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Investigation of cytotoxic and genotoxic effects of olive leaf extract on colon cancer cells and normal cell lines

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Abstract

Colon cancer is a type of cancer that occurs when cells in the mucous layer membrane that surrounds the inner surface of the large intestine sections multiply unevenly. This study aimed to investigate olive leaf extract's cytotoxic and genotoxic effects on HTC-116 colon cancer cell lines and HDF healthy cell lines. In the study, olive leaf extract was prepared in methanol. The passaged HTC-116 and HDF cell lines were then incubated in Dulbecco's Modified Eagle's Medium (DMEM). Olive leaf extract extracted in methanol was applied to these cell lines at 3, 10, 20, 50, and 80 µg/ml and left for incubation for 24 hours. Then, the cytotoxic effect was determined by MTT analysis. The genotoxic effect of olive leaf extracts applied to HTC-116 and HDF cell lines after a 24-hour incubation period was determined by Hoechst stain. DNA damage was visualized with a DAPI filter on a camera-attached trinocular fluorescence microscope 30 minutes after staining with Hoechst. The MTT analysis revealed that the cytotoxic effects of olive leaf extract applied to the HTC-116 cell line were close to each group after the 24-hour incubation period. As a result of the examination of the genotoxic activity with Hoechst DNA staining in colon cancer cells (HCT-116), olive leaf extract at various concentrations treated to the cell line had a minor genotoxic effect at low doses (3, 10, and 20 µg/ml), while at high concentrations (50 and 80 µg). /ml), the genotoxic effects of the applications were determined. In examining the genotoxic impact of Hoechst DNA staining in the HDF cell line, no genotoxic effect was observed at low doses (3, 10, and 20 µg/ml) of olive leaf extract applied to the cell line at various doses. The little genotoxic effects of high doses (50 and 80 µg/ml) were also observed. Olive leaf contains phenolic substances which inhibit cell proliferation and DNA damage in colon cancer cells and has no toxic effects on normal cells.

Keywords: Olive leaf extract, colon cancer, cytotoxicity, genotoxicity

Introduction

Cancer is a disease that involve the proliferation of cell that have lost their genetic stability and cell cycle control through mutation [1]. More than 14 million new cancer cases were reported worldwide in 2012 [2], raising a need to further develop treatments and preventive strategies. Cancer mainly occurs with aging, and, there are factors other than age that contribute to the development of cancer. There is strong scientific support that the traditional Mediterranean diet (MD) protects against some cancers [3,4]. The effects of MD

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on inflammation have not been fully elucidated [5,6]. MD has shown a protective role in cancer as a whole [6]. However, it is important to understand whether any beneficial effects attributed to MD are due to a particular component of the diet rather than the entire diet. As an example, polyphenol bioactive compounds have shown particular promise.

Olive tree leaves (Olea europaea) (OLE) are an alternative medicine agent widely used in traditional medicine in the Mediterranean region [7]. The bioactive properties of this Leaf have formed a basis for its use as an antioxidant, anti-hypertensive, anti-atherogenic, anti-inflammatory, hypoglycemic and hypocholesterolemic agent [7,8]. OLE components not detected in Olive Fruit oil include flavonoids such as luteolin and apigenin, which show anti-cancer properties [9,10].

In this study, we investigated the effect of olive leaf extract on human colon cancer cells. It was aimed to investigate the cytotoxic and genotoxic effects of olive leaf extract on HT-116 cell line and Human Dermal Fibroblast (HDF) cell lines.

Materials and Methods

Chemicals: HTC-116 and HDF cell lines were taken from YUTAM cancer laboratories and used for examinations. DMEM (Dulbecco's Modified Eagle's Medium), PBS, Penicillin, and L-glutamine were used in cell passages and treatment of oil lead extract. Trypsin EDTA and PBS were used for the passage of cells.

Oil Lead extract preparation: The leaves were ground with a grinder. They were then left to dissolve in methanol, and extraction was completed. Then, a rotary evaporator separated the extract from the solvent (Laborota 4001, Heidolph). The temperature of the water bath in the rotary evaporator was set at 40°C and the rotation frequency at 60 rpm. The pressure in the condenser was adjusted with a vacuum valve to evaporate the solvent faster. The evaporating solvent was concentrated via a condenser. Finally, olive leaf extract was obtained to be used in this study.

Cell viability analysis: The viability of Oil leaf extract treated cells was measured using flow cytometry according to the manufacturer's instructions (Ecotech Biotechnology, Turkiye). HCT-116 and HDF cells were seeded in 96-well plates at 1000 cells per well and treated for 24h at concentrations of 3, 10, 20, 50, and 80 µg/mL of Oil leaf extract diluted in the medium. After incubation for the indicated time, the MTT solution has added to the wells and allowed to be incubated for at least 3 hours in darkness. The cell number and viability were measured by the colorimetric absorbance of cells (Thermo Fischer, USA). The absorbance values were used for the cell viability assay.

