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Article

Selective Cytotoxic effect of Probiotic, Paraprobiotic and Postbiotics of *L.casei* strains against Colorectal Cancer Cells: *Invitro* studies

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This study highlights the cytotoxic effect of three *L. casei* strains on colorectal cell lines in *invitro* conditions. Different concentrations of live, heat killed (HK) and cell free supernatant (CFS) of three *L.casei* strains were subjected to CaCo2 and MRC5 cell lines. The viability of the treated and untreated cells was determined after 72 hrs by MTT assay, and IC_{50} estimated. Apoptosis was evaluated by Annexin V-propidium iodide method using flow cytometry. The live, HK and CFS of the *L. casei* strains showed cytotoxic effects on colorectal cell lines with significant differences. The cytotoxicity effects of live cells on CaCo2 cells were significantly higher ($p<0.01$) than the HK cells. A dose dependent response was observed, as higher concentrations resulted in enhanced cytotoxicity effects. Live *L.casei* 1296-2cells inhibited 91% of CaCo2 cell growth, with IC_{50} of less than 10^8 cfu/ml. MRS medium and concentrations of CFS at above 20% v/v, were cytotoxic to the normal cell lines. Flow cytometry analyses of *L. casei* 1296-2 indicated that cytotoxicity effects on CaCo2 cells is related to apoptotic induction. *Invitro* studies indicate that Live and CFS of *L. casei* 1296-2 might be promising candidate for the control of colorectal cancers.

Keywords: *Lactobacillus casei*. Probiotic. Paraprobiotic. Postbiotics. Colon cancer. MTT Assay. Apoptosis. Flowcytometry.

INTRODUCTION

Lactic Acid bacteria (LAB) are the dominant flora residing in the gut of a healthy individual that are recognized as GRAS (generally recognized as safe). Majority of the LAB species are widely used as probiotics in a number of food products and supplements (FAO/WHO, 2006). Probiotic refers to the live bacteria and yeast that provide health benefits to the host when are taken in adequate amounts (Mehra *et al.*, 2012; Howarth, Wang, 2013). Probiotics are also termed as "immunobiotics" as they regulate the immune system of the host (Benson *et al.*, 2010, Spor, Koren, Ley, 2011; Becattini, Taur, Pamer, 2016). Among a number of therapeutic effects correlated to the probiotic LAB, their anticancer and anti-tumor activities are widely studied (Sharma, Shukla, 2016; Kahouli, Tomaro-Duchesneau, Prakash, 2013). The occurrence of cancer is prevented by these bacteria by (i) lowering PH,

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(ii) inhibiting the growth of microbiota involved in the production of carcinogens (iii) reducing the level of pro-carcinogenic enzymes (Donaldson, 2004), (iv)enhancing cell proliferation by inhibiting normal cell apoptosis and by promoting cell differentiation and cytoprotective activities (Lin *et al*., 2008), (v) suppressing inflammation-induced cell apoptosis (Prisciandaro et al., 2011), (vi) enhancing innate immunity, (vii) promoting various gut homeostasis (Tiptiri-Kourpeti *et al*., 2016) and (viii) displaying antioxidant activity (Zhong, Zhang, Covasa, 2014).

Colorectal cancer (CRC) is one of the most important causes of cancer related deaths. Diet is known to play significant role in the pathogenesis of CRC, among which red meat and animal fats are the main enemies. On the other hand, reports have indicated that fruits and vegetables might have preventive effects on the CRC. With the recognition of importance of diet in the control and prevention of a number of diseases, the demand for functional foods that are claimed to have health benefits are highly sought for (Aghajanpour, 2017). In this context, probiotic food products are also of importance as the beneficial bacteria in these products can manipulate the microbiome of the gut in a manner leading to desired health outcomes. A number of studies have indicated that probiotic lactic acid bacteria can lower the risk of a number of cancers. However, the anti-cancer effects of these bacteria is yet controversial and still more experimental studies are required to confirm these effects of probiotic bacteria (Spor, Koren, Ley, 2011; Becattini, Taur, Pamer, 2016; Jacouton *et al*., 2017; Faghfoori, Pourghassem Gargari, Gharamaleki, 2015).

