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ORIGINAL ARTICLE

Saccharomyces cerevisiae inhibits growth and metastasis and stimulates apoptosis in HT-29 colorectal cancer cell line

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Abstract

Colorectal cancer (CRC) is one of the leading causes of cancer-related death worldwide. Accumulating evidence has suggested that probiotics affect cellular pathways and specific genes involved in slow growth. Probiotics reduce the impact or stop the growth of cancer cells and tumors in animal models and human cell lines. The aim of this study was to investigate the effect of *Saccharomyces cerevisiae* (*S. cerevisiae*) on cell growth, metastasis, and apoptosis of the HT-29 colon cancer cell line. The HT-29 cells and *S. cerevisiae* were co-cultured in order to study the effects of *S. cerevisiae* on cell apoptosis, growth, and metastasis using 4′,6-diamidino-2-phenylindole (DAPI) staining, 3-(4,5-dimethylthiazoyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and scratch wound-healing assay, respectively. Real-time PCR was applied to evaluate the expression levels of Akt/PTEN, MAPK, and NF-κB genes. The supernatant obtained from *S. cerevisiae* in HT-29 cell line increased the expression of *PTEN* and *Caspas3* genes in the first 24 h while the *Bclxl* and *RelA* genes showed decreased expression. By using MTT method after 48 h of treatment HT-29 cells with supernatant of *S. cerevisiae*, about 75% of the cells showed stopped growing. Therefore, it could be concluded that *S. cerevisiae* inhibits the growth of the HT-29 cells by inducing apoptosis and reducing metastasis.

Keywords Apoptosis · HT-29 · Probiotics · *Saccharomyces cerevisiae*

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Background

Cancer occurs as a result of uncontrolled cell division caused by multifactor agents, such as environmental factors and genetic disorders (Benchekroun et al. 2010). Colorectal cancer can inflict the entire length of the rectum and colon. CRC is the third common cause of cancer-related death (dos Reis et al. 2017). It has been estimated that about 24.4 million new cases of CRC will be annually detected, by 2035 (Organization WH, Unit WHOMoSA 2014). Resistance to apoptosis and deregulation of cell proliferation are the major features of cancer cells. The agents that trigger apoptosis in cancer cells can be applied as anti-cancer drugs. However, resistance to chemotherapy has become a major problem in CRC treatment. It has been demonstrated that gastrointestinal cancers can be induced by the consumption of dietary products; therefore, some scientists have been attracted to investigate the impact of consuming dietary products on the health of the individuals. Probiotics are non-pathogenic organisms that are present in the human digestive system and exert beneficial effects on their host by affecting the microbial flora of the body. It has been found that certain probiotics exhibit anti-cancer activity

(Daniluk 2012; Foo et al. 2011). Some studies conducted on human have previously proved the anti-cancer activity of probiotics (Commane et al. 2005). Numerous findings have indicated that probiotics may regulate cell division and apoptosis (Elmore 2007). *Saccharomyces cerevisiae*, as a species of yeast, has been widely used in the production of alcoholic beverages, waffles, and baking since ancient times (Feldmann 2011). Many of the major biological proteins, including cell cycle proteins, signaling proteins, and the processing enzymes, that have been initially found in human resemble those of the yeast (Walker et al. 2004). The HT-29 cell line is a human colon adenocarcinoma cell line, which provides a desirable experimental system for studying the effective factors in the differentiation of epithelial cells. These cells form nonpolar layers, when cultured under standard conditions. These morphological characteristics of HT-29 cells make them a suitable model for studying various cell signaling pathways, as well as therapeutic agents or approaches. The polarized phenotypes of the cells are characterized by physiological biochemical markers as well as their specific structure (Cohen et al. 1999). *PTEN* (phosphatase and tensin homolog deleted on chromosome ten), a tumor suppressor gene, plays an important role in cell growth, proliferation, and migration (Saito et al. 2003). AKT, protein kinase B, is a hyperactivated kinase protein found in many tumors. AKT is a key player in both cell survival and resistance to tumor treatment. A recent study has reported that AKT cannot function unaided; however, it plays an important role in inhibiting cell death under severe conditions. Phosphorylation of the AKT gene inhibits the apoptosis pathway (Haier and Nicolson 2002). NF-κB is a protein set that controls DNA transcription, cell survival, cytokine production, and the regulation of immune response to the cancer. It is a heterodimer activator of transcription, which contains the DNA-binding subunit P50 and exuviates the trans-subunit p65/RelA (Vasudevan et al. 2004). The Bcl-2 family proteins, which include both anti- and pro-apoptotic proteins, regulate apoptosis; the Bcl-XL protein is a member of the bcl-2 family that prevents apoptosis.Therefore, extremely small changes in the physiological levels of these proteins may result in either inhibition or promotion of cell death. Abnormal activation of the Bcl-2 gene appears to be an early event in colorectal tumorigenesis that can inhibit apoptosis and may facilitate tumor progression (Sinicrope et al. 1995; Ola et al. 2011). Caspases (CASP) play an essential role in induced apoptosis (Kim et al. 2000; Jänicke et al. 1998; Yakovlev et al. 2001). Studies have demonstrated that activation of CASP3 induces apoptosis of HT-29 cells (Yang et al. 2006). The expression levels of the above mentioned genes were examined in the present study using realtime PCR. Our results indicated that the supernatant obtained from *S. cerevisiae* exerted beneficial effect in the HT-29 cell line by inducing apoptosis and reducing cell growth as well as metastasis.

