Evaluation of cytotoxic properties of *Curcuma longa* and *Tagetes erecta* on cancer cell line (Hep2)

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Cancer is the major public difficulty and one of the top causes of death in prosperous countries. Conventional plants are precious source of novel cytotoxic agents and play a critical role in health concern. Due to its wide range of biological and pharmacological effects and lack of toxicity, curcumin and lutein were selected for this study. Curcumin and lutein were isolated from rhizomes of *Curcuma longa* and petals of *Tagetes erecta*. The isolated pigments were quantified spectroscopically and separated by thin layer chromatography. The active components of the pigments were further purified and identified by high performance liquid chromatography. *In vitro* cytotoxic activity of both extracts against Hep2 cancer cell lines were evaluated. Furthermore, the activities of both pigments in different concentrations against Hep2 cancer cell line were compared. The test sample showing cell viability of more than 97% at 0.078 mg/ml were considered to be less active at minimum concentration. The maximum viability of Hep2 cell line were 3.27% (curcumin) and 8.88% (lutein), respectively, which are most suitable to perform cytotoxic studies. This method suggests that it is suitable for the rapid screening of plant materials and also can be performed without any special sample pretreatment.

**Key words:** Cytotoxic, curcumin, lutein, Hep2 cells.

**INTRODUCTION**

Cancer is a leading cause of death worldwide and had accounted for 7.9 million deaths (approximately 13% of all deaths) in 2007. Most drugs currently available for the treatment of cancer have limited potential, because they are highly toxic, inefficient in treating cancer, or highly expensive. Treatments without these disadvantages are needed. Hence, the identification and synthesis of novel, efficient and less toxic anticancer agents remains an important and challenging task for the cancer treatment. Use of plant extracts as medicine for cancer treatment is certainly the effective method and dozens of plant based products have been reported for cancer treatment progress. Since the plant based products have the natural multi-targeting ability as well as inexpensive and is safe as compared to synthetic agents (Preetha et al., 2008). Among them, plant based products such as curcumin and lutein occupied significant role against cancer, microbial infections and other inflammatory diseases. Due to its wide range of biological and pharmacological effects and lack of toxicity, curcumin and lutein were selected for this study.

Curcumin is a naturally occurring yellow pigment isolated from the rhizome of the perennial herb *Curcuma longa* which has been cultivated for centuries in several Asian countries. In general, the commercially available curcumin is a mixture of curcuminoids, containing...
of TAMILNADU AGRICULTURAL UNIVERSITY (TNAU, COIMBATORE) AND GROW MORE BIOTECH, HOSUR, INDIA. THE PLANT MATERIAL WAS IDENTIFIED AND AUTHENTICATED BY ASSISTANT PROFESSOR DHARMARAJ, DEPARTMENT OF BOTANY, AYYA NADAR JANAKI ANMAL COLLEGE, SIVAKASI, TAMILNADU, INDIA. THE CELL LINE USED IS HUMAN EPITHELIOVA CELL LINE OF LARYNX (HEP2) OBTAINED FROM TISSUE CULTURE SECTION OF Virology Department, King Institute of Preventive Medicine, Guindy, TAMILNADU, INDIA.

Preparation of plant extract

The rhizomes of C. longa and petals of T. erecta were detached and dried for 7 days, and reduced to coarse powder using a hand blender. The powder (910 g) was subjected to continuous Soxhlet extraction using acetone (turmeric) and n-hexane (marigold flowers) (100%) at 50°C for 18 h to obtain the extract, which was concentrated in a rotary evaporator under reduced pressure. A fresh batch powder (500 g) was macerated in distilled water for 72 h, filtered, and freeze-dried.

Biophysical characterization and analysis

Spectrophotometric measurements were carried out using the Beckman DK-2 spectrophotometer inbuilt software. The detector wavelength was set at 420 and 445 nm, at which curcumin and lutein have their maximal spectrophotometric absorption. Similarly, saponification experiments were carried out as described earlier.

Thin layer and high performance liquid chromatography

Thin-layer chromatography was used to detect the individual curcuminoid in turmeric. Samples were dissolved in the appropriate organic solvent, applied to the silica gel G plates (Sigma Aldrich, India) and developed with petroleum ether, methanol and formic acid (3:4:0.5:0.1; v/v) developing solution. Further, the amount of curcumin and lutein was determined by HPLC (LC10 Shimadzu Corp., Tokyo, Japan) using C18 column. Chromatographic peaks of incubation samples were identified by spiking with corresponding authentic standards.

