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Effect of Phytochemical Kaempferol on HCT-15 And Lymphocytes

KEYWORDS: Kaempferol, Anti-proliferative activity, Cytotoxicity, MTT assay.

CHEPURI KALYANI	MANGAMOORI LAKSHMI NARASU	YUMNUM PRIYADARSHINI DEVI
Centre for Biotechnology, JNTUH, Hyderabad	Professor, Centre for Biotechnology, JNTUH, Hyderabad	Centre for Biotechnology, JNTUH, Hyderabad

ABSTRACT *Kaempferol is a flavonoid found abundantly in many plants. Many investigations revealed its anti-microbial, anti-cancer, cardio protective, anti-oxidant activities. In the present study, we screened the effect of kaempferol on human colon cancer cell line (HCT-15) and human normal lymphocytes at different concentrations. The morphological alterations were observed using microscopy and the cytotoxicity was evaluated by MTT assay. Kaempferol showed dose dependent antiproliferative activity on HCT-15 after 24hrs of incubation. The study was compared with chemotherapeutic drugs Doxorubicin and Cisplatin. The IC50 concentrations were found to be 120 µg/ml , 50 ug/ml and 25 ug/ml for Kaempferol, Doxorubucin and Cisplatin respectively. However, normal human lymphocytes were not affected with Kaempferol.*

INTRODUCTION:

Flavonoids are the natural poly phenolic compounds widespread in many vegetables, grains, roots, stems and flowers. These secondary metabolites are reported by many epidemiological, clinical trial and laboratory studies as promising anticancer agents. Besides, anti-cancer activity, flavonoids encompass many biological and pharmacological functions like anti-allergenic, anti-inflammatory, anti-viral, anti-carcinogenic and anti-atherogenic properties. These biological effects of flavonoids could be due to their influence on inflammatory processes, immune functions, cell surface signal transduction and tumor growth. (1, 2 & 8). It has been statistically estimated that the average human daily dietary intake of poly phenols as 10mg/day. Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is one of the flavonoids under the subclass flavonols distributed widely in plant kingdom and commonly used in traditional medicine (3 & 6). Kaempferol has received a great deal of attention in research due to its definite role in reducing many diseases like osteoporosis, diabetes, neurodegenerative diseases, anxiety, infectious diseases, allergies, inflammation and pain (7, 9, 10 &11). Numerous *in vitro* and *in vivo* studies have also reported that kaempferol exhibits anti cancer, anti-inflammatory, anti allergic, anti asthmatic, antimicrobial and antioxidant activities (12). Further, several epidemiological studies evaluated the positive interaction between the consumption of kaempferol rich foods and its role in reducing many types of malignancies like lung, ovarian, gastric and pancreatic cancers in the human population. Although, kaempferol has been evaluated for anticancer activity, many clinical studies indicating its therapeutic and cancer preventive activities. In cancer treatment strategies, kaempferol may be used as adjuvant in combination with chemotherapeutic drugs to sensitize the cells for cytotoxicity.

In the present study, we are investigating the effect of kaempferol on human colon cancer cell line (HCT-15) as well as on human lymphocytes with increasing concentrations at different time intervals.

MATERIALS & METHODS:

Materials:

Phytochemical Kaempferol was purchased from Calbiochem. Culture medium RPMI-1640, fetal bovine serum (FBS), lymphocyte isolation medium (Histopaque), Trypsinversene and all other cell culture related chemicals were purchased from Himedia (India).

Cell culture:

Human colon cancer cell line HCT-15 was procured from NCCS, Pune and the cells were maintained and grown using RPMI-1640 supplemented with 10%FBS, penicillin(50IU/ml) and Streptomycin sulphate (50ug/ml)in a CO_2 incubator which set with optimum culture conditions like 5%CO_2, 37°C temperature and 100% humidity. To maintain sub-confluent state, the cells were sub-cultured twice in a week using 0.1 % trypsin with 0.5 mM EDTA. Cryopreservation procedure was followed to maintain master and working banks for future experimental studies by using 10%DMSO (molecular grade).

Lymphocytes culture:

3ml of venous blood was collected from healthy donors who were not on any medication and JNTU ethical committee had approved the study. Equal volume of Lymphocyte isolation medium (Histopaque 1077) was layered carefully on the whole blood and centrifuged at 400g for 30 min. After centrifugation, upper translucent layer was removed and opaque inter phase layer residing with mononuclear cells was collected into a sterile centrifuge tube. To this 10ml of PBS was added and centrifuged at 1200rpm for 10min. The pellet collected was washed again with PBS. The wash was repeated twice with RPMI-1640 media and the resulting pellet was re suspended in 5ml of fresh RPMI-1640 media.

