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In vitro cytotoxic activity of ethanolic extract of *Triumfetta rhomboidea* against different cancer cell lines

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ABSTRACT

Triumfetta rhomboidea (TR) is being used in traditional medicine for a variety of ailments. It is widely distributed in tropical and subtropical part of India up to an elevation of 1,200 m in the Himalayas. *Triumfetta rhomboidea* is used for tumor, leprosy, gonorrhea, intestinal ulcer, dysentery and various other ailments. The aim of the work is to explore phytochemical constituents present in leaves extract and to evaluate the cytotoxic activity of the plant. The phytochemical investigation of the ethanolic extract of *Triumfetta rhomboidea* revealed the presence of carbohydrates, glycosides, tannins, flavonoids, steroids, saponins and terpenoids. To substantiate the traditional claim of TR for its cytotoxic activity, extract was tested on different cell lines (In vitro). The leaves extract from *Triumfetta rhomboidea* was subjected for cytotoxic activity against HT-29 (Human, colorectal cancer cell line), HeLa (Human, Epithelial cervical cancer) and C_2 C_{12} (Mouth muscle cell line) cell lines by Microculture Tetrazolium (MTT) assay method. The extract exhibited moderate cytotoxic properties towards cancerous cell lines HT-29 and HeLa models. Where as against C_2C_{12}, test extract failed to exhibit cytotoxicity even at higher test concentrations. Cytotoxicity tests indicated that the extract has moderate cytotoxic effect.

Keywords: *Triumfetta rhomboidea* , Cytotoxicity, MTT Assay, HT-29, HeLa, and C_2C_{12} cell lines.

INTRODUCTION

In recent years, considerable interest has been evinced by the public and the medical professional regarding the use of indigenous drugs in the treatment of diseases. Efforts are being made to develop anticancer agents from natural sources by many workers. In the past, plants have provided anticancer compounds like vincristine and taxol. The plant *Triumfetta rhomboidea* (Family: Tiliaceae) is distributed throughout all districts of south India, also reported from Ceylon, Malay, Africa and America. Traditionally, the plant used in treatment of diarrhoea, dysentery, intestinal ulcer and as diuretic. Leaves & Stem are used in tumors, gonorrhoea and leprosy.[1-3] No investigation has been carried out for

its anticancer property till now. In view of these reports and ethno medical uses of *Triumfetta rhomboidea* leaf, we studied the in vitro cytotoxic properties of its crude ethanolic extract (EETR) against HT-29, HeLa, and C_2C_{12} cancer cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolim bromide (MTT) assay.

MATERIALS AND METHODS

Plant Materials

The leaves of *Triumfetta rhomboidea* was collected from Yercaud, Salem district, Tamilnadu. The collected plant was authenticated by comparing it with authentic specimen at Botanical survey of India, Coimbatore.

Preparation of extract

The shade dried leaves was powdered and extracted using ethanol as solvent in a Soxhlet apparatus and after complete extraction (48 hrs) the solvent was removed by distillation under reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator to yield (12.0% w/w) a solid residue.[4,5]

Phytochemical screening

Qualitative phytochemical screening was carried out to identify the phytoconstituents. The ethanolic extract of *Triumfetta rhomboidea* showed the presence of carbohydrates, glycosides, tannins, flavonoids, steroids, saponins and terpenoids.[6-8]

Chemicals

3-(4,5–dimethylthiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA., Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

Cell lines and Culture medium

HT-29 (Human, colorectal cancer), HeLa (Human, Epithelial cervical cancer) and C_2C_{12} (Mouse, Muscle cell line) cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells of HT-29, C_2C_{12} and HeLa were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an 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 either 96 microtiter plates (Tarsons India Pvt. Ltd., Kolkata, India).

Determination of cell viability by MTT Assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM medium containing 10% FBS/NBCS. To each well of the 96 well microtiter 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, washed the monolayer once with medium and 100 µl of different test concentrations were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37° C 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 extract 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° C 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 micro plate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of drug or test extract needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.[9-13]

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

Table 1: *In vitro* cytotoxic properties of ethanolic extract of *Triumfetta rhomboidea* on different cell lines.

S. No	Cell line	Test Concentrations (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	**HT-29**	1000	61.50	701.50 ± 0.245
		500	43.05	
		250	30.95	
		125	17.80	
		62.5	0.00	
		31.25	0.00	
2	**HeLa**	1000	56.75	785.50 ± 0.529
		500	41.35	
		250	36.00	
		125	22.65	
		62.5	9.55	
		31.25	0.00	
3	**C_2C_{12}**	1000	31.65	> 1000
		500	25.60	
		250	14.50	
		125	0.00	
		62.5	0.00	
		31.25	0.00	

Values are mean ± SEM, P < 0.001 (followed turkey Kramer equation).

