Int.J.Curr.Microbiol.App.Sci (2016) *5*(8): 414-421

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 8 (2016) pp. 414-421**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.508.044

In *Vitro* Cytotoxicity Effect of Kaempferol in Breast Cancer Cell Lines MCF-7 and Lung Cancer Cell Lines A459

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Keywords

Kaempferol, cytotoxicity effect, breast cancer, lung cancer.

Article Info

Accepted:
21 July 2016
Available Online:
10 August 2016

A B S T R A C T

In this study cytotoxic effect of kaempferol isolated from *Vigna unguiculata* was evaluated on breast cancer (MCF-7) and lung cancer A459 cell lines. MCF-7 and A459 cells were cultured in DMEM medium and incubated with different concentrations (100, 50, 25, 12.5, 6.25 µg/ml) of kaempferol .The cell viability was assessed by MTT assay. Kaempferol has decreased malignant cells in a concentration dependent manner. The IC50 values in MCF-7 and A459 cells were determined as 90.28 ± 4.2 µg/ml and 35.80 ± 0.4 µg/ml respectively. It may be concluded that *kaempferol* can cause cell death in MCF-7 and A459 cancer cells which is considered as a promising chemotherapeutic agent in breast cancer and lung cancer treatment.

Introduction

Cancer has been thought to be a preventable disease due to its slow development and progression, taking many years to become invasive in a step by- step manner (Sreejaya and Santhy, 2013). Such property provides a greater opportunity not only for early detection, but a particular interest in the role of medicinal plant extracts in cancer prevention. Plants are the rich sources of chemically diverse compounds, many with beneficial properties to human health. Consequently, about 50% of the anticancer therapeutic agents known are derived from plants (Balunas and Kinghorn, 2005).

Institute has screened about 35,000 higher plant species for activity against cancer, where about 3,000 of these plants have demonstrated reproducible activity. As mentioned above, the exploration of nature as a source of new active agents is needed for discovering bioactive chemo- types from natural product for the development and novel molecular diversity of efficacious drugs. In this respect, natural products from plants, used either alone or with combinatorial synthetic methodologies, constitute a multidisciplinary approach to the current drug productivity (Newman *et al.*, 2003) (Cos *et al.*, 2006).

Breast cancer is one of the most aggressive types of cancer that can occur in women of any age and is the most frequently diagnosed cancer in women worldwide and ranks second as a cause of cancer death (Am. Can. Soc., 2012). A number of dietary factors have been linked to the risk for breast cancer. Dietary factors which may increase risk include a high fat diet (Blackburn, 2007).

Lung cancer is the uncontrolled growth of abnormal cells in one or both of the lungs. Cigarette smoking causes most lung cancers when smoke gets in the lungs. Lung cancer kills 1.3 million people each year, more than any other cancer. It is currently the leading cause of cancer death in both men and women, cancer has been regarded as a leading cause of cancer related mortality throughout the world .Its occurrence and development are associated with a variety of factors disorders, dysfunction of lung epithelial cells, inflammation etc (Carpagnano *et al.,* 2011). Lung cancer is the most common cancer worldwide and accounts for 75-80% death. The current chemotherapy treatment method will destroy the normal cells along with cancer cells and also sometime develops resistance to treatment. So, the discovery of novel drugs of natural origin which are less toxic, endowed with fewer side effects and more potent in their mechanism of action are necessary to be discovered (Sumathy Arockiasamy and Vinu Ramachandran, 2012).

According to World Health Organization, 80 % of the people living in rural areas depend on medicinal herb as primary healthcare system, The herbal formulations can be designed to attack the cancerous cells without harming normal cells of the body. Herbal medicines have a vital role in the prevention and treatment of cancer. Cancer can be defined as a disease in which a group of abnormal cells grow uncontrollably by disregarding the normal rules of cell division. It is serious, frightening diseases about a third of humans develop cancer in a lifetime (Marcy *et al.,* 2005) (Minky Mukhija e*t al.,* 2015). It is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions and mutations). Treatment for cancer includes local treatments, such as radiation therapy, surgery and systemic treatments such as chemotherapy and targeted therapy. Despite considerable progress in the management of cancer by conventional synthetic drugs, the search for natural anti-cancer plant products for controlling cancer is very important as synthetic drugs has many side effects (Michael Marmot, 2007). Today much attention has been devoted to natural antioxidant and their association with health benefits. Plants are the potential source of natural antioxidants. Reactive oxygen species (ROS) are generated as byproducts of biological reactions and from exogenous factors (Wiseman, 1996). Excess ROS, if not eliminated by antioxidant system, results in high levels of free radicals which causes oxidative stress (Sreeramulu *et al.,* 2013). Oxidative stress arising from free radicals is the basis of many diseases such as cancer (Reuter *et al.,* 2010) (Durackova, 2010). The curative effects of several medicinal plants are usually due to antioxidant phytochemicals present in it such as polyphenols, flavonoids and phenolic compounds (Yildrim *et al.,* 2001).

