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CYTOTOXIC EFFECT OF GINGER ROOT (*Zingiber officinale*) ON LIVER AND BREAST CANCER

Nashat E.M. El-Sayeh*, S.S. Elsaadany, R.A. Elmassry and H.T.M. Hefnawy

Agric. Biochem. Dept., Fac. Agric., Zagazig Univ., Egypt

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ABSTRACT: Cancer is a big health problem with high morbidity and mortality and possess both economic and phsychological challenges. The aim of the present study is to evaluate methanolic (80%) ginger extract for their anticancer activity using two cancer cell lines: hepatocellular carcinoma cell line (HePG2) and breast carcinoma cell line (MCF7). The results showed that ginger methanolic (extract 80%) exhibited a pronounced cytotoxic effect and was found to possess a very potent inhibitory activities against hepatocellular carcinoma cell line (HePG2) and breast carcinoma cell line (MCF7).

Key words: Ginger root (*Zingiber officinale*), antioxidant and antitumor methanolic extract.

INTRODUCTION

Ginger has been used as a spice and as natural additives for more than 2000 years **(Bartley and Jacobs, 2000)**. Also, ginger has many medicinal properties. Studies have shown that, the long term dietary intake of ginger has hypoglycaemic and hypolipidaemic effect **(Ahmed and Sharma, 1997)**. Ginger has been identified as a herbal medicinal product with pharmacological effect. Ginger suppresses prostaglandin synthesis through inhibition of cyclooxygenase- 1 and cyclooxygenase- 2. In traditional Chinese and Indian medicine, ginger has been used to treat a wide range of ailments including stomach aches, diarrhea, nausea, asthma and respiratory disorders **(Grzanna *et al.,* 2005).** As ginger is widely used both as a spice and for its medicinal properties.

Ginger (*Zingiber officinale* Roscoe) has a long history of being used as a medicine and herbal since ancient time and had been used as an important cooking spice throughout the world. Phytochemical investigation of several types of ginger rhizomes has indicated the presence of bioactive compounds, such as gingerols, which are antibacterial agents and shogaols, phenylbutenoids, diarylheptanoids, flavanoids, diterpenoids and sesquiterpenoids **(Sivasothy *et al.,* 2011)**. Furthermore, there are many studies that proved their beneficial effects against the symptoms of diseases, acting as an anti-inflammatory, anti-tumour, anodyne, neuronal cell protective, anti-fungal and anti-bacterial agent **(Mesomo *et al.,* 2012).**

Cancer is a big health problem with high morbidity and mortality and possess both economic and psycological challenges **(Dossus and Kaaks, 2008)**. Cancers result from cells growing in uncontrolled and abnormal fashions, and the resulting tumors are classified as either benign or malignant. While benign tumors do not invade the surrounding tissue, malignant tumors aggressively invade surrounding tissues, altering the surrounding tissue's natural function. When malignant tumor cells spread to the lymph and circulatory systems, the metastatic cascade begins, spreading cancer cells throughout the body. Control of cancer may be accomplished by a variety of treatments including: suppressing, blocking, and transforming agents. The use of suppression agents prevent the formation of new cancers from procarcinogensis, while blocking agents prevent carcinogenic compounds from

* **Corresponding author: Tel. :** +201019044503
E-mail address: mhnashate@gmail.com

reaching critical initiation sites but transformation agents act to facilitate the metabolism of carcinogenic components into less toxic materials or to prevent the biological actions of the carcinogen. Other methods for controlling cancer involve blocking metastatic cascades through inhibiting cancer cell invasion into surrounding tissues or by inhibiting cancer cell mobility in circulatory systems **(Wattenberg, 1992)**

