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Apoptotic effect of *Capparis Spinosa* extract on breast cancer cell line (MCF-7)

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ABSTRACT

In recent years, the incidence of breast cancer and the associated mortality have increased throughout the world. In general, chemotherapy, surgery, and radiotherapy are the common methods of cancer treatment. It should be mentioned that chemical drugs, other than cancer cells, also effect on healthy cells, their beneficial factors (i.e., enzymes and substrates related to DNA replication. For this reason, researchers have so far attempted to identify new natural/synthetic compounds with anti-cancer characteristics. In the present survey, the effect of the extract of Capparis spinosa on the breast cancer cell line MCF-7 was evaluated. In order to select an appropriate dose and determine the cytotoxicity of theC. spinosa compound, the MTT test was employed. On the other hand, for the evaluation of the effect of this compound on the protein expression and the mRNA of Bcl-2 and Bax, western blotting and real-time PCR were applied, respectively. The evaluation of C. spinosa cytotoxicity showed that this compound has IC50, equivalent to 70 milligram/milliliter. In addition, it was stated that while the expression rate of protein and the mRNA of the Bax gene was significantly increased, the expression rate of protein and the mRNA of Bcl2 gene was not altered. In general, the results obtained from this survey demonstrated the effectiveness of this herbal complement against breast cancer. With regard to the naturalness of this product as well as the low cost and the easy availability, using this compound in the dietary regimencan assist in the treatment of breast cancer.

Keywords: Breast cancer, Apoptosis, *Capparis spinosa*, Bcl-2, Bax.

INTRODUCTION

Breast cancer (BC), the most frequent cancer in females, is a critical public health issue, with the estimated new cases of 1,500,000 all around the world and the associated deaths of approximately 500,000 [1]. The typical treatments for BC are surgery and chemotherapy. In this context, it should be indicated that the chemotherapeutic agents impact on both normal and tumor cells and induce some negative side-effects (e.g., digestive problems, nausea, leukopenia, hair loss, and development of multidrug resistance)[2, 3].

Today, the attraction for natural products is growing and in the light of long-term and safe cancer prevention, present strategies have mainly accentuated the utilization of food and edible medicinal herbs, as the products that may be able to efficiently manage cancers [4-6]. This would be evident by the fact that approximately 74% of new anticancer compounds are either natural products or natural product-derived compounds[7-9]. Therefore, very much focus have been given to the natural substances with minimal or no toxicity in individuals and with the ability to reduce proliferation and stimulate apoptosis in cancer cells [10].

Apoptosis is a very organized and tightly synchronized biological process that performs a critical function in checking the number of non-pathological cellular conditions[11]. Apoptosis is generally controlled by an intrinsic process related to the activation of genes like Bcl-2, which is an anti-apoptotic protein member of the Bcl-2 gene family that participates in programmed cell death [12, 13]. Reduced apoptosis is important in cancer development and a significant barrier to successful therapy [14]. Apoptosis is, actually, the desired mode of cell death in the therapy and management of cancers, due to the programmed nature and fewer sequelae. Recently, apoptosis has received close attention as a potential mechanism that might be focused via the activation of oxidative stress in cancer cells [15, 16].

Capparis spinosa L. of the Hill masaikai genus in the Capparaceae is also known as capers. It has been reported that the fructification of *C. spinosa*is rich in amino acids, trace elements, polysaccharides, and proteins, which are of high nutritional and healthy value. As an ethnic medicine, *C. spinosa* L. has been widely used for rheumatoid arthritis, scapulohumeral periarthritis, dermatitis, and other diseases (such as malaria and carbuncle)[17, 18]. In recent years, many new natural products/compounds have been discovered with the further research of the domestic chemical market, which have gained great interest of the majority of medical researchers. According to a number of reports, *C. spinosa* has many pharmacological effects such as antibacterial, anti-inflammatory, anti-liver-toxic, anti-oxidation, lowering blood glucose and lipids, and so on [19-21]. The purpose of the current investigation was to determine the effect of the *C. spinosa* extract on apoptosis, Bax, and Bcl-2 gene expression in the breast cancer cell line (MCF-7).