The non-viable probiotic bacterial cells are regarded as "Paraprobiotics" or "Probiotic ghost cells" (Clifford, 2010). Similar to live probiotic cells, the dead cells of probiotic bacteria are known to bring about a number of biological responses in the hosts. Although, their exact mechanism of action is yet not fully explored but they are believed to provide health benefits by the ability of their cell wall and other cellular components to boost the immune system, and inhibit the pathogens by adherence to the intestinal walls etc. (Murosaki, 2000). Furthermore, the responses exerted by these live and dead bacteria might also be due to the secretory metabolites released in the cell free supernatant fluids by either the live bacteria or released after the cell lysis, respectively (Aguilar-Toalá *et al*., 2018). These secretory metabolites released by the probiotic bacteria are often termed as "postbiotics" or "metabiotics" and are known to exert beneficial effects in the gastrointestinal tract of the host (Forsyth, 2009). Organic acids, bacteriocin, and H_2O_2 are some of these metabolites from probiotic bacteria that have significant role in decreasing the viability of colorectal cancer cells and the induction of apoptosis by influencing different signaling pathway (Tiptiri-Kourpeti *et al*., 2016).

Owing to the reports available on the cytotoxicity effects of a number of probiotic bacteria, their dead cells and secretory metabolites, in this study we aimed to evaluate the live, dead and cell free supernatant fluid of three probiotic *L. casei* strains, for their i*nvitro* cytotoxicity activity.

MATERIAL AND METHODS

Bacterial culture

Three probiotic *Lactobacillus casei* strains; Razi Type Culture Collection (RTCC) No 1296-1, RTCC 1296-2 and RTCC 1296-3 obtained from Razi Vaccine and Serum Research Institute, Iran, were used in this study. The bacterial cells were grown on MRS broth (Merck, Germany) at 37˚C for 24h under microaerophilic conditions. Stock cultures were maintained by freezing the bacterial suspensions in 20% glycerol at -70 ºC. The cultures were activated by sub-culturing the stock cultures twice in fresh BHI broth at mentioned condition.

Preparation of Probiotic, Paraprobiotic and Postbiotics suspensions

Overnight bacterial cultures grown to the density of 10^9 CFU/ml, were centrifuged (4000 g 20 min) and the collected pellets washed twice with phosphate buffer (PBS, PH=7.2). The washed cells were lyophilized (Martin Christ, GmbH, Germany) and stored until use. For further use, different concentrations (10^7,10^8,10^9 CFU/ml) of the dried bacteria were made in DMEM medium with 10% FBS.

Paraprobiotics were prepared by autoclaving (121˚C, 15min) the freshly grown bacterial culture (10^9 CFU/ml) and collecting the cell debris by centrifugation as above. The inactivated samples were checked for any growth

by plating a drop of the prepared suspension on MRS agar plate. No growth after 48 h confirmed complete inactivation. The cell debris were lyophilized and desired concentrations were prepared for the viability assay.

Postbiotics were obtained by collecting cell –free supernatant (CFS) fluids from the overnight *L.casei* cultures, respectively. The culture (10^9 CFU/ml) were centrifuged (4000g, 20min, 4˚C) and the collected supernatant were filtered using 0.22 µm filter, lyophilized and desired concentrations prepared as above.

Cell lines

Human colorectal adenocarcinoma cancer (CaCo2 ATCC®HTB37™) cells and a normal cell line (MRC5 ATCC® CCL171™) were obtained from Cell Bank at Razi Vaccine and Serum Research Institute, Iran, respectively. The cell lines were cultured in DMEM medium (Sigma, Germany) containing 10% Fetal Bovine Serum (FBS), 1% (v/v) penicillin- streptomycin solution and incubated at 37˚C in humidified atmosphere with 5% CO_2 for a maximum of two weeks till complete differentiation or 70-80% confluence achieved. Cell were detached using Trypsin–EDTA (Sigma, Germany) for further use.

Assesment of cell viability by MTT assay

For quantitative measurement of viable cells, MTT was used which is based on the reduction of the tetrazolium salt, 3-(4,5- dimethyltiazole-2-yl)-2,5-dephenyltetrazolium bromide) by metabolic active cells. MTT assay was performed according to the method of van de Loosdrecht, et al (1994), with minor modifications. In brief, the respective cell lines were seeded individually in 96 well plates (10^4cells/well) in 100µl of standard medium and incubated overnight. After 24 hrs, different concentrations of the live (10^7, 10^8, 10^9 CFU/ml), HK and CFS (5, 10, 20,30,40,50 %) respectively, were added into the wells and incubated further for 24-48 hrs. For control, MRS broth was added to the wells instead of the prepared fractions. Later, 20 µl of 5 mg/ml MTT (Sigma, St. Louis, MO) was aseptically added to each well, and plates incubate for 3.5 hours at 37ºC. Control included wells with MTT without the cells. The media was removed carefully, and 150 µl of MTT solvent was added to the wells and the plates covered with tinfoil and agitated for 15 min on an orbital shaker. Absorbance at 570 nm was measured by ELISA reader and the cellular viability was estimated as the percentage of survival relative to the control cells, according to the following formula:

$$\%Viability = [(OD_{570} \text{ of treated} - OD_{570} \text{ of blank})]/ [OD_{570} \text{ of control} - OD_{570} \text{ of blank})]*100$$