Methods

Cell culture

The HT-29 cancer cells were obtained from the Pasteur Institute's cellular storage (Iran) and stored at − 196 °C. Briefly, the HT-29 cells were cultured in 1640 RPMI, containing penicillin and streptomycin and 10% fetal bovine serum (FBS), and incubated at 37 °C in 5% CO_2 (Wright et al. 1999; Nozari et al. 2016).

Yeast culture

The yeast was isolated from the local yogurt of Tabriz followed by determining its strain prior to being transferred to a plate containing the yeast culture media (peptone and distilled water, dextrose 40%, yeast extract, and agar). It was then incubated at 28 °C for 48 h (Cortes 2004). A yeast colony was then transferred to an YPD liquid culture (peptone and distilled water, dextrose 40%, and yeast extract) and was incubated in a shaking incubator for 48 h at 28 °C.

Co-culture of yeast and HT-29

The HT-29 cells were seeded in six-well plates (5×10^5 cells/cm^2 per well) and incubated in 5% CO_2 at 37 °C for 48 h. A single clone of the yeast was then cultivated in 1640 RPMI, containing 10% FBS, and incubated in a shaking incubator at 37 °C for 30 to 60 min. The culture medium containing the yeasts was then centrifuged at 1200 rpm (400*g*) for 5 min. The supernatant was separated, and its optic density (OD) was measured at 600 nm using a Spectrophotometer (Beckman, MO, USA; model DU-530). The above mentioned steps were repeated at various time intervals, and the ODs were measured at 600 nm. The HT-29 cells were cultured in six-well plates and washed twice with 2.5 ml PBS, prior to being treated with the prepared supernatant with specific ODs (0.3, 0.5, and 1), followed by 2 to 6-h incubation period in 5% CO_2 at 37 °C. The supernatant was filtered through 0.22-μm filter before being added to the HT-29 cells (Westwater et al. 2005).

MTT assay

The HT-29 cells were first seeded in 96-well plates (5×10^3 cells/well) and incubated at 37 °C in 5% CO_2 for 48 h. The cultured cells were treated with various concentrations of the *S. cerevisiae* supernatant (0.5, 0.25, 0.125, 0.0625, and 0.031 mg/ml). 5-Fluorouracil (5-FU) treated HT-29 cells were used as the positive control. The untreated cells were applied as the negative control. Half of the

culture medium in each well (100 μl) was replaced with 100 μl of various concentrations of the *S. cerevisiae* supernatant. All the treatments were performed three times in triplicate. The cells were incubated at 37 °C and 5% CO_2 for 12, 24, 48, and 72 h. The MTT assay was then performed for each treatment group) in this method, the dose-dependent time is used((Bray and Carpenter 2013).

DAPI staining

A total of 5×10^5 cells/cm^2 (HT-29 cells) were seeded in six-well plates and treated with various concentrations of *S. cerevisiae* for 12 h prior to being fixed with paraformaldehyde 4% for 10 min. The fixed cells were then washed three times with PBS and permeabilized with 0.1% Triton X-100 for 10 min. Finally, the cells were washed three times with PBS before being stained with DAPI for 5 min. An Olympus IX81 Inverted Fluorescence Microscope was applied to visualize the cells and acquire images at × 40 magnification (Sabnis n.d.).

