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A comparative study of the activity of Kaempferol on cancer cells and normal cells

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ABSTRACT

Several plants contain flavonoids that show beneficial effects for human health. The present report deals with the chemo-protective activity of kaempferol that is present abundantly in green tea. The activity of kaempferol has been checked on cancer cells and normal cells. The anti oxidant properties of kaempferol has been reported. There are few reports of its anti-cancer activity in some cell lines. The effect of kaempferol on cell proliferation and extent of DNA damage in breast cancer cell line and normal lymphocytes has been studied. The present study indicates that MDA MB 231 cells are sensitive to kaempferol while the lymphocytes are not affected. The DNA damage due to kaempferol is extensive in case of MDA MB 231 cells but is not significant in the case of lymphocytes. The outcome of the present study indicates that kaempferol is a promising anti-cancer agent with minimal toxicity in normal cells.

Keywords: Kaempferol, MDA MB 231, lymphocytes, Comet assay.

1. INTRODUCTION

Carcinogenesis is a multi-step process involving an imbalance of numerous factors which is one of the important areas of research. In spite of all the advances in cancer therapy, the search for the chemopreventive agents is still on as there has been a statistical increase in deaths due cancer related issues [Bode A.*et al*]. GLOBOCAN had reported an estimated 8.2 million cancer related deaths in 2012, which was 7.6 in 2008 [Press Release No 223].

Many epidemiological studies have confirmed the important role played by dietary compounds in reducing the risks of cancer. In fact, several studies have indicated that the populations in South East Asia have lower risk of cancer than their counter parts in the western countries. It is apparent that the food habits of these populations play a key role in preventing the disease [Dorai T *et al*]. More importantly, the use of conventional chemotherapeutic drugs is limited due to its life-threatening side-effects because of which flavonoids and other phytochemicals are being studied for possible use as chemotherapeutic agents [Nair M. K. *et al* and Ramos S *et al*]. Kaempferol is a natural flavonoid present in various natural sources including apples, tea, broccoli onions, leeks, citrus fruits, grapes, red wines, gingko biloba and is considered to have anti-cancer potential and exerts cytotoxic effects in many types of cancer cells [Eun-Jung Park *et al*]. Majority of the studies revealed the importance of kaempferol as a very promising anticancer drug candidate as it is proved to play a key role in many cellular signal transduction pathways like apoptosis, angiogenesis, inflammation, and metastasis. It is also reported that kaempferol inhibits cancer cell growth, simultaneously preserves normal cell viability. In some cases, it is also proved to exert protective effect [J.M. Calderón-Montaño *et al*]. In addition, the protective activities of antioxidants against the toxic chemicals are being studied *in vitro* with the help of cell culture [Duthei S. J *et al*]; and lymphocytes is considered ideal for studying toxicity and cytoprotectivity on a routine basis [Mosmann T. *et al*].

The present study has been initiated with the objective of checking the activity of the kaempferol on the cancer cell lines and normal cells. The result will further lead to the development of kaempferol as an anticancer drug and will be able to disclose its protective effect.

2. MATERIALS AND METHODS

2.1. Materials and Medium

All chemicals used were procured from Sigma Aldrich, USA. Reagents and medium involved in cell culture was from HiMedia, India. Growth medium was prepared by supplementing RPMI 1640 with 10% fetal bovine serum, penicillin (100 IU/ml) and streptomycin (100µg/ml) was used for cell culture.

2.2. Cell culture

The human breast cancer cell line, MDA MB 231, obtained from NCCS, Pune, was used. It was maintained in growth medium at 37^0C in a humidified 5% CO_2 incubator.

2.3. Lymphocyte isolation

Lymphocytes were isolated from fresh blood using Histopaque 1077, Sigma Aldrich, USA. Fresh blood was drawn from a healthy person at the age of 25-30, who was not exposed to any drugs for 6 months. The isolated cells were counted using trypan blue exclusion method in a Neubauer type Hemocytometer. The concentration of the cell was made up to 2 x 10^5cells/ml.

2.4 Treatment

The MDA MB 231 cancer cells and lymphocytes were exposed to kaempferol at the concentration range from 5 to 100 µg/ml. After the desired incubation time, the cells were collected for further processing. The untreated cells served as control.