Apoptotic detection by Flow cytometry

For quantitative measurements of apoptotic cells, Annexin V-FITC apoptosis detection kit (abcam, USA) were used. The CaCo2 cells ($2x10^5$cells/well) were seeded into a six –well culture plate and treated with live, HK and CFS of *L. casei* RTCC 1296-2. After 72 h, the treated/ untreated control cells were detached by trypsin and centrifuged (900g for 10 min at 28˚C). The collected cell pellets were suspended in 500µl of 1x Annexin V. binding Buffer and later, 5µl of Annexin V-FITC and 5µl propodium Iodide were added to the tubes. All tubes were incubated in dark at room temperature for 5 min and the cells analyzed by flow cytometry. The data were analyzed with Flow Jo software version 9.7.5.

Statisticall analysis:

The experiments was performed in triplicate and data were analyzed using ANOVA, which was performed according to SPSS (ver. 20/0). Duncan's multiple ranges test were used to determine the significant differences among the means ($P<0.05$). Values are expressed as mean ± Standard Deviation (x ±SD).

RESULTS

Three probiotic *L. casei* strains were studied for their cytotoxic effect and apoptosis induction in colon cancer cell lines in *invitro* conditions, when used in different concentrations. Different concentrations of the live (probiotic), the dead cells (paraprobiotic) and the cell free supernatant fluid (postbiotic) from the mentioned strains were tested for their cytotoxic effects on colorectal cancer (CaCo2) and normal (MRC5) cell lines.

Table I indicates the cell viability of MRC5 and CaCo2 cell line after 72 hours of exposure to different concentrations (cfu/ml) of the live probiotic bacterial strains, measured by MTT assay. The basis of MTT assay depends on the enzymatic reduction of the tetrazolium salt that is brought about by metabolic active living cells and not the dead cells. According to the results, the actively growing cells of all three *L. casei* strains showed varied level of CaCo2 growth reduction, while all showed in-significant cytotoxicity effects ($P<0.01$) on the tested normal cell line (MRC5). Moreover, a dose dependent response was evident, and the cytotoxicity effects were enhanced with increasing concentrations of the bacteria. *L.casei* 1296-2 at the concentrations of 10^9 cfu/ml resulted in maximum reduction in the cell viability of tested colorectal cell line, compared to lower used cell concentrations. The viable cultures of 1296-1, 1296-2 and 1296-3, at the concentration of 10^9 CFU/ml showed significant ($P<0.05$) reduction in the viability of CaCo2 cell lines (21.91, 9.90 and 27.83%), respectively. The IC_{50} valve for these cells was less than 10^8 CFU/ml. However, the MRC5 cells appeared significantly un-effected by all tested concentrations of the live bacterial cells. The minimum cytotoxicity on the normal cell lines was seen at 10^7 cfu/ml of all three tested live cells ranging from 96 to 98 % viability.

Table II depicts the cytotoxicity effects of different concentrations of heat killed cells on the normal and colorectal cancerous cell lines, respectively. Similar to live probiotic cells, a dose dependent response was demonstrated by the heat killed cells of the three strains on the tested cell lines. According to our observations, 50% (v/v) concentrations of the thermal treated paraprobiotic cells of RTCC 1296-1, 1296-2 and 1296-3, resulted in 75.20, 86.81 and 66.49 % reduction in the viability of CaCo2 cells, respectively. However, same concentrations of the HK cells had insignificant ($p<0.01$) effect on MRC5 cells and approximately 85 % of the human normal cell lines appeared unaffected. The IC_{50} valve of paraprobiotic *L.casei* 1296-1 and 1296-3 was estimated to be 20% v/v, while this was slightly lower (15% v/v) for 1296-2 ($P<0.05$), respectively.

