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Anti-cancer potential of a mix of natural extracts of turmeric, ginger and garlic: A cell-based study

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ABSTRACT

Cancer related morbidity and mortality is a major health care concern. Developing potent anti-cancer therapies which are non-toxic, sustainable and affordable is of alternative medicine. This study was designed to investigate the aqueous natural extracts mixture (NE mix) prepared from common spices turmeric, ginger and garlic for its free radical scavenging potential and anti-cancer property against human breast cancer cell lines (MCF-7, ZR-75 and MDA-MB 231). Qualitative analysis of their bioactive constituents from turmeric, ginger and garlic were done using liquid chromatography-ESI- mass spectrometry (LC-ESI-MS/MS). To the best of our knowledge, NE mix with and without Tamoxifen has not been tested for its anti-cancer potential. We observed that the NE mix induced apoptosis in all the breast cancer cell lines, but it was more prominent in MCF-7 and ZR-75 cell lines in comparison to MDA-MB 231 cell line. The extent of apoptosis due to combined treatment with NE mix-Tamoxifen was higher than Tamoxifen alone, indicating a potential role of the NE mix in sensitizing the ER-positive breast cancer cells towards Tamoxifen. In support to MTT assay, cell cycle analysis, our RT-PCR results also prove that the NE mix 10 μg, Tam 20 μg and combination of NE mix 10 μg-Tam 20 μg altered the expression of apoptotic markers (p53 and Caspase 9) leading to apoptosis in all three cell lines. Our data strongly indicate that our NE mixture is a potential alternative therapeutic approach in certain types of cancer.

1. Introduction

Breast cancer is one of the leading cancers affecting women globally. Incidence of breast cancer is on the rise in countries like India, Japan and other Asian countries. In India, over 50,000 women die of breast cancer every year [1]. Even in western countries, where the rate of incidence is reported to be either stabilized or declining, breast cancer still continues to contribute significantly to cancer-related mortality and morbidity. Adding to existing healthcare concerns of breast cancer, its rising incidence in younger (pre-menopausal) women worldwide [2] and its overall morbidity and mortality underscores a need for developing alternative breast cancer preventive and intervention strategies.

Apoptosis is a conserved and pivotal process of cell death that is required for host defense, suppression of oncogenesis and for normal development and faulty apoptosis is enhances the tumor development and progression. The hallmark of human cancers is evading apoptosis. p53 gene was initially described as an oncogene in 1979, but later it is to known as tumor suppressor gene whose function is to abolish and hinder abnormal cell proliferation, thus preventing neoplastic development [3,4]. It is found in all human cancers that p53 function in controlling the negative growth regulators is lost. Under normal cellular environment, the p53 signalling pathway is always in standby mode in normal cells, but it gets activated in response to cellular stresses, and several autonomous pathways of p53 which are dependent on distinct upstream regulatory kinases. p53's pivotal role in mammary carcinogenesis has been acquired from enormous information collected from mechanistic, molecular pathological and transgenic animal studies [5].

The *in vitro* studies on p53 function, and it is found that it continuously suppresses tumorigenesis. Vousden and Lu [6] suggested that p53 might be involved in preventing tumor development

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especially in humans. The suppression of tumorigenesis is exerted mainly by initiating the apoptosis [7]. Attardi and Jacks [8] demonstrated that loss of p53 activity has accelerated the tumor formation in transgenic mice as a result of apoptosis dysfunction. The integrity and mutation status of p53 decides its qualitative and quantitative activity in different stress-induced signalling pathways. The fact that p53 function is lost due to mutation in breast neoplasia, but its mutation rate is significantly low in other solid tumours [4,9].

Caspases and numerous upstream regulatory factors execute the cell death process by initiating or directing their proteolytic activity, which could act either as tumor suppressors or oncogenes. These regulatory factors do regulate cell survival or repair signalling pathways often in response to cellular stress, apart from being strong apoptotic inducers [10,11]. Hence, loss of function doesn't mean that tumor formation is a result of apoptosis dysfunction due to inactivation of caspases. Any injury to outer mitochondrial membranes triggers the release of caspase-9 enzyme found in the mitochondrial intermembrane space into the cytosol together with cytochrome *c*, hence it interacts with and activates the apoptosis-activating factor (Apaf-1) in a cytochrome *c* and dATP-regulated manner [12]. Caspase-9 is one of the essential caspases which is required for the initiation of apoptosis signalling on the apoptosome complex through mitochondrial pathway and severe pathophysiological events will arise in case of failure to activate caspase-9 [12]. The activation of caspase 9 and downstream caspase cascade usually occur during mitochondrial disruption [13]. Tumor suppressor proteins and proto-oncogenes with a more direct effect on Caspase activity can be found among p53-transregulated genes harbouring apoptosis-specific functions. Despite an obvious central role of p53 and caspases in the hallmarks of cancer, their status is not yet used for the management of breast cancer [9].

