Evaluation of Prepared formulation and hydroalcoholic extract of *Carica papaya* leaf for *in vitro* cytotoxicity activity on Human Liver Carcinoma (HEPG2) cell line

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Abstract

Carica papaya (papaya) of Caricaceae family is a well-known medicinal plant which is being used traditionally for immunomodulatory, anti-sickling and anti-thrombocytopenic property. It is an evergreen herbaceous tree originated from Central America. It is reported to have carbohydrates, amino acids, saponin glycosides, flavonoids, phenolics, tannins and alkaloids. In present study, phytosomes of hydroalcoholic extract of Carica papaya leaves is prepared and assessed for their in vitro cytotoxicity activity by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay on Human Liver Carcinoma (HEPG2) cell line. The performance of prepared formulation is compared with conventional preparation i.e. hydroalcoholic extract. From the results, it was concluded that the novel formulation of hydroalcoholic extract of Carica papaya leaves is more effective than extract.

Keywords: *Carica papaya, phytosomes, MTT assay, HEPG2 cell line*

Introduction

Cancer is a disease which is caused by ruthless growth of abnormal cells. It leads to aggregation of cells and forms tumours in the body. It is distributed worldwide with high mortality rate. One of the major reasons for the disease is damage by free radical, which further leads to damage of Deoxyribonucleic acid (DNA), lipids and proteins and results in mutations. Mutation results in conversion of normal cells into cancer cells[1-5].

Liver cancer

Liver diseases are the major causes for the deaths in India. Liver cancer is the second in terms of mortality due to cancer. The treatments used for the treatment of liver cancer are radiation therapy, anti-hormonal therapy, liver resection and chemotherapy. These treatments on longer term produce various side effects. These anti-cancer therapy causes cytotoxicity which destroys cancer cells along with normal cells. Therefore, it is need of the hour to find an alternative solution to this with no or minimum side effects. To overcome this situation, the use of traditional herbal medicine is screened out for anticancer effects[6-10].

Hepatocellular cancer

HepG2 cell line is an immortalized cell line. It is produced from Caucasian male liver tissue. HepG2 cell line consists of carcinoma cells of human liver. It has 55 chromosomal pairs and it is grown at a higher scale. According to study, around 630,000 new cases of Hepatocellular carcinoma are diagnosed every year making it the most common cancer in the world. The major reasons for Hepatocellular carcinoma are infections of Hepatitis B, Hepatitis C and alcohol-induced liver injury[11-21].

Nowadays, there is great demand of herbal products for liver diseases in the people because of increased awareness for health benefits. But they have poor bioavailability in case of hydrophilic natured phytoconstituents. Hence, incorporation of these hydrophilic herbal drugs in a lipophilic carrier system will increase their permeability, bioavailability and thus better therapeutic effect. Phytosomes are novel formulation formed by complexation with phospholipids. They have better stability in the gastric environment and increase the bioavailability of herbal drug[22-26]. Nanotechnology has opened new possibilities in medical sciences. It offers a novel and efficacious approach to formulate bioenhanced anticancer formulations.

In vitro study

MTT Assay

In latest studies MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay is considered to be reliable for quantification of cell viability. MTT is a yellow coloured water soluble tetrazolium salt. The living cells which are metabolically active convert this salt to dark blue formazan crystals which is water insoluble. Therefore the number of living cells can be calculated by amount of formazan produced and it is directly proportional.

Carica papaya

Carica papaya is economically important plant cultivated globally for their fruit. The phytochemical investigation proves the presence of carbohydrates, amino acids, saponin glycosides, flavonoids, phenolics, tannins and alkaloids. All parts of *Carica papaya* has well known. The health benefit of all parts of *Carica papaya* is well known and considered worldwide[27-54].

Materials and methods

Chemicals like MTT, Trypsin – EDTA, Antibiotics and DPBS were procured from HiMedia, India. Fetal Bovine Serum (FBS) and DMEM-HG (Dulbecco's Modified Eagle Medium-high glucose) were purchased from Gibco, Brazil and Gibco, USA respectively. Dimethylsulfoxide (DMSO) was purchased from SDFCL, India. Ethanol, chloroform, phosphatidylcholine and cholesterol were purchased from Merck. All chemicals used were of analytical grade.

Biosafety Cabinet (Ascension, India), Carbon dioxide Incubator (NuAire, USA), Inverted tissue culture microscope (Nikon, Japan) and Automated micro plate reader (Biotek, USA) were used during the experiment.

Preparation of extract

The leaves of *Carica papaya* were collected from a local area of Kangra district (Himachal Pradesh), India in the month of August, 2020 depending upon its easy availability. The leaves were authenticated by Dr. Anjula Pandey, principal scientist at ICAR- National Bureau of Plant Genetic Resources, National Herbarium of cultivated plants, New Delhi against a voucher specimen NHCP/NBPGR/2013-24, NHCP/NBPGR/2013-25, NHCP/NBPGR/2013-23. The leaves were thoroughly washed with water to remove the impurities and air dried.

The fresh leaves of *Carica papaya* were macerated with hydroalcoholic mixture for 24 hours with occasional shaking. The extracts were then filtered and concentrated to dryness. The resultant extracts were stored for further study.

