ORIGINAL ARTICLE

Anti-Breast Cancer Activity of Bergenin and Naringenin on Michigan Cancer Foundation-7 Cells: An *In-Vitro* Study

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Abstract

Background: Breast cancer is among the biggest causes of mortality for women around the globe. The treatment of breast cancer usually involves surgical procedure or chemotherapy but nature offers potentially safer and more effective treatment options. Using the MTT assay, this study evaluates the cytotoxic effects of Bergenin (Berg) and Naringenin (NG) on Michigan cancer foundation-7 (MCF-7), a human breast cancer cell line. **Materials and Methods:** Breast cancer cells, MCF-7 cells were treated with several concentrations of Berg and NG and the MTT assay was used to measure cell viability, from which half-maximal inhibitory concentration (IC_{50}) values were calculated to measure cytotoxicity. **Findings:** The cytotoxic effect of both drugs was dose dependent. IC_{50} value was of Berg was 135.06 µg/mL, but NG's was 114.59 µg/mL, indicating a higher cytotoxic effect. Potential mechanisms for the observed effects include reactive oxygen species production, cell cycle arrest, apoptosis activation, and modification of the estrogen receptor and survival signaling pathways. **Conclusion:** When it comes to MCF-7 breast cancer cells, Berg and NG exhibit moderate but noteworthy anti-proliferative action. Their promise as lead compounds for additional research in breast cancer therapy is highlighted by their natural origin and multi-targeted modes of action. To confirm their therapeutic value, more mechanistic and *in-vivo* research is advised.

Key words: Bergenin, breast cancer, Michigan cancer foundation-7, Naringenin

INTRODUCTION

Breast cancer continues to be the most common and deadly cancers among women globally. As per the World Health Organization and International Agency for Research on Cancer, 2.3 million new cases of breast carcinoma were diagnosed with almost 670,000 deaths in 2022.[1,2] Current trends continue, new incidences of breast cancer have been estimated to be 3.2 million and with 1.1 million deaths from the disease annually by 2050. However, different parts of the world do not bear an equal burden of breast cancer. Instance rates of breast cancer were lowest in South-Central Asia, Middle Africa, and Eastern Africa, and higher number is observed in New Zealand and Australia following the Northern Europe and Northern America. In breast cancer, cells proliferate uncontrollably and develop into tumor capable of spreading throughout the body and become lethal if untreated. Usually, breast cancer initiates from the milk ducts and/or milk-producing lobules. The earliest type can be detected in its early stages, referred as *in situ* and is not harmful for life but as cancerous cells have the ability to invade neighboring breast tissue, it reaches to invasive, complications arise.[3] Numerous internal and external factors contribute to the development and occurrence of breast cancer. It is associated with poor lifestyle choices, environmental conditions and social-psychological variables. Genetic mutations and family history have been shown to be responsible for 5–10% of breast carcinoma, whereas several other possible factors are accountable for 20–30% of all the cases.[4]

Although numerous approaches such as surgeries, radiation therapy, and chemotherapy are there for the treatment of breast cancer but as they are associated with severe side effects, researchers are exploring phyto compounds for breast cancer treatment.[5,6] Due to their varied pharmacological characteristics and reduced toxicity profiles, natural

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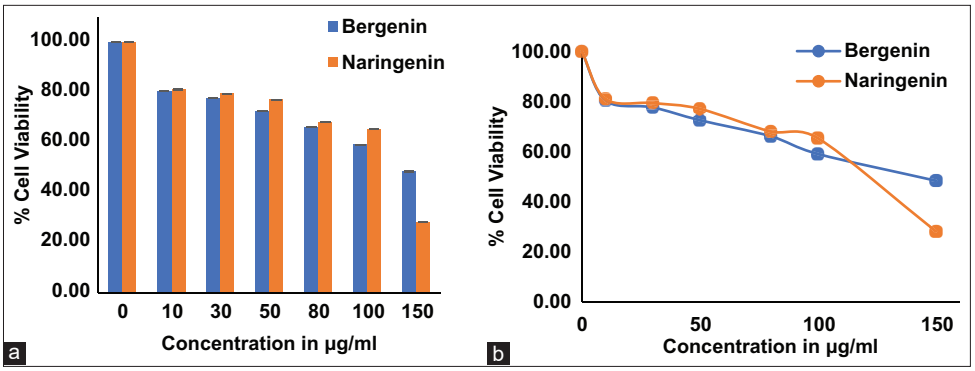

