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

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ABSTRACT: Cancer is a big health problem with high morbidity and mortality and possess both economic and psychological 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 antibacterial agent (Mesomo et al., 2012).

Cancer is a big health problem with high morbidity and mortality and possess both economic and psychological 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...
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 (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 (Gülçin et al., 2004). Percentage of
antioxidant activity of extract calculated as follow:

\[
\text{Antioxidant activity (Inhibition) (\%) = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100}
\]

Where:

- \(A_{\text{control}}\) is the absorbance of the control
  reaction and \(A_{\text{sample}}\) 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
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% CO₂.
- 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.
- IC₅₀ 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), IC₅₀ 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

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH</td>
<td>25%</td>
<td>23%</td>
<td>21%</td>
<td>74%</td>
</tr>
<tr>
<td>TBHQ</td>
<td>39%</td>
<td>82%</td>
<td>82%</td>
<td>84%</td>
</tr>
</tbody>
</table>

![Graph showing DPPH free radical scavenging activity of methanolic 80% ginger extract](image)

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

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>HepG-2 (MA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>250</td>
<td>42.5</td>
</tr>
<tr>
<td>500</td>
<td>12.3</td>
</tr>
<tr>
<td>800</td>
<td>0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>MCF-7 (MA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td>100</td>
</tr>
<tr>
<td>500</td>
<td>75.8</td>
</tr>
<tr>
<td>250</td>
<td>39</td>
</tr>
<tr>
<td>100</td>
<td>5.8</td>
</tr>
</tbody>
</table>
NF-κB 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-κB 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-κB in inducible chemoresistance 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).
REFERENCES


التأثير السمي للمستخلص الميثانولي لجذور الزنجبيل على الخلايا السرطانية للنّكسي والكبد

نشأت السيد مصطفى السايح، سيد عادل السعدي، رجب عبد الفتاح المصري، حفناوي محمد منصور جفناوي

قسم الكيمياء الحيوية – كلية الزراعة – جامعة الزقاقية – مصر

الجميع يعلم بمدى خطورة الإصابة بمرض السرطان على حياة الإنسان ولذا تتضاعف جهود البحث عن
مصادر طبيعية للعلاج لما تمثله من قلة الآثار الجانبية، ولذا كان الغرض من البحث هو دراسة الشتمول للمستخلص الميثانولي لجذور الزنجبيل عن طريق الـ(2,2-diphenyl-1-picrylhydrazyl) DPPH
للنكسي والكبد، سرطان الثدي (MCF-7 carcinoma cell line) وسرطان الكبد (HePG-2 carcinoma)

أظهرت النتائج أن المستخلص الميثانولي لجذور الزنجبيل له نشاط مضاد للأكسدة عالي مما ساعد على تثبيط
الخلايا السرطانية تحت الدراسة.

المؤخرون:

- د. إمام عبدهم د. عبد الرحيم
- د. صلاح الدين محمد لبيب