Cell culture, in vitro cytotoxic and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The cytotoxicity tests define the upper limit of the extract concentration, which can be used in subsequent anticancer studies. Cells were harvested and separated to single cell suspension by gentle pipetting action and the viable cells were counted in a haemocytometer using trypan blue. Viable cell density was adjusted to 5,000 to 40,000 cells/100 µl. Hep2 cells were treated with different concentration of plant extract materials and observed morphological changes were observed under inverted microscope. After the addition of drug, cell death and cell viability was estimated. Furthermore, the cell survival was determined by using the MTT assay.

RESULTS AND DISCUSSION

The organic extraction of lutein from marigold flower and curcumin from turmeric is simple and less time consuming. Lutein extraction with saponification was performed, which does not allow the chlorophyll and other water soluble contaminants. The spectrophotometric
Figure 1. Cytotoxic effects of curcumin and lutein on Hep2 cells. Cells (3×10^5 cells/well), in 100 µl medium were grown in the presence of 0.4% DMSO (vehicle control) and various concentrations of curcumin and lutein. The numbers of viable cells were determined by MTT assay. The number of viable cells is expressed as a percentage of vehicle control. Mean ± standard deviation (SD) of 3 independent experiments.

Figure 2. Microscopic images of cytotoxic effects of curcumin and lutein on Hep2 cells. Control (A) normal Hep2 cells without curcumin and lutein treatment; Test 1: Hep2 cells treated with curcumin (B) and lutein (C) with 0.3 mg/ml. Test 2: Hep2 cells treated with curcumin (D) and lutein (E) with 1.25 mg/ml.

The cytotoxicity study was carried out with the Hep2 cell lines at different concentrations to determine growth inhibition rate (Ju-Hyung et al., 2003). Dose response curves constructed between the range of 10 and 0.078 mg/ml for curcumin and lutein (Figure 1), express decreasing number of viable cells with increasing concentration of extract. The test sample showing cell viability of more than 97% at 0.078 mg/ml were considered to be less active at minimum concentration. The maximum viability of Hep2 cell line were 3.27% (Curcumin) and 8.88% (lutein), respectively, which are most suitable to perform cytotoxic studies. We therefore, can conclude that both plant extracts showed selective in vitro cytotoxicity against Hep2 cancer cell lines and both plants were found to be highly effective against various cancerous cells. The results were in accordance with previous research done in both plants by other authors. In the present study, both extracts proved to have effective cytotoxicity, but the growth inhibitory effect of lutein was maximum than curcumin. The anti proliferative effect strengthens with increase in the concentration of the extract. To verify curcumin-induced and lutein-induced cell toxicity, the changes in cell morphology were examined under an inverted microscope. High doses of curcumin and lutein were associated with increased cell apoptosis. Induction of apoptosis by curcumin appears to be dependent on the formation of reactive metabolites. Curcumin-induced apoptosis mainly involves the...
mitochondria-mediated pathway in various cancer cells. Curcumin causes Hep2 cells to develop characteristic features of cell shrinking, rounding and partial detachment, thus demonstrating the lobulated appearance of apoptotic cells. We also examined the effects of different concentrations of curcumin and lutein on cell viability on Hep2 cells. After treatment, survival was inversely correlated with lutein concentration. When the cells were treated with 0.3 mg/ml of curcumin, 25% of cell death occurred (Figure 2). While decreasing the concentration of curcumin to 0.078 mg/ml and the viability of the cell peaks to 93.3%, respectively showing negligible amount of cell death and minimum lethal dose. We could observe that the 50% cell death could be seen at the concentration of 1.25 mg/ml where the viability was up to 50%. At 5 mg/ml concentration, the apoptosis rate was up to 75%. At 10 mg/ml, only 8.88% of viable were observed. Maximum cell death of 91.2% was observed at the concentration of 10 mg/ml. Very less amount of viable cells (8.8%) were detected at this concentration which shows the maximum inhibition concentration. Likewise when the cells were treated with lutein, the cell death was proportional to the concentration used same as curcumin. When the cells were treated with 0.3 mg/ml of lutein 25% of cell death occurred. Maximum viability of 96.72% was observed in the concentration of 0.078 mg/ml showing the lethal toxic rate. At the concentration of 1.25 mg/ml, the significance of viability was up to 46% leading to cell death of 44%. At 5 mg/ml concentration, the apoptosis rate was up to 78% (Figure 2). This finding will greatly benefit the clinical use of T. erecta in Indian medicine and suggests that lutein could be a potent anti-tumor drug candidate than curcumin. These findings validated the plant T. erecta as an antimicrobial herb.

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REFERENCES