The CFS of the *L. casei* strains were able to significantly ($P<0.05$) reduce the viability of CaCo2 cells (Figure 1). CFS of *L. casei* 1296-2 at 50 % concentrations showed highest reduction in the viability of the CaCo2 cells, compared to other two strains (1296-1, 1296-3) in study, respectively. The IC_{50} valve of CFS of RTCC 1296-2 strain against Caco2 cells was 5% (v/v). As predicted, the dose dependent effect was also exerted by CFS on the tested cancer cell lines, and highest reduction in the cell viability was seen at highest used CFS concentrations (50% v/v). However, increasing concentrations of the CFS also had increasing cytotoxic effects on the normal MRC 5 cell lines. Only 10% v/v of CFS appeared to be non-effective against the tested normal cell lines and higher doses significantly inhibited the viability of these cells. Same CFS concentrations (10%v/v) of *L. casei* 1296-1, 1296-2 and 1296-3 reduced the viability of Caco2 cell lines by 45.66, 59.57 and 51.75%, respectively. While studying the cytotoxic effect of CFS, we also evaluated the cytotoxicity of MRS medium as the collected CFS was in the MRS broth medium. The cytotoxic effect of MRS medium on both the tested cell lines was seen. This cytotoxic effect was more enhanced on MRC5 ($P<0.05$) cell lines then the colorectal CaCo2 cell lines, respectively. However, lower concentrations (> 20% v/v) of the broth medium showed only 5.1 % cell growth reduction compared to 50% which resulted in approximately 85% reduction. This effect was lesser seen in CaCo2 cell lines, and MRS broth up to 50% percent showed only 25 % cell growth reductions. Compared to the other two *L.casei* strains tested, *L.casei* 1296-2 demonstrated maximum inhibitory effects on the tested cell lines. Figure 2 illustrates the effect of cell free supernatant fluids (postbiotic), heat killed cells (paraprobiotic) of *L.casei* 1296-2 and MRS broth medium on the viability of MRC5 and CaCo2 cell lines, determined by MTT assay.

The apoptosis effects of the fraction on CaCo2 cell line was analyzed by Annexin V-FITC/P1 flow cytometry. Figure 3 represented the apoptotic activity of live (probiotic), HK (paraprobiotic), and CFS (postbiotic) of RTCC 1296-2 on CaCo2 cells. The early and late stage apoptosis for the probiotic RTCC 1296-2, were 44.5% and 18.7 %, respectively. While, HK and CFS fractions showeffectsd 48.4% and 20.6 %; 25.4% and 23.3% for early and late stage apoptosis, respectively. The untreated CaCo2 cells showed 14.4% and 4.9% during early and late apoptosis stage, respectively.

TABLE I - Cell viability of MRC 5 (a) and CaCo2 (b) cell lines after exposure to different concentrations of live (Probiotic) *L. casei* strains, after 72 hrs by MTT assay

(a)

***L.casei* strains**	**Colony forming unit per ml**		
	10^7	**10^8**	**10^9**
1296-1	98.52+1.20[a]	95.41±1.20[a]	91.60±1.90[a]
1296-2	96.70±1.28[a]	93.16±0.93[a]	90.60±2.00[a]
1296-3	97.31±0.81[a]	94.23±1.41[a]	92.38±1.04[a]

(b)

***L.casei* strains**	**Colony forming unit per ml**		
	10^7	**10^8**	**10^9**
1296-1	78.19+1.93[b]	38.19±1.92[b]	21.91±2.09[b]
1296-2	60.40±1.70[a]	30.61±2.07[a]	9.90±2.35[a]
1296-3	71.31±2.09[c]	43.26±2.21[c]	27.83±2.11[c]

Means ± SD within a column with the same uppercase letters are not significantly different at $P < 0.05$.

TABLE II - Cell viability of MRC 5 (a) and CaCo2 (b) cell lines after exposure of different concentrations of heat killed bacterial cells (Paraprobiotics) of the three *L.casei* strains, after 72 hrs by MTT assay

(a)

***L.casei* strains**	**Concentrations % (V/V)**					
	5	**10**	**20**	**30**	**40**	**50**
1296-1	98.91+2.03[a]	95.22±2.16[a]	92.10±2.63[a]	89.92±2.95[a]	86.07±1.95[a]	84.50±2.42[a]
1296-2	98.18±1.90[a]	96.15±2.18[a]	93.91±2.43[a]	91.20±2.39[a]	87.16±2.58[a]	85.90±2.08[a]
1296-3	97.00±1.14[a]	93.80±2.86[a]	90.76±2.26[a]	87.90±2.42[a]	85.37±3.10[a]	83.55±2.74[a]

(b)

***L.casei* strains**	**Concentrations % (V/V)**					
	5	**10**	**20**	**30**	**40**	**50**
1296-1	75.71+2.75[b]	61.63±3.04[b]	92.19±3.95[c]	40.10±3.20[b]	30.07±3.43[b]	24.80±2.90[b]
1296-2	69.25±3.03[a]	53.90±2.91[a]	40.44±2.84[a]	30.30±2.49[a]	19.96±3.28[a]	13.19±3.06[a]
1296-3	80.63±2.83[b]	93.85±3.20[c]	58.90±2.98[b]	48.10±3.30[c]	37.62±2.73[c]	33.51±4.05[c]

Means ± SD within a column with the same uppercase letters are not significantly different at $P < 0.05$.