Recently flavanoids have attracted considerable interest because of their potential beneficial effects on human health. They have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor, antioxidant, antithrombotic, hypolipidemic and hypoglycemic activities (Joby jose *et al.,* 2014).

Materials and Methods

Chemicals & reagents

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Minimum Essential Medium (MEM) 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

MCF-7(Human Breast Carcinoma) & A549 (Human Lung Carcinoma) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in MEM 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). flavonoid constituents Minky Mukhija, Mahendra Pal Singh, Kanaya Lal Dhar,

Preparation of Test Solutions

For Cytotoxicity studies, the weighed test drug was separately dissolved in distilled DMSO and volume was made up with MEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT Assay

The ability of the cells to survive a toxic insult has been the basis of most Cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The numbers of cells were found to be proportional to the extent of formazan production by the cells used.

The monolayer cell culture was trypsinized and the cell count are adjusted to 1.0 x 10^5 cells/ml using MEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different concentrations of test drug were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37^o C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37^o C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. 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 (Francis, 1986).

$$\% \text{ Growth Inhibition} = 100 - \left(\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100 \right)$$

Results and discussion

Cancer is a dreadful disease across the worldwide, and the treatment strategies for combating cancer severity have gained more importance to public health. For the development of new anticancer drugs, drug combinations, and chemotherapy strategies by methodical and scientific exploration of the enormous pool of synthetic, biological, and natural products. Cancer chemopreventions with strategies using foods and medicinal herbs have been considered as the main strategy in cancer control (Vennila srinivasahan, 2015).

In conclusion, the plant *vigna unguiculata* seeds of methanolic extract of bioactive compound kaempferol *in vitro* investigated against MCF-7 and A459 -cell lines, and resulted with IC50 = 90.28±4.2, 35.80±0.4 µg/ml respectively. This may be due to the presence of flavonoid compound especially kaempferol which also show high cytotoxicity which isolated from high polar fraction of the methanolic extract, in we study the cytotoxicity of MCF-7,A459 cell lines by isolated compound. Our findings are line line with previous studies (Nguyen *et al.*, 2003).

Table.1 Cytotoxic property of test drug against MCF-7 cell line

Sl. No	Name of Test sample	Test Conc. (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	**RR 2852**	100 50 25 12.5 6.25	52.32±1.1 40.58±0.8 36.21±2.4 31.73±0.8 22.31±4.4	90.28±4.2

Table.2 Cytotoxic property of test drug against A549 cell line

Sl. No	Name of Test sample	Test Conc. (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	**RR 2852**	100 50 25 12.5 6.25	83.72±1.1 71.15±0.8 33.87±1.6 10.15±0.8 0.99±0.6	35.80±0.4

