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ORIGINAL ARTICLE

Antiproliferative and apoptotic effect of *Morus nigra* extract on human prostate cancer cells

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Abstract *Background: Morus nigra* L. belongs to the family *Moraceae* and is frequently used in traditional medicine. Numerous studies have investigated the antiproliferative effects of various extracts of different *Morus* species, but studies involving the *in vitro* cytotoxic effect of *M. nigra* extract are very limited. The purpose of this study was to evaluate the phenolic composition and antioxidant activity of dimethyl sulfoxide extract of *M. nigra* (DEM) and to investigate, for the first time, the probable cytotoxic effect in human prostate adenocarcinoma (PC-3) cells together with the mechanism involved. *Methods:* Total polyphenolic contents (TPC), ferric reducing antioxidant power (FRAP) and phenolic compounds of DEM were evaluated using spectrophotometric procedures and HPLC. The cytotoxic effect of DEM on PC-3 cells was revealed using the MTT assay. Mechanisms involved in the cytotoxic effect of DEM on PC-3 cells were then investigated in terms of apoptosis, mitochondrial membrane potential and cell cycle using flow cytometry, while caspase activity was investigated using luminometric analysis. *Results:* TPC and FRAP values were 20.7 ± 0.3 mg gallic acid equivalents and 48.8 ± 1.6 mg trolox equivalents per g sample, respectively. Ascorbic acid and chlorogenic acid were the major phenolic compounds detected at HPLC analysis. DEM arrested the cell cycle of PC-3 cells at the G_1 phase, induced apoptosis via increased caspase

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activity and reduced mitochondrial membrane potential. *Conclusions:* Our results indicate that *M. nigra* may be a novel candidate for the development of new natural product based therapeutic agents against prostate cancer.

1. Introduction

The genus *Morus*, commonly known as mulberries, contains 10–16 species and belongs to the family *Moraceae*. *Morus alba* L., *Morus rubra* L. and *Morus nigra* L. are the most extensive *Morus* species. *M. nigra* is native to western Asia and has been cultivated in Europe since Pre-Roman times (Ozgen et al., 2009; Kostic et al., 2013). The deep-colored *Morus* fruits are a rich source of phenolic compounds, including flavonoids, anthocyanins and carotenoids (Kostic et al., 2013). Due to their rich anthocyanin contents, the fruits of *M. nigra* in particular exhibit higher antioxidant activity than the other *Morus* species (Ozgen et al., 2009; Kostic et al., 2013). Recent studies have reported antimicrobial, antioxidant, antidiabetic, anti-HIV, anti-inflammatory, hypolipidemic, hepatoprotective, anticancer, antiobesity, and neuroprotective activities of different species of *Morus* and have attributed to those compounds (Sakagami et al., 2007; Khalid et al., 2011; Kostic et al., 2013; Ramesh et al., 2014; Grajek et al., 2015). *M. nigra* is used to treat urinary incontinence, dizziness, sore throat, depression, fever and cancer in traditional medicine (Khalid et al., 2011; Kostic et al., 2013).

Prostate cancer is the fifth most common cancer overall, the second most prevalent malignancy worldwide and the second greatest cause of cancer-related deaths among men after lung cancer (Shahneh et al., 2014; Huo et al., 2015; Kim et al., 2015). A combination of treatment options (radiation therapy, brachytherapy, cryosurgery, chemotherapy, hormonal therapy, and surgery) is often recommended for managing prostate cancer. Of the conventional modalities for prostate cancer treatment, chemotherapeutic drugs lead to various side effects. Natural anticancer drugs derived from medicinal plants that selectively induce apoptosis and/or growth arrest in cancer cells without causing detrimental effect in healthy cells are today available, and these natural products can serve as chemotherapeutic agents (Shahneh et al., 2014).

Numerous studies have investigated the antiproliferative effects of various extracts of different *Morus* species. Eo et al. reported that 80% methanol extract of root bark of *M. alba* L. exhibited a dose-dependent anticancer effect on human colorectal carcinoma cell line (SW480) via induced cell growth arrest and apoptosis (Eo et al., 2014). Fathy et al. demonstrated that *M. alba* extract had a cytotoxic effect on hepatocellular cell line (Fathy et al., 2013). However, studies involving the *in vitro* cytotoxic effect of *M. nigra* extract are very limited. Qadir et al. demonstrated that *M. nigra* leaf extract exhibited cytotoxic effect on human cervical cancer (HeLa) cell line (Qadir et al., 2014). However, to the best of our knowledge no previous study has investigated the cytotoxic effect of *M. nigra* extract on prostate cancer cells. The purpose of this study was to therefore to evaluate the phenolic composition and antioxidant properties of dimethyl sulfoxide extract of *M. nigra* to investigate, for the first time, the probable cytotoxic effect in human prostate adenocarcinoma cells together with the mechanism involved.

2. Materials and methods

2.1. Chemicals and reagents

All phenolic standards, methanol, folin phenol reagent, sodium carbonate, potassium ferricyanide, trichloroacetic acid, iron (III) chloride, gallic acid, trolox, acetonitrile, cisplatin, phosphate buffer saline (PBS) tablet, trypan blue solution, dimethyl sulfoxide (DMSO), and thiazolyl blue tetrazolium bromide (MTT dye) were purchased from Sigma (St. Louis, MO, USA). Kaighn's modification of Ham's F-12 (F-12K) and Eagle's minimal essential medium (EMEM) media were obtained from Lonza (Verviers, Belgium). Fetal bovine serum (FBS) was obtained from Biochrom (Berlin, Germany). Penicillin-streptomycin was purchased from Gibco (Paisley, England) and trypsin-EDTA solution from Biological Industries (Kibbutz Beit Haemek, Israel). All flow cytometry kits were obtained from BD Biosciences (San Diego, CA, USA).

2.2. Drug preparation and treatment

Cisplatin was used as a reference anticancer agent for cytotoxicity experiments due to its use in prostate cancer treatment (Dhar et al., 2011). It was dissolved in absolute DMSO to prepare a 1000 μg/mL stock solution.

External working concentrations of both extract and cisplatin were prepared by further dilution with DMSO. The final concentration of DMSO did not exceed 0.5% in culture media during any experiment, and this concentration did not affect cell morphology or viability.

2.3. Plant collection and extraction

Fully mature fruits of *M. nigra* were harvested from Kelkit town, Gumushane, Turkey. Samples were preserved in cool bags for transportation to the laboratory. The fruits were air-dried at room temperature for 20 days and converted into a fine powder using a blender and milling. 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 prepared 50 mg/mL stock DMSO extract of *M. nigra* (DEM) was filtered with Whatman No. 1 filter paper and a 0.2 μm filter and then stored at −20 °C until used in further experiments.

2.3.1. Estimation of total phenolic content (TPC)

Content of total phenolics of DEM was established by the spectrophotometric method (Slinkard and Singleton, 1977)