Scratch assay

In vitro scratch assay was performed to evaluate the cell migration capability in a two-dimensional space. The cells were seeded in six-well plates (2×10^5 cells/cm^2) and kept in culture to obtain confluent monolayers. The HT-29 cells were treated with supernatant of *S. cerevisiae* for 4, 24, and 72 h. The cells were then scratched using a scraper across the layer followed by two washing steps with PBS in order to remove the floating cells and cellular debris. The culture media were replaced with a serum-free medium (or 1% FBS) to inhibit cell proliferation. Images were then acquired periodically from the same field at 0-, 4-, 24-, and 72-h intervals (Liang et al. 2007).

DNA ladder assay

The treated cells were incubated at 37 °C for 24 h. The DNA ladder assay was performed based on a previously published procedure (Rahbar Saadat et al. 2015). Briefly, the treated cells were incubated in a lysis buffer 10 mM Tris (pH 7.4), 5 mM EDTA, and 0.2% Triton at 37 °C for 10 min. The protein lysate was then denaturized with 700 μl of chloroform/isoamyl alcohol (a ratio of 24:1), and the total DNA was separated by centrifugation at 13,000*g*. The total DNA was then precipitated using an equal volume of isopropranol and was electrophoresed on a 1.5% agarose gel containing 1 μl/100 ml SYBR® Safe DNA gel stain. The gel was finally examined and photographed using an ultraviolet gel documentation system.

Evaluation of the molecular mechanism of *S. cerevisiae* on HT-29

Expression analysis of pro- and anti-apoptosis genes in HT-29

Total RNA was extracted from the conditioned media-treated and untreated cells (Sepideh Zununi Vahed et al. 2016). The RNA yield and purity were determined by using a NanoDrop (NanoDrop, Wilmington, USA) instrument (Fleige 2006).

cDNA synthesis

In order to perform stem-loop quantitative reverse transcription and qRT-PCR analysis, 1 μg of each isolated total RNA sample was reversely transcribed using universal hexamer primers and diethyl pyrocarbonate (DEPC) water by incubating the mixture at 65 °C for 5 min followed by incubation on ice. The reaction solution was then mixed with MMLV, dNTP mix, RT buffer, and RNase inhibitor, and the volume of the solution was adjusted to 20 μl with DEPC water. Reverse transcription of the mRNAs was performed at 25 °C for 10 min followed by incubation at 42 °C for 60 min.

Gene regulation study

Real-time PCR was performed to measure the expression levels of the target mRNAs using SYBR Master Mix (Life Technologies Applied Biosystems, UK) and a Bio-Rad IQ5 Real-Time PCR instrument (Bio-Rad, Hercules, CA, USA). Q-RT PCR was performed at 95 °C for 30 s, followed by 45 cycles at 95 °C for 5 s, 60 °C (Table 1) for 30 s, and 72 °C for 25 s. Melt curves were generated at 95 °C to verify specificity of the amplification. To generate the standard curves, qPCR amplification of cDNA was performed using a serial dilution of the cDNA (10^{-1} to 10^{-4}). GAPDH was used as an internal comparator in parallel with the control sample in order to normalize the expression levels of the intended mRNAs. Cycle number (CT) values were used to calculate the relative expression using the difference in the C_T values of the target RNAs after normalization to the RNA input level. The relative quantification was represented by the standard $2^{-\Delta\Delta CT}$ calculations. All the PCR reactions were performed in triplicate.

Statistical analysis

Numerical data were expressed as the mean ± SD. The Excel spreadsheet software (Microsoft, Redmond, WA) was applied for data analysis. Statistical analyses were performed using the Graphpad Prism (version 5; Graphpad Software, San Diego, CA) and one-way ANOVA test, followed by Tukey's multiple comparison test. 뭣 value < 0.05 was considered as statistically significant (Livak and Schmittgen 2001).

Table 1 Details of the primers used for real-time PCR

mRNA/miR	Sequence	Annealing (°C)
PTEN	Forward: 5′-TCGACTACTTGCTTTGTAGA-3′ Reverse: 5′-TTTACAGCCCCGATTGGGCT-3′	60
BCL-XL	Forward: 5′-ACGTGT TAGCTTATCAGACTGA-3′ Reverse: 5′-GCTCTCGGGTGCTGTATTG-3′	60
RelA	Forward: 5′-CCAGACCAACAACAACCCCT-3′ Reverse: 5′-TCACTCGGCAGATCTTGAGC-3′	61
Caspas3	Forward: 5′-TGATGATGTGGAAGAACTTAGG-3′ Reverse: 5′-ACGGCTCCGCACCTGAGGC-3′	60
GAPDH	Forward: 5′-AAGCTCATTTCCTGGTATGACAACG-3′ Reverse: 5′-TCTTCCTCTTGTGCTCTTGCTGG-3′	63

