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Original Research Article

In Vitro anticancer screening of herbal syrup formulation of allium cepa extract

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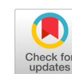

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A B S T R A C T

The formulation of herbal syrup of extract of Allium Cepa was reported in the previous paper. The herbal syrup of extract was evaluated here for *in vitro* anticancer activity. The *in vitro* anticancer activity was evaluated against 2 cell lines namely human lung cancer cell line A-549 and human breast cancer cell line MCF7. The results indicate that there is no significant anticancer activity of the herbal syrup formulation of the extract as compared with the standard Adriamycin.

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1. Introduction

1.1. *Role of allium cepa*

Allium Cepa or the onion has significant role in day-to-day life. Mostly it is consumed as vegetable fruit, spices, and adds delicious flavour to the food.[1] Allium Cepa is well known for its antidiabetic properties. Also, it has been reported to have aphrodisiac along with Safed Musli,[2] blood purifying[3] and as a fibre to the diet.[4] The well know utilities of onion or Allium Cepa can be found in Ayurveda. Thus, it can be consumed as extract and as a nutraceutical for complementary medicine and naturopathic purposes.[5] Further it has been reported to have anticancer properties.[6–8]

1.2. *Medicated herbal syrup of onion or allium cepa extract*

The formulation and evaluation or optimization of formulation of herbal syrup of extract of Allium Cepa was reported by Nerkar et al.[9] in agreement of 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 of Allium Cepa or Onion 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 Allium Cepa extract was coded as APO.

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

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Table 1: *In vitro* anticancer assay of herbal syrup of allium cepa (APO) 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
APO	47.4	32.8	26.7	20.6	40.5	28.1	25.9	20.0	41.4	27.6	18.2	16.3	43.1	29.5	23.6	19.0
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 allium cepa (APO) 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
APO	49.0	47.4	40.0	23.6	56.2	42.6	37.8	33.3	61.8	43.8	35.9	25.9	55.7	44.6	37.9	27.6
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 APO against A549

Drug concentrations (μg/ml) calculated from graph			
MCF7	**LC50**	**TGI**	**GI50**
APO	>80	>80	21.6
ADR	3.7	3.0	0.2

Table 4: *In vitro* anticancer assay showing LC50, TGI and GI50 of APO against MCF7

Drug concentrations (μg/ml) calculated from graph			
MCF7	**LC50**	**TGI**	**GI50**
APO	>80	>80	35.5
ADR	3.7	3.0	0.2

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 ≤10 μg/ml is considered to demonstrate activity in case of pure compounds. For extracts, GI50 value ≤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.[10,11]

3. Result and Discussion

From results as mentioned in Tables 1, 2, 3 and 4., it is exemplified that the herbal syrup formulation of Allium Cepa or Onion 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 Allium Cepa 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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