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Invitro Anticancer Activity of *Rubia Cordifolia* Against Hep G 32 Cell Line

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Abstract : The aim of the present study was to evaluate *in vitro* cytotoxicity of *Rubia cordifolia* against Hep G32 (human Hepatocellular carcinoma) cell line using XTT assay. Methanol fraction of *Rubia cordifolia* extract exhibited potent inhibition of Hep G32 cell line with IC_{50} value of 28.07 µg/ml while was found to be less cytotoxic against normal human kidney cells with IC_{50} value more than 100 µg/ml displaying safety for normal cells.

Keywords: Rubia cordifolia, Cell line, Anticancer, Hep G32

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a primary malignancy of the hepatocyte, generally leading to death within 6-20 months. [1] Rubia cordifolia (Rubiaceae), also known as Indian Madder or Manjistha is traditionally used as anti-inflammatory, antiseptic and galactopurifier. *In vitro* cytotoxicity activity of the plant has been reported against various cancer cell lines like HL 60, U937, HeLa and Hep 2. [2], [3] Studies on various other cell lines are required to explore the value of the plant to treat the dreadful disease like cancer.

MATERIAL AND METHODS

Reagents

Trypan blue (Hyclone, Lot no: JRH27098, 100 ml), Triton X100 (MP Biomedicals, Lot No: 8009H), DMSO cell culture grade (MP Biomedicals, Lot No: R20759), Sodium bicarbonate (MP Biomedicals, Lot No: 2048J), HYQ® Antibiotic/Antimycotic solution, 100X (10000 U/ml Penicillin G,10000µg/ml Streptomycin, 25 µg/ml Amphotericin B) (Hyclone, Lot no: JRM28184, 100 ml), Penicillin and Streptomycin solution (MP Biomedicals, Lot No: R23253), EDTA (MP Biomedicals, Lot No: 6941H), HYQ® DPBS/modified 1X (Dulbecoo's phosphate buffer saline without Ca^{+} & Mg^{+}) (Hyclone, Lot No: ASA28462, 100 ml), 0.25% Trypsin 1X (Invitrogen, Lot No: 1376596), Cyclophosphamide monohydrate (MP Biomedicals, Lot No: 6383H), HBSS –1X (Hank's Balanced Salt solution) (Hyclone, Lot no:ARL27892, 500 ml), Cell proliferation kit (XTT) 2500 tests (Roche, Lot No: 11465015001), Ethanol, Methanol, Petroleum ether, Dichloromethane

Media

DMEM (Dulbecoos Modified Eagels medium, low glucose with glutamine) (US Biological, Lot No: L7020976), FBS (Fetal Bovine Serum, South American origin, 500 ml) (Bioclot, Lot No: 07310), HYQ® SFM HEK-293™ (Hyclone, Lot no: ARF26635, 500ml)

Cell lines

HEK 293 (Human Epithelial Kidney cell line), Hep G 32 (Human Hepatocellular carcinoma)

Collection and Preparation of Plant Material

The plant sample (root) of *Rubia cordifolia* was purchased from Yucca enterprise, Mumbai. For taxonomical identification, it was authenticated by Mr. V.R.Patel (Dept.

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of Pharmacognosy, Baroda college of Pharmacy, Vadodara). After proper identification, the plant samples were cut into small pieces followed by dried and grinded into coarse powder by using high capacity grinding machine and passed through sieve number 14. It was stored in an airtight container.

Extraction and Fractionation Procedure

500 g of dried powder of *Rubia cordifolia* was soaked into ethanol and boiled at 80ºC for 3 hours to get crude ethanol extracts. The extract was then filtered through cotton followed by Whatman No.1 filter paper and the filtrate thus obtained was concentrated at 40ºC with a rotary evaporator. The concentrated extract was dried residue. The yield of the extract was 38.4 g. The crude extract was then dissolved in 10% water in methanol (100 ml) and partitioned between pet-ether (2.8 g), dichloromethane (4.2 g) and methanol fractions (22.9 g).