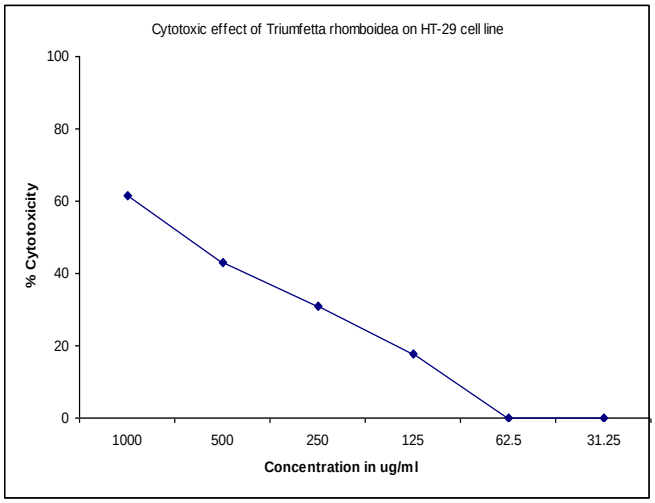

Fig. 1: Cytotoxic effect of *Triumfetta rhomboidea* on HT-29 cell line

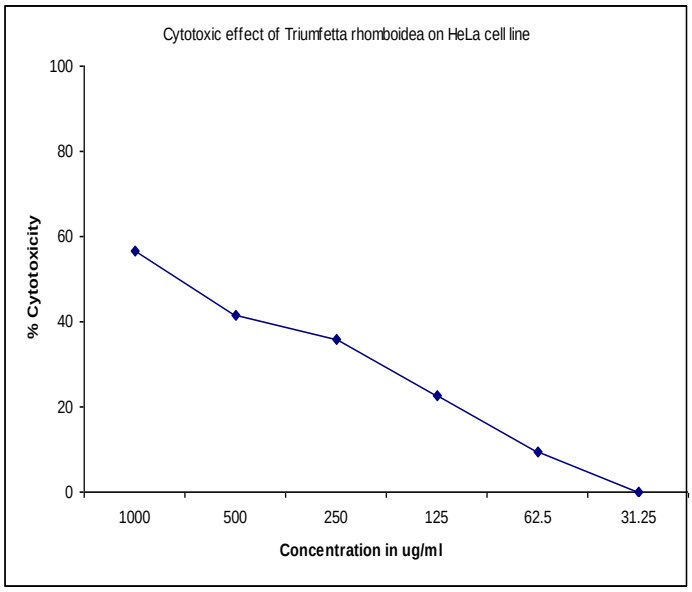

Fig. 2: Cytotoxic effect of *Triumfetta rhomboidea* on HeLa cell line

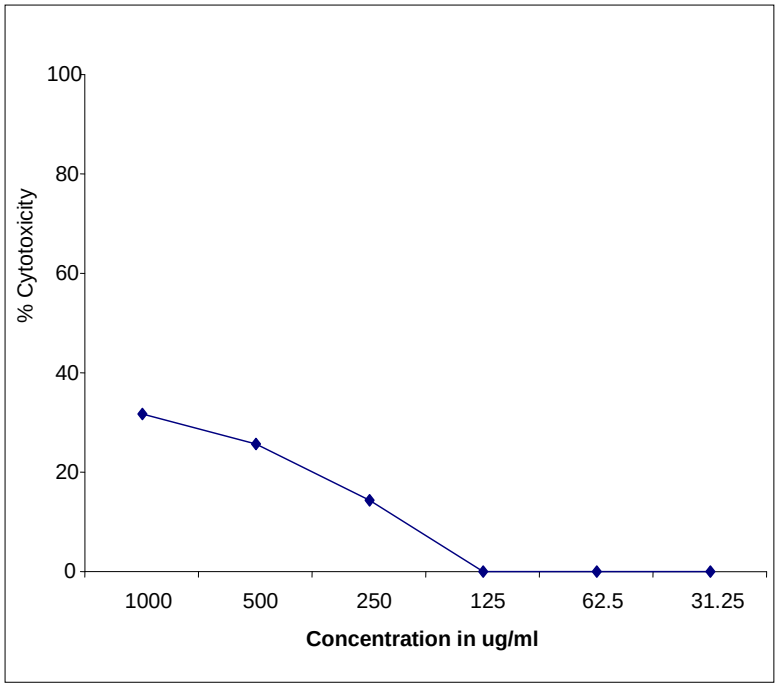

Fig. 3: Cytotoxic effect of *Triumfetta rhomboidea* on C_2C_{12} cell line

RESULTS

The extract from *Triumfetta rhomboidea* was tested for cytotoxic activity against HT-29, HeLa and C_2C_{12} cell lines by MTT assay method. The ethanolic extract of *Triumfetta rhomboidea exhibited* moderate cytotoxic properties towards cancerous cell lines HT-29 and HeLa with CTC_{50} values 701.50 and 785.50 µg/ml, respectively. Whereas against C_2C_{12}, test extract failed to exhibit cytotoxicity at test concentrations with only 32% cytotoxicity at 1000 µg/ml.

DISCUSSION

Treating cells with a cytotoxic compound can result in a variety of cell fates. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death (apoptosis).

Triumfetta rhomboidea extract has been reported to antiulcer, antimicrobial and antigermination activities.[14-16] Srinivisan, et al., isolated the new flavone glycoside Triumboidin from the leaves of *Triumfetta rhomboidea* Jacq.[17]

In terms of their bioactive constituents, phytochemical screening of *Triumfetta rhomboidea* leaves demonstrated the presence of flavonoids, saponins, triterpenes, tannins and steroids, but no alkaloids. Interestingly, flavonoids, tannins, saponins and triterpenes have all been reported to possess anticancer activity.[18] Flavonoids have also been proven to be effective in the prevention of several chronic diseases involving oxidative stress (i.e. cancer).[19]

With regards to the possible anticancer mechanisms involved, flavonoids have been reported to exhibit their anticancer activity via the modulation of cell cycle arrest at the G1/S phase, down-regulation of anti-apoptotic gene products.[20] On other hand, saponins induce anticancer activity via the necrosis cell death, which depends on the types of cancer cells affected.[21] Further more, triterpenes also induce apoptotic response on cancer cells by inhibiting nuclear factor-kappa B or by causing cell cycle disruption (by decreasing the number of cells in G0/G1 phase with initial increase in S and G2/M).[21]

In conclusion, the ethanol extract from *Triumfetta rhomboidea* possess moderate cytotoxic activity against the HT-29 and HeLa cell lines, which could be associated with its antioxidant mechanisms.

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