It is known that different cell lines might exhibit different sensitivities towards an antiproliferative compound, so the use of more than one cell line is therefor-reconsidered necessary in the detection of anti proliferative compounds. Breast cancer starts when cells in the breast begin to grow out of control. The cells usually form a tumor that can often be seen on an x-ray or felt as alump. The tumor is malignant (cancerous) if the cells can grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body. Breast cancer occurs almost entirely in women, but men can get it, too. Cells in nearly any part of the body can become cancer, and can spread to other areas of the body. Breast cancers can start from different parts of the breast. Most breast cancers begin in the ducts that carry milk to the nipple (ductal cancers). Some start in the glands that make breast milk (lobular cancers). In addition, other types of breast cancer are less common. A small number of cancers start in other tissues in the breast. These cancers are called sarcomas and lymphomas and are not really thought of as breast cancers. Although many types of breast cancer can cause a lump in the breast, not all do. There are other symptoms of breast cancer you should watchout for and report to a health care provider. It is also important to understand that most breast lumps are not cancer, they are benign. Benign breast tumors are abnormal growths, but they do not spread outside of the breast and they are not life threatening but some benign breast lumps can increase a woman's getting breast cancer. Any breast lump or change needs to be checked by a health care provider to determine whether it is benign or cancer and whether it might affect your future cancer risk (**Weber, 2008)** liver cancer is that begins in the liver. About 80% of primary liver cancer is hepatocellular carcinoma (HCC). Other subtypes of primary liver cancer include bile duct cancer and angiosarcoma, a cancer of the blood vessels in the liver (**Morimitsu *et al.*, 2000**). The aim of the present study is to evaluate the use of methanolic (80%) ginger extracts for their anticancer activity using two cancer cell lines: hepatocellular carcinoma cell line (HePG2) and breast carcinoma cells lines (MCF7).

MATERIALS AND METHODS

Plant Material

Fresh ginger roots (*Zingiber officinale*) were purchased from local market, washed with distilled water and dried in oven at 40°C, then ground and stored in airtight container under refrigeration.

Chemical reagents and solvents

The chemicals used for the study were purchased from Sigma Company, USA and Gomhoriya Co. They were all of analytical grade. Double distilled water, methanol, were used for extraction.

Extraction of the sample

One hundred gram of sample was weighed accurately and suspended in 100 ml of solvent. It was shaken for 3 hr., in an electric shaker at room temperature, centrifuged at 4000 rpm for 20 min and filtered with Whatman No.1 filter paper. For all experiments, fresh extracts were used.

Determination of Antioxidant Activity of Methanolic Extract

Sample preparation

250 mg of sample were mixed with 25 ml of solvent and extracted for 3 hr., centrifuged at 4000 rpm for 20 min and passed through filter paper (Whatman No.1) to get clear extract.

Method

The electron donation ability of the obtained extract was measured by bleaching of the purple colored solution of DPPH according to the method of **Hanato *et al.* (1988)**, with some modifications. Five hundred ml of each extract were added to 3 ml of 0.1 mM DPPH dissolved in methanol. After incubation period of zero, 30, 60 and 120 min at room temperature, the

absorbance was determined against a control at 515 nm (**Gulcin *et al.,* 2004**). Percentage of antioxidant activity of extract calculated as follow:

Antioxidant activity (Inhibition) (%) = $[(A_{control} - A_{sample})/A_{control}]\times100$

Where:

$A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of plant extract. 0.5 ml of TBHQ (200 µg/ml) was used as a positive control.

Cytotoxic effect By SRB assay

Potential cytotoxicity of the methanolic (80%) extract of ginger was tested for breast cancer carcinoma cell line (MCF7) and hepatocellular carcinoma cell line (HePG2) using the method of **Skehan and Storeng (1990)** as follows: Cells (MCF7) and (HePG2) were plated in 96-multiwell plate (10^4 cells/well) for 24 hours before the treatment with the extract to allow the attachment of cells to the wall of the plate.

- Different volumes of the tested extract were added to the cells monolayer, 6 replicates wells were prepared for dose.
- Monolayer cells were incubated with the extract for 48 hr., at 37°C and in atmosphere of 5% CO_2
- After 48 hr., cells were fixed, washed and stained with sulfo-Rhodamine-B stain. Excess stain was washed with acetic acid and the attached stain was recovered with Tris EDTA buffer.
- Color intensity was measured in an ELISA reader.
- The relation between surviving fraction and extract volume after the specified compound.
- IC_{50} of this extract against both cells lines (liver and breast) were calculated using these survival curves.