Fig.1 Cytotoxic effect of the sample RR 2852 on MCF-7 cell line

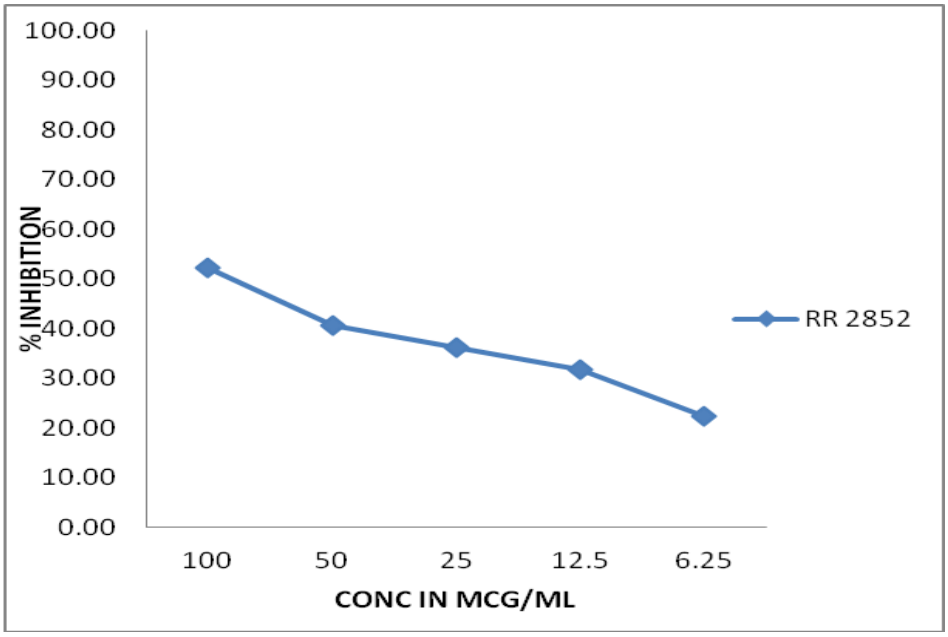


Fig.2 Cytotoxic effect of the sample RR 2852 on A549 cell line

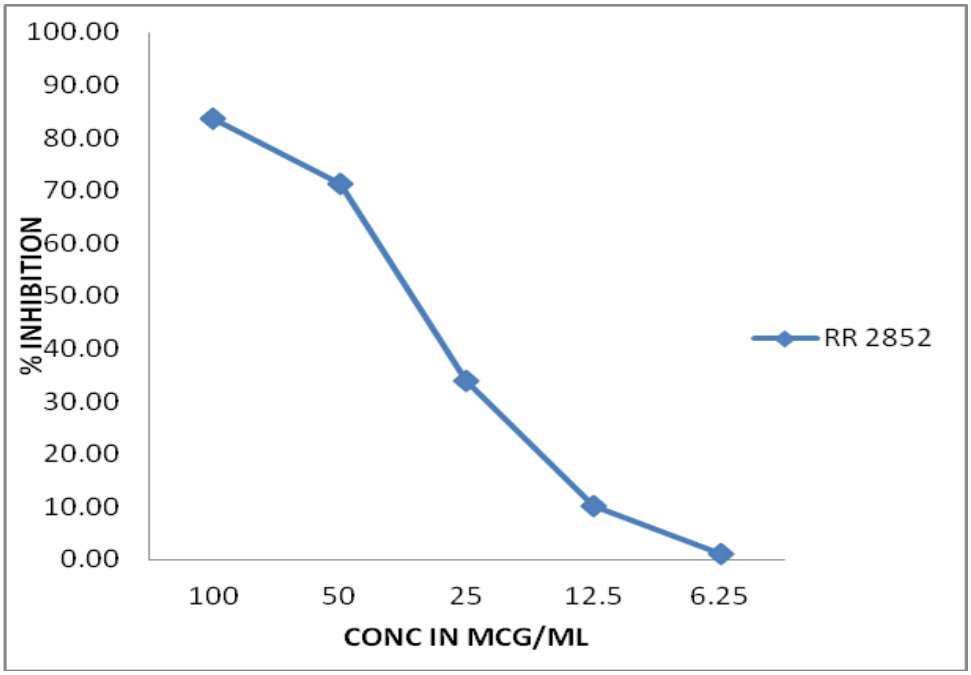
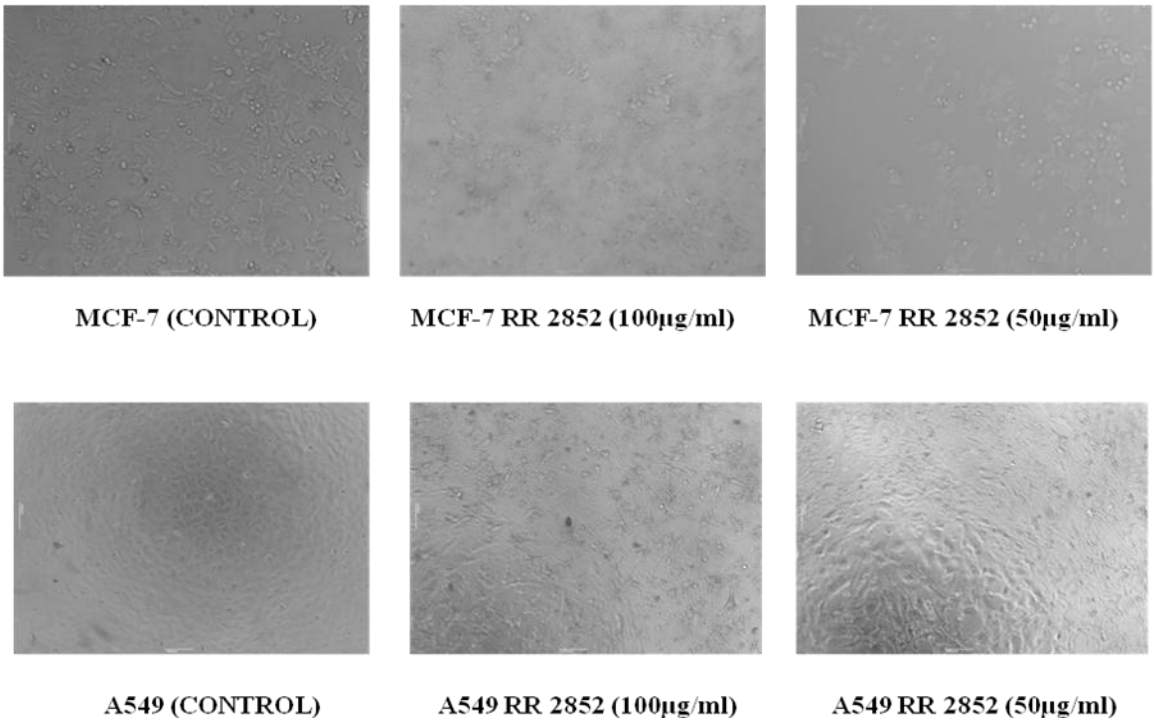


