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Original Research Article

In vitro anticancer screening of herbal syrup formulation of podophyllum (May Apple) extract

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ABSTRACT

The in vitro anticancer screening of the medicated herbal formulation of podophyllum as reported earlier. The formulation of herbal syrup was prepared and optimized as per industrial practice guidelines and was subjected to in vitro anticancer assay against a A549-Human lung cancer cell line and MCF7-Huam Breast cancer cell line. The LC50, TGI and GI50 are reported here against standard Adriamycin used in the test. The result showed comparable activity of the herbal syrup of podophyllum extract.

1. Introduction

1.1. Role of podophyllum or may apple in cancer[1]

Podophyllotoxin has been found to be active on several human cancer cell lines HL- 60, A-549, HeLa and HCT-8.[2] In another study, podophyllotoxin was shown to activate the stress signalling pathway of the pro-apoptotic endoplasmic reticulum. Intraperitoneal injection of podophyllotoxin inhibited the growth of P-815, P-1537 and L-1210 tumour cells [2a]. The anti-tumour activity of podophyllotoxin resembles to paclitaxel [2b]. Podophyllotoxin has also been the active content of podophyllum or may apple extract has demonstrated fewer haematological and biochemical side effects.[3]

1.2. Medicated herbal syrup of may apple or podophyllum

It was concluded from the label claim that the commercially procured herbal plant extract of podophyllum was rich in content of Podophyllotoxin and its derivatives. Further the extract herbal formulation of syrup was prepared as reported by Nerkar et al.[4] and optimized as per the standard guidelines of Indian Pharmacopoeia i.e., the guidelines to optimize the syrup dosage form. Thus, in this paper we report here the in vitro anticancer screening of the formulation against A549 Human Lung Cancer Cell Line and MCF7 Human Breast cancer Cell Line.

2. Materials and Methods

The syrup was dried in petri dishes and 0.5g dried 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 dried formulation of Podophyllum extract was coded as SGM.

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Table 1: In vitro anticancer assay of herbal syrup of May apple (SGM) against A549

	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
SGM	100.0	100.0	96.6	85.1	100.0	98.9	95.7	89.6	100.0	96.3	94.1	89.3	100.0	98.4	95.5	88.0
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 assay of herbal syrup of May apple (SGM) against 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
SGM	100.0	100.0	100.0	100.0	68.8	66.9	64.4	58.3	58.7	46.9	46.5	43.3	75.8	71.3	70.3	67.2
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: In vitro anticancer assay showing LC50, TGI And GI50 of SGM against A549

A549	LC50	TGI	GI50
SGM	>80	>80	>80
ADR	3.7	3.0	0.2

Table 4: In vitro anticancer assay showing LC50, TGI And GI50 of SGM against MCF7

MCF7	LC50	TGI	GI50
SGM	>80	>80	>80
ADR	3.7	3.0	0.2

2.1. In vitro anticancer activity against cancer cell lines (anticancer cytotoxicity assay)

2.1.1. 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 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 were calculated only when the level of activity was observed. The values were expressed as greater or less than maximum or minimum concentration tested when the effect was not reached or exceeded.[5,6]

3. Result and Discussion

From results as mentioned in Tables 1, 2, 3 and 4., it is exemplified that the herbal syrup formulation of Podophyllum or May apple extract does not show any significant 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. However there is a possibility that it may show anticancer activity against other cancer cell lines which is a part of further research.

4. Conclusion

The herbal formulation of syrup of podophyllum extract does not show any significant anticancer activity against Human A549 and Human MCF-7 Cell Line.

5. Source of Funding

None.

6. Conflict of Interest

None.

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