2.5 MTT

The MTT assay was performed using minor modification of Mosmann [11]. The treated cells were washed with PBS (phosphate buffer saline) and treated with MTT **(**3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide**).** After 5 hrs of incubation, DMSO (dimethyl sulfoxide) was used to dissolve the formazan crystals formed resulting in purple color. This was read at 540 nm.

2.6 Comet assay

The Comet assay was executed according to Tice *et al.* [12]. 10 µl of the treated and untreated cells resuspended in 1% low melting agarose in PBS was layered on the slide pre-coated with 1% normal melting agarose. The slides were incubated at 4^0C for 30 min. A third layer was applied with 1% normal melting agarose and again incubated at 4^0C for 30 min. Subsequently, the slides were kept in

cold lysis buffer (10 mM Tris, 2.5 M NaCl, 100 mM EDTA, 1% N-lauroylsarcosine at pH 10, 1% Triton X-100 and 10% DMSO) [13] overnight at 4^0C. For unwinding of DNA these were then incubated in alkaline electrophoresis buffer (300 mM NaOH, 1 mM EDTA at pH 13) for 20 min. Later on, the slides were washed thrice with neutralizing buffer (0.4 M Tris HCl at pH 7.5) after electrophoresis at 0.8V/cm and 300mA for 20 min. 25 µM propidium iodide was used as stain after the slide were fixed with 75% ethanol and observed under fluorescent microscope. OPEN COMET was used to score 50 comets.

3. RESULTS AND DISCUSSION

3.1. MTT Assay

In the present study, the MTT assay was used for assessing the cell viability. The kaempferol was tested for its cell viability assay on the human breast cancer cell line MDA MB 231 and peripheral blood lymphocytes. Moreover, the morphology of the cancer cells, observed under inverted phase contrast microscope, showed signs of apoptosis such as cell shrinkage, nuclear condensation and detachment from substratum, while no significant changes was observed in treated lymphocytes (result not shown). In case of the cancer cells, kaempferol showed a dose dependent increase in cell death (IC_{50} of kaempferol was 64 µg/ml). The anti-proliferative activity of kaempferol was not significant in peripheral blood lymphocytes with approximately 95% viable cells at the highest concentration (Fig. 1). This result is preliminary evident that the kaempferol has anti-proliferative activity against cancer cells no considerable effect on the lymphocytes. All the subsequent experiments were performed at the IC_{50} value of kaempferol (64 µg/ml).
The comet assay was performed to analyse the DNA damage caused by kaempferol (64µg/ml). The exposure of MDA MB 231 cells to kaempferol showed substantial DNA damage with the tail length 72.64, percentage DNA in tail of 16.89 and olive moment of 18.19 when compared to the control untreated cells. By contrast, the lymphocytes did not have significant DNA damage. Treated lymphocyte illustrated tail length of 4.62, percentage DNA in tail of 2.98 and olive moment of 0.92 which was relatively comparable to the untreated control lymphocytes (Fig 2; table 1). This finding indicates that kaempferol induced substantial DNA damage to the cancer cells while having minimum effect on the normal lymphocyte cells.

