Original Article

Cytotoxic Activity of Familact: A Probiotic Supplement

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ABSTRACT

Background: *Lactobacillus* and *Bifidobacterium* species are among the probiotics discussed due to their anti-cancer effects in the treatment of colorectal and breast cancers in recent studies. The aim of this study was to investigate the anticancer effect of Familact, a commercial probiotic capsule containing seven bacterial strains (*L. casei*, *L. acidophilus*, *L. rhamnosus, L. bulgaricus, B. breve, B. longum* and *Streptococcus thermophilus*).

Methods: Various cancer cell lines including Caco-2, HT-29, T47D and normal cell line L929 were treated with different concentrations of Familact. Using MTT assay, the cytotoxicity effect was investigated for each cell line and then flow cytometry analysis of apoptosis was evaluated.

Results: Familact demonstrated inhibitory effects on the proliferation of all tested cancer cell lines in a dose-dependent manner. Although Familact augmented apoptotic cell death in HT-29 human cancer cells, it was less effective in the case of Caco-2 and T47D cells. Moreover, exposure to Familact showed moderate cytotoxicity towards L929 mouse fibroblast cells.

Conclusion: Familact could be considered as a complementary therapy in the treatment of cancers.

Keywords: Apoptosis, Breast Cancer, Colorectal Cancer, Familact, Prebiotic, Probiotics.

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INTRODUCTION

Cancer as the result of uncontrolled growth of the cells has been addressed as a challenging disease during recent years. One of the effective factors in the prevention of cancer is the use of probiotics, which, in addition to their potent properties, have anti-genotoxicity, anti-mutagenicity and anti-carcinogenicity properties which are nowadays considered [1-3].

"Probiotics are the living microorganisms that their sufficient consumption ads to appearance of the health effects of the host body" [4]. Probiotics were introduced as living microbial supplement which acts on the gastrointestinal tract, improves the microbial balance of the intestine and confers health effects [5]. Probiotics compose of various types of lactic acid bacteria (LAB) including *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Streptococcus* and *Pediococcus* [6, 7], of which *Bifidobacterium* is one of the most common probiotic bacteria [8, 9]. *Bifidobacterium* spp. protects the intestinal health and helps useful microbiota in the intestine, such as preventing the colonization of pathogenic bacteria in the intestine [9, 10]. Of special note, LAB including *Lactobacillus* have anticancer activity and prevent metastasis [11]. Moreover, a great attention has been paid to the preventive effects of LAB on colorectal cancer [12]. LAB also exert antitumor activity in breast cancer. Th-1 cytokine production by *L. acidophilus* could harness breast tumors in BALB/c mice mediated through activation of immune responses [13]. Moreover, inhibition of tumor growth was achieved by *L. casei* CRL 431 on a model of breast cancer, confirming the anti-cancer activity of probiotics [14]. The inhibitory mechanism of probiotics includes: (a) the conversion of glucose to lactic acid and the creation of an acidic environment which disrupts desirable conditions for the survival of many pathogenic microorganisms, (b) the production of inhibitor compounds, including hydrogen peroxide, which destroy the pathogens around them, (c) Production of bacteriocins that prevent the growth and proliferation of competing strains, (d) Probiotics with the ability to attach to intestinal epithelial cells, prevent the attachment of pathogens, (e) Using nutrients by probiotics, pathogenic bacteria expose to nutritional deficiency, (f) Probiotics apply

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appropriate protective effects by eliminating the toxin receptors in the intestinal mucus [3, 15].

The benefits of probiotic usage include antagonism against diarrhea caused by antibiotics [16], infections and irritable bowel syndrome [17], decreased lactose intolerance [18], decreased cholesterol level, modulation of bile hydrolysis [19], increased intestinal movements and constipation relief, mucosal tissue survival, vaginal infection reduction [20] as well as anti-inflammatory, anti-mutagenic and anti-cancer activity [21, 22]. The anticancer effects of probiotics are mediated by inhibiting the conversion of procarcinogens to carcinogens, inactivation of mitogens, and decreasing the growth of procarcinogenic bacteria [2, 23].