Figure 1: *In-vitro* cytotoxicity study using MTT assay of Bergenin and Naringenin. (a) % Cell viability of Michigan cancer foundation-7 (b) Plot of % cell viability versus concentration for half-maximal inhibitory concentration value determination

chemicals derived from medicinal plants have become attractive prospects in the development of anti-cancerous agents.[7] Two such phyto constituents are Bergenin (Berg) and Naringenin (NG). A C-glycoside of 4-O-methyl gallic acid named Berg can be derived from numerous species of *Bergenia genus* viz. *Bergenia cordifolia, Bergenia ciliate*, *Mallotus japonicas* has been shown to have a variety of anti-cancer potential against several cancers of cervical, ovarian, prostate, bladder etc.[8] Comparably, NG, a flavanone that is mostly present in citrus fruits, is well-known for its potential in inhibiting the proliferation of many cancer cells and induces apoptosis.[9] The present work incorporated the exploration of the Michigan cancer foundation-7 (MCF-7) human breast cancer cell line to assess the *in-vitro* efficacy of Berg and NG with the aim to clarify the cytotoxic and anti-proliferative effects of these phytochemicals against breast cancer.

MATERIALS AND METHODS

Materials

Berg and NG were procured from Yucca Enterprises, Mumbai and TCI Chemicals, India, respectively. MCF-7, a breast cancer cell line was purchased from NCSS, Pune. Dulbecco's Modified Eagle's Medium ([DMEM]; pH 7.4) and 10% fetal bovine serum (FBS) were purchased from HiMedia Laboratories while pen-strep was procured from Gibco, Thermo-Fisher. MTT dye, i.e., 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide, and all other reagents for culturing were obtained from Sigma Aldrich Chemicals Ltd. India.

Cell culture

MCF-7 was regrown in DMEM nourished with FBS (10% v/v) and penicillin/streptomycin (1% v/v).Cells were them kept in incubator at 37°C, 5% carbon dioxide (CO_2), and 95% relative humidity until they reached 80% confluency.

Table 1: IC_{50} values of Bergenin and Naringenin on Michigan cancer foundation-7 cell lines

Compounds	24 h (µg/mL)
Bergenin	135.06±0.08
Naringenin	114.59±0.03

Each value represents mean±standard deviation (*n*=3)

Cytotoxicity study

The MTT-based colorimetric assay was employedto determine the cytotoxic effect of Berg and NG. MTT, a colorful water-soluble tetrazolium salt, also known as (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide),was utilized to measure the cytotoxic effect as a percentage of cell viability or proliferation. It works on the phenomenon when MTT comes in contact with metabolically active cells it transforms yellow MTT salt into blue formazan crystals which is dissolvable in organic solvents and its concentration can be measured spectrophotometrically.[10]

The cytotoxicity of Berg and NG was evaluated against estrogen receptor+ breast cancer cell line MCF-7. After the 80% confluency was reached, trypsin was used to separate the cells which were then counted to obtain the viable number through hemocytometer. MCF-7 cells at a density of 1×10^4 cells/well were seeded in an Eppendorf 96-well culture plate in DMEM media provided with 10% FBS. The cells were then incubated for 24 h at 37°C with 5% CO_2. After prescribe incubation period, media was withdrawn and cells were treated with drug and positive control (dimethyl sulfoxide [DMSO]). The free drug concentrations for both the drugs ranged from 10 to 150 µg/mL and were used to determine % cell viability. Following 24 h of incubation, wells were aspirated and then cells were thoroughly cleaned with phosphate-buffered saline (PBS) 7.4. Each well was further treated with 20 µL of MTT solution (5 mg/mL in PBS) followed by incubating for 4 h to enable the formation of formazan crystal. The excess dye solution was aspirated and 200 µL of culture-grade DMSO was added for dissolving the developed formazan crystals. Before measuring the optical density, the plate was