MARTIAL AND METHOD

Cell lines

The MCF-7 cell line was purchased from Pasteur institute, Tehran, Iran. The cells were cultured in RPMI-1640 medium (PAA, Austria) supplemented with 10% fetal bovine serum and 1% antibiotics (penicillin/ streptomycin) in a humidified atmosphere containing 5%CO2 and 95% air, at 37°C.

Plant extract

Young whole plants were collected from Ahvaz Province, Iran in May 2015. The plant was botanically identified and authenticated by local Plant Biotechnologist, Department of Natural Resources, khuzestan, Iran. The plants were dried at ambient temperature (30–40°c) for 25–30 days then plants were into fine powder. The dried and powdered plants (100 g) were extracted with ethanol 90% v/v through soxhlet apparatus. The crude extracts were filtered and concentrated under reduced pressure at approximately 40°C.

MTT assay

The viability of the cells was assessed by MTT (3,4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide) assay, which is based on the reduction of MTT by the mitochondrialdehydrogenase of intact cells to a purple formazan product Yellow MTT is reduced to purple formazan in the mitochondria of living cells. This reduction takes place only when mitochondrialreductase enzymes are active and, therefore, the conversion can be directly related to the number of viable (living) cells.[22]

Cells were seeded into 96-well culture plates at densities of 3×10^5 cells per well. After 24 hours, they were treated with the concentrations (12.5, 25, 50, 100 and 200 mg/ml) of *Capparisspinosa* L for 24, 48 and 72 hours. After the treatment, 10 ml of MTT solution (5 mg/mL) was added to each well of 96-well plates and incubated for 3 hours at 37.6C. After incubation, the purple MTT-formazan crystals were dissolved by adding 100 ml of 0.04N HCl in isopropanol. The absorbance of the samples was measured with an ELISA reader (OD570nm). MTT reduction is used to estimate cell proliferation at the end of the assay.

Real time polymerase chain reaction

The expression level of *bax and bcl2*, a widely established apoptotic-related gene, was analyzed using real time PCR assay. MCF-7 cells were treated with IC_{50} concentration of extract (70 mg/mL) during 6 and 12 h periods.

Total cellular RNA was extracted from the treated and untreated cells using the TriPure Isolation Reagent (Roche, USA) based on the manufacturer's instructions. RNA quantification was performed by spectrophotometer (UNICO 2100, USA) using routine procedures. The extracted RNA was immediately used in RT-PCR to generate first-strand cDNA (cDNA Synthesis Kit, Thermo Scientific, USA).

Real time PCR was carried out to quantify the amount of mRNA in the treated and untreated cancer cells. A PCR reaction mixture of 50 µL containing 5 µL of ddH_2O, 25 µL of Taq Man, Universal Master Mix, 5 µL of forward primer, 5 µL of reverse primer, 5 µL of labeled probe (FAM/MGB and or JOE/TAMRA), 1 µL of reverse transcriptase, 2 µL of random hexamer and 2 µL of purified RNA were used. Two pairs of primers were separately applied: one pair to amplify *bcl2 and bax* genes, the other for the endogenous control gene, *β-actin*. Primers and probes were selected according to previous study [23] and purchased from metabion incorporation (Table 1)

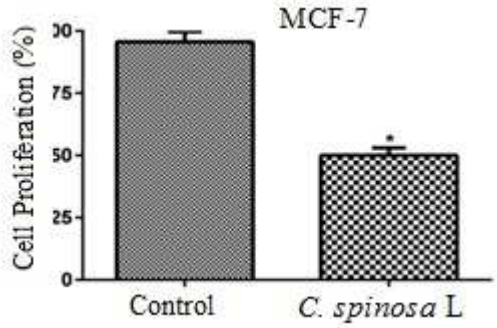
Gene	Forward primer (5'–3')	Reverse primer (5'–3')	Size (bp)
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC	226
Bax	CGAGAGGTCTTTTTCCGAGTG	GTGGGCGTCCCAAAGTAGG	242
Bcl-2	CGGTGGGGTCATGTGTGTG	CGGTTCAGGTACTCAGTCATCC	90

Western blotting analysis

MCF-7 cells were treated with 45.7 mg/mL of *Capparisspinosa* L extract for 48 h. The cells were harvested and rinsed with ice-cold PBS. The cell pellet was resuspended in a lysis buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% triton-X100, 1 mM EDTA, 0.2% SDS, 1% protease inhibitor cocktail, 1% phosphatase inhibitor cocktail and 1 mM phenylmethylsulfonyl fluoride and left on ice for 30 min. After centrifugation at 13000 rpm for 20 min at 4 °C, the cell lysate was collected and protein concentration was determined according to the Bradford method [24].