RESULTS AND DISCUSSION

Antioxidant Activity

The results of radical scavenging capacity of methanolic (80%) ginger extract were showed in Table 1. The inhibition percentages were 25, 23, 21 and 74% at zero, 30, 60 and 120 min., respectively.

Ait M'barek *et al.* (2007) reported that the methanolic 80% ginger extract which contain oxygenated monoterpenes and/or sesquiterpenes have greater antioxidative properties. Hence, many aromatic plants are today considered as the most important sources for the extraction of compounds with strong antioxidant activity. Ginger is too spices widely used in folk medicine, cosmetics and the flavoring of food products (**Tepe *et al.,* 2004**). Antioxidant activity exhibited by methanolic (80%) ginger extract justifies traditional uses of ginger roots.

Cytotoxic effect of ginger

The results showed that methanolic (80%) ginger extract exhibited a pronounced cytotoxic effect and was found to possess a very potent inhibitory activities against hepatocellular carcinoma cell line (HePG2) and breast carcinoma cells lines (MCF7), IC_{50} of this extract against (HePG2) and (MCF7) cell line decreases reactive oxygen species.

Gingerol and Paradol which are most important component in ginger extract, induces endogenous antioxidant until its activity. It is known for its function as an exogen antioxidant to prevent cell damage and inhibits cancer cell growth by binding with free radical agents. The Gingerol and Paradol has a role to inhibit Cell Lines (HepG2) growth through oxidation-reduction reaction by trapping free radical agents that eventually decreases reactive oxygen species. An antioxidant is a molecule that can slow or prevent oxidation reactions with other chemicals. The action mechanism of Gingerol and Paradol as chemo-preventive is to inhibit free radical called antioxidant. Oxidation is a chemical reaction redox move electrons from a substance to an oxidizing agent. The oxidation reaction, can cause the onset of free radicals, may give rise to a dangerous chain reaction. Antioxidants may terminate these chain reactions by removing radical substance, and inhibit other oxidation reactions by oxidizing the substances themselves. Therefore, most of the antioxidant substances called reducing agents such as thiols or phenols. Antioxidants can be produced in the body or obtained from the diet (**Badreldin *et al.,* 2008**).

Several mechanisms have been postulated for the tumor growth-inhibitory effects of flavonoids, including, but not limited to, the inhibition of NF-kB signaling pathway (**Sarkar *et al.*, 2009**).

Table 1. DPPH free radical scavenging activity of methanolic (80%) ginger extract

Time (min)	0	30	60	120
DPPH	25%	23%	21%	74%
TBHQ	39%	82%	82%	84%

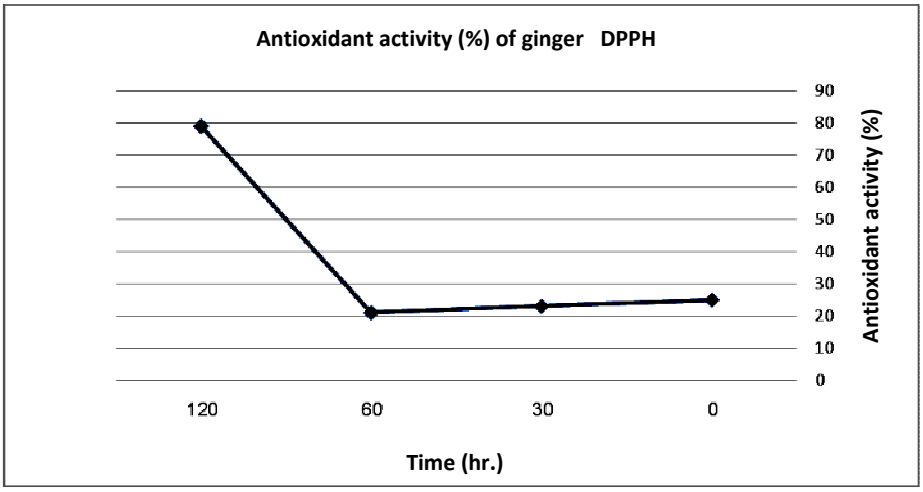

Fig. 1. DPPH free radical scavenging activity of methanolic 80% ginger extract

Table 2. Cytotoxicity effect of ginger methanolic extract on hepatocellular carcinoma cell line

