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Inhibitory effects of cinnamon-water extract on human tumor cell lines

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ABSTRACT

Objective: To question the inhibitory effect of cinnamon-water extract (CWE) on four human tumor cell lines (AGs, HeLa, MCF-7 and MDA-MB234). Naturally, compounds are an important source for clinical proposes. Cinnamon, a plant-derived spice, is widely used as a food additive and has been attracted many researches in recent years to find its pharmaceutical benefits.
Methods: In order to find the answer to this subject, the water extract of cinnamon was prepared and cell proliferation was evaluated using MTT assay. The effect of apoptosis was investigated by DNA fragmentation analysis.
Results: The inhibitory effect of CWE on the growth of the cells was significant. DNA fragmentation was found in cultured AGs and MCF-7 cell lines treated by CWE.
Conclusions: This study showed the anti-neoplastic activity of CWE on tumor cell lines.

1. Introduction

Spices and herbs have been used both as therapeutic foods and as medicines from olden time[1]. These compounds have also been defined as herbal medicines. They are plant-derived products which have been used as traditional folk medicine and food additives. Recently, their medicinal properties are under extensive investigation and become a main part of complementary and alternative medicines [cinnamon (*Cinnamomum cassia*)-water extracts (CAMs)][2].

Cinnamon, one of the favorite spices, has been the focus of research and approved to be a compound that generally recognized as safe status by the Food and Drug Administration recently.

Cinnamon bark is the outer skin of an evergreen tree belonging to the family Lauraceae. This oriental herbal medicine has been used not only as a food additive spice and tea, but also as proposed as an herbal remedy in many countries from ancient times[3]. It is believed as a benefit for common cold, cardiovascular, neurodegenerative diseases and gastrointestinal disorders[4,5]. The pharmacological properties of cinnamon have been studied extensively, signifying contribution in various biological functions including anti-oxidant, anti-microbial, anti-inflammation[6], anti-diabetic and anti-tumor activity[7,8]. Some of its active components such as essential oils (cinnamic aldehyde and cinnamyl aldehyde), tannin, mucus and carbohydrate have been studied in detail. The effects of extracted essential oil and water-based extracts of cinnamon against pathogenic microbes, viruses, and various types of tumor cell lines and the regulation of the level of serum glucose have been approved[9-11].

Since cancer is one of the common diseases causing death in the world population, the discovery of novel anti-cancer drugs has been targeted[12]. Because of relatively low adverse effects of naturally originated agents in common belief, these are the most desired.

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The idea that herbal extracts could help fight against disease is not a new one and some of the cancer drugs come from the natural world. Previously, the anti-tumor properties of the aqueous cinnamon extract in a human cervical cancer cell line (SiHa) have been found[10,13]. Due to differences between the cell lines, these sorts of studies must be extended.

However, since cinnamon bark has been usually used in the form of a water extract, we aimed to study the outcome of CWE on the growth of another four human cancer cell lines (AGs, HeLa, MCF-7 and MDA-MB234).

2. Materials and methods

2.1. Cells and chemicals

The cell lines were obtained from National Cell Bank of Iran affiliated to Pasteur Institute of Iran. The description fo the used cell lines in this study were all human cell lines with epithelial-like morpholpgy. AGs were collected from stomach, HeLa were collected from cervix, MCF-7 and MDA-MB234 were collected from breast. Cell culture medium (RPMI 1640), ampicillin, MTT, fetal bovine serum and other reagents were acquired from Sigma (USA).

2.2. Preparation of CWE

A fine powder of cinnamon barks was made using a mortar. Then the distilled water (10 mL/g) was added to the powder and the suspension was incubated and stirred for 4 h followed by boiling for 2 h. The obtained slurry extract was centrifuged in 7 500 *g* for 30 min. The supernatant was subjected to filtration using Whatman No. 2 filter paper, and then lyophilized. Dried extracts were later dissolved in serum-free cell freezing medium (RPMI) (20 mg/mL) by mixing at 37 °C for 20 min. The obtained solution was sterilized by passing through 0.22 μm filter.