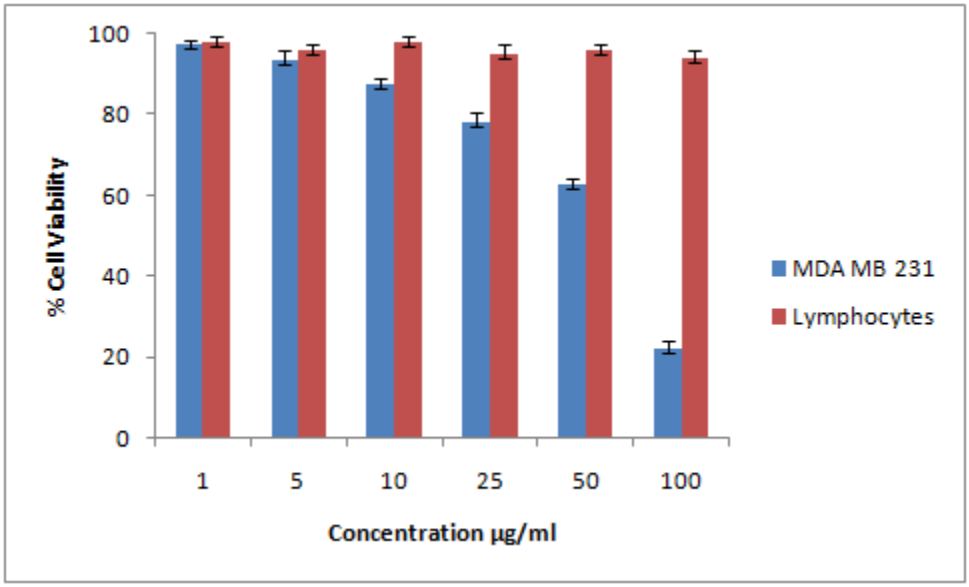

Figure 1
Cell viability assay of kaempferol on MDA MB 231 and Lymphocytes. Data represents the mean +/- SD, n = 5

Present scenario in chemotherapy stipulates selective killing of cancer cells [Tice R. R *et al*]. Hence, the effect of the anticancer agent should be limited to the cancer cells and not the normal cells of the body. Several studies on the effect of phytochemicals on numerous cell lines and animal models have illustrated the potential of phytochemicals in preventing cancer [Tice R. R *et al*]. kaempferol, being of the flavonoid having anticancer activity on vast number of cancer cell lines, was considered for the comparison of its effect on cancer and normal cells.

The MTT assay, a gold standard technique used for checking the cell viability, revealed that kaempferol has potential anticancer activity against MDA MB 231 cells and minimal effect on the lymphocytes. Moreover, as membrane blebbing and DNA

fragmentation is considered the hallmark of apoptosis, the MDA MB 231 cells were believed to undergo apoptosis. This assumption was further supported by the comet assay which illustrated the extent of DNA fragmentation in the cells. Comet assay is a rapid and reliable technique and among all the parameters estimated tail length, percentage DNA in tail and olive moment are the most vital [Kwon K. H., *et al*]. The extent of DNA damage was prominent in the kaempferol treated MDA MB 231 cells in contrast to the lymphocytes which did not elucidate substantial DNA damage.

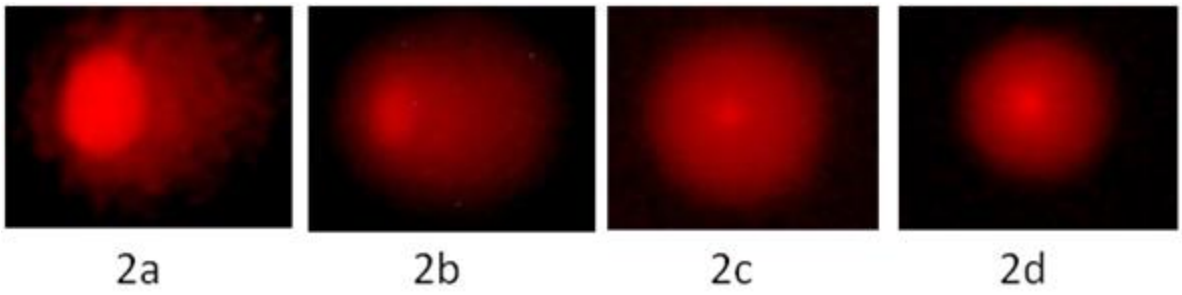

Figure 2
Comet assay showing DNA damage by kaempferol in MDA MB 231 and lymphocytes, (a) untreated MDA MB 231, (b) MDA MB 231 treated with kaempferol (64µg/ml), (c) untreated lymphocyte and (d) lymphocyte treated with kaempferol (64µg/ml).

Table 1 Effect of kaempferol (KA) on the extent of DNA damage on MDA MB 231 and lymphocytes

	MDA MB 231 Control	KA on MDA MB 231	Lymphocytes Control	KA on lymphocytes
Tail length	39.33+/-2.17	72.64+/-2.18	3.10+/-1.02	4.62+/-1.96
% Tail DNA	12.22+/-1.90	16.89+/-0.98	1.96+/-0.96	2.98+/-1.25
Olive moment	6.38+/-1.64	18.19+/-2.27	0.79+/-0.49	0.92+/-0.63

4. CONCLUSION

Thus, the outcome of the comparative study was that kaempferol is a potential anticancer agent as it targets the cancer cells without having any detrimental effect on the normal lymphocyte cells. This study can further be carried out at a molecular level to find out the possible mechanism involved in this contradictory effect on cancer cells and normal cells.

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