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Original Article

STUDY ON *IN VITRO* CYTOTOXICITY OF PAPAIN AGAINST LIVER CANCER CELL LINE HEP G2

M. AKILA, A. SUSHAMA, R. KUMARESAN*

Department of Biotechnology, Periyar Maniammai University, Vallam, Thanjavur 613403, India
Email: kumaresanramanatha@gmail.com

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ABSTRACT

Objective: Cancer is a devastating public health problem all over the world. Herbal medicines have a vital role in the prevention and treatment of cancer. Papain is a proteolytic enzyme and a phyto therapeutic agent which highly appreciated in the medical field that prevents several chronic diseases such as cardiovascular disease, cancer and diabetes. The aim of the study describes about the cytotoxicity of papain against liver cancer cell line HepG2.

Methods: In different concentrations of samples have been taken for cytotoxicity analysis using the MTT assay and calculate the percentage of cell viability.

Results: The 50% inhibition concentration (IC_{50}) of a sample was 125µg/ml and their cell viability percentage was 49.20. The 85% of cytotoxicity has been observed in 1000 µg/ml of papain against HepG2 cell line than other concentrations.

Conclusion: 1000 µg/ml of papain has maximum (85%) cytotoxicity effect against liver cancer cell line hepG2.

Keywords: Papain, HepG2, Cell viability, Cytotoxicity.

INTRODUCTION

Nature is a source of medicinal agents for thousands of years. Various medicinal plants have been used for years in daily life to treat disease all over the world [1]. The commonest liver cancer is the hepatocellular carcinoma which is third most frequent cause of cancer death in India and fifth commonest cancer in the world [2]. Various parts of papaya include fruits, shoots, leaves, rinds, seeds, roots or latex have been traditionally used as ethno medicine for a number of disorders, including cancer **[**3].

Papaya latex constitutes proteolytic enzyme papain, chymopapain, glutamine cyclotransferase, chymopapain A, B and C, peptidase A and B and lysozymes. Papain is an endolytic plant cysteine protease enzyme which is the major constituents of papaya [4]. It preferentially cleaves peptide bonds involving basic amino acids, particularly arginine, lysine and residues following phenylalanine [5]. Many cancer cells having a protective coating of fibrin. That is why the development of cancer may be undetected for many months and year. Due to this proteolytic activity of papain, it may break down the fibrin coat of cancer cell wall. Thus, ultimately it may help against the cancer. Hence, we aimed to study the efficacy of papain in cytotoxic activities against liver cancer cell line HepG2. To best of our knowledge, there are no literature available in this aspect and the present study may be the first kind of attempt in the global arena.

MATERIALS AND METHODS

Cell line (Hep G2) and culture

A HepG2 cell line *was* obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

MEM was purchased from Hi Media Laboratories Fetal Bovine Serum (FBS) was purchased from Cistron laboratories. Trypsin, methylthiazolyl diphenyl-tetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) were purchased from Sisco research laboratory chemicals, Mumbai. All the other chemicals and reagents were obtained from Sigma Aldrich Mumbai. Commercial papain has been purchased from Sigma Aldrich to study the cytotoxic activity against hep G2 cell line.

In vitro assay for anticancer activity (MTT Assay) [6]

Cells (1×10^5/well) were plated in 24-well plates and incubated at 37^0C with 5% CO_2. After the cells reached the confluence, the various concentrations of the samples were added and incubated for 24 hours. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100 µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1 ml of DMSO was added to all the wells.

The absorbance at 570 nm were measured with UV- Spectro photometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC_{50}) was determined graphically. The % cell viability was calculated using the following formula:

% cell viability = (A570 of treated cells / A570 of control cells) × 100

RESULTS

The Figure 1(a) indicates the control Hep G2 cell line. It indicates the cell viability of the HepG2 cells (i. e) 100%. Fig 1(b) has shown the feasible cell after 1000µg/ml of sample. It point out the cell viability of the Hep G2 cells when 1000µg/ml of papain added to it. The cell viability is 15.87% out of 100 % and in this concentration, lesser amount of cells are viable. Fig 1(c) has shown the viable cells after adjoining 125µg/ml of sample. It illustrates that the feasible Hep G2 cells, when 125 µg/ml of sample noted up.

The percentage of cells feasible are 49.20% which we can admit as consistent sum of cells demise. Fig1 (d) has shown the total cell survived adjoining 62.5 µg/ml of sample. It points up the conscious cells, when 62.5 µg/ml of sample transfer to it. The total cipher of cells exist are 61.90%, where another half of the cells are viable. It shows potential of that particular concentration of sample when added to it. Fig 1(e) has shown the existing cells subsequent to addition of 31.2 µg/ml of sample and it proves that 69.84%of cells are feasible (i. e.) nearly $3/4^{th}$ cells are viable. It is not a vowing concentration of sample when we compare all the other.