Morphological analysis:

To observe the morphological alterations, the viable human colon cancer cells (HCT-15) and human lymphocytes at a density of $2.5X10^5$ cells per well were seeded onto 6 well treated and untreated plates respectively. The freshly isolated lymphocytes were treated with increasing concentrations of kaempferol (1, 5, 10, 25, 50, 100 and 150ug/ml). After 24hrs of incubation, the morphological analysis was done by examining the cells under inverted phase contrast microscopy. Ethanol at 10% served as the negative control.

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MTT Assay:

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was used to screen cytotoxic activity of Kaempferol on HCT – 15 and human lymphocytes. Briefly, the viable cancer and lymphocyte cells at a density of 2.5X 10^4 to 3.5X10^4 cells/well were seeded onto treated and non treated 96-well plates respectively. The freshly isolated lymphocytes were treated with increasing concentrations of kaempferol(10,25,50,100 and 150 ug/ml) and incubated at 37^0C for 24 hrs. The cancer cells were incubated for 24-h prior to treatment with kaempferol for the proper attachment. After 24 hrs, the attached cancer cells were treated with the increasing concentrations of kaempferol and incubated for 24 hrs. Following washing with PBS, both the cells were incubated with MTT solution (1ml) for 4 hrs. Blue colored crystals indicate the formation of formazan salts. To dissolve the crystals, MTT was removed and 1ml of DMSO was added. The absorbance was measured after 1hr using microplate reader (Wallac 1420 Multilabel counter, PerkinElmer) at a wavelength of 560 nm. Ethanol at 10% served as the negative control. The data were presented as percent post treatment recovery (% live cells), whereas the absorbance from untreated control cells was defined as 100% live cells.

RESULTS:

Phase Contrast Microscopic analysis:

In the present study, we examined the morphological alterations of human colon HCT-15 cancer cells after 24 hrs of treatment with kaempferol, doxorubicin and cisplatin at increasing concentrations by using phase contrast microscopy. The treated cancerous cells showed a gradual change of cellular morphology from healthy round shining cells to irregular shape, sparse cell density population in a dose dependent manner (Fig 1) in comparison with untreated cells. In contrast, the normal lymphocytes treated with Kaempferol, did not show any significant morphological changes (Fig 2).

Cytotoxicity:

Exponentially growing cell lines (2.5–5 × 10^3 cells) were exposed with increasing concentrations of kaempferol for 24 hrs in 96-well tissue plates as described above, and a dose/response curve was performed. The same experiment was performed with increasing concentrations of the chemo preventive drugs Doxorubicin and Cisplatin. Figure 3 depicts absorbance results when HCT-15 colon cancer cells were treated with kaempferol, Doxorubicin and Cisplatin for 24 hrs. These results showed a significant decrease in absorbance, mainly in the 25-150 µg/ml concentration range, with an IC_{50} concentration of 120 µg/ml , 50 ug/ml and 25 ug/ml for kaempferol, doxorubicin and cisplatin respectively. As shown in (Table 1), there was a significant decrease in the final number of viable cells, with a growth inhibition of 70–100% versus control cells. MTT Assays were carried out to determine the IC_{50} value of the kaempferol against HCT 15 cells (Fig 3). The IC_{50} value was determined from this assay by plotting the graph of percentage cell viability against the concentration, using MS Excel spreadsheet and simultaneously there was no observable effect of the said compound on human lymphocyte cells (Fig 4).

Compound	Concentrations (µg/mL)							IC_{50} (µg/mL)
	1	5	10	25	50	100	150	
Kaempferol	-	95.04+ /-0.70	80.48+ /-1028	77.49+ /-1.18	65.94+ /-1.68	59.91+ /-0.6	37.31+ /-1.86	120+ /-3.2
Doxorubicin	84.63+ /-3.49	72.29+ /-1.20	62.91+ /-0.7	55.52+ /-2.21	49.44+ /-1.10	-	-	49.6+ /-0.5
Cisplatin	85.19+ /-1.2	76.06+ /-1.6	56.95+ /-2.5	48.88+ /-1.5	40.08+ /-1.9	-	-	25.4+ /-2.9

Table 1: Effect of Kaempferol, Doxorubicin and Cisplatin on HCT 15. Data represents the mean +/- SD, n = 5.

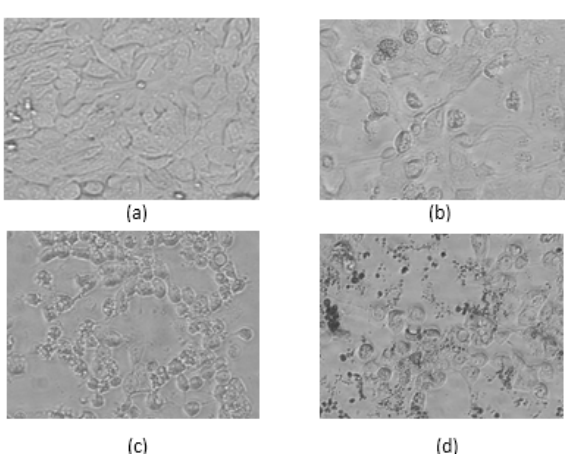

Figure 1. Photomicrographs of the HCT- 15 cell line showing the morphological changes. (a) Control cells (b) Doxorubicin 50µg/ml, (c) Cisplatin 25µg/ml, (d) Kaempferol 120µg/ml respectively.

