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Research Article

Anti Cancer Activity of *Plumeria rubra* (Flowers) Against Human Liver Cancer

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ABSTRACT
The present study has been performed experimentally by in vitro method to examine the anti cancer activity of various concentrations of ethanolic extract of flowers of Plumeria rubra. The report on to the experiment reveals a significant anti cancer activity at different concentrations of the extract. The ethanolic extract of flowers of Plumeria rubra was tested for its anti cancer activity against liver cancer HePG2 cell line by MTT assay. The CTC_{50} value of sample was 98.14µg/ml against liver cancer HePG2 cell lines. Significant results were observed thereby justifying the use of this plant in the traditional system of medicine

Keywords: MTT assay, Anticancer activity, Plumeria rubra, Liver cancer HePG2 etc

INTRODUCTION

Cancer is a general term for uncontrolled growth of abnormal cells medically known as neoplasm characterized by autonomous growth of tissues and loss of differentiation. Cancer is one of the leading causes for mortality worldwide and inadequacy of conventional chemotherapy to effect major reduction in mortality indicates that new approaches are critically needed. It is estimated that 7.6 million people died of cancer in 2007 worldwide and it is projected to increase to 11.5 million deaths by 2030. The low efficacy of current chemotherapy accompanied with severe adverse reactions has been driving an increasing number of patients towards alternative medicine. In the United States, half of all patients with cancer have tried complementary and alternative medicine. Therefore, there is an urgent need to develop new anticancer agent with minimum side effects. From the earliest times, herbs have been prized for their pain –relieving and healing abilities and today we still relay largely on the curative properties of plants.

According to world health Organization, 80% of people living in rural areas depend on medicinal herbs as primary health care system. The synthetic anticancer remedies are beyond the reach of common man because of cost factor. Herbal medicines have a vital role in the prevention,treatment of cancer and medicinal herbs are commonly available and it is comparatively economical. Of the 92 anticancer drugs approved between 1983 and 1994 for commercial use, approximately 62% are directly related to natural origin. Plant derived natural products such as flavonoids, terpenoids, alkaloids etc., have received considerable attention in recent years due to their diverse pharmacological properties including anti-oxidant and cancer chemo-preventive effects. Free radical damage may lead to cancer. Some of the natural products are rich anti-oxidants. Anti-oxidants interact with these radicals and may prevent the damage caused by them.

Plumeria rubra trees are found throughout in India and in tropical areas. Though the plant and its extracts have been extensively used in the folklore medicines, information from organized search of published literature does not provides the evidence for its antitumor activities. Also the increase in the use of medicinal plants and their phyto-constituents in recent times, as well as the scarcity of scientific studies on their safety and efficacy have raised concerns in the scientific community and there is a need to assess the potential effects of these plants. Keeping this in view, the present study has been undertaken to investigate the anticancer potential of ethanolic extract of Plumeria rubra flowers.

MATERIALS AND METHODS

Collection of Plant materials: Fresh flowers of Plumeria rubra were collected from Roever Engineering College garden, Perambalur district, TamilNadu, India, during the month of August and Identified by Head, PG & Research Department of Botany, Periyar E.V.R.College, Trichy. TamilNadu.

Flower extraction: 2 kg Fresh flowers were soaked with 90% ethanol at room temperature (25°C-30°C) After 72 hrs the ethanolic extract was filtered. This extract was concentrated in vacuum and the dry powder obtained was dissolved in ethanol to get required concentrations and were used for screening anti cancer activities.

MTT ASSAY method:

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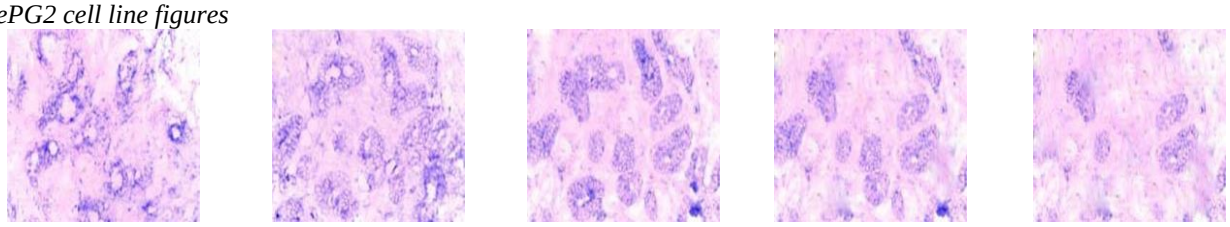
HePG2 cell line figures



1. 62.5 µg/ml 2. 125 µg/ ml 3.250 µg/ml 4. 500 µg/ml 5. 1000 µg/ml

Figures of Plumeria rubra f lower extract against human Liver cancer HePG2 Cell line in different concentrations.

Table.1: The IC50 values of Plumeria rubra flower extract against human Liver cancer HePG2 Cell line.
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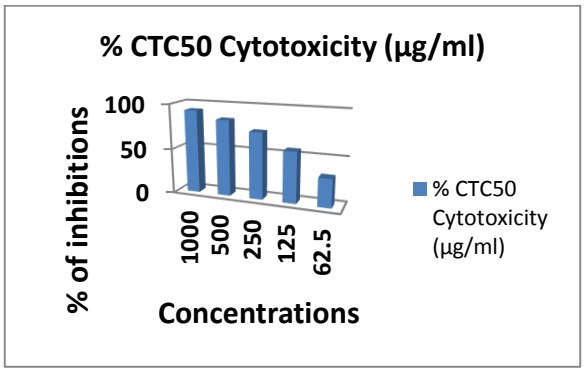
S.No	Concentration of extracts (µg/ml)	% CTC_{50} Cytotoxicity (µg/ml)	CTC_{50}
1	1000	92.43	
2	500	84.12	
3	250	73.61	98.14
4	125	56.48	
5	62.5	31.62	



Graphical representation of the IC_{50} values of Plumeria rubra flower extract against human Liver cancer HePG2 Cell line.

MTT-Assay-Chemicals: 3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. 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: HePG-2 (Liver cancer cell line) cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in Dulbecco's modified Eagle's medium (DMEM). Medium was 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 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions: For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized. Serially two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of Cell Viability by MTT Assays**:** The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using medium containing 10% FBS and were used for the determination of cell viability by MTT assays as described by Francis and Rita (1986). 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.

% Growth inhibition =

$$100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

RESULT AND DISCUSSION

The MTT assay is based on the reduction of MTT (3-(4,5-dimethyl thiazolyl)-2,5-diphenyl-tetrazolium bromide) by mitochondrial dehydrogenase to purple formazan product. The different concentration of whole plant Plumeria rubra flowers were subjected for MTT assay and results are presented in table.1. The photographs (Fig. 1 to Fig. 5) of different concentration of Plumeria rubra flowers shows the human Liver cancer HePG2 Cell line.

CONCLUSION

The MTT assay of ethanolic extract of flowers of Plumeria rubra shows that all concentrations are having anticancer activity. The sample concentrations of 1000µg/ml, 500 µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml show 92.43µg/ml,84.14µg/ml,73.61µg/ml,56.48 µg/ml,31.62µg/ml IC50 value against the human Liver cancer HePG2 Cell line respectively. These concentrations were able to induce apoptosis on human cancer cell lines and its anticancer activity was found to be specific. Further work is required in order to establish the identity of the chemical constituent responsible for anticancer activity. Studies are in progress in our laboratory to elucidate the molecular structure. which contributes towards the development of potent anticancer drug.

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