Fig.3 Photos



Kaempferol is a polyphenol antioxidant found in fruits and vegetables. Many studies have described the beneficial effects of dietary kaempferol in reducing the risk of chronic diseases, especially cancer. . Not only the kaempferol is a potent promoter of apoptosis (Ramos, 2007), but it also modifies a host of cellular signaling pathways. In addition, kaempferol is much less toxic to normal cells in comparison to standard chemotherapy drugs (Allen *et al.*, 2013).

In the present time herbal products are considered to be symbols of protection of cells in comparison to the synthetic product that are regarded as unsafe to human life and environment. Although herbs have been priced for their medicinal importance. But now everyday phytochemical and pharmacological studies are conducted on different parts of plants. Several mechanisms of action were detected in MCF-7 cells and A459 cells. In the present study, isolated compound from methanolic extract of vigna unguiculata was found to be cytotoxic towards human MCF-7 and A 459 in MTT assay and the concentration required for 50% cell death was found to be 90.28g± 4.2µg / ml and 35.80 ±0.4µg/ml. Hence present study shows the efficacy of *kaempferol* for the cytotoxicity towards MCF-7 cells and A459 cells are highly significant (Blackburn, 2007).

Medicinal plants are able to act through several mechanisms to provide protection against cancer. The percentage of cytotoxicity to MCF-7 cells and A459 cells which are exposed to the kaempferol to wide concentrations at 100, 50, 25, 12.5, 6.25 µg/ml was found to be 52.32±1.1, 40.58±0.8, 36.21±2.4, 31.73 ±0.8, 22.31±4.4, and 83.72±1.1,71.15± 0.8, 33.87±1.6, 10.15± 0.8 0.99±0.6 respectively. The dose-dependent anti-proliferative effect

on the cell viability of MCF-7 and A459was observed. The results have been summarized in Table 1 and2 . Inhibitory concentration 50 (IC50) of MCF 7 is 90.28±4.2µg/ml and A459 is 35.80±0.4µg/ml value indicated that the maximum cytotoxic effect ofkaempferol which is a isolated fraction of *vigna unguiculata* showed 50% reduction in cell viability upon treatment with highest concentration.

Thus it can be concluded that the kaempferol is acting as an anticancerous agent to kill the MCF-7 and A-459 cells without harming the normal cells .hence this can be for mutated as a new anticancer drug.

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How to cite this article:

Ramesh L. Londonkar and Basavarajeshwari S. Awanti. 2016. In *Vitro* Cytotoxicity Effect of Kaempferol in Breast Cancer Cell Lines MCF-7 and Lung Cancer Cell Lines A459. *Int.J.Curr.Microbiol.App.Sci.* 5(8): 414-421. doi: http://dx.doi.org/10.20546/ijcmas.2016.508.044