Genotoxicity analysis: For preparing Hoechst dye, 20 µl of Hoechst stock dye was added to 50 ml of PBS. For Hoechst staining, the HCT-116 and HDF cell lines were seeded in 24 well-plates and the concentration of Oil lead extract 3, 10, 20, 50, and 80 µg/mL incubated the cells and then, the cells were fixed with 4% paraformaldehyde in phosphate-buffered saline at 4 °C for 30 min. The cells were washed with phosphate-buffered solution (PBS), and samples were incubated with 1 mM Hoechst 33258 fluorescent dye (Sigma-Aldrich®, USA) for 5 min at room temperature. Nuclear abnormalities were observed under the fluorescent microscope (Leica® DM IL LED, excitation/emission wavelength = 365/420 nm) on a total of 1000 cells in each well. The scored nuclear alterations (NAs) were divided into the following categories: lobed (L), notched (N), and micronuclei (MN) [11].

Results

Cell viability results: MTT analysis was performed

to observe whether olive leaf extract has cytotoxic effects on both healthy human fibroblast cell line and colon cancer cell line.

Table 1. MTT (Cell viability) values after 24 hours of incubation with olive leaf extract treated to HCT-116 and HDF cell lines

Group	HCT-116		HDF	
	Mean	S.D.	Mean	S.D.
Control	0.174	0.010	0.175	0.050
3 µg/mL	0.154	0.006	0.174	0.022
10 µg/mL	0.157	0.015	0.187	0.077
20 µg/mL	0.153	0.008	0.203	0.006
50 µg/mL	0.162	0.016	0.193	0.032
80 µg/mL	0.160	0.011	0.179	0.036

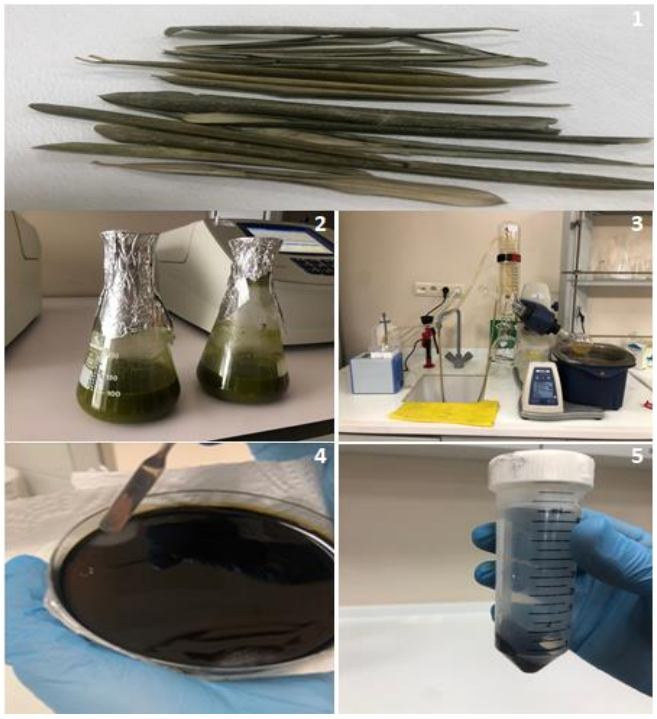

Figure 1. Process of Oil leaf methanol extract preparation; 1: Olive leaves, 2: Homogeneous extract, 3: Evaporator device, 4: Planning by scraping the dried extract, 5: Extract transferred to test tube.

Cytotoxicity was determined after incubation with the olive leaf extract at concentrations of 3, 10, 20, 50 and 80 µg/ml to colon cancer cells (HCT-116) and human dermal fibroblast cells (HDF) for 24 hours. While it was observed that the cytotoxic effects of olive leaf extract concentrations were close to each other in the groups, the most effective cytotoxicity value was found in the group administered at a dose of 20 µg/ml. It was determined that the cytotoxicity of 50 and 80 µg/ml concentrations was less than the other concentrations. After the incubation period, no cytotoxic effect was detected in any of the doses of olive leaf extract on the HDF cells for 24 hours in the MTT analysis. However, it was determined that olive leaf extract at a concentration of 20 µg/ml showed a proliferative effect (Table 1, Fig 2 and 3).

