Marmara Pharmaceutical Journal 21: 121-126, 2017
DOI: 10.12991/marupj.259889

ORIGINAL RESEARCH

Cytotoxic Effect of *Laurocerasus officinalis* Extract on Human Cancer Cell Lines

Selim DEMİR, İbrahim TURAN, Feyza DEMİR, Elif AYAZOĞLU DEMİR, Yüksel ALİYAZICIOĞLU

ABSTRACT

Laurocerasus officinalis Roem. (*Prunus laurocerasus* L.), also locally known as "karayemis or taflan", is a popular black summer fruit belonging to the genus *Prunus* and the family *Rosaceae*. *L. officinalis* exhibits numerous biological activities, such as anti-inflammatory, antinociceptive, antioxidant, neuroprotective, and antidiabetic effects. The aim of this study was to determine the total phenolic content and cytotoxic effect of dimethyl sulfoxide extract of *L. officinalis* (DEL). The total phenolic content and cytotoxic effect of DEL were investigated using the Folin-Ciocalteu method and thiazolyl blue tetrazolium bromide (MTT) assay, respectively. Total phenolic content value was 33.7±0.13 mg gallic acid equivalent per g sample. DEL exhibited selective cytotoxic effects against four human cancer (lung, colon, liver, and cervix) cell lines. The most selective cytotoxic effect of the extract was observed in colon cancer cells (IC_{50}=265.2±8.1 µg/mL) compared to normal fibroblast cells. These data demonstrate that *L. officinalis* extract exhibits selective cytotoxicity towards some cancer cells. Further studies are now needed to clarify the molecules involved and their mechanisms.

Keywords: Antioxidant activity, Cherry laurel, Cytotoxic effect, *Laurocerasus officinalis*, Polyphenolic compounds

Selim Demir
Karadeniz Technical University, Faculty of Health Sciences, Department of Nutrition and Dietetics, 61080, Trabzon.

İbrahim Turan
Gumushane University, Faculty of Engineering and Natural Sciences, Department of Genetic and Bioengineering, 29100, Gümüşhane.
Gumushane University, Medicinal Plants, Traditional Medicine Practice and Research Center, 29100, Gümüşhane.

Feyza Demir, Yüksel Aliyazıcıoğlu
Karadeniz Technical University, Faculty of Medicine, Department of Medical Biochemistry, 61080, Trabzon.

Elif Ayazoğlu Demir
Karadeniz Technical University, Faculty of Sciences, Department of Chemistry, 61080, Trabzon.

Corresponding Author:
Selim Demir
e-mail: selim-demir@hotmail.com

Submitted / Gönderilme: 15.08.2016 **Revised / Düzeltme:** 28.09.2016
Accepted / Kabul: 03.10.2016

INTRODUCTION

Laurocerasus officinalis Roem. (*Prunus laurocerasus* L.) belongs to the genus *Prunus* and the family *Rosaceae*. It is a popular dark blue or black fruit growing particularly in the Black Sea region of Turkey. The plant is also known as cherry laurel in the English-language literature, and is grown in some Balkan, Western Europe, Middle Eastern and Mediterranean countries. This fruit is very popular in Turkey as a foodstuff and is consumed not only in fresh form, but also in the forms of jam, marmalade or fruit juice (1-4). It is regarded as a good source of nutrients due to the content of monosaccharides, ascorbic acid, dietary fiber, various minerals, and phenolics, such as chlorogenic, benzoic, vanillic, and caffeic acid (5). This fruit is used to treat several diseases in Turkish traditional medicine, such as eczema, sore throat, asthma, coughs, stomach ache, and hemorrhoids (1-4, 6). Recent studies have reported anti-inflammatory, antinociceptive, antioxidant, anti-atherosclerotic, and antidiabetic activities of *L. officinalis,* and these beneficial activities have been attributed to the compounds listed above (1, 4-7).

