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Effects on Oxidative Stress of Next Generation Pharmabiotics in Colorectal Cancer Cells: Postbiotics

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Abstract : *There is a serious imbalance in the human body, especially in the intestines, where many epigenetic and nutrigenetic factors increase oxidative stress and reactive oxygen species (ROS), and metabolic activities have difficulty eliminating this situation. Many studies in recent years have reported that probiotics have positive effects on various cancer cells by affecting different pathways. Probiotics are live microorganisms that create a health benefit in the host when used in sufficient amounts and can also show anti-tumoral activity. However, their use may pose a risk for individuals receiving immunosuppressive treatment and those with chronic diseases. For this reason, new generation pharmabiotic agents (postbiotics) have become important. In our study, the effect of postbiotics on oxidative stress in colorectal cancer cells (HT29) was examined. In our study, postbiotics obtained from Lactobacillus rhamnosus EnA 17 strain and HT29 and L929 cell lines were used. MTT method was used to determine cytotoxicity.* The total oxidant status (TOS) levels were measured spectroscopically with the autoanalyzer in cell culture. *The data obtained showed that postbiotics had significant cytotoxicity on HT29 cell lines. It was also determined that postbiotics played a balancing role in oxidative stress parameters.*

Keywords – *Colorectal cancer, Cytotoxicity, HT29 cell, Oxidative stress, Postbiotics*

I. INTRODUCTION

Colorectal cancer is the 4th deadliest cancer in the world, with approximately 700,000 deaths per year. Poor nutrition, especially consumption of low-fiber, excessively fatty foods, is an important factor in the development of intestinal diseases and the increase in the incidence of colorectal cancer [1, 2]. Colorectal cancer is a slowly emerging disease, and early detection increases survival rates. For this reason, early diagnosis is the basis for colorectal cancer prevention [2,3].

Oxidative stress is a serious imbalance between production and excretion of reactive oxygen species (ROS), causing significant health problems. Excessive production of ROS or long-term exposure leads to several intestinal diseases such as inflammatory bowel diseases (IBD), intestinal infections, and cancers [4].

Epigenetic and nutrigenetic factors, pathogenic microorganisms, are considered as an important source of ROS. Increased oxidative stress in the intestines plays an important role in the early stages of intestinal damage [5].

The gut microbiota represents an important group of the gastrointestinal system [6]. This microbial flora found in the gut contributes significantly to the protection of host health. [7]. A balanced intestinal micro-flora is directly related to the establishment of healthy intestinal homeostasis [8]. Intestinal microbiota has an important effect on the regulation of intestinal functions by breaking down non-metabolizable nutrients and secreting some important bioactive compounds such as SCFA and bacteriocins [5]. It is known that postbiotic metabolites, especially SCFAs, exhibit anticarcinogenic properties in the development of colon cancer, while butyrate is effective in preventing the progression of the proliferation cycle in cancer cells [9]. Studies show that ROS in the host are related to the balance of the microbial community in the intestines is maintained by the metabolites produced by the flora elements there.

The purpose of our study is to determine the cytotoxic activity and cellular oxidative stress status of postbiotics on in vitro colorectal cancer cells.

II. MATERIALS AND METHODS

In our study, postbiotics were obtained from *Lactobacillus rhamnosus* EnA 17 strain. Postbiotics were obtained by collecting the supernatant (CFS) portions obtained after centrifugation (4000g, 20min, 4˚C) from bacterial cultures that were activated and incubated for 1 night and the density was adjusted to 10^9 cfu/ml. The collected supernatant was filtered using a 0.22 µm filter and lyophilized at room temperature. It was stored at $+4^0$C until the study was performed.

In cytotoxicity studies, colorectal cancer cell line (HT-29, ATCC® HTB-38™) and healthy fibroblast cell line (L929) were used. HT-29 and L929 cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 1% penicillin-streptomycin for 24 h at 5% CO2 and 37℃ in 25 cm2 cell culture flasks. The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT) method was used to determine cytotoxicity [10]. Postbiotics were added to confluent HT-29 and L929 cell lines at different concentrations (500-250-125-62.5-31.25 mg/ml) and incubated. Cell viability was examined at 12 and 24 hours after incubation. EC_{50} (half-maximal effective concentration) values were calculated.

To investigate the changes in oxidant events in colorectal cancer cells and healthy control cells with postbiotic application, total oxidant status (TOS) and total antioxidant status (TAS) levels were measured spectroscopically with the autoanalyzer according to the manufacturer's instructions [11].

Statistical Analysis

As a result of the studies, the data obtained were evaluated statistically with the SPSS program. The results were used graphically as mean values ± standard deviation.