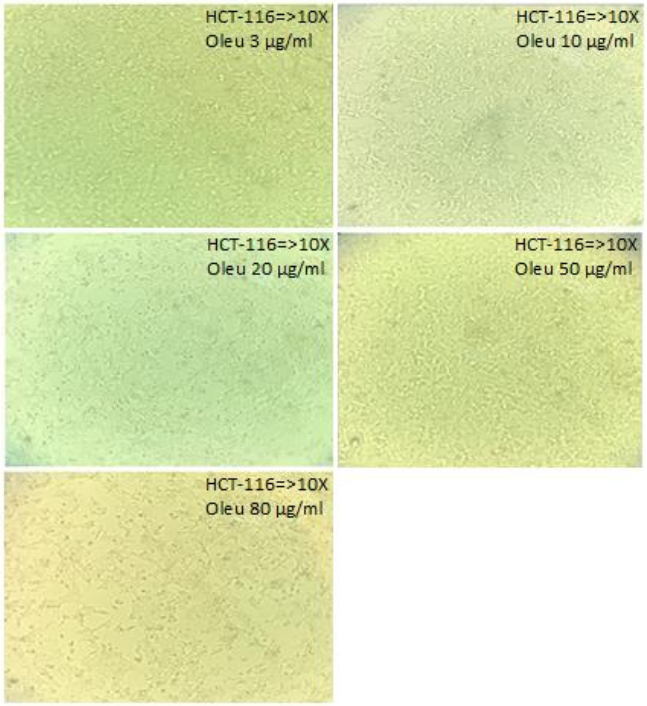

Figure 2. Illustration of inverted Microscope after the application of olive leaf extract at various concentrations to HCT-116 cell lines.

Genotoxic analysis: In evaluating genotoxic activity with Hoechst DNA staining, a small amount of genotoxic effect of olive leaf extract applied to colon cancer cell line at various concentrations was determined at low doses. In contrast, the genotoxic effects of applications at high concentrations (50 and 80 µg/ml) were determined. In the evaluation of genotoxic activity, no genotoxic effect was observed at low doses of olive leaf extract at various doses applied to the human dermal fibroblast cell line. In contrast, genotoxic effects were determined at high doses (50 and 80 µg/ml) (Fig. 4 and 5).

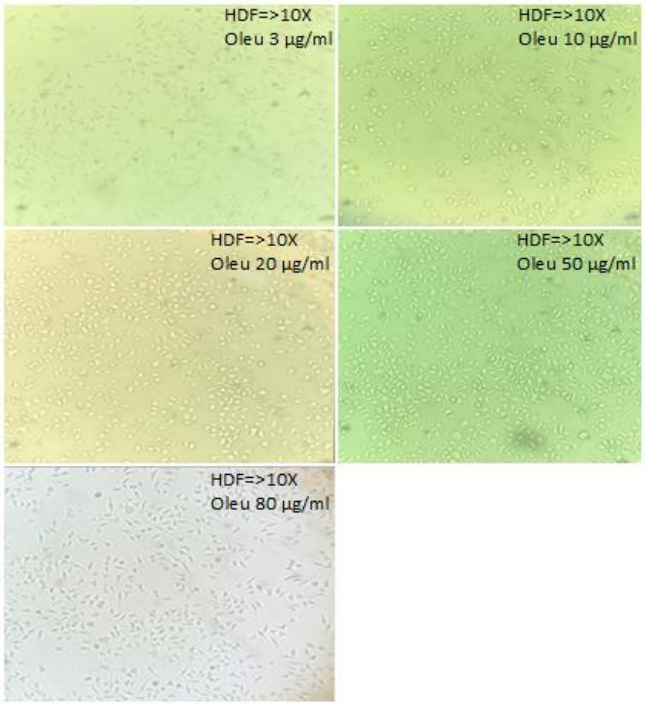

Figure 3. Illustration of inverted Microscope after the application of olive leaf extract at various concentrations to HDF cell lines.

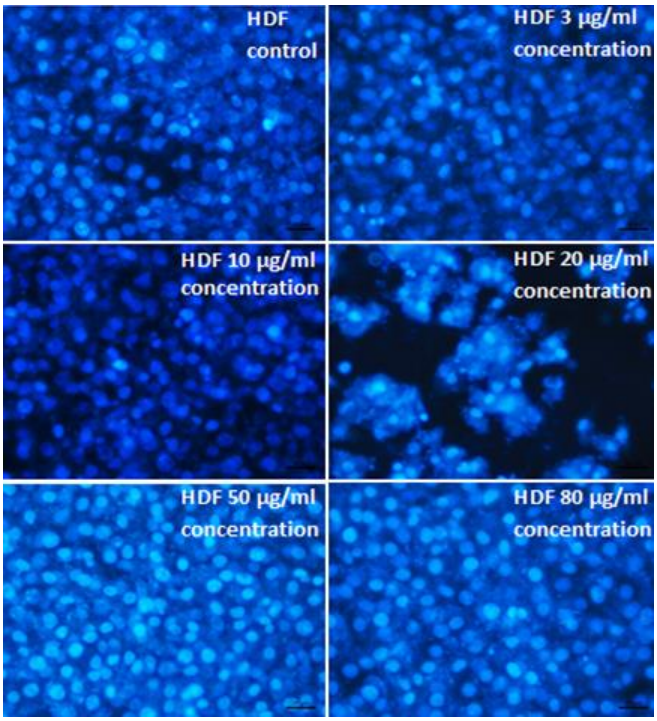

Figure 4. Fluorescent microscope images of the HDF cell line after hoechst dye application