Conc. : mg/ml	HepG-2 (MA)
100	75
250	42.5
500	12.3
800	0

Table 3. Cytotoxicity effect of ginger methanolic extract on breast carcinoma cells line

Conc. : mg/ml	MCF-7 (MA)
800	100
500	75.8
250	39
100	5.8

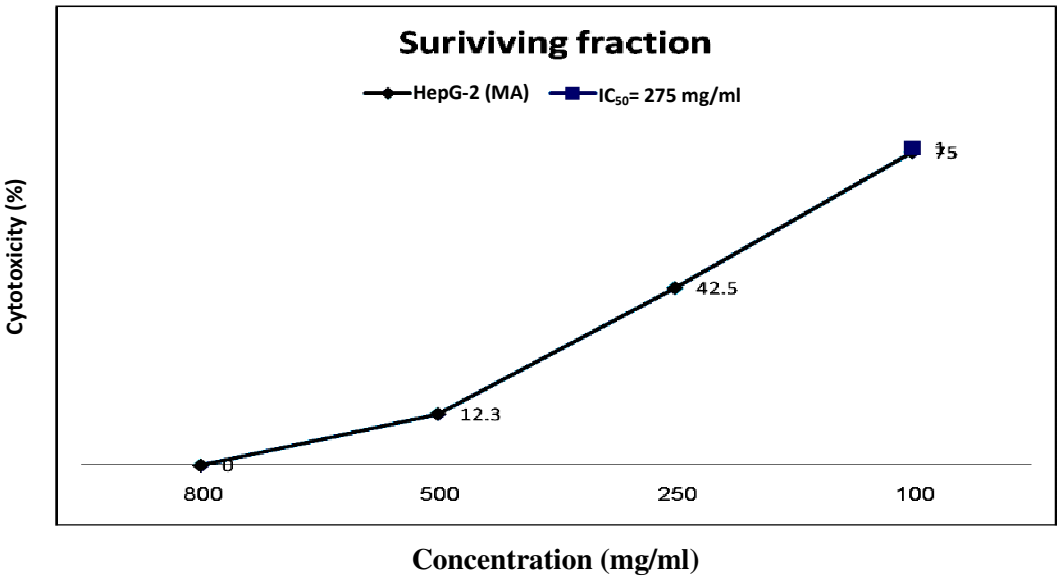

Fig. 2. Cytotoxicity effect of ginger methanolic extract on hepatocellular carcinoma cell line