2.3. Cell culture

Four different cell lines were grown in RPMI 1640 complemented with 10% fetal bovine serum in flasks at 37 °C in 5% humidified CO_2 incubator until confluency and propagated after trypsinization. Trypan blue was used to assess the viability of cells and the proliferation was measured by counting viable cells. Direct microscopy observation was used for finding any changes in morphological form of cells, detachment, shrinkage, and colony forming of cells by inverted microscope (Nikon, Japan).

2.4. DNA fragmentation analysis

The cultured cells were trypsinized and transferred to microtubes. Then the cells were pelleted using centrifugation (13 000 *g* for 10 seconds). The pellets were washed by phosphate buffer solution, kept in liquid nitrogen for 10 min and thawed by heating at 95 °C. This freeze and thawing cycle was repeated four times and then proteinase K (0.1 mg/mL) in DNA lysis buffer (2% sodium dodecyl sulfonate and 10 mmol/L ethylene diamine tetraacetic acid in 10 mmol/L Tris-HCl, pH 8.5) was added to the pellets and incubated at 37 °C for 4 h. The DNA was precipitated in two steps using ethanol (70%) and isopropanol. The obtained suspension was centrifuged and RNA was removed with RNase A (0.5 mg/mL in 200 μL of 10 mmol/L Tris-HCl, pH 7.5). The analysis of samples was performed by electrophoresis on agarose gel in Tris-HCl-ethylene diamine tetraacetic acid buffer containing SyberSafe. The gel was observed under UV light.

2.5. MTT colorimetric assay

MTT colorimetric assay was performed in 96-well microplates as described before[14,15]. Briefly, the cells were seeded (5 000 cells/well in 200 μL) and cultured for 24 h. Then, the medium was removed, and the media containing CWE extract at different concentrations (ranging from 1 to 8 mg/mL) were added. The incubation was continued for 72 h. A control group (RPMI without extract) and blank groups (without cells) were also included.

3. Results

In this study, the inhibitory effect of CWE on four tumor cell lines (AGs, HeLa, MCF-7, and MDA-MB234) was studied. All of these cell lines were adherent epithelial-like and originated from human. After the cells reached to about 90% confluency, CWE were added to the wells. After 24 h, detachment and shrinkage of cells were found in treated cells compared with negative control cells without CWE. These changes were observed further in higher concentration of CWE. The inhibitory effect of CWE on proliferation of cells was assessed by MTT test (Figure 1).

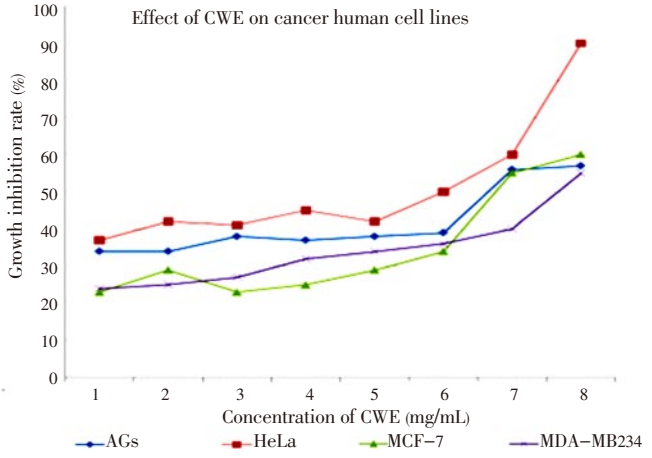

Figure 1. Effect of CWE on the cell lines.

The inhibitory effect of CWE on proliferation of cells

was assessed by MTT test. IC_{50} (mg/mL) of the cell lines were found 7.06, 4.034, 7.51, and 8.65 for AGs, HeLa, MCF-7 and MDA-MB234, and confluency percentages of these were detected 40%-50%, 60%-70%, 30%-40% and 60%-70%, respectively. Regarding to these, the HeLa cell line was detected the most sensitive cell line.

Existence of anti-proliferative activity in CWE was confirmed but to know more about the mechanism of this inhibition effect, the extraction and evaluation of the DNA of the cells by gel electrophoresis were needed. One of anti-proliferation mechanism induced by drugs is apoptosis. During apoptosis, DNA is fragmented and a ladder pattern of DNA is seen on gel electrophoresis.