PTEN phosphatase and tensin homolog deleted on chromosome ten

Results and discussion

MTT assay

Viability of the HT-29 cells was significantly reduced in response to treatment with the supernatant obtained from *S. cerevisiae* (Fig. 1). The cell death rate was more than 75% post-treatment with *S. cerevisiae* for 48 h ($P < 0.05$). All the in vitro results were expressed as the inhibition ratio of HT-29 cell proliferation as follows: an ELISA reader was applied to read the absorbance at 600 nm, and the inhibitory effect of *S. cerevisiae* on cell proliferation was then calculated to determine the IC50 values. The following formula was used to calculate the inhibitory rate:

$$\text{Inhibition ratio } (\%) = [(\text{OD control} - \text{OD} - \text{treated})/(\text{OD control})] \times 100\%\text{OD}$$

: *optical density*

Evaluation of the nuclear morphology using DAPI staining

DAPI staining was applied to determine the nuclear morphology and integrity. The capability of the *S. cerevisiae* to induce apoptosis in the HT-29 cells cultured in the conditioned media was investigated using the microscopic analysis of the DAPI-stained cells (Fig. 2). As illustrated in Fig. 3, the control cells showed intact nuclei of uniform shape and size with smooth edges, whereas the nuclei of treated cells showed chromatin condensation and fragmentation.

Evaluation of cell migration using scratch assay

To study the effect of *S. cerevisiae* on the motility of HT-29 cells, scratch assay was performed by measuring the extent of cell migration into the scratched area. The cells were treated with the conditioned media for 24, 48, and 72 h. Cell migration was significantly inhibited in response to the *S. cerevisiae* treatment as shown in Fig. 3.

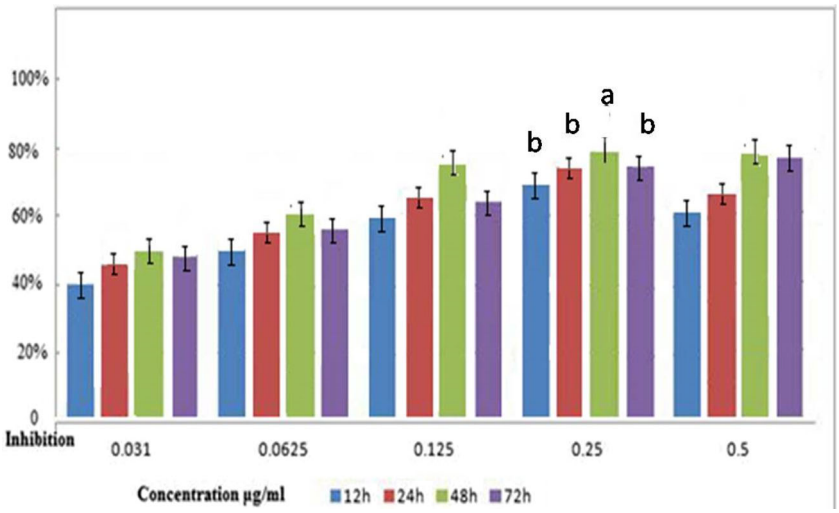

Fig. 1 The inhibitory effects of *Saccharomyces cerevieses* on proliferation rates of the HT-29 cell during various exposure period (12, 24, 48, and 72 h). In this method, the dose-dependent time is used. All the experiments were performed, at least, in triplicate. All the data are presented as mean ($n = 3$) ± 1 standard deviation. Different lowercase letters show intragroup difference ($P < 0.05$)