The aim of this study was to identify the inhibitory effect of Familact on the proliferation of a panel of human cancer cell lines, colorectal adenocarcinoma (Caco-2), colon carcinoma (HT-29), breast carcinoma (T47D), and mouse fibroblast cells (L929).

Familact capsule is a probiotic-prebiotic (synbiotic) complex which contains high levels (10^9 CFUs) of seven beneficial bacteria (*L. casei*, *L. acidophilus*, *L. rhamnosus*, *L. bulgaricus*, *B. longum*, *B. breve*, *S. thermophilus*), along with prebiotic fructooligosaccharide (contributing to the growth and activity of probiotics) and other components (lactose-magnesium acetate-talc) [24].

MATERIALS AND METHODS

Chemicals and Reagents

Familact capsules were purchased from Zist Takhmir (Tehran, Iran). Dulbecco's Modified Eagle's Medium (DMEM-F12), RPMI 1640, Penicillin/Streptomycin and Trypsin-EDTA 10X were from Biosera (East Sussex, UK). Fetal Bovine Serum (FBS) was obtained from Gibco (USA). Dimethyl sulfoxide (DMSO) and propidium iodide (PI) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3- [4,5-dimethylthiazol-2-yl] -2,5-diphenyl tetrazolium bromide (MTT) was obtained from Carl Roth (Karlsruhe, Germany). Annexin V-FITC was provided by Invitrogen Inc (USA).

Cell Culture

Human cancer cells Caco-2, HT-29, T47D and normal L929 cells were from the Pasteur Institute (Tehran, Iran). Caco-2 cells were propagated in 35% DMEM/F12, 50% RPMI1640 containing 15% FBS and 1% Penicillin/Streptomycin solution. However, HT-29, T47D, and L929 were cultured in 89% RPMI1640 supplemented with 10% FBS and 1% Penicillin-Streptomycin. Cells were incubated at 37 °C in a humidified incubator with 5% CO_2.

Cell Viability Assay

The cells were incubated at a density of 3×10^4 cells/well in 24-well plates for 48 h. Subsequently, cells were treated with various concentration of Familact (0.39-100 µg/ml) for additional 48 h. Afterward, the medium was replaced with 150 µl of MTT (5 mg/ml in PBS) and incubated for 4 h to form purple formazan crystals. Then, DMSO (450 µl) as the solvent was added to each well, and the absorbance was measured by a microplate reader (Anthos 2020, Biochrom, UK) at 570 nm vs. 690 nm.

Apoptosis Assay

To determine Familact-induced apoptosis in Caco-2, HT-29, T47D and normal L929 cell lines, flow cytometry was applied using Annexin V-FITC and PI staining. Cells were cultured in 6-well plates at the density of 3×10^5 cells/well. Following 48 h after seeding, cells were treated with concentrations of the drug-induced 25% and 50% inhibition, which were obtained from the MTT assay. Thereafter, the trypsinized cells were washed with PBS and suspended in 100 µl binding buffer. The cell suspension was labeled with Annexin V-FITC (5 µl) and PI (2 µg/ml) following by incubation for 15 min at room temperature in the dark. Then, 400 µl binding buffer was added and analyzed by FACSCalibur flow cytometer (BD Biosciences, USA).

Statistical Analysis

All experiments were performed with three independent repeats and the results were expressed as mean ± standard deviation (SD). One-way ANOVA was used to analyze the variance followed by the Tukey post hoc test for inter-group comparisons. Statistical analysis was performed using GraphPad Prism ver. 5.01 software (San Diego, CA).

RESULTS

Cytotoxicity Effect of Familact

The cytotoxic activity of Familact on L929, Caco-2, HT-29, and T47D cell lines was investigated using MTT assay. After treatment with serial concentrations of the drug for 48 h, the 50% inhibitory concentration (IC_{50}) was assessed.