Cancer is a major health problem and the second most important cause of death after cardiovascular diseases (8). According to the World Health Organization, cancer accounted for 13% of the total 58 million deaths worldwide in 2005 (9). Although chemotherapy is one effective means of treating several types of cancers, it also has a number of disadvantages, such as toxic effects on normal cells, ineffectiveness and gradual drug resistance occuring in cancer cells (10). In order to solve these problems, the scientific community has begun looking for efficient chemotherapeutics, and natural products are regarded as novel candidates for the development of new chemotherapeutics due their high anticancer activity and lower levels of toxicity in normal cells (11).

Several studies have investigated the cytotoxic effect of different species of the genus *Prunus*. Nabende *et al*. demonstrated that methanolic extract from the leaves of *Prunus africana* exhibits selective cytotoxic effects in mouse breast and colon cancer cells compared to monkey kidney (Vero) cells (12). Poongodi *et al*. reported that methanolic *Prunus angustifolia* leaf extract has a cytotoxic effect in human breast cancer cells (13). Peach polyphenolics isolated from *Prunus persica* have recently been reported to inhibit *in vivo* tumor growth by the inhibition of metalloproteinase (MMP) gene expression (14). However, to the best of our knowledge there have been no previous studies of the cytotoxic effect of *L. officinalis*. The purpose of this study was to determine the cytotoxic effect of dimethyl sulfoxide (DMSO) extract of *L. officinalis* (DEL) in human prostate (PC-3), breast (MCF-7), colon (WiDr), lung (A549), liver (HepG2), and cervix (HeLa) cancer cell lines and human normal foreskin fibroblast cells.

MATERIALS AND METHODS

Plant Material

The fruits of *L. officinalis* were collected from Yomra, Trabzon-Turkey in Summer 2015, and identified by Dr. Ibrahim Turan. These were air-dried at 25°C and powdered using a blender and milling into fine powder.

Plant Extraction

The fruit powder (1 g) was extracted with 20 mL DMSO in a mechanical shaker (Shell Lab, Cornelius, OR, USA) in the dark for 24 h at 45°C. The suspension was subsequently removed by centrifuging at 3000 rpm for 10 min. Supernatants were filtered with Whatman No.1 filter paper and 0.2 µm filters. Prepared 50 mg/mL stock extract was used for further experiments.

Chemicals

DMSO, sodium carbonate, Folin reagent, gallic acid, chlorogenic acid, cisplatin, trypan blue solution, thiazolyl blue tetrazolium bromide (MTT dye), and phosphate buffer saline (PBS) tablets were purchased from Sigma (St. Louis, MO, USA). Penicillin-streptomycin and trypsin were obtained from Biological Industries (Kibbutz Beit Haemek, Israel). Eagle's Minimum Essential Medium (EMEM) was obtained from Lonza (Verviers, Belgium). Fetal bovine serum (FBS) was purchased from Biochrom (Berlin, Germany).

Drug Preparation and Treatment

Cisplatin was dissolved in DMSO and used as a reference compound due to its frequent use in previous cytotoxicity studies (11, 15). Chlorogenic acid was dissolved in ethanol and used as a single phenolic since it is one of the major phenolics of *L. officinalis* (16, 17). Final solvent concentrations were no higher than 0.5% in culture media in any experiment. That concentration was not sufficient to affect cell morphology or viability.

Determination of Total Phenolic Content

The total phenolic content was established using the spectrophotometric method (18) adapted to microscale using gallic acid as a standard. Briefly, 12.5 µL DEL was mixed with 62.5 µL Folin reagent and 125 µL 20% sodium carbonate. This was incubated at room temperature for 30 min, after that absorbance was measured at 760 nm. The results were calculated using a standard gallic acid chart and were expressed as milligrams of gallic acid equivalent (GAE) per g sample. Experiments were performed in triplicate, and data are represented as mean±SD.