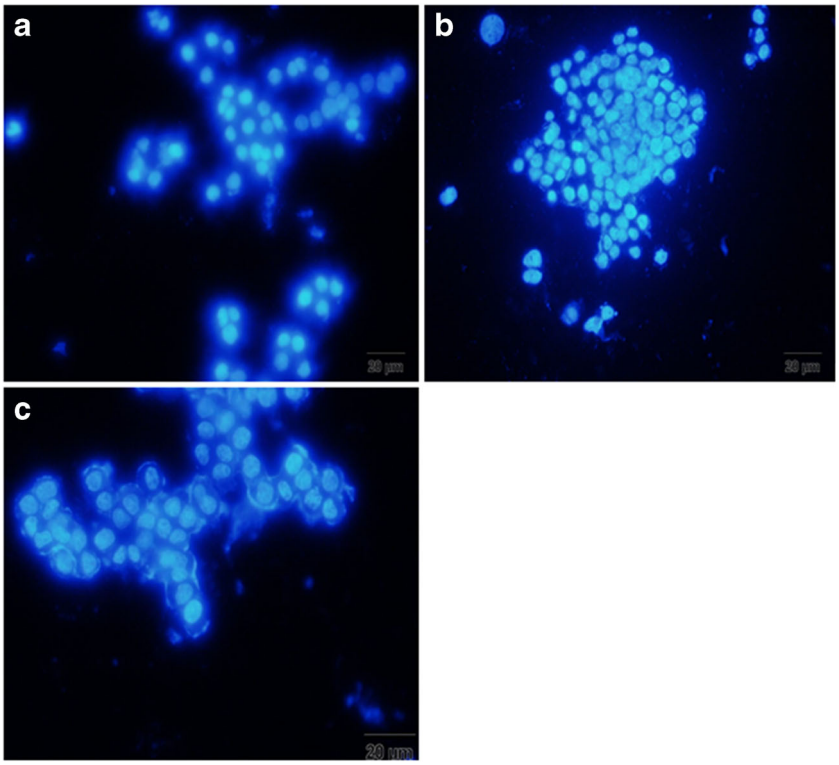

Fig. 2 Effects of *Saccharomyces cerevisiae* on induction of cell apoptosis in the HT-29 cells. The fluorescent microscopy of DAPI-stained HT-29 cells of **a** untreated control cells, **b** treated cells with *Saccharomyces cerevisiae*, and **c** treated cells with 5-FU. HT-29 human colorectal adenocarcinoma cell line, DAPI 4′,6-diamidino-2-phenylindole, 5-FU 5-fluorouracil

Detection of DNA fragmentation by DNA ladder assay

The DNA fragmentation is considered a hallmark of cell apoptosis. The results of the DNA ladder assay demonstrated that treatment of the HT-29 cells with the conditioned media resulted in the formation of the typical DNA ladder pattern (Fig. 4) indicating cell apoptosis.

S. cerevisiae treatment altered the expression of apoptosis genes in the HT-29 cells

To analyze the expression variation of certain pro-oncogenes that are reported to be over-expressed in cancer, their expression levels were measured in the *S. cerevisiae*-treated HT-29 cells 12 and 24 h post-treatment. *S. cerevisiae* significantly downregulated the expression levels of the intended pro-oncogenes in the HT-29 cells. Therefore, *S. cerevisiae* acted as a pro-apoptosis agent (Figs. 5, 6, 7, and 8).

Discussion

The results of the present study indicated that the expression levels of *RelA* and *bcl-XL* genes were significantly downregulated in the HT-29 cells treated with *S. cerevisiae*, while those of *PTEN* and *CASP3* genes were markedly upregulated 24 h post-treatment. CRC is one of the deadliest cancers. Therefore, it has attracted global attention as it results in a high mortality rate (Liu et al. 2015; Ting Shuang et al. 2016; Gaikwad et al. 2015). Cognition of the molecular mechanisms underlying chemo-resistance is a critical step in improving the survival rate of the CRC patients (Sato and Itamochi 2015; Chen et al. 2009). Recent developments in the field of anti-cancer drug discovery are often related to drug design targeting a specific molecule or a signaling pathway. Various targets are used in anti-cancer drug development, including growth signaling cascades, cell division, DNA replication, angiogenesis, and apoptosis processes (Bernardes et al. 2010; Saber et al. 2017). Even though the gold standard of ovarian cancer management is still chemotherapy, employing adjunctive therapies are being rigorously investigated (Maria Muccioli 2014). The microbial-based therapy of cancer is one of the recently developing cancer treatment modalities (dos Reis et al. 2017; Bernardes et al. 2010). The protective and anti-cancer effects of probiotics have been reported in an in vitro, in vivo, epidemiologic, and clinical trials study conducted by Kim et al. The effects of the cellular components of the probiotics on 11 different cancer cell lines have been studied (Maria Muccioli 2014). It has been reported that probiotics significantly inhibited proliferation of the cancer cell lines (Kim et al. 2003). Ghoneum and Gollapudi have investigated the effect of heat-killed *S. cerevisiae*, isolated from yeast tablets, on non-metastatic and metastatic breast cancer cells. They have found that heat-killed *S. cerevisiae* induced apoptosis in their cells of interest (GHONEUM 2004). Moreover, Ravi Subbiah et al. have found that ergosterol (a 28-carbon sterol