III. RESULTS

In our study, it was determined that postbiotics obtained from *L. rhamnosus* EnA 17 strain had a proliferative effect on the healthy fibroblast cell line (L929) and a cytotoxic effect on the colorectal cancer cell line (HT-29) with a correlation in parallel with the increasing concentration (Fig.1).

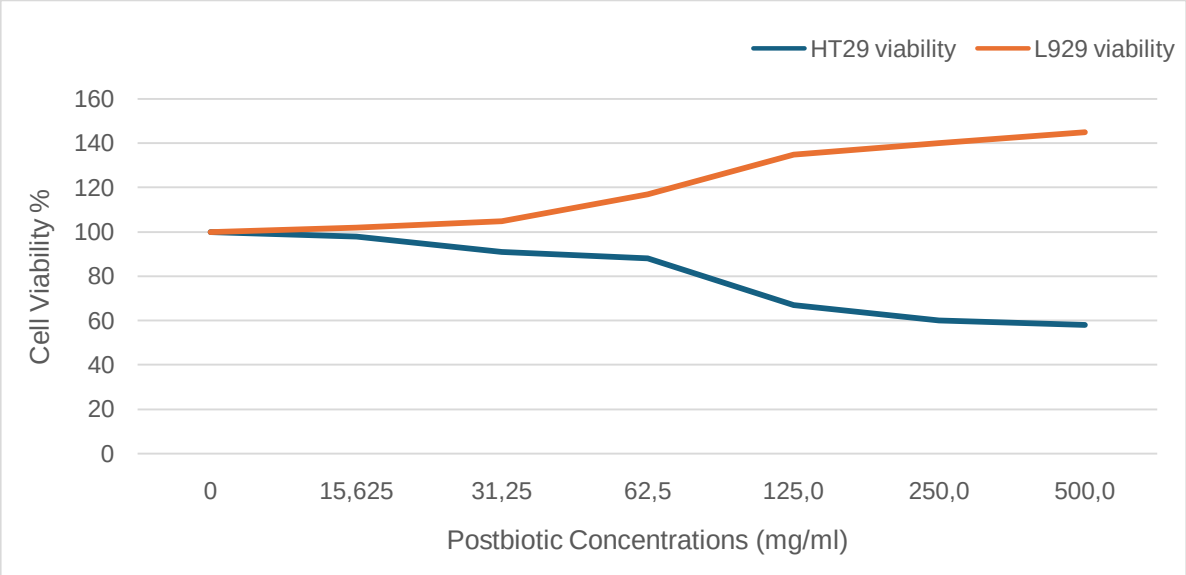

Figure 1. Effect on viability in HT-29 and L929 cell lines after postbiotic application

When oxidative stress parameters were evaluated in our study, TAS values were lower in HT-29 cell line than L929 cell line. The TOS values were higher in HT-29 cell line than L929 cell line. In the postbiotic applied groups, it was determined that total antioxidant status (TAS) increased in HT-29 colorectal cancer cells in a concentration and time dependent manner.

IV. Discussion

Colorectal cancer is closely related to epigenetic and nutrigenetic factors. Many studies have shown that inflammatory bowel disease and the imbalance of intestinal flora, 'dysbiosis', are closely related to the colorectal cancer process [12]. It is thought that probiotics and their metabolites can seriously prevent the progression of most cancer processes when they have positive effects on the intestinal flora [13, 14]. In a study, it was observed that Lactobacillus casei and Bifidobacterium bifidum species promoted ROS formation and initiated the apoptosis signaling pathway in HT-29 colon cancer cells [15]. In another study, it was reported that *Lactobacillus paracasei* subsp. *paracasei* suppressed the proliferation of HT-29 cancer cells and promoted cell apoptosis with ROS production [16]. Shen et al. In a study by [17], it was shown that postbiotics of *Bifidobacterium animalis* 01 strain scavenged hydroxyl radicals and superoxide anion in vitro while increasing antioxidant activity in vivo. In the study conducted by Song et al., it was shown that the living form and heat-inactivated forms of *L. brevis* have antioxidant activity [18]. Recent studies have indicated that probiotics, which are claimed to be beneficial for health, may have some negative effects in terms of use in individuals with suppressed immune systems. For this reason, new literature has focused on paraprobiotics and postbiotics.

Since the intestines are constantly exposed to food substances and microbial pathogens, they are considered an important source of ROS. Oxidative stress developing in the intestines plays a serious role in the early stages of intestinal damage [19, 20]. Our study showed how oxidative stress in colorectal cancer cells can have positive effects with its postbiotic effect.

V. Conclusion

Many studies are investigating the role of probiotics in the prevention, treatment and prognosis of colorectal cancer. However, it is very important to demonstrate the effectiveness of postbiotics for patients who cannot use live organisms due to immune system suppression. In our study, the cytotoxic activity of postbiotics in colorectal cancer cells and the balancing of oxidative stress are important findings. More similar studies are needed.

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