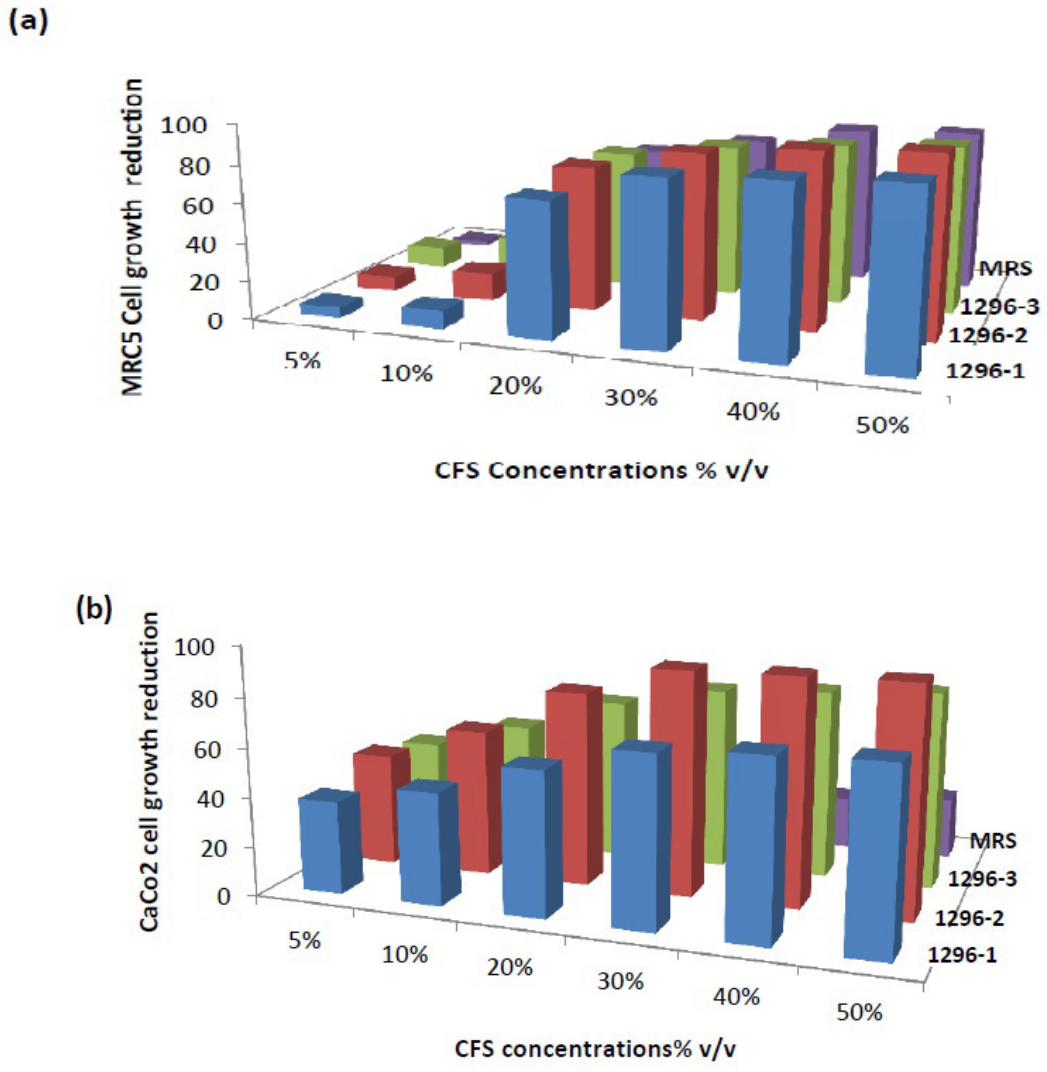

FIGURE 1 - Cell growth inhibition of MRC5 (a) and CaCo 2 (b) cell lines after treatment with different concentrations of cell free supernatant fluids of *L.casei* RTCC 1296-1, 1296-2 and 1296-3 strains and MRS broth, and determining the number of viable cells after 72 hrs by MTT assay. The results were recorded as mean (SD±) of three individual experiments.

CFS: cell free supernatant fluids (postbiotics)

