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In vitro anticancer screening of herbal syrup formulation of vinca extract

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ABSTRACT

In continuation of previous research, the anticancer screening of formulation of herbal syrup of Vinca extract was performed. The formulation of herbal syrup was performed as per reported literature method. The anticancer cancer screening was performed on two Human cancer cell lines. These cell lines were Human Lung Cancer cell line A549 and Human Breast Cancer Cell line MCF7. The formulation was found to be devoid of anticancer properties.

1. Introduction

Cancer is a complex disease that results from the uncontrolled growth and division of abnormal cells. It can occur in any part of the body and spread to other parts of the body, making it difficult to treat. The impact of cancer on society is significant both in terms of human suffering and financial costs.[1]

1.1. Here are some ways cancer affects society

1. Human suffering: Cancer can cause physical pain, mental suffering and emotional trauma to patients and their families. It can affect all aspects of a person's life, from work to relationships and daily activities. The impact of cancer on a patient's quality of life can be devastating.
2. Mortality: Cancer is one of the leading causes of death worldwide. In 2020, it caused an estimated 9.9 million deaths worldwide. Certain cancers, such as pancreatic and lung cancer, have particularly high death rates.
3. Financial costs: Cancer treatment can be expensive and the financial burden of cancer is significant. The costs of cancer treatment include not only the cost of treatment, but also the loss of productivity and quality of life. In 2020, the global economic cost of cancer was estimated at $1.16 trillion.
4. Health system: The high incidence and prevalence of cancer places a significant burden on health systems. Cancer patients require special care and the resources needed to treat cancer can be limited. The demand for cancer treatment is expected to continue to grow as the population ages and the incidence of cancer increases.[2]
5. Research and development: the search for new and better cancer treatments is an ongoing process. Research and development in the field requires significant resources and investments. Although advances in cancer treatment have been made in recent years, much remains to be done to improve outcomes for cancer patients.[3]

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2. Vinca[4,5]

Vinca alkaloids are used clinically as chemotherapy agents for various types of cancer, including leukemia, lymphoma, and solid tumors such as breast, lung, and ovarian cancer.

1. Vinca alkaloids prevent the formation of microtubules necessary for cell division. This leads to the arrest of cell growth and induction of apoptosis or programmed cell death.
2. Vinca alkaloid has an immunomodulatory effect in addition to its anticancer effect, which can improve the body's ability to fight cancer.[6]

Overview of vinca alkaloids and their mechanism of action

Vinca alkaloids are a group of natural compounds derived from the Madagascar periwinkle (Catharanthus roseus). They are widely used in chemotherapy for many types of cancer, including leukemia, lymphoma, and solid tumors such as breast, lung, and ovarian cancers. The two most commonly used vinca alkaloids in clinical practice are vincristine and vinblastine.[7,8]

The mechanism of action of periwinkle alkaloids involves the inhibition of microtubule formation, which is essential for cell division. Microtubules are tubular structures composed of the protein tubulin, which play an important role in maintaining the structural integrity of the cell and in the formation of the mitotic spindle during cell division. Vinca alkaloid binds to tubulin and prevents its polymerization into microtubules, resulting in cessation of cell growth and division. Specifically, vinca alkaloid binds to the beta subunit of tubulin, inhibiting the formation of microtubule bundles and leading to the formation of abnormal microtubule structures. In addition to their effects on microtubules, periwinkle alkaloids have immunomodulatory effects, which may contribute to their antitumor activity. Specifically, they have been shown to boost the activity of natural killer cells, which play a key role in the immune response to cancer.

The specific mechanism of action of vinca alkaloids may vary depending on the type of cancer and the stage of the cell cycle. For example, in white blood cells, the alkaloid vinca can induce apoptosis (programmed cell death) by disrupting the assembly of the spindle apparatus during cell division. In solid tumors, vinca alkaloids can inhibit the formation of new blood vessels (angiogenesis) by disrupting microtubule function in endothelial cells.

2.1. Chemical constituents of vinca[5] (Figure 1)

3. Materials and Methods

3.1. Formulation of medicated herbal syrup of vinca extract

This was prepared as reported literature methods.[9–12] Further the syrup was dried in petri dishes and 0.5g dried

