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Anticancer Activity of *Parthenium hysterophorus* Linn and *Oldenlandia corymbosa* Lam by Srb Method

Khushbu Pandey[1]*, Pramod K. Sharma[1] and Rupesh Dudhe[1,2]

[1]*Department of Pharmaceutical Technology, M.I.E.T, Meerut- 250005, U.P, India*
[2]*Uttarakhand Technical Universities, Dehradun- 284007, U.K, India*

Abstract

Aim: To study the anticancer activity of ethanolic extract of leaves of *Parthenium hysterophorus* Linn and *Oldenlandia corymbosa* Lam by SRB assay method on K562 human leukemia cancer cell line.

Materials and Methods: Anticancer activity of ethanolic extracts leaves of *Parthenium hysterophorus* Linn and *Oldenlandia corymbosa* Lam and also in combination of both the plant extract was performed on K562 cancer cell lines by the Advanced Centre for Treatment Research and Education in Cancer (ACTREC) Mumbai, India.

Results: *Parthenium hysterophorus* and *Oldenlandia corymbosa* showed significant anticancer activity on K562 human leukemia cancer cell line. Cell line were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine with the help of SRB assay and the absorbance was recorded on an Elisa plate reader at a wavelength of 540 nm with 690 nm.

Conclusion: *Parthenium hysterophorus* Linn and *Oldenlandia corymbosa* Lam has been showed anticancer activity individually but when they are given in combination of both plants extract on K562 human leukemia cancer cell line has been showed potent anticancer activity.

Keywords: K562 cell line; Leukemia; SRB assay

Introduction

Ethno historical accounts shows that medicinal plants have been used as a remedy for various human ailments, the reason of using these plants is that they contain certain types of chemical constituent which is having greater therapeutic value that produces a definite pharmacological actions on human body with lesser side effects [1]. Cancer is one of the most life threatening diseases and possess many health hazard in both developed and developing countries [2].

Leukemia is one of the most common cause of cancer occur throughout the world due to the lack of effective chemotherapeutic agents and side effects of anticancer drugs on prolong therapy of chemoprevention so anticancer patients switch over to the herbal medicine for their curement.

Parthenium hysterophorus Linn and *Oldenlandia corymbosa* Lam is the two herbaceous weed and they are known for their various pharmacological activity. Medicinal uses of both the plant extract show remarkable antipyretic, CNS stimulating, skeletal muscle relaxant [3] also the anti-oxidant [4], hypoglycemic activity [5]. *Oldenlandia corymbosa* are used as an herbal medicine for the treatment of hepatitis among the people of Southern India [6]. Thus, the present study was done to evaluate the anticancer potential of ethanolic extract of leaves of *Parthenium hysterophorus* Linn and *Oldenlandia corymbosa* Lam.

Materials and Method

Plant material

The fresh leaves of *Parthenium hysterophorus* Linn was collected from the Meerut (India) and *Oldenlandia corymbosa* Lam was collected from Chhattisgarh region of M.P. These plants were identified and authenticated by the research officer of botany at National Bureau of Plant and Genetic Resources (Pusa campus) New Delhi and a voucher specimen had been kept in the department of Pharmacology, Meerut Institute of Engineering and Technology, Meerut.

Preparation of ethanolic extract: For the preparation of ethanolic extract, the leaves of *Parthenium hysterophorus* Linn and *Oldenlandia corymbosa* Lam was collected, cleaned, air dried and grinded into coarse powder, then 22 gm powder was packed into the soxhlet extractor with 95% ethanol at a temperature 45°C. The extract was concentrated and dried by using rotary evaporator and the percentage yield was found to be 26.8% and 20.53% extract was stored in a refrigerator at 5°C.

Anticancer activity on k562 cell lines

The anticancer activity of ethanolic extracts leaves of *Parthenium hysterophorus* Linn and *Oldenlandia corymbosa* Lam and also in combination of both the plant extract was performed on K562 cancer cell lines by the Advanced Centre for Treatment Research and Education in Cancer (ACTREC) Mumbai, India. The cell viability was measured using SRB assay. All the environmental conditions were maintained throughout the experiment for all the groups. The assay was performed in triplicate for each of the extracts. The growth curve was plotted against molar drug concentration of plant extract and % control growth. The 50% reduction in net protein increase was observed on the combination extract of Oldenlandia and Parthenium with the positive control when compared compound i.e. adrinamycin.