DNA extracted from the treated cells was electrophoresed on agarose gel (1.5%), and interestingly, DNA ladders were found only for AGs and MCF-7 cells (Figure 2).

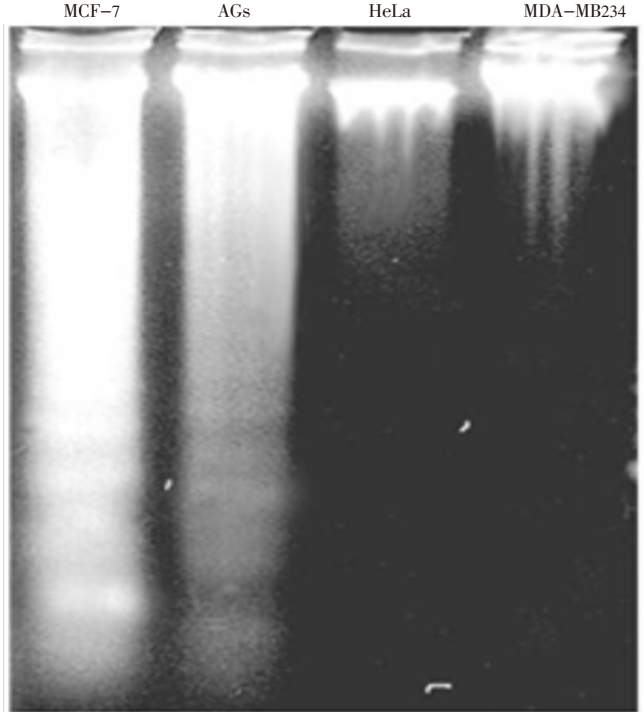

Figure 2. Genomic DNA fragmentation of treated cells with CWE.

4. Discussion

One of the mentioned potency of cinnamon in literature is to treat cancers. Our preliminary results indicate that cinnamon has anti-proliferation activities.

Cancer stands for the largest cause of mortality in the world. The use of natural agents with chemoprevention effects is one of the promising strategies to combat cancer. Since cinnamon is used as a spice for food preparation and folklore medicine as well as in modern pharmacy, it has been reputed to be useful in the cure of numerous diseases; therefore, scientific analysis of its anti-tumor potential will be useful in making new anti-cancer drugs[9]. The anti-mutagenic activity of cinnamon has been reported by Sharma *et al*. in animals using the Ames test[16].

Evaluation of treated HL-60 and SiHa cell lines confirmed the anti-neoplastic activity of cinnamon extract. An active compound, cinnamaldehyde, isolated from cinnamon barks was studied on the potential cytotoxicity on human promyelocytic leukemia cells. Cinnamaldehyde is known as an inducer of apoptosis and mitochondrial permeability transition, the activity of caspase-3 and cytochrome C release to the cytosol.

The anti-neoplastic proposed mechanism for cinnamon was described by Koppikar *et al*[13]. Cinnamon can down-regulate matrix metalloproteinase-2 and HER-2 oncoprotein expression and increase in intracellular calcium signaling as well as the loss of mitochondrial membrane potential of cervical cancer cells. They suggested that cinnamon may augment the pro-apoptotic activity and the inhibition of NF-kappaB and AP1 activities resulted in anti-tumor activity. Cinnamon can modulate effector function of $CD8^+$ T cells[13,17].

In this study, we found the apoptotic effect of CWE on AGs and MCF-7 cells. To explain this different behavior of CWE on cell lines, the deeper scrutiny is needed with the focus on the characters of these cells and the mechanisms of cell death. One of the attractive differences between these cells is the presence of estragon receptor (ER+) on the AGs and MCF-7 cell lines that their DNAs were fragmented and went under death by apoptosis. But the HeLa and MDA-MB234 cell lines are negative estrogen receptor (ER-). These ER- are estrogen-independent and anti-estrogen resistant. It seems that estrogen receptor status of these cell lines contribute to the quality of their response to the CWE. While our study would not be expected to find specific genes or molecular mechanisms, it is evident that RELA (NFkappaB p65) is implicated in estrogen independence and acquired anti-estrogen resistance in cell culture models, and is readily detected by immunochemistry[18-20].

