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Investigation of The Antiproliferative Effect of *Camelia sinensis* on Liver Cancer (Hepg2) Cell Line

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Original article

Abstract

Liver cancer is the second leading cause of death among other cancer types in the world. The use of preclinical models to develop new diagnostic technologies and new therapies for the prevention of liver metastasis is inevitable. Tea is a beverage that attracts the attention of many researchers because of the bioactive substances in its composition. In recent years, intensive researches have been conducted to examine the effects of green tea on health. The study aimed was to investigate the antiproliferative effect of green tea (*Camelia sinensis*) extract on liver cell lines. XTT test was performed to investigate the cytotoxicity of HepG2 and WI-38 cell lines. In the study, IC_{50} was found to be 150 µg / mL in the WI-38 cell line of Green tea and 72 µg / mL in the HepG2 cell line. Green tea has an effect on HepG2 cell lines. And more work is needed in this area.

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INTRODUCTION

Liver cancer is the second leading cause of death among other cancer types in the world. Every year 500,000 people die from this disease in the world. The five-year survival rate of these patients is less than 15%. It is also highly resistant to chemotherapy and radiotherapy. The use of preclinical models to develop new diagnostic technologies and new therapies for the prevention of liver metastasis is inevitable. According to research conducted by the Ministry of Health; If no measures are taken against cancer and a systematic control program is not implemented, the direct treatment costs of cancer in the 2030s will reach a size that will not be covered by the Ministry of Health budget. Therefore, researchers are also looking for adjuvant therapies or new herbal sources to improve cancer. The interest in plant-derived foods and their active components has increased considerably in recent years, and intensive studies have been conducted on their effects on cancer protection [1]. In the latest World Cancer report prepared by the World Health Organization, epidemiological studies conducted since the 1970s are examined and the importance of nutrition and diet in cancer prevention is emphasized [2].

Tea is a beverage that attracts the attention of many researchers because of the bioactive substances in its composition. In recent years, intensive researches have been conducted to examine the effects of green tea on health. In these studies, which are generally performed on microbial systems and mammalian cell systems and experimental animals, it has been reported that green tea may have the effect of preventing and supporting various cancer types such as stomach, colon, breast, prostate and pancreas cancers, cardiovascular diseases, inflammatory and neurodegenerative diseases [3, 4].

Healthy eating is very important to prevent disease. Antioxidant nutrition is especially important to avoid the effects of free radicals Various "antioxidant defense systems" have been developed under normal physiological conditions to prevent the formation and removal of free oxygen radicals and to ensure their removal. Antioxidant nutrients are nutrients that reduce electron-hydrogen and free radicals and reactive compounds are taken by the environment and nutrition and reduce them and significantly reduce the negative effects [5-7].

The leaves harvested from the tea plant are immediately subjected to thermal treatment (usually steam application) together with curling to dry green tea. It accounts for approximately 1% of the world's tea consumption and is widely consumed in Japan, China and other Asian countries [8, 9]. Green tea, which has been reported to have beneficial effects on health in recent researches, is rapidly increasing in popularity worldwide. According to the studies, green tea has antioxidant, anti-inflammatory, antimutagenic, anticarcinogenic, antiangiogenic, apoptotic, anti-obesity, hypolipidemic, antiarteriosclerotic, antidiabetic, antibacterial, antiviral, and anti-aging effects have been shown [10, 11]. It is stated in these studies that the beneficial effects of green tea on health are due to catechins in its composition.

This study aimed was to investigate the antiproliferative effect of green tea (*Camelia sinensis*) extract on liver cell lines.

MATERIALS and METHODS

Preparation of Ethanolic *Camelia sinensis* Extract (50 mg/mL)

Camellia sinensis plant were collected from Turkey Rize. The plants were dried and ground and 0.5 g of dried plant material was added in 10 ml of pure ethanol. After vortexing, it was incubated for 24 h on a mechanical shaker at 45 ° C, 150 rpm. The supernatants were filtered through 0.22 µm filters. Aliquots for later use and stored in the dark at -20 ° C.

Cell Culture

In our study, HepG2 (liver cancer) cell line was used for anti-proliferative effect and WI-38 human fibroblast cell line was used for determination of toxic effect. DMEM (with phenol red, with L-glutamine, with 4.5 high glucose) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS) was prepared as medium.

Cytotoxic Analysis

XTT test was performed to investigate the cytotoxicity of HepG2 and WI-38 cell lines. Cells were added 200 µl (1x10 4 cells / well) in each well (n=6). The 96-well Plate was incubated at 37 ° C for 24 hours. Extract solutions were prepared and at concentrations 200, 100, 50, 25, 12.5, 6.25, 3,125, 1.56 µg / ml) of 2 µl was added to the wells. DMSO was added as a negative control. After completion of the procedure, plates were incubated at 37 ° C for 24 h. The reagents will be 50/1 XTT (2,3-bis [2-methoxy-4-nitro-5-sulfophenyl + -2H-tetrozolium-5 -caboxa-nilide salt) agent (Labeling reagent) / Activating agent (electron coupling reagent) XTT solution was prepared by stirring. After removing the medium from the plates, 50 µl of XTT solution was added to each well. 150 µl of medium solution containing colorless DMEM was added. After incubation at 37 ° C for 4-6 hours, it was read at a wavelength of 450 nm to measure the optical density.