The effect of the samples on the proliferation of HepG2 were expressed as the percentage cell viability, using the following formula

% cell viability = (A570 of treated cells / A570 of control cells) × 100

The HepG2 cell line with its anticancer activity of papain at various concentrations is listed below. The dilution rate at various ratio shows the feasibility of cells. These cells were absorbed under UV Spectrometer at 570 nm. In table.1 at the dilution rate of 1:4 the cell feasibility is consistent and its absorbance is 0.31 nm.

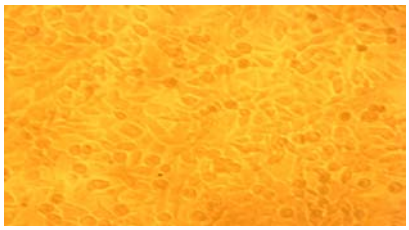

Fig. 1(a): indicates the control Hep G2 cell line

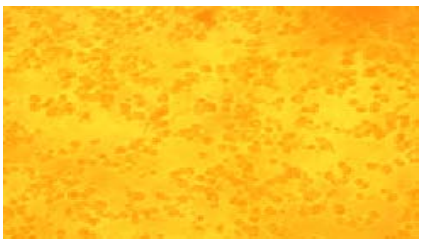

Fig. 1(b): has shown the feasible cell after 1000µg/ml of sample

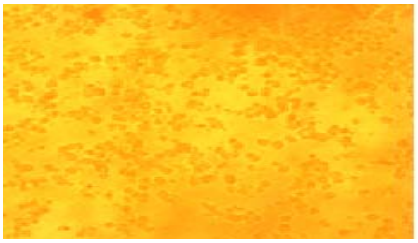

Fig.1(c): has shown the viable cells after adjoining 125µg/ml of sample

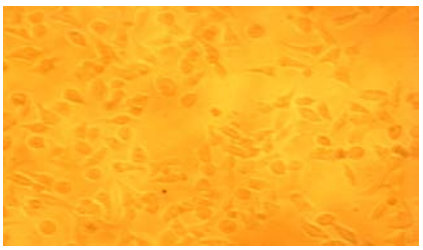

Fig1. (d): has shown the total cell survived adjoining 62.5 µg/ml of sample

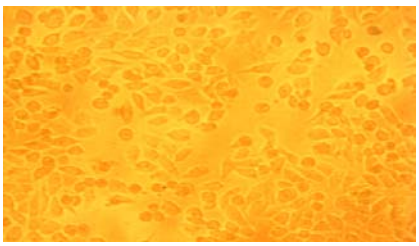

Fig. 1(e): has shown the existing cells subsequent to addition of 31.2 µg/ml of sample

Table: 1.

S. No.	Concentration (µg/ml)	Cell viability (%)
1	1000	15.87
2	500	30.15
3	250	42.85
4	125	49.20
5	62.5	61.90
6	31.2	69.84
7	15.6	77.77
8	7.8	84.12
9	Cell control	100

DISCUSSION

Papain is a proteolytic enzyme which extracted & purified from papa ya latex by advanced biochemical technology. The drugs containing papain have the functions of anti-cancer, anti-tumour, anti-lymphatic leukemia, anti-lysogenic bacteriums and anti-parasite, anti-tubercle bacillus and etc. It can diminish inflammation lidan pain helps digestion, treatment women's illnesses, glaucoma, osteo proliferation. Gun knife wound healing, Blood type identification, Mosquito bites and so on. No clinical or animal cancer studies were identified and only seven in vitro cell-culture-based studies were reported [7].

The present study observed that papain has anticancer activity against liver cell carcinomas. This observation could gave a new dimension towards the curing of cancers using herbal medicine. This result may be the first kind of report because to best of our knowledge there are no data available to compare our results. But one earlier study [3] has revealed that many cancer cells having a protective coating of fibrin and papain breaks down that fibrin coat of cancer cell wall and protein into amino acid form. So ultimately it helps against the cancer.

Really, the cytotoxicity of papain against liver cancer was a very good approach to bring out the platform for new drug discovery and delivery technologies. The present study results gave a remarkable finding and added a new feather to the papain family.

In conclusion, papain has 85 % of cytotoxicity against HepG2 liver cancer cell line with the concentration of 1000µg/ml. Larger study needs to confirm our results.

CONFLICT OF INTERESTS

Declared None.

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