Recently, several groups have shown that the G-protein coupled estrogen receptor G proteincoupled receptor 30, now called G protein-coupled estrogen receptor (GPER), is able to mediate the rapid actions of estrogen[21,22]. It has been showed that GPER1 can suppress MCF-7 cell (GPER and ER+) proliferation in a time dependent manner by mobilization increasing of intracellular calcium, activate PI3 kinase in MCF-7 cells, resulted in inhibition of these cells[23]. Meanwhile, the breast cancer cell line MDA-MB234 has been used by several groups to investigate the role of GPER on breast cancer cells because this cell line does not express GPER or expresses GPER at a very low level[24]. More than two-thirds of breast cancers express the ER and depend on estrogen for growth and survival[25]. Therapies targeting ER function plays a central role in the treatment of ER+ breast cancers of all stages[26].

Recent studies showed that the novel estrogen receptor GPER was associated with different cancers. It has been reported that activation of GPER can stimulate cancer cell proliferation, so cinnamon might have the ability to block this receptor as our data shown. It is evident that human cancer cell lines reflect important phenotypic characteristics in human disease, and they have been central to discovering and extending new knowledge in

many areas of cancer research.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Serafini M, Stanzione A, Foddai S, Anton R, Delmulle L. The European role on traditional herbal medicinal products and traditional plant food supplements. *J Clin Gastroenterol* 2012; **46**(Suppl): S93-S94.

[2] Gruenwald J, Freder J, Armbruester N. Cinnamon and health. *Crit Rev Food Sci Nutr* 2010; **50**(9): 822-834.

[3] Woehrlin F, Fry H, Abraham K, Preiss-Weigert A. Quantification of flavoring constituents in cinnamon: high variation of coumarin in cassia bark from the German retail market and in authentic samples from Indonesia. *J Agric Food Chem* 2010; **58**(19): 10568-10575.

[4] Akilen R, Tsiami A, Devendra D, Robinson N. Glycated haemoglobin and blood pressure-lowering effect of cinnamon in multi-ethnic type 2 diabetic patients in the UK: a randomized, placebo-controlled, double-blind clinical trial. *Diabet Med* 2010; **27**(10): 1159-1167.

[5] Khasnavis S, Pahan K. Sodium benzoate, a metabolite of cinnamon and a food additive, upregulates neuroprotective Parkinson disease protein DJ-1 in astrocytes and neurons. *J Neuroimmune Pharmacol* 2012; **7**(2): 424-435.

[6] Bandara T, Uluwaduge I, Jansz ER. Bioactivity of cinnamon with special emphasis on diabetes mellitus: a review. *Int J Food Sci Nutr* 2012; **63**(3): 380-386.

[7] Sleha R, Mosio P, Vydrzalova M, Jantovska A, Bostikova V, Mazurova J. *In vitro* antimicrobial activities of cinnamon bark oil, anethole, carvacrol, eugenol and guaiazulene against *Mycoplasma hominis* clinical isolates. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2012; **158**(2): 208-211.

[8] Leach MJ, Kumar S. Cinnamon for diabetes mellitus. *Cochrane Database Syst Rev* 2012; doi: 10.1002/14651858.CD007170.pub2.

[9] Kwon HK, Hwang JS, So JS, Lee CG, Sahoo A, Ryu JH, et al. Cinnamon extract induces tumor cell death through inhibition of NFkappaB and AP1. *BMC Cancer* 2010; **10**: 392.

[10] Tung YT, Yen PL, Lin CY, Chang ST. Anti-inflammatory activities of essential oils and their constituents from different provenances of indigenous cinnamon (*Cinnamomum osmophloeum*) leaves. *Pharm Biol* 2010; **48**(10): 1130-1136.

[11] Vafa M, Mohammadi F, Shidfar F, Sormaghi MS, Heidari I, Golestan B, et al. Effects of cinnamon consumption on glycemic status, lipid profile and body composition in type 2 diabetic patients. *Int J Prev Med* 2012; **3**(8): 531-536.

