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Research Article

In-vitro Anticancer and Cytotoxic Activity of Ginger Extract on Human Breast Cell Lines

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Abstract

Breast cancer is the leading cause of cancer related mortality in women. Breast cancer is highly metastatic and can spread to various parts of the body. Ginger, the rhizome of Zingiber officinale, one of the most widely used species of the ginger family, is a common condiment for various foods and beverages. Ginger has a long history of medicinal use. The aim of this study was to evaluate the effect of in vitro anticancer and cytotoxic activity of ginger ethalonic extract against breast cancer cell lines using MTT assay method. Results revealed that ginger extract possesses dose dependent cytotoxic effects on breast cancer cell lines. Whereas, IC_{50} values for 48hours of treatment found to be 9.68 mg/l, 2.47 mg/l and 12.81 mg/l on MCF-7, MDA-MB-231 breast cancer cell lines and non-tumorigenic, normal breast epithelial MCF-10A cell line, respectively with relatively high selectivity (SI= 5.1) against MDA-MB231. It could be concluded and recommended that ginger exhibits anticancer activity on breast cancer cell lines, hence ginger can be further evaluated for potential promising anticancer activity.

Keywords: Ginger, Breast cancer, Cancer cell lines, MTT assay.

Introduction

Cancer is a broad term used for a class of diseases characterized by uncontrolled growth of abnormal cells. It is the most crucial health problem of the current era [1]. Breast cancer is the leading cause of cancer related mortality in women. Breast cancer is highly metastatic and can spread to various parts of the body [2]. Different treatments for breast cancer have been proposed and they include chemotherapy, surgery, radiation therapy , hormonal therapy and anti HER2 therapy [3]. Unfortunately most of these treatments are associated with multi-drug resistance [4], toxicities [5] and side effects such as hair loss and vomiting [6]. In addition, the currently and commonly used cancer therapies such as targeted therapy and immunotherapy are not affordable in low and middle-income country due to its high cost and thus, there is need to search for novel cancer therapeutics such as plant extracts or plant derived compounds since they are natural, relatively non-toxic, easily assessable and affordable [7]. Administration of antioxidant rich compounds could be a promising chemopreventive and chemotherapeutic approach in the management of breast cancer [8,9]. Crude natural plant extracts or their active constituents

therein may be able to exert significant role in treating cancers in combination with conventional chemotherapeutic drugs. Thereby improving their efficacy or reducing their toxicity [10]. Among natural products, ginger (*Zingiber officinale*), a member of Zingiberaceae family, has attracted the interest of medical scientists. Ginger is cultivated as a spice and for medicinal purposes [11]. The rhizome of this plant has been used as a medicine in Asian, Indian, and Arabic herbal traditions since ancient times [12]. It has been prescribed for the treatment of various conditions, including arthritis, rheumatological conditions, muscular discomfort atherosclerosis, migraine headaches, rheumatoid arthritis, high cholesterol, ulcers, depression, and impotence. In addition, a significant number of *invitro* and *invivo* studies provide substantial evidence that ginger and its organic pungent vallinoid compounds are effective inhibitors of the carcinogenic process [13,14].

Methods

Ginger Rhizomes extraction

Identified *Zingiber officinale* Roscoe family *Zingiberaceae* dried rhizomes were purchased from Wad Medani local market, Sudan. The plant material was cleaned, air dried and was milled into a coarse powder. 100 g of powdered plant was macerated in pure ethanol(1:10) at room temperature for seven days with occasional shaking. The liquid extract obtained was filtered and dried at 60°C under vac. using rotary evaporator. The resulting oily mass was stored in refrigerator until use.

Cell culture

The human breast cancer cell lines MCF-7 and MDA-MB-231 and non-tumorigenic, normal breast epithelial MCF-10A cell line were obtained from American Type Culture Collection (ATCC) and used to evaluate the cytotoxic activity of ginger extract. Cells were seeded in low glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 2 mM glutamine, 0.01 mg/ml insulin and 1% penicillin/ streptomycin mix and incubated at 37°C in an atmosphere of 5% CO_2. [15]

MTT assay and IC_{50} determination

The cytotoxicity of ginger extract on the cells was measured by the MTT method. Cells were seeded at densities of 5×103cells/well in 96-well tissue culture plates. On day two, cells were treated with or without various concentrations of ginger extract. After 48 hours incubation, 20 µl of MTT (10 mg/ml stock solution of saline) was added to each well for 4 h. Subsequently, the supernatant was removed, and the insoluble formazan crystals were solubilized with 150 µl anhydrous DMSO in each well. Thereafter, cell viability was measured by Model 550 microplate reader at 540 nm. The 50% inhibitory concentration (IC_{50}) was determined as the concentration causing 50% reduction in cell viability; it was calculated from the cytotoxicity curves using Originpro software (Origin Lab Corporation, Northampton, USA).

The inhibitory rates were calculated using the following formula:

Inhibition rate (%) = (mean control absorbance - mean experimental absorbance) / (mean control absorbance) x 100. Inhibition rates % were plotted for various concentrations and time treatments [15].

