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Short Communication

In vitro toxicity screening of polyphyto combination using Caco-2-cell line

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The aim of the research work is polyphyto combination compression of different antilithiogenic agent and to screen its toxicity by Caco-2-cell line. Polyphyto combination was prepared by geometrical dilution method; it was subjected for aqueous and alcoholic extract. The formulation and extract were screened for its initial *in vitro* toxicity study using Caco-2-cell line by 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay method for aqueous extract of formulation. It showed nontoxic to Caco-2-cell line, even at high dose, that is, 1000 µg/ml, whereas aqueous extract (FH_1) exhibited moderate toxicity CTC 50 value 875±35.3 µg, FH_1 crude formulation showed significant toxicity against Caco-2-cells with low concentration with CTC 50 value 382.75 based on this result the conclusion was drown that the formulation FH_2 and aqueous extract of FH_1 is devoid of toxicity. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS).

Key words: Cytotoxicity, dimethyl sulfoxide (DMSO), flavones glycosides.

INTRODUCTION

The Caco-2 cell line is a continuous line of heterogeneous human epithelial colorectal adeno-carcinoma cells, developed by the Sloan-Kettering Institute for Cancer Research through research conducted by Fogh and Trempe (1975). Although derived from a colon (large intestine) carcinoma, when cultured under specific conditions the cells become differentiated and polarized such that their phenotype, morphologically and functionally, resembles the enterocytes lining the small intestine (Pinto, 1983; Hidalgo, 1989). Human colon adenocarcinoma (Caco-2) cells have been widely used as *in vitro* models to evaluate the transport of drug candidates across the intestinal epithelial barrier.Caco-2 cells when grown on semi permeable filters (Hilgers et al., 1990), spontaneously differentiate in culture to form confluent monolayers which both structurally and functionally resemble the small intestinal epithelium. These cells resemble various biological membrane properties, including enzymatic and transport systems. The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used (Francis and Rita, 1986). The current objective of the research work was to formulate and screened *in vitro* cell line toxicity using Caco-2-cell line by MTT assay inert to determine the dose response against the concentration of the formulation and its extraction (Ahmed et al., 1993).

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MATERIALS AND METHODS

Chemicals

3-(4, 5– dimethyl thiazol– 2–yl)– 5– diphenyl tetrazolium bromide (MTT), fetal bovine serum (FBS), phosphate buffered saline (PBS),

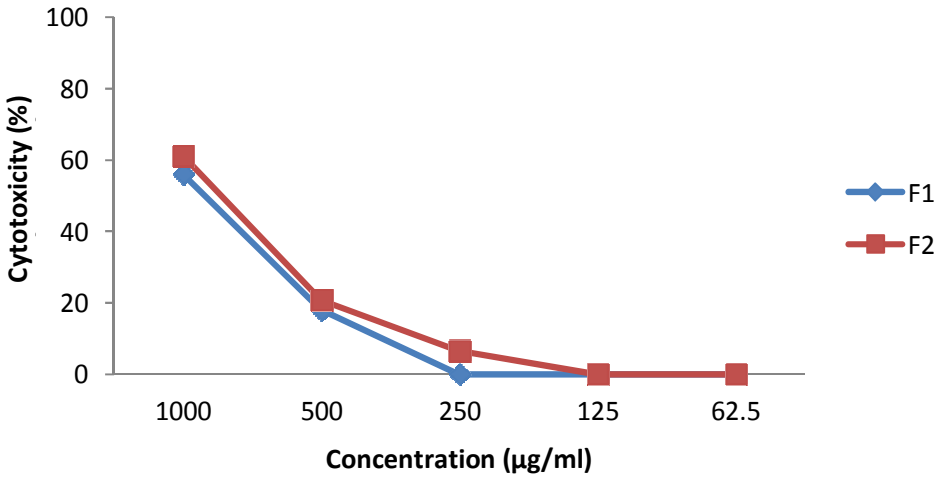

Figure 1. Cytotoxic effect of Form-1 on Caco-2 cell line.

Eagle's minumum essential medium (MEM) and trypsin were obtained from Sigma Aldrich Co, St Louis, USA; EDTA, glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai, and dimethyl sulfoxide (DMSO) and propanol from E. Merck Ltd., Mumbai, India.

Cell lines and culture medium

Caco-2 (Human, Epithelial colorectal adenocarcinoma) was cultured in MEM supplemented with 10% inactivated fetal bovine serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 µL plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of test solutions

Plant material for cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with MEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Preparation of crude FH_1

The contents of this preparation were *Kegelia africana*, *Bergenia ligulata*, *Asparagus rosemosus*, *Boerhaavia diffusa* etc. and which were dried under sun shade dried, powdered.

Preparation of aqueous FH_1

Polyphyto mixture was collected from the Forest Research Institute Dehradun (UK) and authenticated which were dried under sun shade, powdered and macerates with distill water and allowed to stand for 24 h and then filtered and evaporated.

Preparation of alcoholic FH_1

Polyphyto mixture was collected from the Forest Research Institute of Dehradun (UK) and authenticated before drying under sun shade. It was then powdered and macerated with ethanol and allowed to stand for 24 h, after which it was filtered and evaporated (Mukharjee PK.).

Determination of cell viability by MTT assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using MEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37℃ for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37℃ in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line (Anonymous, 1959; Duncan, 1951; Hidalg, 1989):

$$\% \text{ Growth Inhibition} = 100 - \left(\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100 \right)$$

RESULTS

The result of the study is shown in Figure 1 and Table 1.

Table 1. Cytotoxic properties of test drugs on Caco-2 cell line.

S/N	Name of drug	Test concentration (µg/ml)	% Cytotoxicity		CTC_{50} (µg/ml)		Average CTC_{50} (µg/ml)±SEM
			F1	F2	F1	F2	
1	Sample 1 (Crude FH_1)	1000	76.63	71.50	375.00	390.50	382.75 ± 10.9
		500	71.96	68.52			
		250	22.31	19.15			
		125	0.00	0.00			
		62.5	0.00	0.00			
2	Form1 (Aqueous FH_1)	1000	56.08	61.25	900.00	865.00	875.00 ± 35.3
		500	17.99	20.85			
		250	0.00	6.50			
		125	0.00	0.00			
		62.5	0.00	0.00			
3	Form-1 (Alcoholic FH_1)	1000	21.87	33.65	>1000	>1000	> 1000
		500	0.00	0.00			
		250	0.00	0.00			
		125	0.00	0.00			
		62.5	0.00	0.00			

Conclusion

It was concluded from the study that the alcoholic extract of polyphyto combination is less toxic than aqueous extract and aqueous extract is less toxic than crude sample against coca-2-cell line. The alcoholic and aqueous fraction can be further screened for investigating its antilithogenic property by both *In vitro* and *In vivo* method.

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