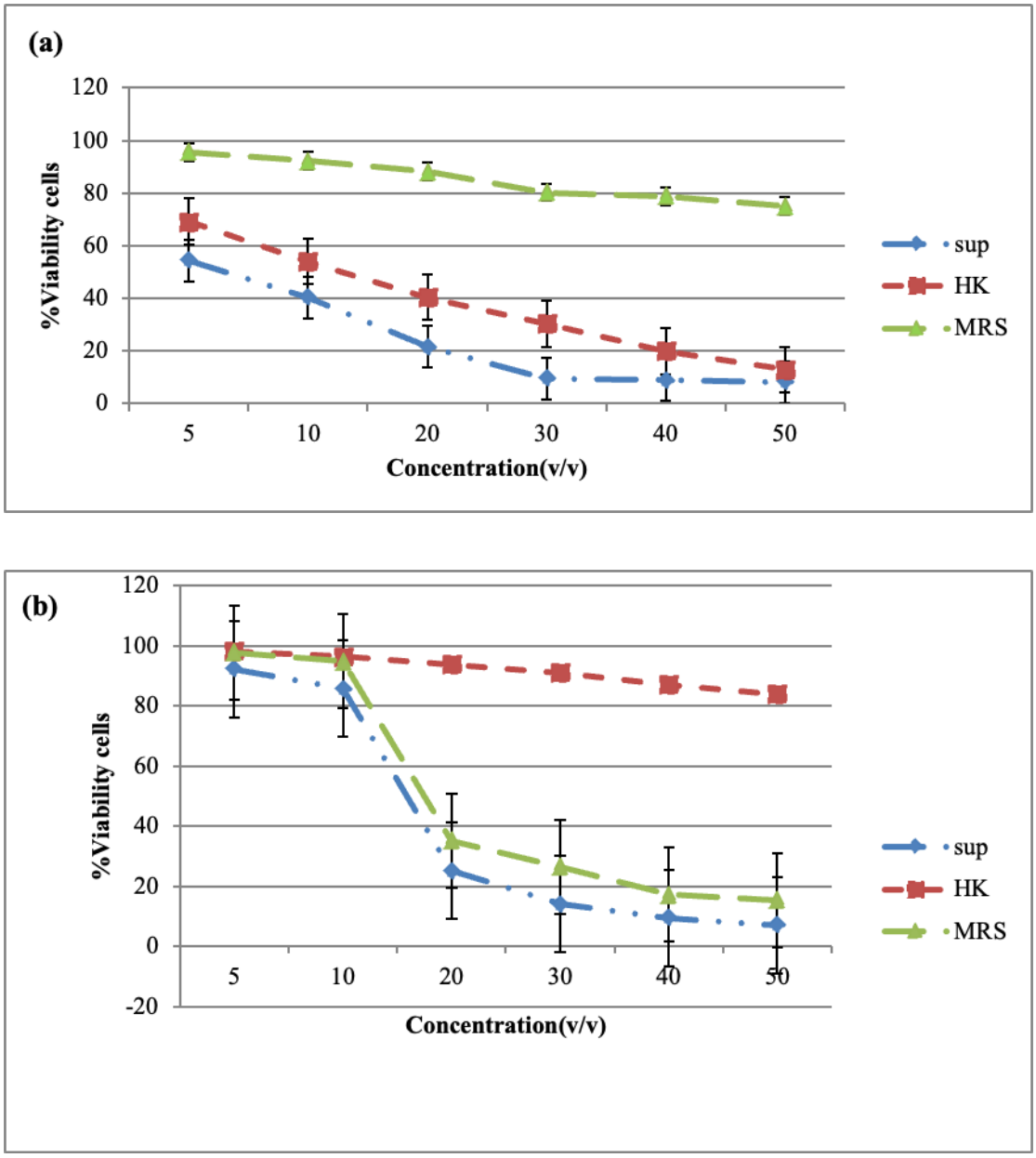

FIGURE 2 - Cell viability of MRC5 (a) and CaCo2 (b) cell lines after treatment with cell free supernatant fluids, heat killed cells of *L.casei* RTCC 1296-2 and MRS medium, analyzed by MTT assay.

Sup: cell free supernatant fluid, HK: heat killed cells; MRS: MRS broth medium.
All results are mean (SD±) of three individual experiments

