Cytotoxic Activity Of Crude Extracts From Nerium Indicum (Apocynaceae)

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ABSTRACT

The study was aimed to evaluate the cytotoxicity activity of the flowers extract of *Nerium indicum* on the HeLa and L929 cell line. The flowers of *Nerium indicum* methanolic extract were tested for its inhibitory effect on HeLa and L929 cell line. The cytotoxicity of *Nerium indicum* on HeLa cell was evaluated by the MTT assay. *Nerium indicum* methanolic extract has significant cytotoxicity effect on HeLa cell line in concentration range between 12.5 µg/ml to 100 µg/ml by using MTT assay. IC_{50} value of *Nerium indicum* on HeLa and L929 cell were 64.3 and > 300 µg/ml respectively. From the performed assay, methanolic extract of these shows greater activity on HeLa cell line and little activity on L929 cell line and these values indicates that *Nerium indicum* shows significant cytotoxicity activity.

KEYWORDS

Cytotoxicity Activity, HeLa cell line, L929 cell line, MTT Assay, *Nerium indicum*

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INTRODUCTION

In recent years the general public has shown an increased interest in the use of herbal medicines in preference to synthetic drugs. This is based on the belief that natural products are intrinsically less dangerous and can be obtained at a lower cost\(^1\). Many diseases, such as cancer, atherosclerosis and
inflammation are caused by free radicals and lipid peroxidation inside human bodies. This kind of risk can be reduced by an appropriate dietary pattern including a great portion of fruit and vegetables\textsuperscript{2, 3} because of the great amount of natural antioxidants in these plant foods\textsuperscript{4}. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of which based on their use in traditional medicine. It has been noted that the original source of many important pharmaceuticals currently in use have been plants used by indigenous people\textsuperscript{5}.

Conventional plants are valuable source of novel cytotoxic agents and are still in performance better role in health concern. Throughout history and crosswise the world, the plant kingdom has provided a diversity of medicines for cancer treatment. In current period, plants have been a source of Antioxidants, Analgesics, Anti-inflammatory, Antiasthmatics, Antiarrhythmic agents, Antihypertensive, Antimicrobial agents known to be frequent.

Cancer chemotherapy now plays a significant role in the treatment of many malignancies, either curative (by itself or as an adjuvant to surgery and/or radiation) or palliative care, depending upon the specific tumor situation\textsuperscript{6}. The objective of cancer chemotherapy is to kill cancer cells with as little damage as possible to normal cells\textsuperscript{7}. Therefore, any discovery of anticancer agents must be related to novel molecular targets; i.e. they should be effective against specific types of cancer cells but less toxic to normal cells, or have a unique mechanism of action for specific types of cancer\textsuperscript{8}.

A HeLa cell is an immortal cell line used in medical research. The cell line was derived from cervical cancer cells taken from Henrietta Lacks, who died from her cancer in 1951. Initially, the cell line was said to be named after a "Helen Lane" in order to preserve Lacks's anonymity\textsuperscript{9}.

Cancer is the third leading cause of death worldwide, only preceded by cardiovascular disease, infectious and parasitic disease\textsuperscript{10}. Drug discovery from medicinal plants has played an important role in the treatment of cancer and indeed, over the last half century most of the plant secondary metabolites and their derivatives have been used toward combating cancer\textsuperscript{11, 12}. 
Cancer is a class of disease in which the body cells become abnormal and divide indiscriminately. Cancer cells may become invasive and transform normal adjacent cells into malignant cells. They may also spread through the blood stream and lymphatic systems to other parts of the body to form metastatic tumors in distant organs. Cancer is caused by abnormalities in the genetic material of the transformed cells. Cancer may also be initiated by carcinogens, tobacco smoke, radiation, chemicals or infectious agents, especially some viruses. Death of the cells in any case is mediated by an intracellular activity either of two distinct mechanisms, necrosis or apoptosis.

*Nerium indicum* belongs to family Apocynaceae and commonly well known as Arali in Tamil. It is an important herbal drug used as Analgesic,
Anticonvulsant,
Anti-anxiety,
Antioxidant,
Antidiabetic,
Anticancer,
Antibacterial,
Anti fungal and Insecticidal. Thus the present study was design to evaluate the in vitro cytotoxicity activity of methanolic extract of *Nerium indicum* flower.

**MATERIALS AND METHODS**

**Materials**

**Cell line and culture**

The human cervical cancer cell line (HeLa) and murine connective tissue cell lines (L929) were obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 5% fetal bovine serum (FBS). All cells were maintained at 37°C, 5% CO₂, 95% air and 100% relative humidity. Maintenance of cultures was passaged weekly, and the culture medium was changed twice a week.

**Methods**

**Preparation of plant extracts**
Accurately weighed 20 g of *Nerium indicum* flower powder was extracted with 500 ml methanol by stirring at 50°C for 3 hr. The extracts were then filtered through whatmann filter paper and the filtrate was concentrated with a vacuum rotary evaporator under low pressure.