gently shaken, and readings were taken at 570 nm using a microplate reader (Synergy™ H1 multimode microplate reader). Using the viability of the control cells as 100%, the percentage of cell viability was computed three times.[11] The concentration required to lower the cell viability by 50% (IC_{50}) quantifies how various drug doses affect cell proliferation and was determined using the given formula:

Percentage of Viable Cells = A570 of Treated Cells/A570 of Control Cells × 100

Statistical analysis

All the results were taken in triplicate and displayed as means ± standard deviation. The data were statistically analyzed using a two-tailed Student *t*-test using data analysis tools in Microsoft Excel. Less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The MTT assay was used to assess the antiproliferative efficacy of Berg and NG, where relative effectiveness of these substances in lowering the cell viability was calculated from the half-maximal inhibitory concentration (IC_{50}) values. *In-vitro* cytotoxicity study on MCF-7 cells was conducted for 24 h time interval with an equivalent dose of 10–150 µg/mL.

Although both the drugs showed sufficient cytotoxicity but Naringenin showed a somewhat higher cytotoxic effect with an IC_{50} value of 114.59 µg/mL, than Bergenin, which had an IC_{50} value of 135.06 µg/mL with p significant <0.05. All the results are depicted in Table 1 and Figure 1. The results of both the substances showed the dose-dependent cytotoxicity as the number of viable cells continuously declined with increasing the dose.

The reason attributable to the cytotoxic potential of NG could be its flavonoid structure which may offer improved cell permeability or greater interactions with intracellular targets linked to cell survival and proliferation. In MCF-7 cell, NG triggers programmed cell death through extrinsic and intrinsic routes, activating caspases 3 and 9 due to overexpression of pro-apoptotic proteins and down regulation of anti-apoptotic ones. It disrupts redox equilibrium, causing oxidative stress, cell cycle arrest, estrogen signaling modification, and inhibiting pro-survival signaling pathways, including PI3K/Akt, nuclear factor-kappaB, and mitogen-activated protein kinases, which are essential for cell survival, inflammation, and proliferation.

While Berg being glucoside derivative has moderate cytotoxic effects on cancer cells by multiple pathways including oxidative stress induction, mitochondrial apoptosis, cell cycle arrest, and signaling pathway modification. It can induce reactive oxygen species (ROS) in cancer cells, disrupt survival pathways in breast cancer cells like MCF-7, and may interact with hormone receptor signaling, although its exact anti-estrogenic effect is yet to explore.

CONCLUSION

The cytotoxic potential of Bergenin and Naringenin was explored using MTT assay on human breast cancer cell line MCF-7. The observations portrayed that Berg had an IC_{50} value of 135.06 µg/mL and this cytotoxicity might be due to cell cycle arrest, pro-survival signaling pathway modification, ROS generation, and apoptosis induction. While NG being a flavonoid showed comparatively greater potency with an IC_{50} value of 114.59 µg/mL by inducing oxidative stress, cell cycle arrest, estrogen signaling modification, and inhibiting pro-survival signaling pathways. Both the drugs showed dose-dependently decreased cell viability on MCF-7, indicating their anticancer properties which could be further enhanced by transforming in a suitable dosage form. Despite their modest cytotoxicity, these compounds are attractive prospects for additional research due to their low toxicity profile, multi-targeted mechanisms, and natural origin. Although to confirm their therapeutic efficacy and investigate their potential in conjunction with well-established chemotherapeutic drugs, more research about gene expression analysis, mechanistic assays, and *in-vivo* study would help in confirming the therapeutic relevance.

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