Selectivity Index

The degree of selectivity of compound/extract can be expressed by its selectivity Index(SI). High SI value (≥2) gives the selective toxicity against cancer cells, (SI= IC_{50} normal cell/ IC_{50} cancer cell). While a compound/extract with SI value less than 2 are considered to produce general toxicity which causes cytotoxicity in normal cells [10].

Statistical analysis

This was done by using SPSS version 20.0 (SPSS Inc. Chicago, IL.USA).All values were expressed as mean± SD. Data were analyzed by one- way ANOVA and difference between means was

assessed by a two-tailed Student's T-test. P≤ 0.05 was considered statistical significant.

Results:

Ginger extract concentrations in a range of 0.175 mg/L to 30 mg/L were added to the tested cell lines for 48 h incubation, in order to measure IC50 of these drugs in different tested cells. MTT assay results on cytotoxicity of ginger extract showed various cytotoxic effects on breast cancer cell lines. The calculated IC_{50} values were 9.68 mg/l, 2.47 mg/l and 12.81 mg/l against MCF-7, MDA-MB-231 breast cancer cell lines and non-tumorigenic, normal breast epithelial MCF-10A cell line respectively in dose dependent studies(Fig.1-3). Ginger extract showed selectivity on MDA-MB231 $(ER)^-$; $(PR)^-$ cells whereas, SI= 5.1 while it possessed cytotoxicity on the MCF-7 $(ER)^+$; $(PR)^+$ cells with SI=1.3.

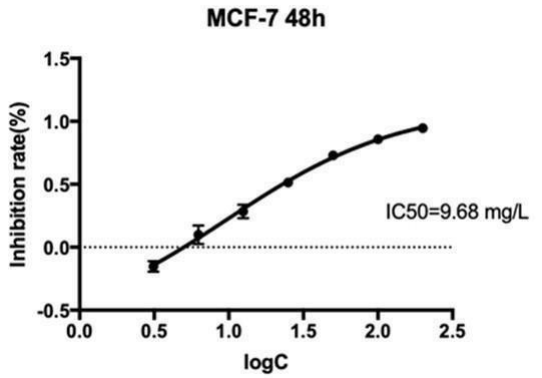

Figure 1. Effect of ginger extract on cell viability of breast cancer cells MCF7

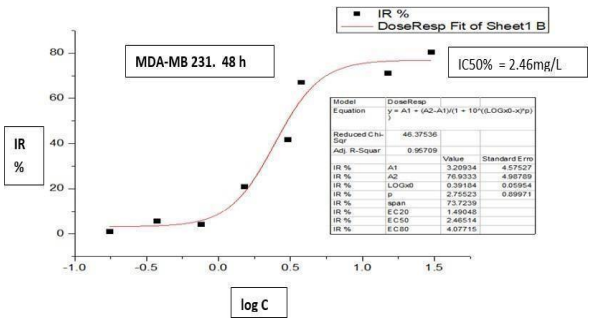

Figure 2. Effect of ginger extract on cell viability of breast cancer cells MDA-MB231

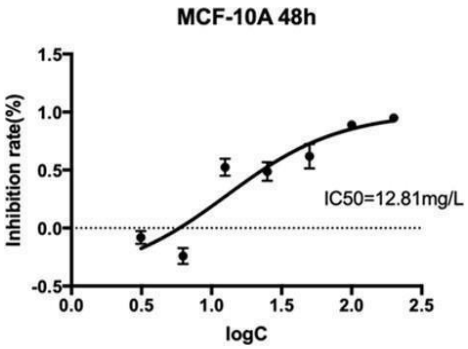

Figure 3. Effect of ginger extract on cell viability of normal breast cells MCF-A10

Discussion

A large number of novel anticancer drugs have been discovered from natural products[10] . Two well known human breast cancer cell lines MCF-7, $(ER)^+$; $(PR)^+$ and MDA-MB231, $(ER)^-$; $(PR)^-$ and non-tumorigenic normal mammary epithelial cell line MCF-10A, were used as a model to evaluate the cytotoxicity of ginger extract. The IC_{50}values represent the overall activity of ginger extract and were used to calculate the SI of the extract.

The IC_{50} of ginger extract values for 48 h of treatment were 9.68 mg/l (SI= 1.3) against MCF-7 and 2.47mg/l (SI=5.1) against MDA-MB 231.These results indicated that ginger extract possesses cytotoxic effects on breast cancer cells. However, it was reported earlier that ginger components proven to exhibit antioxidant and cytotoxic properties [9,16-19]. Moreover ginger has been listed in "generally recognized as safe" (GRAS) document of The United State Food and Drug Administration (FDA) [20].

The ginger extracts should be searched in detail for its anticancer activity against breast cancer cell lines and other cultured cancer cells and to find the compounds responsible for the anticancer activity by various phytochemical studies.

Conclusion and Recommendation

It could be concluded and recommended that ginger exhibits anticancer activity on breast cancer cell lines, hence ginger can be further

evaluated for potential promising anticancer activity.

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