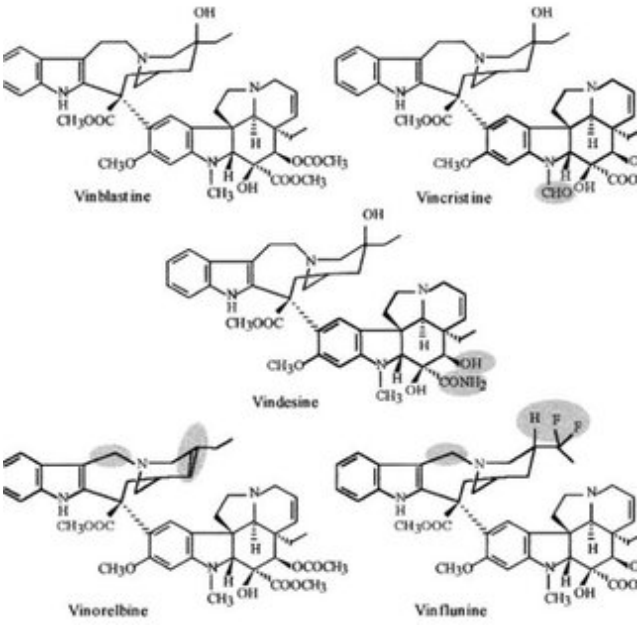

Fig. 1:

syrup was dissolved in 5 ml of water to give a concentration on 100mg/ ml. Further anticancer cytotoxicity assay was performed on 2 Cell lines viz Human Lung Cancer Cell line A549 and Human Breast cancer Cell Line MCF7. The sample was coded as RNV.

3.2. In vitro anticancer activity against cancer cell lines (anticancer cytotoxicity assay)[13,14]

3.2.1. SRB assay

The assay relies on the ability of SRB to bind to protein components of cells that have been fixed to tissue-culture plates by trichloroacetic acid (TCA). The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 μL at plating densities. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. Experimental drugs were initially solubilized in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate (1mg/ml) was thawed and diluted to 100μg/ml, 200μg/ml, 400μg/ml and 800μg/ml with complete medium containing test article. Aliquots of 10μl of these different drug dilutions were added to the appropriate microtiter wells already containing 90μl of medium, resulting in the required final drug concentrations i.e.10μg/ml, 20μg/ml, 40μg/ml, 80μg/ml. After compound addition, plates were incubated at standard conditions for 48 hours and assay

Table 1: In vitro anticancer cytotoxicity assay on human lung cancer cell line A549

Code	Human Lung Cancer Cell Line A549															
	% Control Growth															
	Drug Concentrations (μg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
RNV	100.0	90.2	87.2	79.2	91.4	88.1	84.7	84.1	96.6	88.3	87.2	74.1	96.0	88.9	86.4	79.1
ADR*	3.7	3.0	0.2	-1.2	4.4	3.4	0.5	-4.6	2.1	2.1	1.2	0.3	3.4	2.8	0.6	-1.9

Table 2: In vitro anticancer cytotoxicity assay on human breast cancer cell line MCF7

	Human Breast Cancer Cell Line MCF7															
	% Control Growth															
	Drug Concentrations (μg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
RNV	100.0	100.0	100.0	100.0	95.9	94.3	88.9	78.7	100.0	95.3	87.3	87.3	98.6	96.5	92.1	88.7
ADR*	3.7	3.0	0.2	-1.2	4.4	3.4	0.5	-4.6	2.1	2.1	1.2	0.3	3.4	2.8	0.6	-1.9

Table 3: Parameters for anticancer in vitro screening LC50, TGI, GI50 of RNV against A549 Cell Line

Code	Drug concentrations (μg/ml) calculated from graph		
A549	**LC50**	**TGI**	**GI50**
RNV	>80	>80	>80
ADR*	3.7	3.2	0.2

*Adriamycin

Table 4: Parameters for anticancer in vitro anticancer screening lc50, tgi, gi50 of rnv against mcf 7 cell line

Code	Drug concentrations (μg/ml) calculated from graph		
MCF-7	**LC50**	**TGI**	**GI50**
RNV	>80	>80	>80
ADR*	3.7	3.2	0.2

*Adriamycin

was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50μl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50μl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10mM trizma base, and the absorbance was read on an plate reader at a wavelength of 540nm with 690nm reference wavelength. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells x 100. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as: [Ti/C] x 100% Percentage growth inhibition, total growth inhibition TGI) and LC50 was calculated. GI50 value of $\leq$10 μg/ml is considered to demonstrate activity in case of pure compounds. For extracts, GI50 value $\leq$20 μg/ml is considered to demonstrate activity. Above three parameters.

From results as mentioned in Tables 1, 2, 3 and 4. It is found that the herbal formulation of Vinca is devoid of anticancer activity at concentrations of 10μg/ml, 20μg/ml, 40μg/ml and 80μg/ml as compared to parameters of LC50, TGI and GI50 and standard drug Adriamycin used for the assay.

4. Conclusion

The herbal formulation of syrup of Vinca extract is devoid of anticancer activity against Human A549 and Human MCF-7 Cell Line.

5. Conflict of Interest

None.

6. Source of Funding

None.

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