RESULT

IC_{50} values were calculated according to cytotoxicity analysis results for Green Tea and the results are given in Table 1.

Table 1. IC50 values of Green Tea

Cell Line	IC_{50} (µg/mL)
HepG2	72.0
WI-38	150

In the study, IC_{50} was found to be 150 µg / mL in the WI-38 cell line of Green tea and 72 µg / mL in the HepG2 cell line.

DISCUSSION

Catechins are found in kernels and shells of fruits [12]. It has an important place among tea components and constitutes about one-third of the dry weight of tea. In recent years, due to its strong antioxidant and anti-cancer effects, the number of studies on catechins has increased significantly [13, 14].

Numerous studies have been conducted on the molecular effects of EGCG, the major catechin of green tea, and anti-tumor effects have been demonstrated in both different cancer types and various in vitro and in vivo models. It has been reported that various apoptotic pathways are activated by the effect of EGCG [15, 16]. Green tea catechins have been shown to induce apoptosis and suppress cell growth in prostate carcinoma cells. Regarding the mechanism of action, it increases pro-apoptotic (Bax, Bak) proteins and reduces anti-apoptotic (Bcl-2, Bcl-xl) proteins, inhibits the NF-kB pathway, and reduces IAP levels and as a result, caspase-3 and caspase-6 activation have been increased [17]. Similar results were obtained in a study performed in human melanoma cells [18]. While EGCG causes apoptotic cell death in pancreatic cancer cells, it also increases the amount of Bax, decreases XIAP levels and stimulates the JnK signaling pathway [19].

The protective effect of green tea and its catechins against cancer formation; inhibiting cell proliferation, stopping the cell cycle [20], suppressing active receptors, reducing the release of cytokines [21], suppressing mitotic stimuli [22], preventing mutagenicity and genotoxicity, activating detoxification enzymes, free radical scavenging, accelerating apoptosis of cancer cells [23] and angiogenesis inhibitor [24]. Green tea polyphenols can affect certain enzymes and receptors with their binding properties to proteins. EGCG, green tea catechin, inhibits prooxidant enzymes such as nitric oxide synthase and cyclooxygenase-2, limiting the release of nitric oxide and prostaglandins, which are important mediators of inflammation and tumor formation.

It has been reported that green tea can prevent the progression of cancer by inhibiting tumor metastasis. In vitro studies have shown that green tea accelerates apoptosis of cancerous cells by stimulating the stopping of the G1 cell cycle in oral wounds [10]. In studies carried out on experimental animals, it has been reported that green tea shows anticancerogenic effects against the development and metastasis of carcinogenic tumors in the digestive system organs, mammary glands, liver, lung, and skin [25]. Green tea flavonols, quercetin, kemferol and myricetin have also been found to inhibit the formation of carcinogen-induced tumors in mice and rats [9].

Green tea, a powerful antioxidant, binds polyphenols to reactive oxygen and nitrogen species. It also exhibits antioxidant activity by triggering the synthesis of (endogenous) antioxidant enzymes present in the cell, such as superoxide dismutase, glutathione reductase, Glutathione-S-reductase, catalase, and Quinone reductase. With these effects, green tea prevents lipid peroxidation and DNA structure damage. Green tea and EGCG, which are not separated into their fractions, also bind metal ions and reduce the formation of reactive free radicals in later stages [10]. Weinreb et al. [26] reported that EGCG found in green tea may have protective effects against neuronal diseases such as Alzheimer's and Parkinson's by regulating the activity of free radical scavenging, iron-binding activity, and antioxidant enzymes. Bayer et al. [27] reported that green tea polyphenols show a strong antioxidant effect against lipid peroxidation induced by free radicals in solution, micelles, red blood cells in humans and low-density lipoproteins. In another study, it has been reported that green tea can prevent vascular obstruction and stiffness by preserving paraoxonase (PON1) activity and inhibiting the oxidation of lipoproteins [11]. Studies have shown that when green tea is consumed regularly, the risk of heart disease and cancer is reduced [9]. EGCG, especially in the composition of green tea, exhibits an antiangiogenic effect by regulating protease activity during endothelial morphogenesis. Thus, it has been reported that it can help prevent diseases such as tumor growth, rheumatoid arthritis, diabetic retinopathy and hemangioma associated with pathological angiogenesis [24]. Also, it is stated that green tea can be used as an adjunctive treatment in preventing rejection of the transplanted tissue in humans by the immune system [27].

Green tea inhibits the biological activities of bacteria such as *Streptococcus mutans* and *Streptococcus sobrinus*, which cause tartar and caries formation in the teeth due to its antibacterial effect. It prevents them from sticking to the enamel and helps prevent bad breath. Green tea extracts decrease methicillin resistance of methicillin-resistant *Staphylococcus aureus*. It also prevents the development of *Helicobacter pylori*, which causes diseases such as gastric, and duodenal ulcers. It has been reported that catechins in tea inhibit cellular DNA and RNA polymerases by reverse transcriptase enzyme of HIV [9, 10]. Green tea extracts and polyphenols (especially EGCG, ECG) at microbial systems (*Salmonella typhimurium* and *Escherichia coli*), mammalian cell systems and in vivo animal experiments have been reported to show antimutagenic effects against various mutagens [25].

Conclusion

Green tea has an effect on HepG2 cell lines. And more work is needed in this area.

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