Cell Culture

Prostate adenocarcinoma (PC-3), hepatocellular carcinoma (HepG2), colon adenocarcinoma (WiDr), cervix adenocarcinoma (HeLa), breast adenocarcinoma (MCF-7), lung carcinoma (A549) human cancer and normal foreskin fibroblast cells were purchased from the America Type Culture Collection (Manassas, VA, USA). Cells were cultured in EMEM supplemented with 10% FBS, and 1% penicillin-streptomycin. Cultures were maintained at 37°C in 5% CO_2 and 95% air.

Cytotoxicity Analysis

The cytotoxic effects of DEL, chlorogenic acid, and cisplatin on cell proliferation were determined using MTT assay (19) with minor modifications. Briefly, 5×10^3 cells were seeded into a flat-bottomed 96-well cell culture plate and incubated for 24 h. The cells were then treated with various

concentrations of DEL (0-500 µg/mL), chlorogenic acid (0-500 µg/mL) and cisplatin (0-10 µg/mL) for 72 h. Next, 10 µL of MTT solution (0.25 mg/mL) was added into the wells and incubation continued at 37°C for 2 h. The supernatants were then removed, and 200 µL of DMSO was added into the wells for 2 h. Optical density (OD) at 570 nm was determined using a microplate reader (Versamax, Molecular Devices, California, USA). The percentage of cell viability was calculated using the equation: OD of the treated group/OD of the control group × 100 (11). Experiments were performed in triplicate, and data are represented as mean±SD.

RESULTS

Total polyphenolic content was 33.7±0.13 mg GAE per g sample. All cells were treated with various concentrations of DEL, chlorogenic acid and cisplatin, and their effects on cell growth were determined using MTT assay after 72 h (Figure 1). The IC_{50} values are shown in Table 1. DEL exhibited moderate selective cytotoxicity against WiDr and A549 cells compared to fibroblast cells.

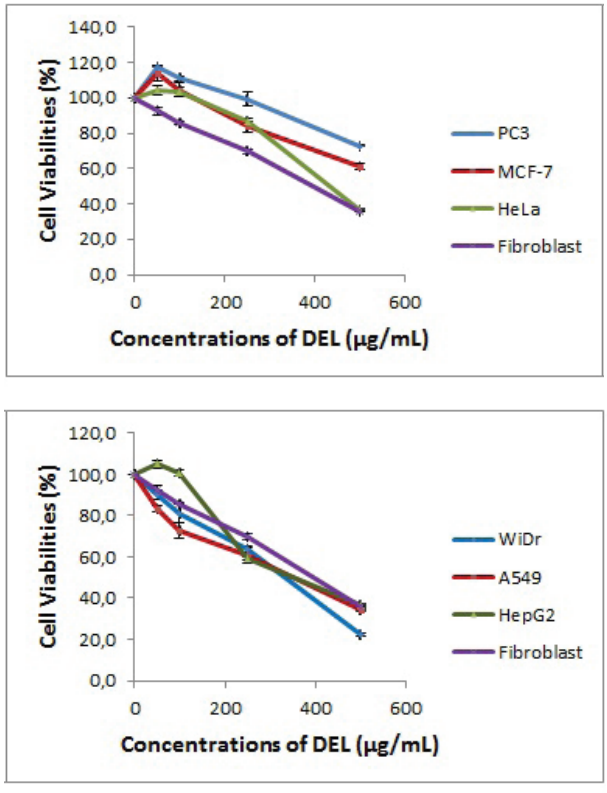

Figure 1. The anti-growth effect after the treatment with the DEL for 72 h against human cancer and normal fibroblast cells by the MTT assay (n=3).

Table 1. Cytotoxic activities (IC_{50}, µg/mL) of DEL and other test compounds (mean±SD, n=3).

