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In vitro cytotoxic effect of selected plants of Verbenaceae on Dalton's Ascites Lymphoma cell lines

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Abstract

The cytotoxic effects of various plant extracts on DAL cell lines were evaluated using the Trypan blue dye exclusion method. The study identified that ***Clerodendrum infortunatum*** and ***Vitex negundo*** induced 100% cell death at a concentration of 200 µg/ml, demonstrating potent cytotoxicity. ***Lantana camara*** exhibited moderate cytotoxicity with 80% cell death at the same concentration, while ***Premna serratifolia*** showed the least cytotoxic effects among the tested extracts. A dose-dependent cytotoxic response was observed across all plant extracts, with increasing concentrations resulting in a progressive decrease in cell viability over a 3-hour period. The findings indicate that DAL cells were most sensitive to ***Clerodendrum infortunatum*** and ***Vitex negundo***, suggesting these extracts as the most effective in targeting cell viability. These results provide insights into the potential use of these plant extracts for further cytotoxicity studies and therapeutic applications.

1.Introduction

Cancer is the abnormal growth of cells in our body and it forms the major health problem in both developed and developing nations. It is estimated that cancer is the second leading cause of death after cardiovascular diseases, at a rate of 350 million people annually as recorded by the American cancer society. Gastrointestinal cancer is the most common of all the cancers associated with aging. Because of the serious side effects such as toxicity and high cost associated with chemo and radiotherapy and also due to the high death rate associated with cancer, many people around the world now adopt alternative approaches of treatment which mainly rely on plant-based drugs or herbal medicines (Kaur *et al*., 2011). The phytochemicals, in particularly secondary metabolites possess strong immune modulatory activities through its antioxidant properties. These antioxidants may prevent and cure cancer and other degenerative diseases by protecting the cells from damage caused by free radicals (Madhuri & Pandey, 2009). Many researchers investigated a diversity of compounds having shown anti-tumor activity and/or cytotoxicity which also varies with plant parts (e.g. root, stem bark, twig, etc.) and conditions under which samples are obtained, such as the location or soil type and developmental stage of the plant (Croom,1983). Plant derived cytotoxic constituents have played an important role in the development of clinically useful anticancer agents (Cragg & Newman, 2005). Various cytotoxic constituents have been isolated from plants and which are used as anti-cancer agents.

Verbenaceae is a family which comprises many plants traditionally used against many ailments some of which are listed in the Ayurvedic and Unani system of medicine. With this backgrounds, four plants namely

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Clerodendron infortunatum, Vitex negundu, Lantana camera and *Premna serratifolia* are selected from this family for its cytotoxicity screening against DAL cell lines, using Trypan blue dye exclusion method which form the preliminary procedure to access the cytotoxic nature of plant extracts or metabolites. Trypan blue, a diazo dye, used in bioscience to colour dead cells because this dye cannot be taken by cells with an intact membrane. So, this dye cannot colour the live cells with intact membrane. Upon entering into dead cells, Trypan blue bind with the intracellular proteins and renders the cells a distinct blue colour. Since the live cells are selectively excluded from staining, this method can be specifically used to measure percentage cell viability lose in a sample (Strober, 1997; Tolnai, 1975).

2. Materials and Methods

2.1 Reagents and apparatus

Ethyl alcohol, Distilled Water, PBS, Trypan blue dye, hemocytometer, Dalton's Ascites Lymphoma cell lines, Whatman No.1 paper, rotary evaporator, weighing balance, Eppendorf 's tubes.

2.2 Preparation of extracts

The fresh leaves of *Clerodendron infortunatum, Vitex negundo, Lantana camara* and *Premna serratifolia* were collected during the month of October, 2018 from the botanical garden of St. Joseph's College for Women, Alappuzha. The plant materials were shade dried for three weeks and then ground into coarse powder using a mechanical grinder. The powdered samples were labelled and stored in air tight container for future use. For the present cytotoxic studies, cold extraction was made. 10gms of fruit powder is accurately weighed and dissolved in 200 ml of 70% ethanol and kept for 2-3 days with gently shaking at intervals followed by filtering. By using a rotary evaporator, the extract obtained was concentrated and transferred to Eppendorf's tubes for further analysis.

2.3 Determination of cytotoxicity by Trypan blue exclusion dye method

Trypan blue exclusion method (Gupta & Bhattacharya, 1978) is the preliminary screening tool used to detect the *in-vitro* cytotoxicity of a plant extract or a drug. In this method the test compounds were studied for short term *in vitro* cytotoxicity using Dalton's Ascites Lymphoma (DAL) cells, originally procured from Amala Cancer Research Institute, Thrissur, India. Trypan blue is not permeable to live cell due to the presence of intact plasma membrane. When cells are dead, they will take up the dye and appear as blue colour. The method is an index of the dead cells in a cell population. The tumor cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Viable cell Suspension (1×10^6 cells in0.1 mL) was added to tubes containing various concentrations (10, 20, 50, 100 and 200 µg/ml) of ethanolic extract of *Clerodendron infortunatum, Vitex negundu, Lantana camera and Premna serratifolia* and the volume was made upto 1mL using phosphate buffered saline (PBS). Control tubes contained only cells suspension. These assay mixtures were incubated for 3 hours at 37°C. Further 0.1mL of cell suspension was mixed with 0.1mL of 1% Trypan blue, kept for 2-3 minutes and loaded on a hemocytometer. The number of

stained and unstained cells was counted separately.

$$\% \text{ of dead cells} = \frac{\text{Number of dead cells}}{\text{Number of viable cells + Number of dead cells}} \times 100$$