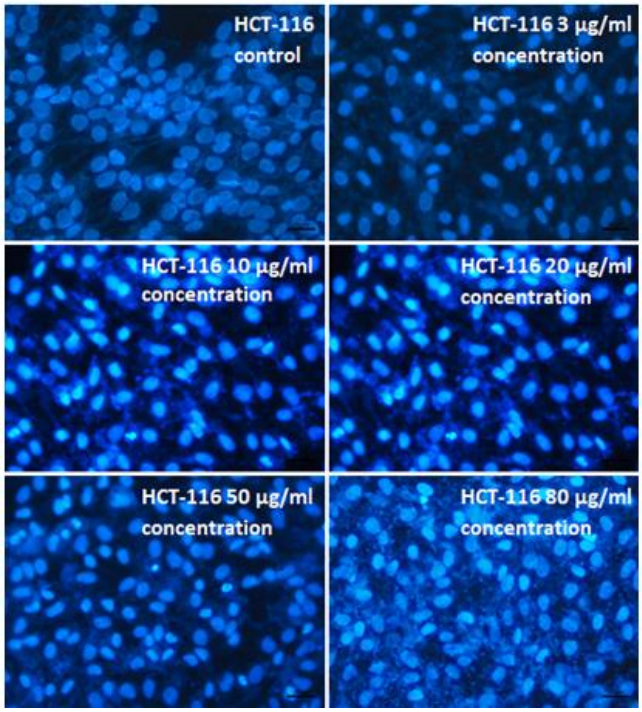

Figure 5. Fluorescent microscope images of the HCT-116 cell line after hoechst dye application

Discussion

Colon cancer is the third most common type of cancer in the world and in Turkey, and ranks third in cancer-related deaths. It is known that most of the colon cancer starts with the abnormal growth of healthy epithelial cells in the mucous layer of the colon or rectum [12]. In this study, it was aimed to investigate the cytotoxic and genotoxic effects of olive leaf extract on colon cancer cells, and additionally, this preliminary study aims to assess the effectiveness of methanol extraction from olive leaves and investigate olive leaf extracts as anticancer agents.

The emergence of colon cancer arises with the interaction of many hereditary, environmental and genetic factors. For these reasons, the susceptibility to mutations and excessive consumption of red meat, insufficient intake of vitamins, bile acids, and mineral intakes, HNPCC is a hereditary type of colon cancer observed at young ages (before the age of 50) and caused an average of 4% of all colon cancer types. This variety is considered genetically predisposed [13].

According to previous research, one of the most important factors in the emergence of colon cancer is the presence of a family history of colon cancer. More than 90% of colon cancer cases were determined accidentally, without any particular symptoms. In the remaining colon cancer cases, it has been determined that there is a high degree of genetic factors. It is known that many such critical genetic factors come together and cause colon cancer [13].

The study showed that olive leaf extract had cytotoxic effects at 20 µg/ml but not cytotoxic at other concentrations on the HCT-116 cell line. Additionally, olive leaf extract was not found to have a toxic effect on HDF cells. Therefore, it was thought that olive leaf extract could accelerate cell death in cancer cell lines. Previous researches were reported that olive leaf extract have cytotoxic effects on many different cancer cells including breast and colon cancers [14,15]. Also, Barbaro et al. [16] reported that the antitumor activity of olive leaf and oleuropein may be associated with reactive oxygens species (ROS).

Antigenotoxic agents often show expected therapeutic effects that may effectively control cancer [17]. A significant number of nuclear abnormalities was determined in the higher concentration of treated colon cancer cells in this study. This result may be explained by olive leaf extract its capacity to act as a potent free radical scavenger [18,19,20,21].

This study's limitations include being limited to one cancer cell line, and the molecular mechanisms underlying activity were not investigated. However, this study provides a platform for further research to evaluate the molecular mechanisms involved in the anticancer activity of the phenolic compounds of olive leaf extract in colon cancer cells.

Declaration of Interest: No potential conflict of interest relevant to this article was reported.

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