Research Article

Cytotoxic potential of *Ocimum basilicum* seed extract on human epidermoid carcinoma (KB cell lines) – An *in vitro* study

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ABSTRACT

Background: Cancer, till today, remains on the unsolved mystery in the phase of prognosis. The death rate of this disease has been increasing in both developing and developed countries. Various researchers are working on the cure for this disease without any side effects. Hence, this study is aimed at to analyze the cytotoxic potential of *Ocimum basilicum* (OB) seed extract on oral carcinoma KB cell lines. **Materials and Methods:** KB Cell line: KB cell line was procured from NNSCCS, Pune and maintained in Dulbecco's Modified Eagle Medium (DMEM). Preparation of test drug: OB seed was uniformly grinded and serially extracted in methanol, using Soxhlet apparatus. Cytotoxic assay: Cytotoxicity of OB seed was analyzed by 3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide assay and compared with standard drug cisplatin. **Results:** The aqueous extract of OB has exhibited significant cytotoxic potential against oral cancer cell line. **Conclusion:** Aqueous extract of OB was effective as an antiproliferative agent which causes apoptosis in the oral cancer cell line.

KEY WORDS: Cytotoxic potential, KB cell line, Natural, *Ocimum basilicum*

INTRODUCTION

Cancer is the second most disease which causes global deaths. Conventional therapies for cancer include surgery, cytotoxic chemotherapy, immunotherapy, and radiation therapy which are used as a single or combinational therapy have its own side effects. Furthermore, a small subpopulation of cancer cells called cancer stem cells is resistant to conventional cancer therapy. Therefore, it is essential to design an alternative method for cancer treatments.[1] Plants are of the important sources of medicine and a large numbers of drugs which are being used today are derived from plant origin. *Osimum basilicum* (OB) commonly known as Sweet basil is used in both Ayurvedic and Unani system of medicine.[2] OB is a culinary plant belonging to the Lamiaceae family that is extensively used as an flavoring agent in a wide variety of fields in its native region and as a popular traditional folk remedies or in complementary and alternative medical therapy.[3] The aromatic herb is about 20–60 cm long with white/purple flowers, ovate/lanceolate leaves, and a hairy-petiole.[4] The plant is native to India and Iran and grows throughout the temperate, tropical, and subtropical regions of the world.[5] Basil seed is a tiny black, ellipsoid seed. These seeds are popularly used in traditional desserts (such as sherbet and falooda) and have several functional characteristics including carminative, stimulant, diaphoretic, diuretic, dyspepsia, antiseptic, anesthetic, flatulence, gastritis, anti-spasmodic, anthelmintic, antidiarrheal, analgesic, and antitussive. Other medicinal uses of OB include treatment of some gastrointestinal disorders, gastrodynia, diarrhea, and vomiting.[3,6] India is considered to be the oral cancer capital of the world. Oral squamous cell carcinoma (OSCC) is a multifactorial disease with tobacco and alcohol being considered major risk factors. However, there is a growing incidence of non-habit (i.e., the absence of tobacco or alcohol) associated with oral cancer. Difference is noted in demographics, site

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predilection, grade, and stage while comparing habit associated and non-habit associated oral carcinoma. OSCC is the eight most common type of cancer.[7-9] A recent trend seen in India is the increased incidence of OSCC in females without a deleterious habit history.[9]

The development of resistance to multiple drugs is a common clinical problem in the treatment of various cancers, and many current therapeutic procedures have shown multiple side effects. In addition, common cytotoxic therapies primarily target rapidly dividing cells including malignant cells as well as certain normal cells, leading to significant death and limited clinical benefits for ill patients. Therefore, it is mandatory to improve anticancer therapies that effectively and specifically target tumor cells and at the same time minimise the toxic side effects on physiologically proliferating cells.[10] There is a new spotlight for naturally acquired plant products.[11] The renewed interest in natural substances, rather than in synthetic agents focused attention on plants used as food or spices, which are a rich source of bio-nutrients or bio-active phytochemical compounds and more detailed studies are needed to establish their safety.[12] Hence, this study conducted to determine the cytotoxic potential of basil seed extract on the oral epidermoid cell (kB cell line) which could create an out-breaking sequel in the field of oncology.

MATERIALS AND METHODS

Cell Culture

KB cells were procured, placed in 25 cm^2 culture flasks and cultured in Roswell Park Memorial Institute medium 1640 culture medium, with 10% fetal bovine serum, L–glutamine, 1% penicillin (100 U/ml), and streptomycin (100 µg/ml) at 37°C in a humidified CO_2 (5%) chamber, and 95% air. The cells were detached using 0.25% EDTA trypsin. Neutralization of the trypsin was achieved using Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and PSGF, and cells were mechanically separated using a pipette. There were 96-well plastic culture plates filled with 200 µl of medium containing in each well. The plates were then incubated at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air for 24 h to permit attachment of the cells to the plates.

Preparation of Test Drug

The OB seeds were uniformly grinded using a mechanical grinder to make a fine powder. The powder was serially extracted in methanol, separately using a Soxhlet apparatus. The solvent present in the extract was then dried at room temperature and stored at 4°C. The stock solution was prepared by dissolving 1 mg of the extract in 1 ml of 10% dimethyl sulfoxide (DMSO) and serially diluted with different concentration (12.5–200 µg/ml) for testing.