Experimental design

A cytotoxicity property of extracts of roots of *Rubia Cordifolia* was carried out by XTT method against HEK293 and Hep G32 cell lines. 2 mg of each plant extract was dissolved in 200µl of DMSO (dimethyl sulfoxide) then 100µl of this solution was diluted to 10ml with DMEM (Dulbecoos Modified Eagels medium, low glucose with glutamine). Thus, final concentration of this stock solution was 100µg/ml. Then by serial dilution varying concentrations were prepared from the stock solution. Thus the concentrations of the solutions obtained were 100 µg/ml, 33.33 µg/ml, 11.11 µg/ml, 3.70 µg/ml, 1.23 µg/ml, 0.411 µg/ml, 0.137 µg/ml, 0.045 µg/ml, 0.015 µg/ml, 0.005 µg/ml. 2 mg of Cyclophosphamide monohydrate (served as the positive control) was dissolved in 200µl of DMSO (dimethyl sulfoxide) then 100µl of this solution was diluted to 10ml with DMEM (Dulbecoos Modified Eagels medium, low glucose with glutamine). Thus, final concentration of this stock solution was 100µg/ml. Then by serial dilution varying concentrations were prepared from the stock solution. Thus the concentrations of the solutions obtained were 100 µg/ml, 33.33 µg/ml, 11.11 µg/ml, 3.70 µg/ml, 1.23 µg/ml, 0.411 µg/ml, 0.137 µg/ml, 0.045 µg/ml, 0.015 µg/ml, 0.005 µg/ml. As for negative control 100µl of DMSO was diluted to 10 ml with DMEM (Dulbecoos Modified Eagels medium, low glucose with glutamine). [4]

Cells were preincubated at a concentration of 1×10^6 cells/ml in culture medium for 3 h at 37°C and 5% CO_2. Cells were seeded at a concentration of 5×10^4 cells/well in 100 µl culture medium and various amounts of compound (final concentration e.g. 100µM - 0.005µM) into microplates (tissue culture grade, 96 wells, flat bottom). Cell cultures were incubated for 24 h at 37°C and 5% CO_2. 50 µl XTT labeling mixture was added and incubated for 18 h at 37°C and 6.5% CO_2. The spectrophotometrical absorbance of the samples was measured using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product was 450 nm according to the filters available for the ELISA reader, used. The reference wavelength was more than 650 nm. [5] [6]

All experiments were performed using three wells for each concentration of each compound tested. The cytotoxicity data was standardized by determining absorbance and calculating the correspondent compound concentrations. Dose response curve was developed for each concentration of each compound tested. IC_{50} value was determined for each concentration of each compound tested. [7]

RESULT AND DISCUSSION

In this in vitro cytotoxicity assay, the root extract of *Rubia cordifolia*, exhibited significant cytotoxic activity against Hep G 32 cell line with IC50 values of 28.07 µg/ml, 37.13 µg/ml and 41.96 µg/ml for methanol fraction, pet-ether fraction and dichloromethane fraction respectively. None of the fraction of the extract was found to be cytotoxic against HEK293 cell line in the concentration range of 0.05 µg/ml to 100 µg/ml.

Table.1 IC_{50} values (µg/ml) of standard Cyclophosphamide monohydrate and three different extracts of *Rubia cordifolia* (Rubiaceae) against HEK 293 and Hep G 32 cell lines.

Sample	IC_{50} VALUES (µg/ml)	
	CELL LINES USED	
	HEK293	Hep G 32
Cyclophosphamide monohydrate*	>100	0.697
Dichloromethane fraction	>100	41.96
Methanol fraction	>100	28.07
Pet-ether fraction	>100	37.13

CONCLUSION

Study results (Table 1) show that root extracts of *Rubia Cordifolia* is promisingly cytotoxic and they might have antitumor activity against hepatocellular carcinoma. None of the fraction of the extract was found to be cytotoxic against the normal cell line (HEK293) in the given range of concentration. We conclude this study with a view that *Rubia cordifolia* will be a potential herbal candidate for treatment of cancer provided further investigations.

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