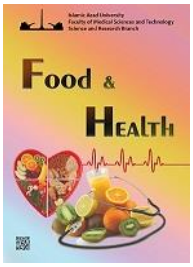

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Primary evidence on the potential of *Lactobacillus paracasei* in treatment of hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is the 6th human cancer with high morbidity and mortality rate. Owing to the recent finding of anticancer effects of probiotics in various types of human cancers, the current study was conducted to assay the potential of *Lactobacillus paracasei* as a main microbial ingredient of dairy products in inhibition of HCC cell growth. HepG2 cell line was treated with different concentration of supernatant isolated from *L. paracasei* strain TD3 and MRL/MRS broth mediums as a negative control. MTT assay was performed to assess cell viability, 24 hours following treatment. MTT results demonstrated a significant decrease in HepG2 cells viability, which was directly correlated with the concentration of bacterial supernatant. Similar to the other human cancers, *L. paracasei* can be considered as a potential anticancer probiotic in restriction of HCC cells growth. Further studies are needed to clarify active components of *L. paracasei* supernatant and its potential as adjuvant therapy in HCC patients' treatment, as well.

1. Introduction

Hepatocellular carcinoma (HCC) is the most frequent primary liver tumor which usually arises following cirrhosis with high mortality rate and it is considered as the 6th of human cancers. Main risk factors of HCC are included chronic hepatitis B virus infection, fatty liver, dietary related factors such as carcinogenic mycotoxins as well as aflatoxin, alcohol, insulin resistance and food compounds with the potential to produce reactive oxygen species. Food-related risk factor elements, as well as aflatoxin, can attenuate critical cancer network genes including p53 and BCL2 genes expression and thereby accelerates tumorgenesis evolution (1-4). Owing to the more frequency in the younger population and the pivotal role of dietary habits on the risk of HCC, including the best supplementary food elements as well as probiotics in routine human diet have shown to be effective in the prevention of various diseases such as different types of human cancers.

Probiotic is a phrase of the modern era, denotation “for life” and is in use to name bacterial association with beneficial effects on human and animal health (5). Probiotics actually are viable micro-organisms which have been demonstrated to be effective in improving human body microbiome as well as the microbial flora of intestine and vagina (6).

Lactobacilli as one of the major traditional probiotic classification were frequently used to be included in dairy products and it is considered as GRAS (generally recognized as safe) organism, which can be incorporated in pharmaceutical products (7). The promising effects of various strains of *Lactobacillus* have been demonstrated on augmentation of immune system function, antimutagenic and antitumor effects and also decreasing in serum cholesterol and sugar levels which can indirectly reverse the carcinogenic process in all cancer patients especially cirrhotic ones (8, 9).

In this regard, we were aimed to investigate the effect of *Lactobacillus paracasei* strain TD3 supernatant on HepG2 cell

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line viability to primarily determine its anticancer effects on HCC.

2. Materials and methods

2.1. Probiotic culture and preparation

Lactobacillus paracasei strain TD3 was subjected to be cultured in de Man Rogosa Sharpe (MRS) broth medium (pH=6.5, Merck, Germany) for 48 h. Optical density (OD) of bacterial culture was then determined using spectrophotometer to be optimized as 0.7-0.8 at 600 nm of UV wavelength which was determined by plate counting on MRS agar (equals to 10^9 CFU/ml bacterial number). Bacterial cell cultures were centrifuged at 1100×g for 15 minutes at 4˚C to separate bacterial supernatant from cell pellets and their pH was adjusted to be neutralized (pH =7.2). The separated bacterial supernatant was filtered using 0.2 µm membrane filter to be sterilized and free of any bacterial cells and debris before cell line treatment.

2.2. *Cell culture*

HepG2 cell line was purchased from Iranian Biological and Genetic Resources Center. It was cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with L-glutamine, 10% (v/v) fetal bovine Serum (FBS, Gibco, USA) and %1 antibiotic solution (10.0 U/ml penicillin G sodium, 10.0 g/ml streptomycin sulfate (Gibco, USA). Harvested HCC cells were transferred to 96-well tissue culture plates at the density of 1×10^4 cells per well. Monolayers of HepG2 cells were incubated for 24 h to be confluent before treatment step. The supernatant of *L. paracasei* culture preparation (10^9 CFU/ml) was added to the monolayer of HepG2 cells with concentration of 5, 10, 20, 30 and 40 % (v/v) in triplicates and then was incubated in a humidified incubator (5% CO_2 at 37˚C). Negative control wells were treated with serial dilutions of MRS and MRS broth adjusted with lactate (MRL, pH=4.35) mediums.

2.3. MTT assay

To measure the cytotoxic effect of Lactobacillus paracasei TD3, 20 µl of MTT solution [5 mg/ml in phosphate-buffered saline (PBS)] was added to the cell culture medium following treatment of cells with bacterial supernatant after 24 h. The cell plates were incubated for 4 hours and then their solutions were replaced by 100 µl DMSO to solubilize formazan blue crystals. Absorbance was measured at 570 nm using ELISA Reader instrument (Biotek, Absorbance Microplate Reader, USA) after 15 minutes. The cell viability was calculated as the following formula:

Viability (percentage of control) = [(absorbance sample-absorbance blank)/(absorbance control-absorbance blank)]×100.