*Arali* (*Nerium indicum*) as identified and authenticated by a Dr. P.N Sudha, Department of chemistry, Thiruvallur University and was collected in Jan 2013.

**Cytotoxicity Assay**

**Micro culture tetrazolium (MTT) assay**

**Principle**

The assay was carried out using 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells and inversely proportional to the degree of cytotoxicity.

**Procedure**

The Cytotoxicity of *Nerium indicum* flower extracts on HeLa cell line was determined by the MTT assay. The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1x10^5 cells/ml. one hundred microliters per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO₂, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample
concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations. Following drug addition the plates were incubated for an additional 48 h at 37°C, 5% CO₂, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

\[
\% \text{ cell Inhibition} = \left(\frac{100 - \text{Abs (sample)}}{\text{Abs (control)}}\right) \times 100
\]

Nonlinear regression graph was plotted between % Cell inhibition and Concentration (µg/ml) and IC₅₀ was determined using Graph Pad Prism software.

RESULTS

In vitro cytotoxicity activity of crude methanol extract of *Nerium indicum* was carried out by using MTT assay on the growth of HeLa and L929 cell line. Extract response curves constructed between the range of 12.5 µg/ml to 100 µg/ml for *Nerium indicum*, express percentage of cell inhibition increasing with increasing concentration of crude methanolic extract.

Cytotoxicity activity

The cytotoxicity study was carried out for plant extract of *Nerium indicum* flowers. *Nerium indicum* extract was screened for its cytotoxicity against HeLa and L929 cell lines at different concentrations to determine the IC₅₀ (50% growth inhibition) by MTT assay.

Results are tabulated in table 1 and graphically represented in Figure 1 and Figure 2. The percentage growth inhibition was found to be increasing with increasing concentration of test compounds,
as shown in Figure 1. *Nerium indicum* effect on HeLa cell line from 12.5 µg/ml to 100 µg/ml and L929 cell line from 18.75 µg/ml to 300 µg/ml (Table 1 and Figure 1, 2) and that IC₅₀ value on HeLa cell line was 64.3 µg/ml, while *Nerium indicum* has no effect on L929 cell line, so its IC₅₀ found to be > 300 µg/ml.

In this preliminary study, we have focused our interest on crude plant extracts, the cytotoxic activity could be due to the presence in the methanolic extracts of active products that could probably have highly anti-growth effects. That means *Nerium indicum* flowers extract has no effect on normal healthy body cell. If drug has more effect on L929 cell line that denotes it affects normal healthy body cell and turn out with side effect. While in case of *Nerium indicum* superior result on HeLa cell but no effect on L929 cell. So *Nerium indicum* shows significant cytotoxicity activity.

Table 1: Determination of cytotoxicity by MTT assay

<table>
<thead>
<tr>
<th>Plant Extract</th>
<th>Conc µg/ml</th>
<th>% of Inhibition</th>
<th>IC₅₀</th>
<th>R²</th>
<th>Conc µg/ml</th>
<th>% of Inhibition</th>
<th>IC₅₀</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nerium indicum</em></td>
<td>12.5</td>
<td>0.281</td>
<td>13.27</td>
<td>64.30</td>
<td>0.982</td>
<td>0.339</td>
<td>0.20</td>
<td>0.48</td>
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<td></td>
<td>25.0</td>
<td>0.245</td>
<td>24.49</td>
<td>75.0</td>
<td>0.327</td>
<td>3.82</td>
<td>3.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>0.198</td>
<td>38.89</td>
<td>150</td>
<td>0.331</td>
<td>2.75</td>
<td>2.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.127</td>
<td>60.91</td>
<td>300</td>
<td>0.32</td>
<td>5.00</td>
<td>5.00</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

The cytotoxic effect of plants is principally contributed by the presence of secondary metabolites like alkaloid, glycoside, steroid, tannin, phlobatannin, terpenoids and flavonoid. The *Nerium indicum*
flowers extract is includes many chemical constituents, such as cardiac glycosides, pregnanes, terpenes and flavonoids etc. The plant which has been reputed as therapeutic agents, has varieties of biological activities including heart failure, cancer, anti-neoplastic, anti-inflammatory, sedation, anti-bacterial and anthelminthic effects.

Plant based compounds have been playing an important role in the development of useful anti-cancer drugs. Studies on the isolation, structure elucidation and biological activities of the components of this plant are now on-going. Our study indicates the scope of developing anticancer drugs from *Nerium indicum*.

**CONCLUSION**

We tested the cytotoxicity to HeLa cell for different concentration of crude flowers extract. Crude *Nerium indicum* flowers extract strongly inhibited HeLa cell and was non-toxic to normal cells. However, further study on investigation of the active components of these plants may provide useful information in the future.

**REFERENCES**