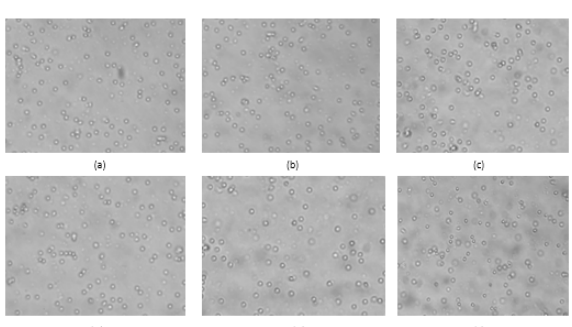

Figure 2. Photo micrographs showing the effect of kaempferol on lymphocytes. (a) Control cells (untreated); (b), (c), (d), (e) & (f) treated with 10, 25, 50, 100 and 150µg/ml kaempferol respectively.

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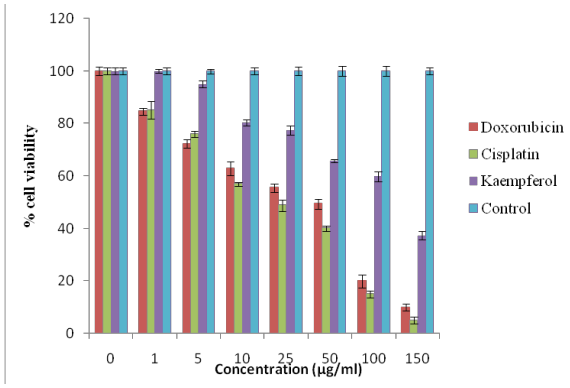

Figure 3. Cell viability assay of HCT15 with Kaempferol, Doxorubicin, Ciplatin drugs. Data represents the mean +/- SD, n = 5.

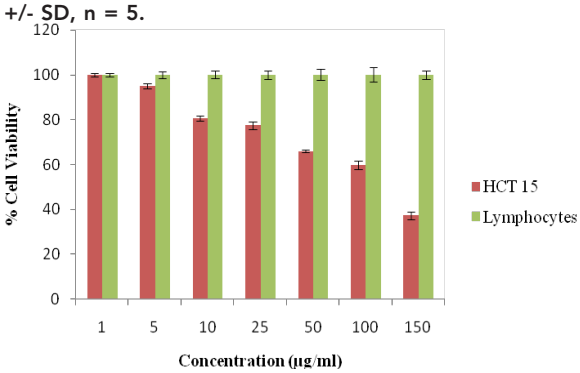

Figure 4. Cell viability assay of HCT15 and Lymphocytes with Kaempferol. Data represents the mean +/- SD, n = 5.

DISCUSSION:

The side-effects of chemotherapeutic drugs during the treatment of cancer are known. As a result a great deal of attention has been focused on flavonoids for their specific anticarcinogenic properties. Many studies have demonstrated the anticancer activity of kaempferol on various human cancer cell lines, including osteosarcoma, breast cancer, lung cancer, colorectal cancer, leukemia, oral cancer and ovarian cancer (3, 5, 6 & 14). In the present study, the effect of a flavanoid, kaempferol on human colon cancer (HCT-15) cell lines has been checked and the cytotoxicity compared with chemotherapeutic drugs doxorubicin and cisplatin. Further, the toxicity of kaempferol on normal human lymphocytes was also checked and found to be low. Our results confirm morphological alterations of human colon HCT-15 cancer cells after 24 hrs of treatment with kaempferol, doxorubicin and cisplatin in a dose dependent manner by using phase contrast microscopy. The same concentrations of kaempferol have been used to examine the effects on human lymphocytes which showed insignificant morphological alterations. The observed antiproliferative activity of kaempferol was consistent with reports that kaempferol inhibits cancer cell growth in various cells (7-10 & 14). MTT assay was performed in order to confirm the antiproliferative activity of all the compounds against cancer cells and IC_{50} concentrations were calculated by considering non treated cells as 100% viable cells.

CONCLUSION:

In conclusion, the results from this study suggest that Kaempferol could be a significant cytotoxic agent against the human colon cancer cell population HCT15. Moreover, our study proved the nontoxic nature of kaempferol on normal cells by exposing the normal human lymphocytes with the same concentrations which were used for cancerous cells. However, further studies are required to investigate the antiproliferative activity of kaempferol at molecular level.

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