3 h, the culture medium

with MTT was carefully removed followed by addition of 100µL dimethylsulfoxide to dissolve formazan crystals and then incubated for 1 h before recording the optical density (Emax-Molecular Devices Corporation, California, USA) at 595 nm. Data analysis The percentage viability and percentage mortality were calculated from the OD values using Microsoft Excel 2010. The mean results of the percentage mortality were plotted against the logarithms of concentrations using the Fig P computer program Ver 4.189/07 (BiosoftInc, USA). Regression equations obtained from the graphs were used to calculate the fifty percent cytotoxic concentration (CC₅₀), which is the concentration killing fifty percent of the cells. An extract with CC₅₀>30 µg/mL is considered non-toxic⁶.

RESULTS AND DISCUSSION

Calculation

$$\% \text{ cell viability} = \frac{A540 \text{ of treated cells}}{A540 \text{ of control cells}} \times 100\%$$

Cytotoxicity studies with normal cell culture systems of plant extracts or folk medicinal plant extracts has not been studied extensively and this is vital for the safety evaluation or any herbal preparation. Therefore, the objective of this study was to evaluate the potential cytotoxic activity of polyherbal formulation against Vero cell line⁷. In-Vitro assay of chloroformic extract of polyherbal formulation were carried out for their confirmation of cytotoxic effect on vero cell line. Percentage of viable cell can be obtained by performing trypan blue dye exclusion technique. The cytotoxicity activity is carried out by using MTT assay. Cell lines derived from NCCS, Pune were free from any kind of bacterial and fungal contamination. Percentage cell viability of cell line was carried out by using trypan blue dye exclusion technique. (Table and figure) it shows that the % viability of Vero cell line is 90.85%, which is most suitable to perform cytotoxicity study. The cytotoxicity study was carried out for plant extracts. These extracts were screened for its cytotoxicity against Vero cell line at different concentrations to determine the IC₅₀ (50% growth inhibition) by MTT assay (IC₅₀ = 9.375 µg) Results of %

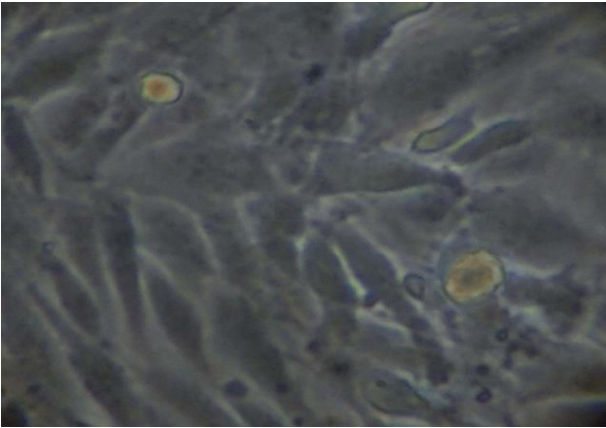


Figure 2.1: Control VERO Cell Lines.

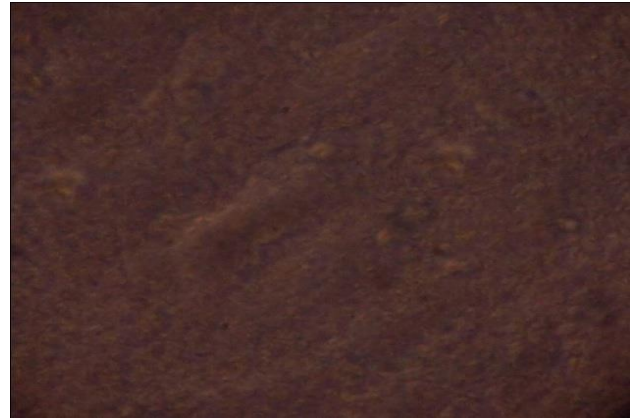


Figure 2.2: 3.12 µg/ml of Novel polyherbal formulation.



Figure 2.3: 6.25 µg/ml of Novel polyherbal formulation.