Currently, Tamoxifen is one of the strategies in hormone therapy. Tamoxifen is a selective estrogen receptor modulator (SERM) used for in treatment of breast cancers. However, Tamoxifen-based prevention strategy is limited to a selective group of women who are Estrogen Receptor (ER) positive. Moreover, prolonged use of the drug predisposes women to ovarian [14] uterine and endometrial cancers [15] apart from causing other serious complications such as retinal vein occlusion [16] deep vein thrombosis, pulmonary embolism [17], stroke [18] and cataracts [19], highlighting an urgent need for developing safer scalable approaches. Strategies to lower the required dose of anti-cancer drugs (sensitization) are useful. Identifying the anti-cancer properties of commonly used dietary products and other household herbs [20,21] may offer probable solutions for prevention. Natural anticancer agents exert significantly lower toxicity, safe and easily available; hence a combinational therapy using natural anticancer drug along with commercially available anticancer drugs should be encouraged in order to reduce the limitations in controlling the metastatic cancers [22]. Such a notion is supported by the observed beneficial impact of natural compounds with anti-cancer treatment modalities [23–25].

A multitude of studies have shown beneficial effects of phyto and marine extracts for intervention and treatment in cancer [26–29]. In view of the above, it may be rationalized that exploiting anti-cancer potential of commonly used food ingredients and easily accessible herbs may be useful in controlling the cancers. Thus in the present study, we have investigated the potential effect of conventional hormone therapy drug i.e. tamoxifen when supplemented with natural extracts mixture made from commonly used dietary spices in inducing cell death and sensitization of immortalized cells i.e. breast cancer cells which are ER-positive (MCF-7 and ZR-75) and ER-negative (MDA-MB 231).

2. Materials and methods

2.1. Materials

Human breast cancer cell lines, MCF-7, ZR-75 and melanoma cell line MDA-MB 231 were obtained from a National cell line repository (National Centre for Cell Science, Pune, India). Hi-Gluta XL™ Dulbecco's Modified Eagle's Medium (High Glucose) cell culture medium, Hi-Gluta XL™ RPMI-1640 cell culture medium, L-Glutamine-Penicillin-Streptomycin solution, Dulbecco's Phosphate buffered saline (DPBS), 0.22 μm sterile syringe driven filters, sterile cell scrapers, 0.25% Trypsin-EDTA solution, Bovine serum albumin (BSA) were obtained from Hi-Media, India. Fetal bovine serum was obtained from Seralab, USA. Sterile cell culture plastic ware was purchased from Thermo Fisher, USA. Flow-cytometer BD FACS caliber Apoptosis Kit – Annexin V Alexa Fluor 488 and propidium iodide (Thermo fishers) and Ultrapure water was generated using Millipore RiOs-DI®3 system.

2.2. Chemicals

Acetonitrile ULC/MS Grade purchased from Biosolve Chimie SARL (Dieuze, France) and formic acid (Optima LC/MS grade) was purchased from Fisher Scientific (Geel, Beljium, Germany). Methanol (LiChrosolv) was purchased from Merck (Darmstadt, Germany). Deionized water was prepared by passing distilled water through a Milli-Q water purification system (Millipore, Milford, MA, USA).

2.3. Methods

2.3.1. Preparation of natural extracts (NE) and tamoxifen solution

Turmeric, ginger and garlic were brought from local markets and the natural extracts mixture was prepared in-house by adding 20 g of each i.e. turmeric, garlic and ginger paste into 500 ml of ultrapure water and heating it at 60 °C for 6 h. Later the mixture was shaken overnight at room temperature followed by centrifugation at 4 °C for 10 min. The supernatant was then separated and filter-sterilized using a 0.22 μm syringe filters and lyophilized.

Tamoxifen stock solution was prepared by dissolving 10 mg of Tamoxifen into 500 μl of absolute ethanol and then adding 4.5 ml of ultrapure water. The solution was then filter-sterilized using a 0.22 μm syringe filter. The required dosages were prepared from diluting this stock solution.

2.4. Antioxidant assays

2.4.1. Superoxide anion scavenging assay

The assay for superoxide anion radical scavenging activity was supported by riboflavin-light-NBT system [30]. Briefly, 1 ml of sample was taken at different concentrations (25–500 μg/ml) and mixed with 0.5 ml of phosphate buffer (50 mM, pH 7.6), 0.3 ml riboflavin (50 mM), 0.25 ml PMS (20 mM), and 0.1 ml NBT (0.5 mM). Reaction was started by illuminating the reaction mixture using a fluorescent lamp. After 20 min of incubation, the absorbance was measured at 560 nm. Ascorbic acid was used as standard. The scavenging ability of the plant extract was determined by the following equation:

$$Scavenging\ activity(\%) = \frac{[1 - \text{absorbance of sample}]}{[\text{absorbance of the control}]} \times 100 \quad (1)$$

2.4.2. Phosphomolybdate assay (total anti-oxidant capacity)

The total anti-oxidant capacity of the fractions was determined by Phosphomolybdate method using ascorbic acid as a standard [31]. An aliquot of 0.1 ml of sample solution was mixed with