	TEST COMPOUNDS		
	DEL	**Chlorogenic acid**	**Cisplatin**
PC-3	>500	>500	0.55±0.02
MCF-7	>500	30.9±1.2	0.39±0.02
WiDr	265.2±8.1	185.8±1.5	1.28±0.01
A549	314.5±10.8	456.6±3.8	0.73±0.03
HepG2	357.1±4.8	117.5±1.5	2.47±0.21
HeLa	396.1±5.4	178.3±4.7	0.62±0.02
Fibroblast	359.1±5.5	92.5±2.4	4.79±0.14

DISCUSSION

This study investigated both the phenolic content of DEL and the cytotoxic effect of DEL on human prostate, breast, colon, lung, liver, and cervix cancer cells and human normal fibroblast cells. Total phenolic content is often used to determine the antioxidant properties of plant extracts since it constitutes an effective, rapid and inexpensive assay (20). A direct relationship has been observed between the total polyphenolic contents and antioxidant activity of many natural product extracts (21). We therefore elected to determine antioxidant capacity of DEL using the Folin-Ciocelteau method. The total polyphenolic content of DEL in this study was 33.7±0.13 mg GAE per g sample. The total polyphenolic content values of various extracts of *L. officinalis* fruits from different regions are reported range between 23.6 and 64.6 mg GAE per g extract (4, 22). In that respect, our results are consistent with the literature. Small differences may have been due to the type of extraction method and solvent used, environmental factors, soil, geographic region, and the maturity levels of the fruits.

Cancer is one of the most important malignant diseases. The most commonly diagnosed cases involve the lungs (13%), breast (11.9%), and colon (9.7%) (12). Chemotherapy is often used in treatment, but this can result in some side-effects, such as increased drug resistance in cancer cells and harmful effects in healthy cells over time. Natural products already

play critical roles in cancer chemotherapy based on their ability to produce apoptosis and/or growth arrest in cancer cells without causing cytotoxic effects in healthy cells (11, 23). *L. officinalis* is one of the most important species of the genus *Prunus*, the fruits containing substantial levels of phenolics, flavonoids, and ascorbic acid (5, 16, 17). Various experiments have evaluated *in vitro* antiproliferative characteristics of different *Prunus* species in recent years (12, 13). However, there are no previous studies of the cytotoxic effect of *L. officinalis* extract in the literature. In order to study new therapeutic approaches, cell lines are used to investigate novel compounds and their effects on tumor cells. If positive results are obtained from *in vitro* experiments, then *in vivo* studies are recommended (11). Effectiveness (high efficacy against multiple cancers) and the absence of deleterious effects on normal cells are two of the main properties desired in an effective chemotherapeutic agent (24). The current study was therefore performed on six different cancer cells and normal fibroblast cells under *in vitro* conditions. The findings show that DEL has no significant antiproliferative effect on PC-3 and MCF-7 cells up to concentrations of 500 µg/mL. However, it exhibits selective cytotoxic effects, particularly on WiDr and A549 cells, compared to fibroblast cells. Lee *et al.* demonstrated that methanolic extract of *Prunus serrulata* exhibits a cytotoxic effect on human colon cancer (HT-29) cells. The highest inhibition in that study was observed at 38.8% at a concentration of 500 µg/mL of extract (23). Fujii *et al.* reported that *Prunus domestica* extract exhibits a selective cytotoxic effect on human colon cancer cells compared to normal colon cells via induction of apoptosis (25), while Adachi *et al.* reported that DMSO extract of *Prunus mume Sieb. et* Zucc has a cytotoxic effect on human stomach and promyelocytic leukemia cancer cells with no harmful effects on human blood cells (26). Studies have also investigated the antiproliferative activity of various compounds isolated from *Prunus* species. Jeong *et al.* reported that n-hexane fraction of methanolic extract of *Prunus mume* inhibits the growth of human larynx carcinoma (Hep-2) and ovary adenocarcinoma (SK-OV-3) cells in a concentration-dependent manner. Moreover, a new compound known as B-1 was purified from the n-hexane fraction, and this exhibits a powerful cytotoxic effect on human kidney hypernephroma (SW-156), uterus adenocarcinoma (HEC-1-B), SK-OV-3, and Hep-2 cancer cells. The IC_{50} values of this compound range from 39 to 58 µg/mL (27). Sunaga *et al.* reported that MK615, a compound isolated from *Prunus mume* fruits, exhibits a cytotoxic effect on human lung cancer cells via induction of autophagy and G_0/G_1 cell cycle arrest and the downregulation of interleukin-8 expression (28). Chlorogenic acid was used as a single polyphenolic compound in our study since it is one of more abundant polyphenols in *L. officinalis* (16, 17). Our findings show that the IC_{50} values of DEL were generally higher than those of chlorogenic acid (except for A549 cells). For this reason, the cytotoxic effect of DEL on four cancer cell lines may not derive only from chlorogenic acid, and this result may explain the synergistic effect of all fruit constituents.