Preparation of phytosomes

The phytosomes of hydroalcoholic extract of *Carica papaya* leaves was prepared by rotary evaporator method. Accurately weighed quantity of phosphatidylcholine, hydroalcoholic extract of *Carica papaya* leaves and cholesterol were dissolved in 10 ml of chloroform in round bottom flask (RBF) and dehydration of organic solvent was done by Rotary evaporator. After complete removal of solvent thin layer of phospholipids mixture was formed. This film was rehydrated with phosphate buffer 7.4. After hydration, mixture was sonicated for 20 minutes. The prepared phytosomes were stored in freezer (2-8^{0}C) until further use.

The *in vitro* MTT assay was performed to determine the level of cytotoxicity for the test substances on Human Liver Carcinoma (HEPG2) cell line.

Preparation of test substances for cytotoxicity screening

10 mg of test substances were weighed separately and made up with DMEM-HG medium (Dulbecco's Modified Eagle Medium-high glucose) supplemented with 2% inactivated FBS

to obtain a stock solution of 10 mg/mL. Furthermore, serial two-fold dilutions were prepared from the stock solution to prepare lower concentrations for cytotoxicity testing.

Cell line and culture medium

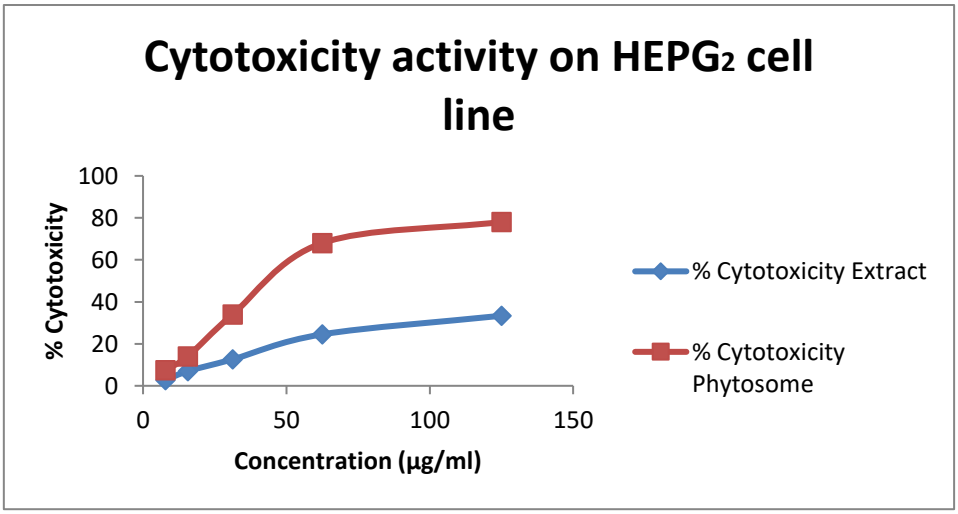
Human Liver Carcinoma (HEPG2) cell line was procured from NCCS, India. Stock cells were cultured in DMEM-HG (Dulbecco's Modified Eagle Medium-high glucose) supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL) and amphotericin B (5 µg/mL) in an humidified atmosphere of 5% CO2 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 cm2 culture flasks and cytotoxicity studies were carried out in 96-well micro titre plate (Tarsons India Pvt. Ltd., Kolkata, India).

Determination of cell cytotoxicity by MTT Assay

The cell culture monolayer was trypsinized and the cell count was adjusted to 1,00,000 cells/mL using DMEM-HG containing 10% FBS. To each well of the 96-well micro titre plate, 0.1 mL of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once with DPBS (Dulbecco's Phosphate Buffered Saline) and different test concentrations were added in the micro titre plate. The untreated cells were maintained as cell control for comparison. The plate was then incubated at 37°C for 24 h in 5% CO_2 atmosphere. After 24 h, microscopic examination was carried out and observations were noted, the test solutions in the wells were discarded and 100 µL of MTT diluted with DPBS was added to each well. The plate was gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100µL of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a micro plate reader at a wavelength of 570 nm[55].

RESULTS

Table 1: Analysis of the *in vitro* cytotoxicity of the test substances against Human Liver Carcinoma (HEPG2) cell line by MTT assay



DISCUSSION AND CONCLUSION

Test substances were assayed for *in vitro* cytotoxicity study against Human Liver Carcinoma (HEPG2) cell line by exposing the cells to different concentrations ranging from 125µg/ml to 7.8µg/ml. MTT assay was employed to test the cytotoxic effect of selected concentrations of given test substances on Human Liver Carcinoma (HEPG2) cell line by measuring the metabolic activity through a colorimetric determination. The MTT assay is usually carried out to detect the cells with constant mitochondrial activity, thereby an increase or decrease in the number of viable cells is linearly related to mitochondrial activity. The CTC_{50} of hydroalcoholic extract of *Carica papaya* leaves were >300 µg/mL and for phytosomes of hydroalcoholic extract of *Carica papaya* leaves were found to be <200µg/mL on Human Liver Carcinoma (HEPG2) cell line. Therefore, the given test substances (hydroalcoholic extract and phytosomes of *Carica papaya* leaves) were considered to be cytotoxic in Human Liver Carcinoma (HEPG2) cell line.

The present study showed that phospholipid complex of *Carica papaya* leaf has better therapeutic efficacy at low concentration as compared to the extract itself because of its improved release property. Hence, better results were obtained for phytosomes of *Carica papaya* leaf at laboratory scale.

The study also indicates that there is an ample scope of using *Carica papaya* leaf as phospholipid complex in the management of liver ailments. However, further studies are required to implement this treatment technique to humans. Therefore, long term *in vivo* toxicity studies as per OECD (The Organisation for Economic Co-operation and Development guidelines) for phytosomes as well as clinical trial studies may be considered in future.

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