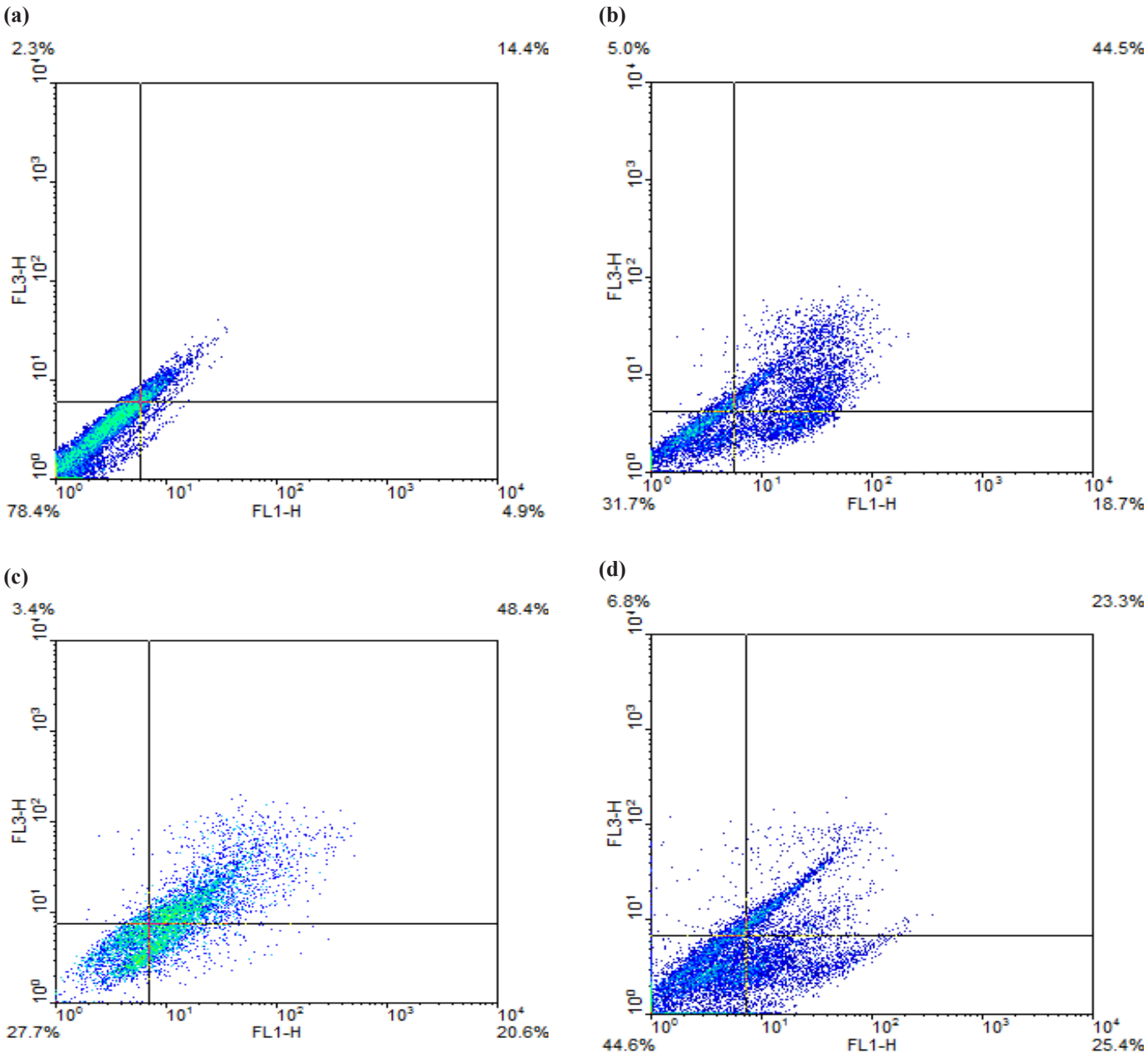

FIGURE 3 - Flow cytometry analysis of untreated (a) and treated CaCo2 cells with 10^8 CFU/ml of live (b), 30% HK *(*c) and 30% CFS (d) of *L.casei* RTCC 1296-2 strain.

DISCUSSION

Colorectal cancer (CRC) is known to affects the colon and rectum of both man and women (Concetta, Andriulli, Pazienza, 2018). According to World Health Organization (WHO), by 2030 approximately 75 million people would be affected by CRC (Salva, Alvare, 2017). The most common treatment options for CRC includes surgery, radiotherapy and chemotherapy. However, these treatments are known to have limitations several side effects which has provoked researchers to search for alternative treatment strategies for developing novel safe and more effective interventions to combat CRC. In this context, probiotics, prebiotics, synbiotics, paraprobiotics and metabiotics or postbiotics are the most widely studied alternatives (Lee, Seto, Bielory, 2008; Fotiadis *et al*., 2008; Orlando *et al*., 2009).

In this research work, strong evidence supporting the anti-colon cancer effects of *L. casei* strain was collected. Although all tested fractions (live, dead and cell free supernatant fluids) of the three *L. casei* strains showed cytotoxicity effects against CaCo2 cell lines, but the level of cell inhibition varied among the strains of same species. Furthermore, a dose- dependent cytotoxic effect was observed, and increasing concentrations of the fractions showed increasing cytotoxicity effects in CaCo2 cells. In agreement with these findings, some LAB strains have been reported to possess dose dependent response for their anticarcinogenic abilities (Salminen *et al*., 1998; Sevda, Koparal, Kivan, 2015).