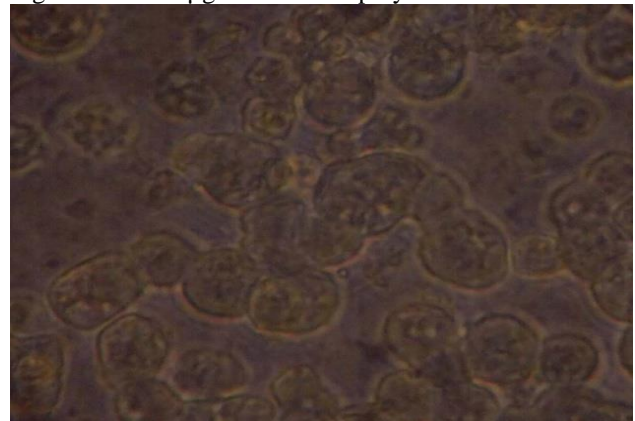


Figure 2.4: 12.5µg /ml of Novel polyherbal formulation.

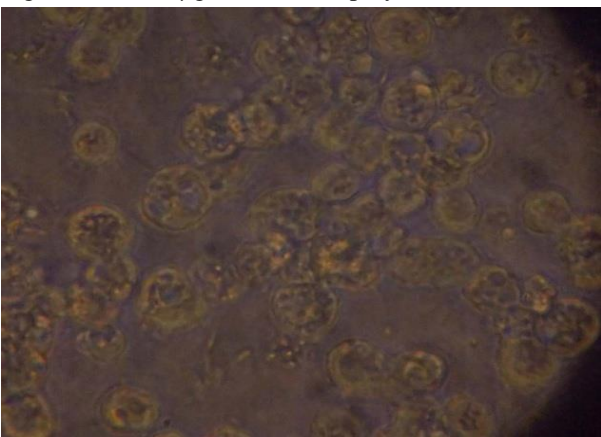


Figure 2.5: 25 µg/ml of *Nalla marunthu*.

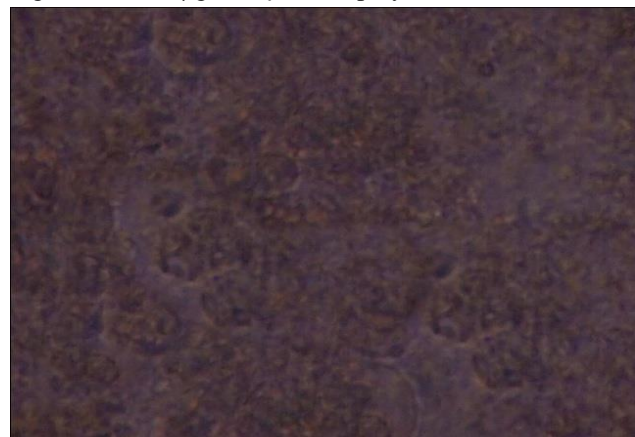


Figure 2.6: 50 µg/ml of Novel polyherbal formulation.

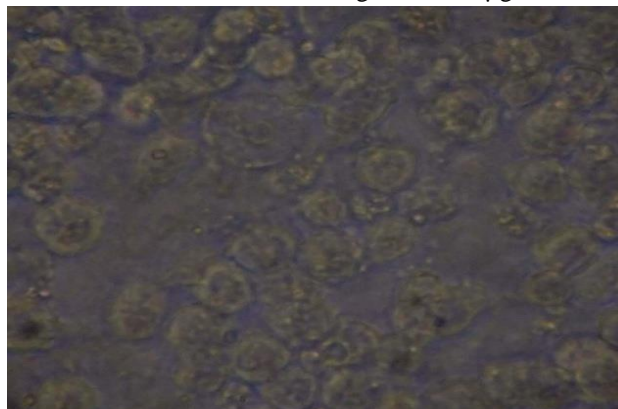


Figure 2.7: 100 µg /ml of *Nalla marunthu*.

Figure 2: Electron microscopic photographs of various concentrations of Novel polyherbal formulation on Vero Cell Lines.

Viability of vero cell line of polyherbal formulation were presented in Table and graphically represented in Figure. The percentage viability was found to be increasing with decreasing concentration of test compounds. The above study shows that selected plants do not have any significant cytotoxicity on the normal vero cell line. The study suggests that, the extracts might have increased the proliferation of the kidney cells, which can be further studied by cell proliferation assay. Thus, the toxicity of the plant has to be studied for further exploration of various biological activities⁸. The study was conducted to assess the possibility of this plant extract to have a non potent cytotoxic potential. From the preliminary assay it is clear that this plant can be further studied for its activity as a non cytotoxic agent. Further studies into their way of action, phytochemicals involved etc., need to be explored.

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