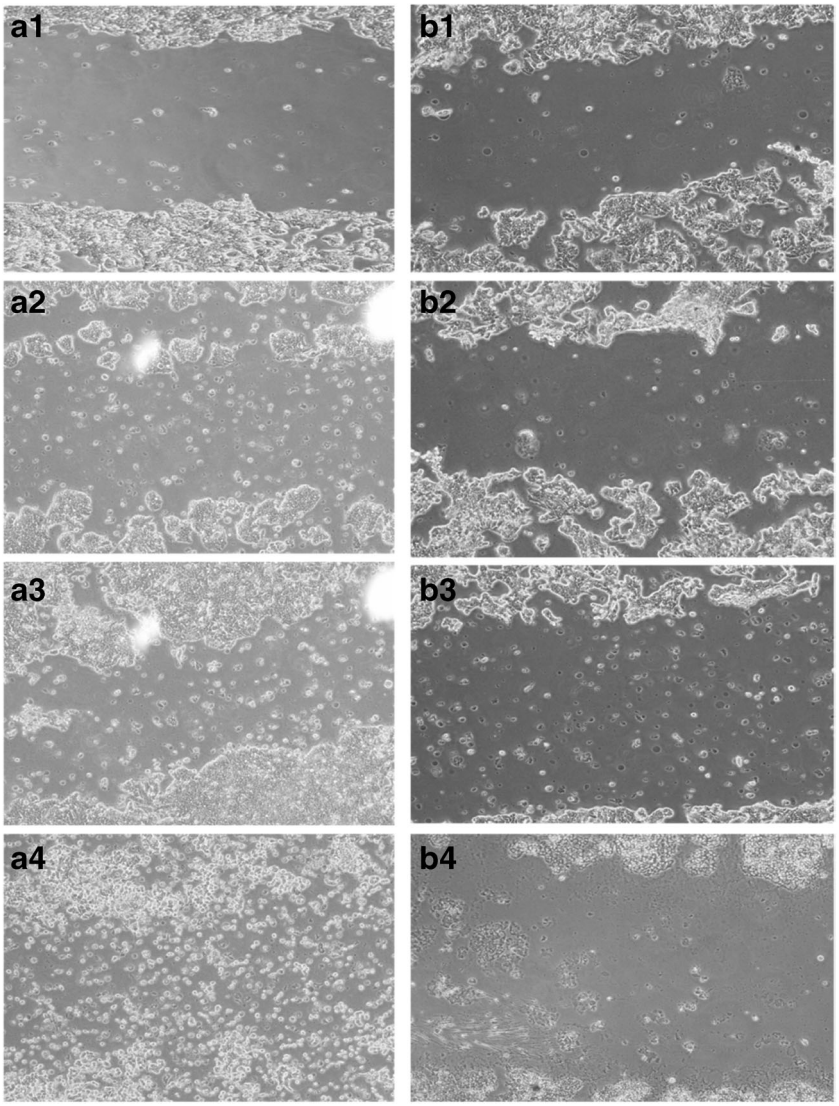

Fig. 3 Cell migration was evaluated using scratch assay. The confluent cells were treated with the supernatant obtained from *Saccharomyces cerevisiae* (containing 1% FBS) for 0, 4, 24, and 72 h. a1, a2, a3, and a4 illustrate untreated cells, while the treated cells are presented in b1, b2, b3, and b4. FBS fetal bovine serum

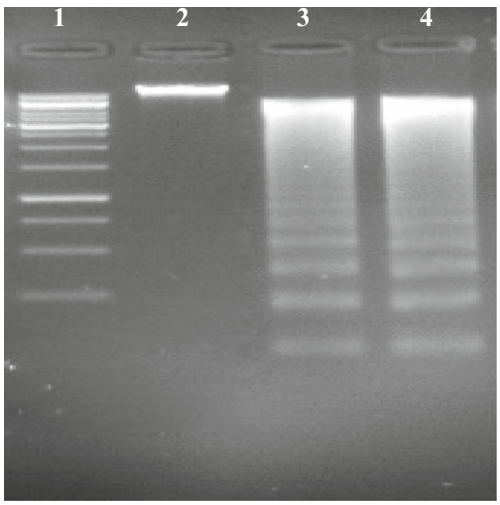

Fig. 4 DNA ladder assay performed on the HT-29 cells. Lane 1, standard molecular size marker (1 kb); lane 2, untreated control cells; and lanes 3and 4, *Saccharomyces cerevisiae*-treated cells