Familact inhibited cell viability of HT-29, Caco-2, T47D and L929 in a dose-dependent manner with an IC_{50} value of 37.4 ± 4.25, 75.41 ± 1.85, 75 ± 9.69 and 78.45 ± 6.8 µg/ml, respectively (Figure 1). Overall, Familact exhibited remarkable cytotoxicity to HT-29 while other aforementioned cell lines experienced moderate toxicity towards this drug.

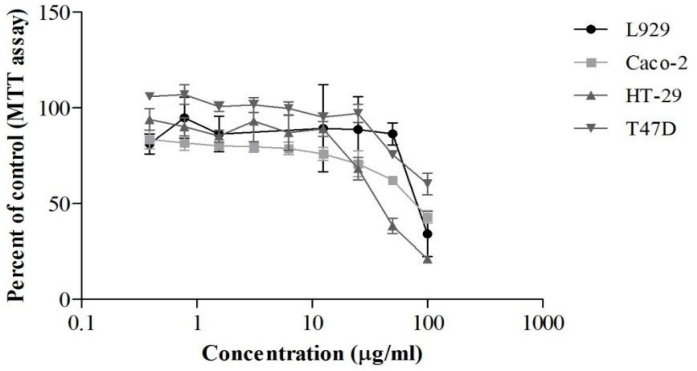

Figure 1. Effect of various concentrations of Famlact on cell survival of L929, Caco-2, HT-29 and T47D cells after 48 h incubation.

Flow Cytometric Analysis of Apoptosis

The effect of Famlact on induced apoptosis in L929, Caco-2, HT-29 and T47D cells was evaluated by Annexin V-FITC and PI staining. The flow cytometry analysis showed that in L929 normal cells, exposure to Famlact increased the percentage of late apoptotic cells in comparison to untreated control cells while HT-29 cells experienced a two-fold increase in late apoptotic cell death ($P<0.01$). In the case of Caco-2 and T47D cells, Famlact was less effective in this regard, implying different mechanisms of cell death may be involved (Figure 2).