Polyphenols are a major class of secondary herbal metabolites. The antioxidant effect of phenolic compounds is attributed to their ability to donate electrons to reactive oxygen species (ROS) chelating metal ions and stimulating antioxidant and detoxifying enzymes (29, 30). There are also several reports concerning their antioxidant, anticarcinogenic, antimutagenic, anti-atherosclerotic, antimicrobial, and anti-inflammatory activities (31, 32). It has been suggested that the anticancer effect of phenolics may derive from their ability to modulate carcinogen metabolism, regulation of gene expression, induction of cell cycle arrest and apoptosis, and inhibition of signal transduction pathways (9). The presence of many phenolics (gallic, protocatechuic, *p*-hydroxybenzoic, chlorogenic, syringic, vanillic, caffeic, *p*-coumaric, and hydroxycinnamic acids, catechin, and rutin) has been shown in extracts of *L. officinalis* (16, 17), and there are many reports of antiproliferative activities of these phenolics in different cancer cells (33-35). We therefore think that the antiproliferative activity of DEL in cancer cells may derive from its phenolic content.

CONCLUSIONS

This study is the first comprehensive study to investigate the cytotoxic effect of *L. officinalis* extract on cancer cells *in vitro* conditions. From that perspective, this study opens up new pharmacological avenues. Further studies are now necessary to obtain a more detailed understanding of the exact interaction of the signaling pathways involved.

Conflicts of interest statement

None of the authors had any financial or personal relationships with other individuals or organizations that might inappropriately influence their work during the submission process.

***Laurocerasus officinalis* Ekstraktının İnsan Kanser Hücre Serileri Üzerindeki Sitotoksik Etkisi**

ÖZ

Laurocerasus officinalis Roem. (*Prunus laurocerasus* L.), yerel olarak karayemiş veya taflan olarak da bilinir, *Prunus* cinsi ve *Rosaceae* familyasına mensup, koyu renkli bir yaz meyvesidir. *L. officinalis*, anti-inflamatuvar, antinosiseptif, antioksidan, nöroprotektif ve antidiyabetik gibi çeşitli biyolojik aktiviteler göstermektedir. Bu çalışmanın amacı, *L. officinalis'in* dimetil sülfoksitli ekstraktının antioksidan ve sitotoksik etkilerini belirlemektir. Ekstraktın toplam polifenol içeriği Folin-Ciocalteu metodu, sitotoksik etkinliği ise MTT yöntemiyle belirlendi. Ekstraktın toplam polifenol içeriği 33.7±0.13 mg gallik asit ekivalanı/g örnek olarak bulundu. Ekstrakt akciğer, kolon, karaciğer ve serviks kanser hücre serilerinde seçici bir sitotoksik etki gösterdi. Normal fibroblast hücreleri ile karşılaştırıldığında en etkili seçici sitotoksik etkinin kolon kanseri hücrelerinde olduğu belirlendi (IC_{50}=265.2±8.1 µg/mL). Sonuçlar *L. officinalis* ekstraktının bazı kanser hücre serilerinde seçici bir sitotoksik etki gösterebildiğini ortaya koymaktadır. Ekstrakttaki etken molekülleri ve bunların etki mekanizmalarını belirleyebilmek için daha ileri çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Antioksidan aktivite, Karayemiş, *Laurocerasus officinalis*, Sitotoksik etki, Polifenolik bileşikler

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