which is present in bread and yeast tablet) in vitro and in the presence of 17β-estradiol inhibited the proliferation of breast cancer cells (Ravi Subbiah and Abplanalp 2003). Hence, Chan et al. have studied the effect of *S. cerevisiae* on human liver cell line (HepG2) and found that it induced an anti-tumor effect as well as apoptosis in the intended cell line (Chan et al. 2004). Investigating the effects of heat-killed *S. cerevisiae*, isolated from yeast tablet, on the human tongue cancer cells (SCC-4 and SCC-9) and human colon cancer cells (Caco-2, DLD-1) indicated that *S. cerevisiae* induced apoptosis in these cells (Ghoneum et al. 2005). Demiret et al., in a study on treating breast cancer patients, have found that β-glucan extracted from *S. cerevisiae* induced peripheral blood monocyte proliferation and activation in the advanced breast cancer patients (Demir et al. 2007). The protective effects of β-glucan, extracted from *S. cerevisiae*, on damaged DNA and cellular toxicity have been investigated in the wild cell line, CHO-k1, and)xrs5(CHO using live cell survival tests, and it has been

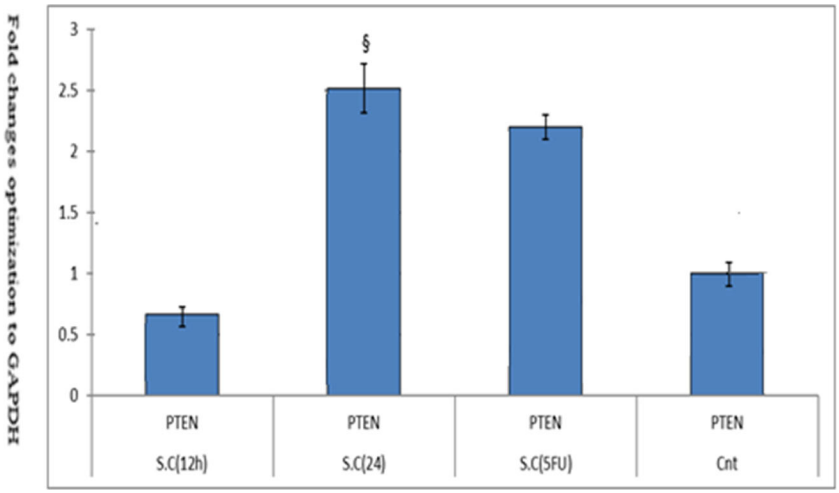

Fig. 5 The expression levels of *PTEN* gene in the *Saccharomyces cerevisiae*-treated HT-29 cells. The data are expressed as fold changes. *S.C* (12 h), HT-29 cells treated with *Saccharomyces cerevisiae* for 12 h; *S.C* (24 h), HT-29 cells treated with *Saccharomyces cerevisiae* for 24 h; *S.C* (5FU), HT-29 cells treated with 5FU; and Cnt, untreated HT-29 cells (used as the negative control). All the experiments were performed, at least, in triplicate. All the data are presented as mean ($n = 3$) ± 1 standard deviation ($P < 0.05$). *PTEN* phosphatase and tensin homolog deleted on chromosome ten, 5FU 5-fluorouracil, HT-29 human colorectal adenocarcinoma cell line

found that β-glucan maintained cell viability in both cells and prevented apoptosis (Oliveira et al. 2007a). Moreover, Ghoneum et al. have performed a similar experiment on mice and found that heat-killed *S. cerevisiae* reduced tumor volume in mice (Oliveira et al. 2007b; Ghoneum et al. 2008). Furthermore, Joon and his colleagues have reported that β-glucan, isolated from *S. cerevisiae*, enhanced the innate immune system, but little evidence was found for its anti-cancer activity (Yoon et al. 2008). Lee et al. have investigated the effects of *S. cerevisiae* on the survival and proliferation rates of the HT-29 cells using MTT and [3H] thymidine assays and found that it positively affected the cells (Lee et al. 2005a; Lee et al. 2005b).The results of a study conducted by Javmen et al. have indicated that β-glucan, derived from *S. cerevisiae*, induced cell death in the mouse hepatoma cells (Javmen et al. 2015). Baricault et al. have examined the effects of fermented milk on the growth and proliferation rates of the HT-29 cells and found that it induced an inhibitory effect on both the growth and proliferation (Baricault et al. 1995). Moreover, Tiptiri et al. have reported that *Lactobacillus casei* prevented growth and initiated apoptosis in the HT-29 cells (Tiptiri-Kourpeti et al. 2016). In a study performed by Zhung-Yuan