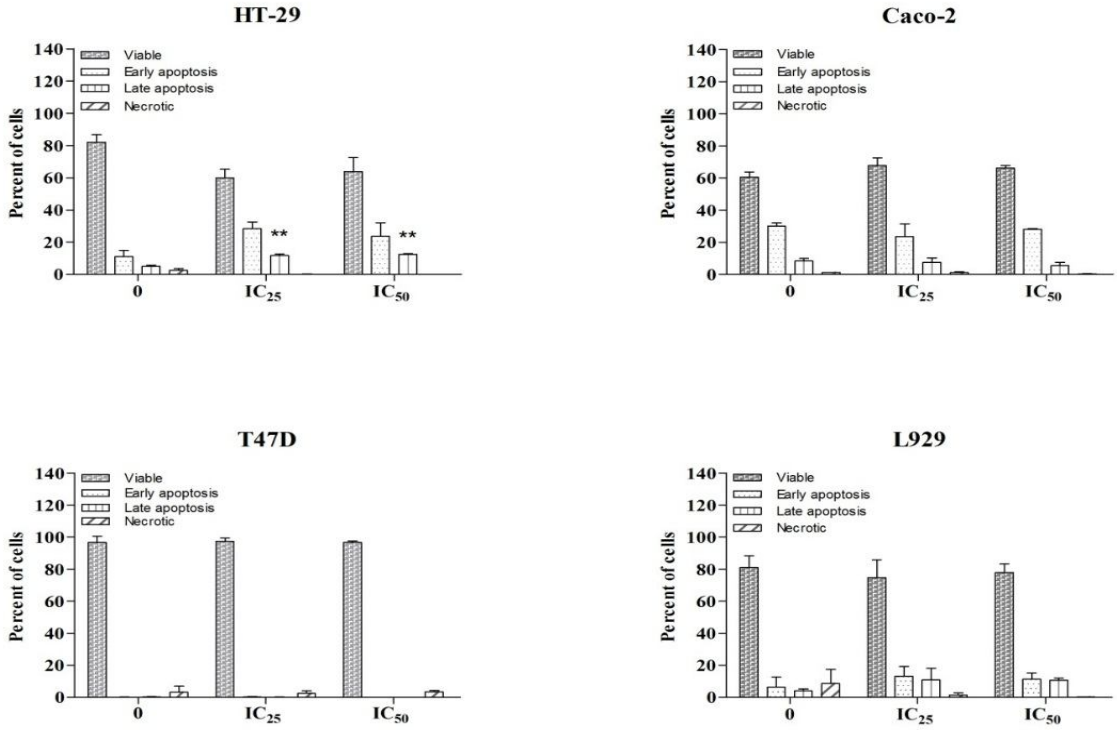

Figure 2. Effect of Famlact on apoptosis-mediated cell death after treatment for 48 h. The data were expressed as mean ± SD. (**$P<0.01$).

DISCUSSION

Cancer is a complicated and multifactorial disease for which researchers have conducted a number of studies for many years. The emergence of drug resistance in cancer patients and the need to discover new drugs for the treatment of these patients has always been a concern for researchers. This problem has led to the use of probiotics for the treatment and prevention of cancer. In human studies, the use of probiotics and dairy products containing *Lactobacillus* or *Bifidobacterium* is associated with the prevention of colon cancer progression [25]. Probiotic bacteria have the ability to harness cancer cell growth, tumor size, and advanced colon cancer [12, 26]. The presence of *Lactobacillus* and *Bifidobacterium* as the intestinal flora is associated with intestinal health [27]. Today, consumption of probiotics including LAB in the range of 10^9-10^{10} per day can reduce the incidence, duration, and severity of some digestive diseases [28].

In this study, the anticancer effect of Famlact was studied on a panel of cell lines: Caco-2, HT-29, T47D and normal L929 cells. Earlier studies have discussed the anticancer effects of probiotics. *Bacillus polyfermenticus* exhibits growth inhibitory

effect on human colon cancer cells (HT-29, DLD-1, and Caco-2 cells) mediated through inhibition of ErbB2 and ErbB3 receptors [29]. This effect was also observed for *B. bifidum* supernatant on Caco-2 cancer cells [30].

The results of our MTT assay showed that Familact had acceptable cytotoxic properties on HT-29 cells with moderate cytotoxicity for Caco-2 and T47D cells. Two probiotic bacterial strain, *L. plantarum* 15HN and *L. lactis* subsp. *lactis* 44Lac, isolated from dairy products exhibited acceptable cytotoxic activity against cancer cell lines of HT-29, AGS, MCF-7, and HLA as well as HUVEC normal cells through the secretory metabolites, suggesting probiotics have inhibitory effects on cancer cells especially colon cancer cells [31].

The supernatant of *Lactobacillus* strains was able to augment LDH release and apoptosis by modulating Bax/Bcl-2 and NO pathway in HT-29 cells [32]. Moreover, obtained data from analysis of three species of *L. casei* and *paracasei* also indicated that their cell wall extract led to apoptosis induction via impairing mitochondrial membrane potential of HT-29 cells [33]. Here, the apoptosis percentage increased in Familact-treated HT-29 cells compared to their normal counterparts. Familact induced statistically significant apoptosis in HT-29 relative to the untreated control cells. However, in Caco-2 and T47D cells, Familact did not significantly induce apoptosis programmed cell death, which probably indicates an involvement of other mechanisms of cell death and further studies need to be performed. In addition, HT-29 cells were more sensitive to Familact than Caco-2 cells.

CONCLUSION

Promising results would be obtained using this probiotic complex as complementary therapy in the treatment of colon cancer. In addition, for the prevention and treatment of cancer, the discovery of new probiotic bacteria with anti-cancer properties seems necessary.

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