3.Results and Discussion

Percentage cell viability of DAL cell lines treated with the selected plant extracts were carried out by using Trypan blue dye exclusion technique. Cytotoxicity effects of all these extracts against DAL cells was analyzed and the results are shown in Table-1 & Plate-1 and the morphological changes are represented in Plate 1. It showed that the % cell death of DAL cells were100% for *Clerodendrum infortunatum* and *Vitex negundu* at 200 µg ml, whereas that of *Lantana camara* was 80% at the same concentration. Least cytotoxicity was noticed for *Premna serratifolia*. All the plant extracts showed a dose dependent cytotoxic effects as the concentration of drug gradually increased in the test condition over a period of 3h. During this time tenure all the four extracts exhibited a progressive lose in cell viability in all the tested DAL cell lines. The present investigation demonstrated that DAL cell lines are most sensitive to *Clerodendrum infortunatum* and *Vitex negundu*.

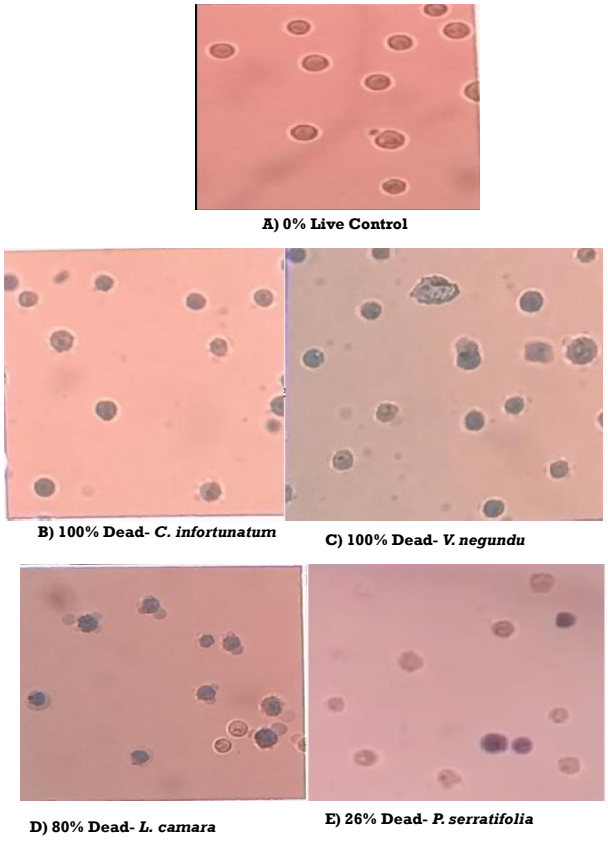
Table1. Cytotoxic effect of *P. serratifolia* on DAL cell lines by Try panblue dye exclusion method.

Conc. (µg/ml)	*C. infortunatum*	*V. negundu*	*L. camara*	*P. serratifolia*
10	20	14	22	1
20	35	20	30	2
50	45	38	42	9
100	85	92	64	17
200	100	100	80	26

Khairunnisa and Karthik (2014) reported that *Hymenodictyon excelsum* extract is capable of exerting its cytotoxic effect on DAL cells using Trypan blue dye. A dose dependent inhibition was noticed when *Cansjera rheedii* J.Gmelin used by the tribals of Auroville village near Puducherry for various liver disorders, is screened for its anti-cancerous property and revealed that the extract possesses 50% inhibition at 150µg/ml on HT-29 cell lines (Rashina, 2018). Similarly, while investigating the cytotoxic potential of ethanol and aqueous extracts of *Drosera indica* at 250mcg/ml dose on DAL cell lines, Asirvatham *et al*. (2013) reported 90% and 86% loss in cell viability. Ethanolic and aqueous extracts of *Portulaca pilosa* was also demonstrated to possesses 56.76% and 49.02% cytotoxicity in Trypan blue method against HT-29 at 200 µg/mL (Ramalingam *et al*., 2017). Comparing to these findings, cytotoxic potential of *Clerodendrum infortunatum* and *Vitex negundu* is investigated to be higher (100% at 200µg/ml) in the present study. This may be due to some active metabolites in these

plants that may be more active either in isolated form or in their crude form that in turn need to be characterized later. Moreover, Trypan blue dye exclusion method is only a preliminary procedure to access the cytotoxicity, MTT assay is to be carried out in these cell lines using the plant extracts tested in the present study, to ascertain their anti-tumorous potential.

Plate1.Cytotoxic effects of plant extracts on DAL Cell Lines at 200 µg/ml



A) 0% Live Control

B) 100% Dead- *C. infortunatum*

C) 100% Dead- *V. negundu*

D) 80% Dead- *L. camara*

E) 26% Dead- *P. serratifolia*

4. Conclusion

The cytotoxic effects of several plant extracts on DAL cell lines were assessed using the Trypan blue dye exclusion method. The study revealed that *Clerodendrum infortunatum* and *Vitex negundo* induced 100% cell death at a concentration of 200 µg/ml, exhibiting strong cytotoxic effects. *Lantana camara* demonstrated moderate cytotoxicity with 80% cell death at the same concentration, while *Premna serratifolia* showed the least cytotoxicity. All plant extracts exhibited dose-dependent cytotoxicity, with increasing extract concentrations leading to a progressive reduction in cell viability over a 3-hour period. The DAL cells were most sensitive to *Clerodendrum infortunatum* and *Vitex negundo*, making these extracts the most potent in terms of cytotoxic effects.

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