3. Results

Bacterial supernatant concentrations which were used in treatment were calculated in log10 to be analyzed in GraphPad Prism (version 7.03). The equivalent calculated logs were 0.69, 1.00, 1.30, 1.47, 1.60 for 5, 10, 20, 30 and 40 % of HepG2 cells concentration, respectively (Fig.1). MTT assay graph demonstrated that TD3 had a significant inhibitory effect on HepG2 cell growth in comparison with negative controls including cells treated with control MRL and MRS solutions. MRL control result was a confirmation of this fact that the acidity was not the main cause of HepG2 cell growth inhibition.

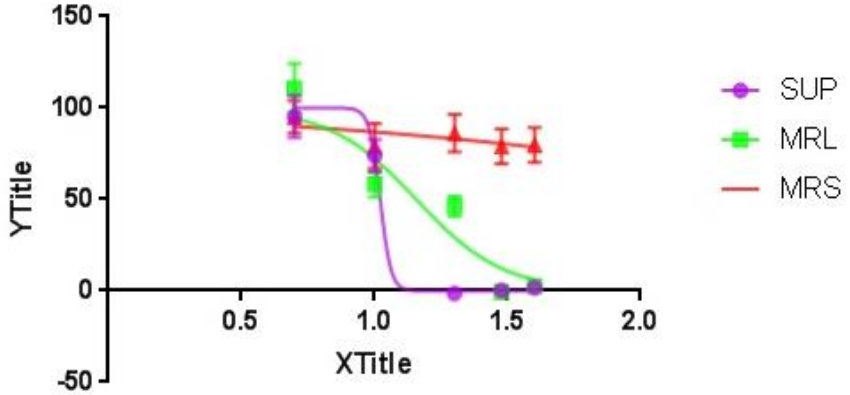

Fig. 1. Inhibitory effect of *Lactobacillus paracasei* TD3 on HepG2 cell growth. Y-axis is indicating cell viability percentage (%) whereas X-axis is showing the $\log_{10}$ of bacterial supernatant concentration; SUP: supernatant, MRS: Man Rogosa Sharpe, MRL: MRS broth adjusted with lactate.

4. Discussion

Due to the possibility of including probiotics in dietary schedules and regimens and even as an ingredient of routine foods as well as various types of cakes and confectionaries, their nutritious values in prevention and control of different human diseases cannot be negligible. In this regard, various studies have investigated the effects of different strains of

probiotics on cancer cells growth and toxicity to assess their potential as an anticancer supplement or maybe therapeutic drugs in adjuvant therapy. Herein, it was primarily shown that HCC cells toxicity has been increased in higher concentrations of *L. paracasei* TD3 treatment. Intestinal microbiome could affect liver health and function through the liver-gut axis and transportation of lipopolysaccharides (LPS) absorbed from pathogenic bacteria via the portal vein which can be modulated by probiotics microbial flora (10). To the best of our knowledge, the literature on the treatment of HCC cell lines with probiotics are limited and their potential as either preventive or therapeutic food supplements is only its primary focus (11). The capability of cancer cell growth confinement by *Lactobacillus paracasei* alone or in combination with other chemotherapeutic drugs has been replicated in many studies performed on other cancer cell lines. Faghfoori et al. (12) in the study on the treatment of Lactobacillus isolated from yogurt including *L. paracasei* on human colon cancer cell lines (HT-29 and CACO-2) have demonstrated that cancer cells viability has been significantly decreased compared to control cells. Chang et al. (13) have shown the same results when treated with another colorectal cancer cells (CT26) with *L. paracasei* combined with 5-fluorouracil (5-FU). In another study that performed on erythroleukemia derived cancer cells (K562 cell line), significant suppression of cancer cells proliferation has been shown to be induced by *L. casei* and *L. paracasei* treatment (14). Hu et al. (15) also have demonstrated that *L. paracasei* caused cell cycle arrest and induced apoptosis in colorectal cancer cells through activation of reactive oxygen species (ROS) meanwhile decreasing in antioxidant enzymes as well as superoxide dismutase (SOD). 14 Duarte et al. also have shown promising inhibitory effects on human breast cancer cell lines (T47D and MDA-MB-231) induced by treatment with *L. paracasei* (16).

5. Conclusion

Given that most studies investigated the effects of *L. paracasei* on cancer cell inhibition have found hopeful results, the next steps will be determining active components of bacterial supernatant with anticancer characteristics. In this way, elucidating the involved signaling pathways and enrolled genes not only will describe the molecular mechanisms behind *L. paracasei* anticancer effects but also may reveal novel active molecules in the carcinogenesis process with the potential of targeting. Further investigation in the aforementioned horizons is strongly warranted to be performed on HCC cells and animal model to precisely determine the anticancer potential of *L. paracasei* as an active bacterial constituent of dairy products.

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