Experimental procedure or SRB assay

The cell lines were grown in RPMI 1640 medium containing 10%

***Corresponding author:** Khushbu Pandey, Department of Pharmaceutical Technology, Meerut Institute of Engineering & Technology, NH-58, Baghpat bypass crossing, Meerut- 250005, UP, India, Tel: 09758709158; E-mail: pandeykhushbu19@gmail.com

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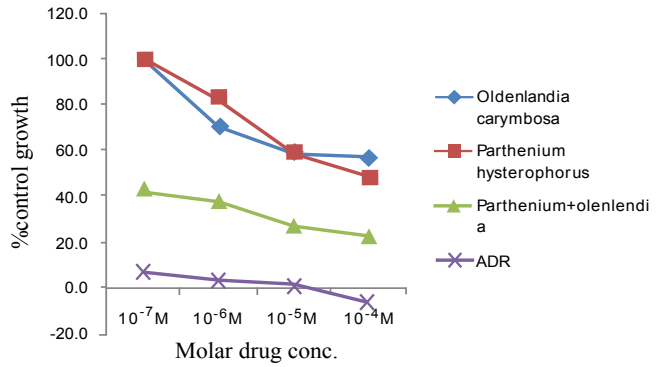

Figure 1: The Plot of percentage control growth vs. molar drug concentration shows the effective drug concentration on the K562 human leukemia cell line.

fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well micro titer plates in 90 µL at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the micro titer plates were incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, one plate of each cell line was fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in appropriate solvent at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum test concentration with complete medium containing test article at a concentration of 10^{-3}. Additional three, 10-fold serial dilutions were made to provide a total of four drug concentrations plus control. Aliquots of 10 µl of these different drug dilutions were added to the appropriate micro-titer wells already containing 90 µl of medium, resulting in the required final drug concentrations.

After compound addition, plates were incubated at standard conditions for 48 h and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl 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 (50 µl) 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 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength [7].

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 *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 [8].

Percentage growth inhibition=

For concentrations for which Ti>/=Tz (Ti-Tz) positive or zero = [(Ti-Tz)/(C-Tz)] × 100

For concentrations for which Ti<Tz. (Ti-Tz) negative = [(Ti-Tz)/Tz] × 100

Growth inhibition of 50%

GI_{50} = [(Ti-Tz)/(C-Tz)] × 100

GI_{50} is that value of the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC_{50} is the drug concentration resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning. During this there is a net loss of 50% cells following treatment is calculated from [(Ti-Tz)/Tz] × 100 = -50 [9].

Statistical analysis

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less

	Human Leukemia Cell Line															
	% Control Growth															
	Molecular Drug Concentration															
	Experiment 1				Experiment 2				Experiment 3				Experiment 4			
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
Oldenlandia carymbosa	100.0	72.0	47.0	48.9	100.0	74.1	66.3	63.6	100.0	68.4	67.8	59.6	100.0	71.5	60.4	57.4
Parthenium hysterophorus	100.0	77.7	63.2	44.9	100.0	97.7	61.7	52.2	100.0	73.9	50.7	48.5	100.0	83.1	58.5	48.5
Parthenium+olenlendie	32.9	27.6	20.6	20.5	50.6	43.7	36.7	33.6	46.5	43.8	24.1	15.7	43.3	38.4	27.1	23.3
ADR	7.7	2.0	1.8	-6.7	5.6	4.2	1.0	-6.1	7.8	4.9	0.9	-7.0	7.0	3.7	1.2	-6.6

Table 1

	µ Molar drug concentration		
K562	LC 50	TGI	GI50
Oldenlandia carymbosa	>100	>100	>100
Parthenium hysterophorus	>100	>100	91.3
Parthenium+olenlendie	>100	>100	<0.1
ADR	67.2	40.6	<0.1

Table 2

than the maximum or minimum concentration tested. The experiment data were estimated using linear regression method of plots of the cell viability against the molar drug concentration of tested compounds.

Results

The non toxic dose of *Parthenium hysterophorus* Linn and *Oldenlandia corymbosa* Lam showed anticancer activity individually but when the combination of both the plant extract is taken it showed more significant anticancer activity as compared to the positive control group i.e. adriamycin (Figure 1 and Table 1).

Experimental groups are compared with positive control group i.e. adrinamycin (ADR) (Table 2).

GI_{50} = Growth inhibition of 50% ($GI5_{50}$) calculated from [(Ti-Tz)/(C-Tz)] × 100 = 50, drug concentration resulting in a 50% reduction in the net protein increase.

TGI = Drug concentration resulting in total growth inhibition (TGI) will calculated from Ti = Tz

LC_{50} = Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of 50% cells following treatment is calculated from [(Ti-Tz)/Tz] × 100 = -50.

Conclusion

The result of this study shows that the ethanolic extract of *Oldenlandia corymbosa* Lam and *Parthenium hysterophorus* Linn was non toxic to normal cell and also had both anticancer activities individually and in combination of both the plant extract. This study points to the probable anticancer potentials of ethanolic leaf extracts of *Parthenium hysterophorus* and *Oldenlandia corymbosa* Lam. The results of the study will also need to be confirmed using *in vivo* models.

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