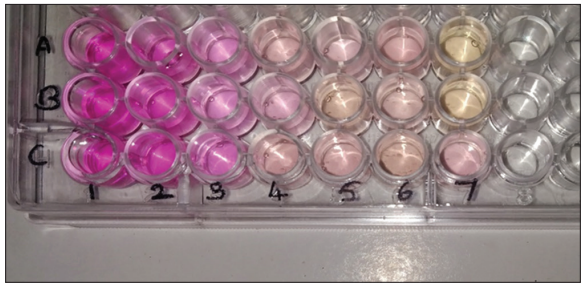


Figure 1: Microplate images showing the color changes for different test groups. 1-KB cells untreated negative control; 2-KB cells treated with OBE (12.5); 3- KB cells treated with OBE (25); 4- KB cells treated with OBE (50); 5- KB cells treated with OBE (100); 6- KB cells treated with OBE (200); and 7- KB cells treated with cisplatin (10)

Cytotoxicity Assay

3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay is a colorimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase. The KB cells were seeded at the density of (1 × 103 cells/ml) and treated with OSE for 24 h. The cells were permitted to adhere for 24 h, and the growth medium minimum essential medium (MEM) removed using micropipette and the monolayer of cells washed twice with MEM without FBS to remove dead cells and excess FBS. Cell culture medium (DMEM) was used as negative control for assessment of cell viability. 1 ml of medium (without FBS) containing different dilution of drugs (12.5–200 µg/ml) were added in respective wells; 200 µl of MTT (5 mg/ml in PBS) were added to each well, and the cells incubated for a further 6–7 h in 5% CO_2 incubator. After removal of the medium, 1 ml of DMSO was added to each well. The effect of the drug on cell growth inhibition was assessed as percent cell viability, where vehicle-treated cells were taken as 100% viable. The supernatant was removed, and 50 µl of propanol was added, and the plates were gently shaken to solubilize the formed formazan. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product. Since the reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. The plates were placed on a shaker for 15 min, and the absorbance was read on an enzyme-linked immunosorbent assay reader at 570 nm.

Statistical Analysis

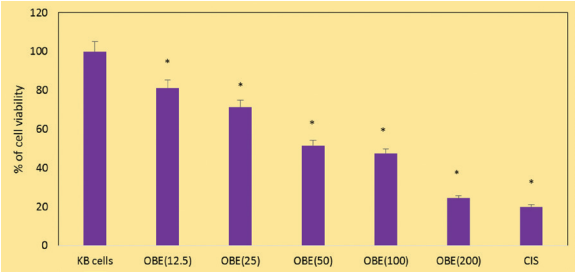
Results were expressed as mean ± SD. Statistical significance was determined by one-way analysis of variance and *post hoc* least significant difference test. $P < 0.05$ was considered significant.

RESULTS

Table 1: Cytotoxicity assay

Treatment	Concentration (µg/ml)	Absorbance 570 nm
KB cells untreated	-	0.389±0.11
OBE	12.5	0.316±0.10*
	25	0.278±0.08*
	50	0.201±0.05*
	100	0.185±0.02*
	200	0.095±0.01*
Cisplatin	10	0.078±0.01*

Values are expressed as Mean±SD (n=3); *P<0.001, as compared with negative control



This graph depicts the cell viability changes. Results were expressed as Mean ± SD (n = 3). *P < 0.05 significantly different as compared with KB cells untreated.

DISCUSSION

Cancer is a very compound disease, and the occurrence and development of tumor cells are closely related to abnormal intracellular signal transduction system.[13] Due to its high malignancy rate with a potent capacity to invade locally and metastasize distantly an approach to decrease tumor's ability to invade and metastasize may facilitate the development of effective adjuvant therapy.[14] OSCC is highly resistant to conventional chemotherapy and radiation therapy and in the current treatment protocol for carcinomas attempts to target only the homogenous cancerous tissues; thus, the development of novel anticancer drugs is a simple method for remedy of cancer.[15] The variability of clinical outcomes in oral cancer patients and the heterogeneity of the disease are the main challenges for the improvement of current treatment modalities.[16]

Nowadays, one of the main methods of modern cancer treatment is chemotherapy. Most chemotherapeutic agents for cancer have different substantial short and long-term unwanted side effects on the human body. Thus, in recent years, tremendous researchers are focused on herbs and plants which have been considered for being nontoxic and for the prevention and treatment of certain types of cancer.

Research into food-derived bioactive components for cancer prevention as well as cancer therapy is a growing area of interest because of relatively lower or no detectable toxicity and better bioavailability. This was a preliminary in Virgo study wherein we evaluated the effectiveness of basil as a cytotoxic agent against oral cancer cells. There was an overwhelming positive response of aqueous basil extract on the oral cancer cell. The cytotoxic effect was mainly due to the apoptosis caused by the aqueous solution. This effect can be attributed to the presence of phytochemical compounds which are abundant in basil seed. In the present study, OB seeds extracts were evaluated for cytotoxic activity on KB cell lines. It showed a cytotoxic effect on KB cell lines. MTT cell growth inhibition assay was taken as *in vitro* measure of anticancer activity of OB seeds using KB cell lines. The cell viability percentage showed maximum activity at the lower concentration, i.e., 12.5 µg/ml [Table 1] and showed maximum cell viability in its highest concentration, i.e., 200 µg/ml [Figure 1]. There was more death of cell line or cell deterioration with an increase of the concentration of OB seeds. Therefore, this plant may be a candidate for further studies. The leaves and flowers of plants contain numerous aroma chemicals, which are widely used in folk medicine and modern aromatherapies.

CONCLUSION

We recommend more studies especially randomized control trails among patients with oral premalignant and malignant lesion to confirm the effectiveness of OB herb. The time has come where we all as clinicians and researchers should explore more into nature and find solutions faced by the community.

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