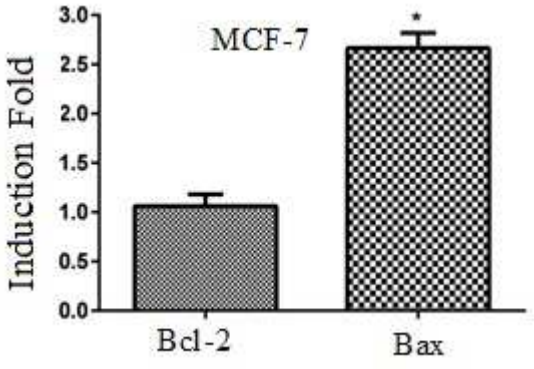
RESULTS

Resulted IC50 for Cpparis Spinosa extract that inhibits the growth of MCF-7 comparing with control group (not treatment) was equivalent to 70 mg/mL (figure 1).



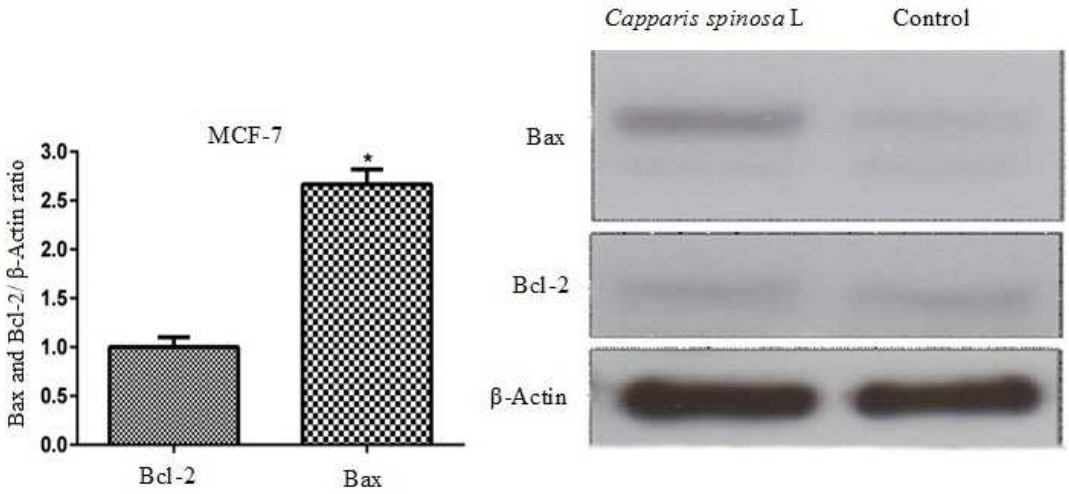
Real time PCR

The cells exposed to 0.5% methanol demonstrated significant differences ($P< 0.05$) in fold changes when compared to the untreated controls for *BAX* ($P< 0.05$) but not for *BCL-2* ($P< 0.05$). In the 0.5% methanol exposed cells, *BAX* was significantly Up-regulated (fig 2).