[12] Davis DD, Díaz-Cruz ES, Landini S, Kim YW, Brueggemeier RW. Evaluation of synthetic isoflavones on cell proliferation, estrogen receptor binding affinity, and apoptosis in human breast cancer cells. *J Steroid Biochem Mol Biol* 2008; **108**(1-2): 23-31.

[13] Koppikar SJ, Choudhari AS, Suryavanshi SA, Kumari S, Chattopadhyay S, Kaul-Ghanekar R. Aqueous cinnamon extract (ACE-c) from the bark of *Cinnamomum cassia* causes apoptosis in human cervical cancer cell line (SiHa) through loss of mitochondrial membrane potential. *BMC Cancer* 2010; **10**: 210.

[14] Hanelt M, Gareis M, Kollarczik B. Cytotoxicity of mycotoxins evaluated by the MTT-cell culture assay. *Mycopathologia* 1994; **128**(3): 167-174.

[15] Vahedi F, Najafi MF, Bozari K. Evaluation of inhibitory effect and apoptosis induction of *Zyzyphus jujube* on tumor cell lines, an *in vitro* preliminary study. *Cytotechnology* 2008; **56**(2): 105-111.

[16] Sharma A, Chandraker S, Patel VK, Ramteke P. Antibacterial activity of medicinal plants against pathogens causing complicated urinary tract infections. *Indian J Pharm Sci* 2009; **71**(2): 136-139.

[17] Sajankila SP, Manthena PV, Adhikari S, Choudhury S, Izumi K, Roy R. Suppression of tumor suppressor Tsc2 and DNA repair glycosylase Nth1 during spontaneous liver tumorigenesis in Long-Evans Cinnamon rats. *Mol Cell Biochem* 2010; **338**(1-2): 233-239.

[18] Brendel A, Felzen V, Morawe T, Manthey D, Behl C. Differential regulation of apoptosis-associated genes by estrogen receptor alpha in human neuroblastoma cells. *Restor Neurol Neurosci* 2013; **31**(2): 199-211.

[19] Hussain S, Lawrence MG, Taylor RA, Lo CYW, BioResource APC, Frydenberg M, et al. Estrogen receptor β activation impairs prostatic regeneration by inducing apoptosis in murine and human stem/progenitor enriched cell populations. *PLoS One* 2012; doi: 10.1371/journal.pone.0040732.

[20] Hattori Y, Kojima T, Kato D, Matsubara H, Takigawa M, Ishiguro N. A selective estrogen receptor modulator inhibits tumor necrosis factor-α-induced apoptosis through the ERK1/2 signaling pathway in human chondrocytes. *Biochem Biophys Res Commun* 2012; **421**(3): 418-424.

[21] Kim SY, Lee IS, Moon A. 2-Hydroxychalcone and xanthohumol inhibit invasion of triple negative breast cancer cells. *Chem Biol Interact* 2013; **203**(3): 565-572.

[22] Sukocheva OA, Wee C, Ansar A, Hussey DJ, Watson DI. Effect of estrogen on growth and apoptosis in esophageal adenocarcinoma cells. *Dis Esophagus* 2013; **26**(6): 628-635.

[23] Wang C, Lv X, Jiang C, Davis JS. The putative G-protein coupled estrogen receptor agonist G-1 suppresses proliferation of ovarian and breast cancer cells in a GPER-independent manner. *Am J Transl Res* 2012; **4**(4): 390-402.

[24] Cao WQ, Ma ZF, Rasenick MM, Yeh SY, Yu JZ. N-3 poly-unsaturated fatty acids shift estrogen signaling to inhibit human breast cancer cell growth. *PLoS One* 2012; doi: 10.1371/journal.pone.0052838.

[25] Kummer S, Jeruschke S, Wegerich LV, Peters A, Lehmann P, Seibt A, et al. Estrogen receptor alpha expression in podocytes mediates protection against apoptosis *in-vitro* and *in-vivo*. *PLoS One* 2011; doi: 10.1371/journal.pone.0027457.

[26] Bailey ST, Shin H, Westerling T, Liu XS, Brown M. Estrogen receptor prevents p53-dependent apoptosis in breast cancer. *Proc Natl Acad Sci U S A* 2012; **109**(44): 18060-18065.