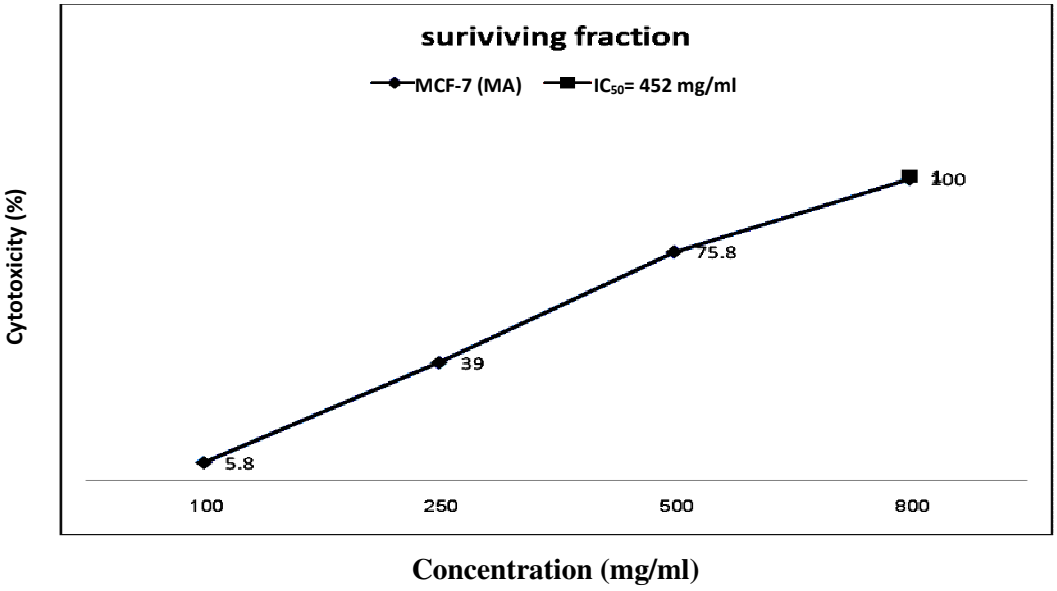

Fig. 3. Cytotoxicity effect of ginger methanolic extract on breast carcinoma cells line

NF-kB plays an essential role during inflammation, immune responses as well as in other physiological functions such as cell growth, apoptosis **(Park *et al*., 2013).** Recent studies have shown that inactivation of the NF-kB in the hepatic compartment inhibits liver tumor formation through induction of cell death inhibition of compensatory proliferation. Furthermore, mounting evidence has illustrated a major role of NF-kB in inducible chemo-resistance of HCCs **(Wang *et al*., 2007)**.

Flavonoid induction of liver enzymes may also ultimately affect the metabolism of endogenous substrates, *e.g.* steroid hormones **(Dai *et al*., 1997)** and thus indirectly influence a great number of biological processes in humans. Typically, inducers of liver enzymes can be divided into 2 classes: 1) bifunctional indications that induce phase I enzymes, *e.g.* cytochrome P450 isozymes, involved in the synthesis of metabolites responsible for the activation of genes encoding phase II enzymes, and 2) monofunctional indications that induce phase II enzymes directly without influencing the levels of phase I enzymes **(Yannai *et al.*, 1998).**

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التأثيـــر الســـمي للمستخلـص الميثـانـولـي لجذور الزنجبيل على الخلايا الســرطانيـة للثـــدي والكبـــد

نشأت السيد مصيلحى السايح- سيد سليمان السعدنى- رجب عبد الفتاح المصري- حفناوي طه منصور حفناوي

قسم الكيمياء الحيوية – كلية الزراعة – جامعة الزقازيق – مصر

الجميع يعلم بمدى خطورة الإصابة بمرض السرطان على حياة الإنسان ولذا تتضافر جميع الجهود فى البحث عن مصادر طبيعية للعلاج لما تمثله من قلة الأثار الجانبية، ولذا كان الغرض من البحث هو دراسة النشاط المضاد للأكسدة للمستخلص الميثانولى ٨٠% لجذور الزنجبيل عن طريق الـ DPPH (2,2-diphenyl-1-picrylhydrazyl) (إحدى طرق قياس مضادات الأكسدة) وتأثير هذا المستخلص على نوعين من الخلايا السرطانية، سرطان الكبد hepatocellular carcinoma cell line (HePG-2 carcinoma)، سرطان الثدى Breast carcinoma cell lines (MCF-7)، وأظهرت النتائج أن المستخلص الميثانولى ٨٠% لجذور الزنجبيل له نشاط مضاد للأكسدة عالي مما ساعد على تثبيط الخلايا السرطانية تحت الدراسة.

المحكمـــون:

١- أ.د. إمام عبدالمبدئ عبدالرحيم أستاذ الكيمياء الحيوية المتفرغ – كلية الزراعة – جامعة القاهرة.

٢- أ.د. صـــلاح الدين محمد لبيب أستاذ الكيمياء الحيوية المتفرغ – كلية الزراعة – جامعة الزقازيق.