Western blot

Bcl-2 and Bax protein levels in untreated and treated MCF-7 cells were studied using western blot technique to examine the involvement of Bcl-2 family proteins in *C. spinosa* L mediated apoptosis. As showed in the Figure 3, *Capparisspinosa* L dramatically increased the expression of Bax (pro-apoptotic) protein in a time dependent manner while there is no change in the expression of Bcl-2 (anti-apoptotic) protein. This result agrees with the Real time PCR results



DISCUSSION

Breast cancer is the most common malignancy among women throughout the world, responsible for 33% of total female cancers and 20% of the mortalities caused by cancer [25]. The incidence of this malignancy in most countries (including Iran) is rapidly increasing. Among the related reasons for the increasing gradient of cancer incidence, the environmental factors, such as air pollution, stress, life style, and dietary regimen can be mentioned [26, 27]. On the other hand, despite using different therapeutic approaches (such as surgery, chemotherapy, and radiotherapy), the mortality rate of cancer is still high. Furthermore, the damaging effect of chemotherapy and radiotherapy on normal cleaving cells is also among the other disadvantages, related to this therapeutic process [28]. Hence, according to the aforementioned notes, the tendency and attention to utilizing natural products and nutritional supplements with anti-cancer characteristics have increased during past years.

Apoptosis is a highly regulated process, with an important role in maintaining the homeostasis of multicellular organisms [29]. It should be denoted that apoptosis is controlled via many extra and intracellular factors.Amongthe intracellular factors, the balance between Bcl-2 (apoptosis inhibitor) and Bax (proapoptotic protein) is the most important determinant of cell survivalin response to the extracellular stimulator. Bax acts as a key protein in apoptosis, induced by different factors in the intrinsic apoptosis pathway. This protein, while interacting with the mitochondrial membrane proteins, increases mitochondrial permeabilitytransition, releases cytochrome C from mitochondria, and activates caspases and apoptosis. On the other hand, Bcl-2 has an anti-apoptotic influence against different apoptosis stimulators via preventing the release of cytochrome C from mitochondria [30]. For this reason,in order to evaluate the effect of the *C. spinosa* extract on inducing apoptosis in the MCF-7 cell line, YYY was calculated. The results showed that the *C. spinosa* extract significantly increases the expression of the mRNA gene of Bax, whereas has no effect on the expression rate of Bcl-2. Furthermore, the expression rate of Bax protein reflected a significant increase, according to the result of the mRNA expression, while no alteration in the amount of the Bcl-2 protein was observed.

Capparis spinosa, because of possessing a variety of chemical compounds (including β-sitosterylglucoside-60-octadecanoate, 3-methyl-2-butenyl-glucoside, isorhamnitine-3-O-rutinoside, 1-tetradecanol, p-hydroxybenzaldehyde, 6,10,14-trimethyl-2-pentadecanone, ursolic acid, glycerol monotetracostanoate, 4-coumaric acid, nicotinamide, methyl hexadecanoate, β-sitosterol, n-butanol, β-sitosterylglucoside, cadabicine, octadecanoic acid, rutin, and stachydrine), has various pharmacological characteristics and is widely utilized in the treatment of different diseases [17, 31].

Various studies have assessed the effect of the *C. spinosa* extract and its chemical compounds on different cancer cell lines. Al-Asady et al. (2012) stated that the extract of *Capparis spinosa* on Hep-2 and HeLa tumor cell lines decreases the growth and proliferation of these cell lines [32].

Yu et al. (2011) showed for the first time that n-butanol (one of the compounds of *Capparis spinosa*), decreases the growth of the cancer cell lines of SGC-7901 and induces apoptosis in them [33]. Some studies presented that n-butanol induces apoptosis in different cancer cell lines through activating cytochrome C and caspase cascade[34]. Recently, Ji and Yu (2015) portrayed in their study, conducted by PCR and western blotting, that n-butanol induces apoptosis by decreasing and inhibiting the expression rate of Bcl-2 and increasing the expression of the Bax gene [34]. It is worth signifying that these findings are consistent with the results of the present study.

CONCLUSION

The results of this study exhibited that the *Capparis spinosa* extract and the other various compounds possess apoptotic properties and are greatly beneficial in inhibiting the growth of cancer cells. In this regard, the naturalness, low cost, and easy availability of this compound are among the significant advantages. However, for a detailed assessment of this compound, a future study of the constitutive parts of this compound using in-vivo studies can be highly helpful in evaluating the effectiveness of this compound.

AUTHORS' CONTRIBUTIONS

All authors had equal role in design, work, statistical analysis and manuscript writing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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