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Full Length Research Paper

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

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diferuloylmethane, demethoxycurcumin and bisdemethoxycurcumin. Curcumin is known for its antioxidant, anti-inflammatory, anti-fatigue, antiparasitic, antiallergic, anti-microbial, anti-mutagenic and anticancer properties. It exhibits wide therapeutic potential due to the multi-targeting nature against variety of different cancers including leukemia, gastrointestinal cancers, genitourinary cancers, breast cancer, etc. Curcumin has been shown to suppress transformation, proliferation, and metastasis of tumors. It also inhibits proliferation of cancer cells by arresting them in various phases of the cell cycle and by inducing apoptosis. It is obvious that curcumin's multitargeting ability may be the key to its therapeutic potential against cancer.

Carotenoids are a subclass of phytonutrients which are prominent in fruits and vegetables. Xanthophylls are a family of oxygenated carotenoids that contain hydroxyl or carbonyl groups that contribute to enhance their solubility and hence their distribution in animal tissues. Lutein is a xanthophyll that, together with zeaxanthin, has gathered increasing attention on the grounds of recent studies that show how an adequate intake of this product might help to prevent or ameliorate the effects of degenerative human diseases, such as age-related macular degeneration. Human plasma lutein has been inversely associated with cytochrome activity and human cancer. It is a known fact that humans do not synthesize lutein and depend entirely on dietary sources such as vegetables or supplement lutein pills. Marigold flower (*Tagetes Erecta* L) petals are a significant source of the xanthophyll, mainly lutein and have a much higher concentration of this pigment as compared to other plant materials. Marigold extracts have been commercialized internationally and are used as additives for poultry feed as they provide bright colors in egg yolks, skin and fatty tissues. It plays enormous biological role for them as chemopreventive agents which include cancer prevention, enhanced immune function, inhibition of the autooxidation of cellular lipids, etc. It is worth mentioning that we have little knowledge on the curcumin and lutein isolated from the Indian sub content plants. *C. longa* and *T. erecta* plants used for this study were collected from the local farmer place in Tamilnadu. The metabolically active compounds curcumin and lutein were isolated from *rhizomes of C. longa* and petals of *T. erecta.* The isolated active compounds were purified by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Further, we have evaluated the cytotoxic activity of both the active compound against Hep2 cancer cell lines. Furthermore, we compared the activity of both pigments in cancer cell line with different concentrations.

MATERIALS AND METHODS

Plant and animal cells

The plant *C. longa and T. erecta* were collected from the premises 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 Ammal College, Sivakasi, Tamilnadu, India. The cell line used is human epithelioma 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 detective 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

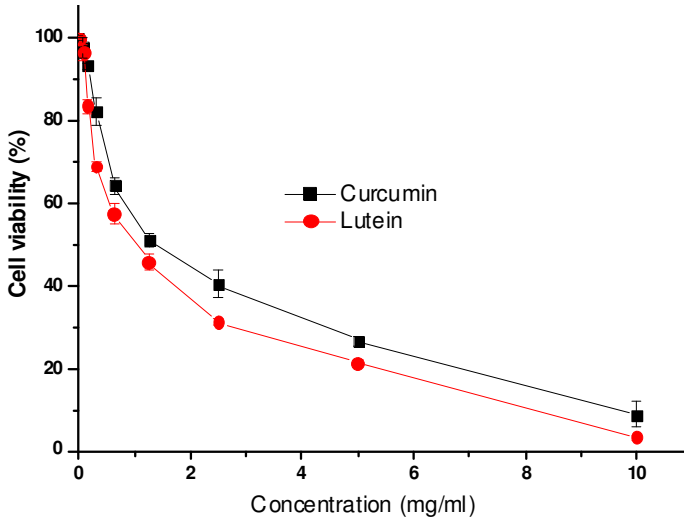

Figure 1. Cytotoxic effects of curcumin and lutein on Hep2 cells. Cells (3×105 cells/well), in 100 µl medium were grown in the presence of 0.4% DMSO (vehicle control) and various concentrations of curcumin and lutein. The numbers of viable cells were determined by MTT assay. The number of viable cells is expressed as a percentage of vehicle control. Mean ± standard deviation (SD) of 3 independent experiments.

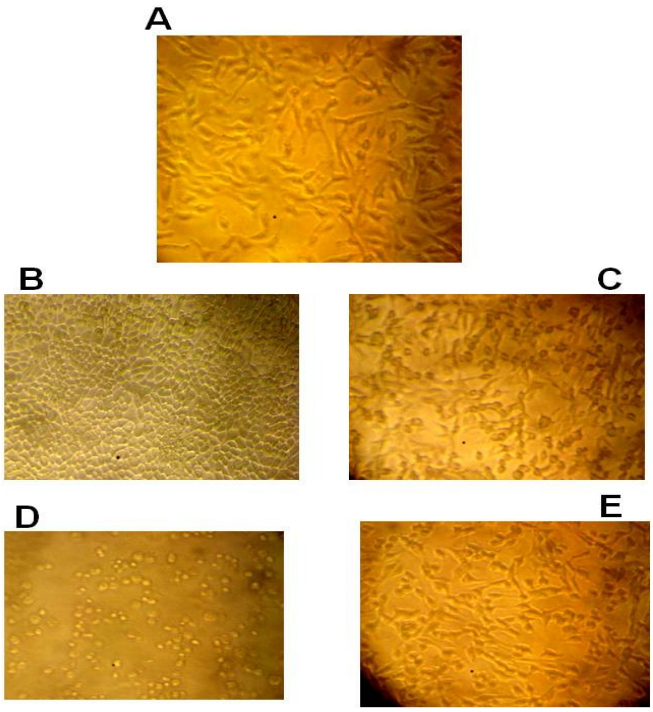

Figure 2. Microscopic images of cytotoxic effects of curcumin and lutein on Hep2 cells. Control (A) normal Hep2 cells without curcumin and lutein treatment; Test 1: Hep2 cells treated with curcumin (B) and lutein (C) with 0.3 mg/ml. Test 2: Hep2 cells treated with curcumin (D) and lutein (E) with 1.25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

method indicates the presence of several fractions from the curcumin and lutein isolated. Interestingly, strong peak was identified at 420 nm which is identical to those of curcumin standard solution (Ruby et al., 1995; Deshpande et al., 1997; Braga et al., 2003; Leal et al., 2003). We could observe the slight depression in the spectrum observed at ultraviolet (UV) region in comparison to lutein standard identified. It indicates that small amounts of polyphenols present in the hexane extract may interrupt during the spectrophotometric analysis. Furthermore, HPLC and TLC were used for the determination of individual curcuminoid in turmeric compounds as described earlier (Gupta et al., 1991). The isolated curcuminoids showed single spots on TLC plate and gave a single peak. The Rf value of curcumin extract is 0.74 and that of the standard is 0.77. Lutein content of the extract was determined by first saponifying the lutein esters in the extract which does not allow the chlorophyll and other water soluble contaminants except lutein with negligible amount of β-carotene. The separated spots of the saponified marigold color standard on TLC plate were identical with the spot of lutein standard in terms of Rf value and color. The Rf value of lutein extract after saponification is 0.45 (before saponification is 0.28) and the standard is of 0.44. Furthermore, the TLC purified samples were subjected to HPLC analysis. The HPLC analysis of curcumin and lutein showed the single peak and its retention time was 3.730 and 4.527 min, respectively. The retention time was comparable and exact to that of the standard curcumin and lutein peak. The solvents used for the elution of lutein are more suitable and reproducible with single peak (Thammanna et al., 2010).

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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