Live probiotic cells influence both the gastrointestinal microflora and the immune response whilst the components of heat killed cells exert an anti-inflammatory response in the gastrointestinal tract (Clifford, 2010). The cell wall components of *Lactobacillus spp*. are known to stimulate the inflammatory reaction involving macrophages in the mammalian gastrointestinal tract (Baken et al., 2006, Oelschlaeger, 2010). Similar findings were reported by another group of researchers (Faghfoori, Pourghassem Gargari, Gharamaleki, 2015), who described that live and HK-sonic protein of *L. casei* induced cytotoxicity effects *invitro* on CT-26 and HT-29 cells and were able to reduce the viability of murine CT-26 (colon cancer cell line) and human HT-29 (colon cancer cell line). From these reports, it is obvious that both live and HK cells in probiotic products can generate a wide range of biological responses that are mainly immunomodulatory effects (Clifford, 2010). Similar to these findings, the cytotoxicity and pro-apoptotic effects of the live and dead bacterial cells of *L. casei* strains was seen on CaCo2 cell lines. Although the HK cells were able to inhibit the CaCo 2 cell viability, but their cytotoxicity effect was significantly lower than the viable cells. The cytotoxicity of HK cells of *L. rhamnosus GG* on cancer cell lines has been reported earlier (Choi, 2006). According to the reports, *L.GG* triggered cytotoxicity of Caco2 and HT-29 colon cancer cells and were able to reduce the cell viabilities to 73 and 62.7 % at the highest used concentration, respectively.

Postbiotics, are referred to the metabolites secreted by the probiotic bacteria during metabolism. These metabolites are known to maintain homeostasis in the gut leading to a decrease in nitroreductase, β-glucuronidase and β-glucosidase enzymes that inhibit the conversion of procarcinogens into carcinogens (Verma, Shukla, 2013). Apart from these, the short chain fatty acids (SCF) in the postbiotics are known to induce chemo-preventive enzymes glutathione S transferase and Glutathione transferase pi (Scharlau, Borowicki, Habermann, 2009; Johnson *et al.*, 2012). Comparable to these reports, our studies indicated the cytotoxic effect of the CFS samples on the colon cancer cell lines. At the concentrations of 20% v/v, the CFS of *L. casei* 1296-2 showed significant viability loss (60.8%) of CaCo 2 cells, compared to the CFS of 1296-3 and 1296-1 strains, respectively. Orlando *et al*., (2009) reported cytotoxic effects of *L.GG* on gastric and colon cancer cells. They found that *L.GG* homogenate and cytoplasm extracts reduced the percentage of cell viability to nearly 55 and 65 % on CT-26 and HT-29, in colon (DLD-1) and gastric (HGC-27) cancer cell lines. Another published report showed that the CSFs produced by *L. fermentum* NCIMB 5221 had significant cytotoxicity effect against CaCo2 (60%, 72h, $p<0.001$) compared to the control cells (Kahouli *et al*., 2015).

One of the most important factors in cancer therapy is that the therapeutic agent poses least damage to the host normal cells and tissues (Motevaseli, Dianatpour, Ghafouri-Fard, 2017). *L. acidophilus* 36YL strains metabolites studied on HeLa, MCF-7, AGS and HT-29 cells and compared to the normal cells (HUVEC), showed that the metabolites of this bacteria decreased viability of the cancerous cell lines while had no toxic effect on the tested normal cells. In contrast to these reports, we observed that high concentrations (<20% v/v) of CFS have cytotoxic effects on MRC-5 cells (normal cell line). However, this undesirable effect might be attributed to the MRS broth medium components and not the secretory metabolites of the tested probiotic strains, as MRS broth itself showed cytotoxicity in the normal cell lines. The presence of acetate in the MRS broth medium is probably one of the reasons for its cytotoxic effects (Marques *et al*., 2013). Hence, in order to pass this obstacle, careful consideration for selecting the appropriate culture medium with strict monitoring of media components is essential. Additionally, under such conditions we might

suggest use of lower doses of CFS of *L. casei* 1296-2 for treating colorectal cancer cells, as 20% of the CFS reduced CaCo2 cell growth by 78.4%, compared to only 14% reduction of normal cells, respectively.

The apoptosis assay performed by others (Soltan Dallal *et al.*, 2015) showed that CFS of a number of probiotic lactobacillus strains reduced cell proliferation of CaCo2 cell lines and showed increased cell apoptosis leading to cell necrosis. Triptiri-kourpeti and his co-workers (2016), by flow cytometry showed that the live *L. casei* (10^8 and 10^9 CFU/ml) cells induced apoptotic cell death in HT-29 and CT-26 colon cancer cell lines. In consistent with these reports, our results showed that *L.casei* RTCC 1296-2 strain while reducing the cell viability of CaCo2, also showed increased cell apoptosis leading to cell necrosis.

In conclusion, the results of this study demonstrated that live and CFS of *L.casei* strains 1296-2 are more effective on CaCo2 cell lines than their HK cells, and most probably the mechanism of the prophylactic effect is related to the apoptosis induction in the mentioned cancer cells. The selected strain might be a promising candidate for prophylactic or therapeutic purpose for the control of CRC. However, further detailed investigation including *invivo* models are required for assuring these results.

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