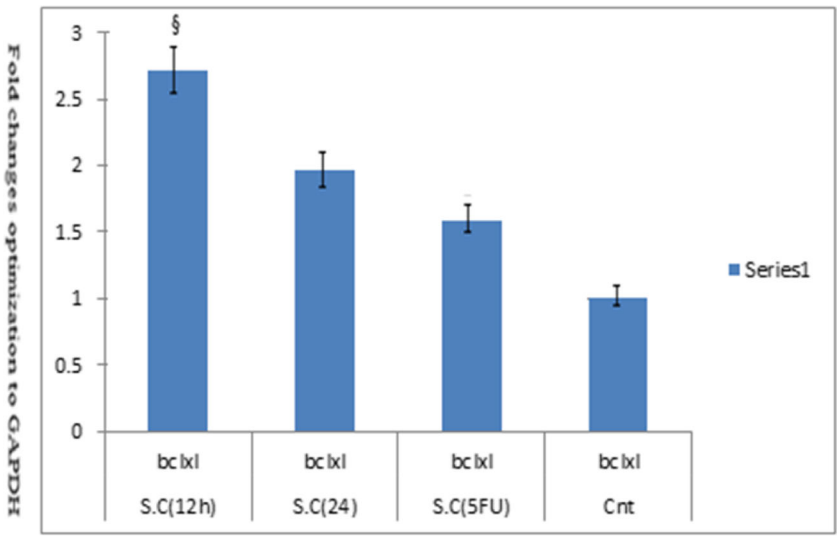

Fig. 6 The expression levels of *bcl-XL* gene in the *Saccharomyces cerevisiae*-treated HT-29 cells. The data are expressed as fold changes. *S.C* (12 h), HT-29 cells treated with *Saccharomyces cerevisiae* for 12 h; *S.C* (24 h), HT-29 cells treated with *Saccharomyces cerevisiae* for 24 h; *S.C* (5FU), HT-29 cells treated with 5FU; and Cnt, untreated HT-29 cells (used as the negative control). All the experiments were performed, at least, in triplicate. All the data are presented as mean ($n = 3$) ± 1 standard deviation ($P < 0.05$). 5FU 5-fluorouracil, HT-29 human colorectal adenocarcinoma cell line

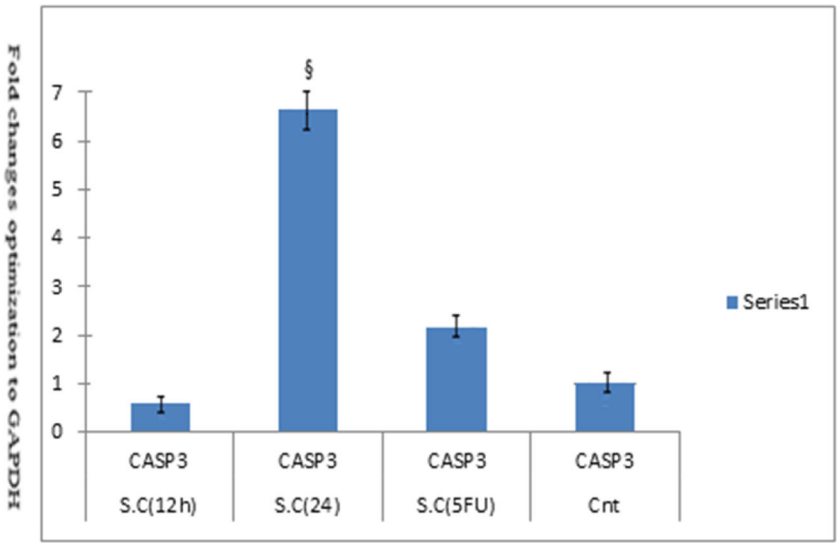

Fig. 7 The expression levels of CASP3 gene in the *Saccharomyces cerevisiae*-treated HT-29 cells. The data are expressed as fold changes. *S.C* (12 h), HT-29 cells treated with *Saccharomyces cerevisiae* for 12 h; *S.C* (24 h), HT-29 cells treated with *Saccharomyces cerevisiae* for 24 h; *S.C* (5FU), HT-29 cells treated with 5FU; and Cnt, untreated HT-29 cells (used as the negative control). All the experiments were performed, at least, in triplicate. All the data are presented as mean ($n = 3$) ± 1 standard deviation ($P < 0.05$). 5FU 5-fluorouracil, HT-29 human colorectal adenocarcinoma cell line

Chen, it has been demonstrated that the *Lactobacilli*, isolated from fermented products, induced inhibitory effects on the growth of the human colonic carcinoma cell line, HT-29 (Chen et al. 2017). Furthermore, Lee et al. have found that *S. boulardii* effectively inhibited the survival and proliferation of the HT-29 (Lee et al. 2005b). The results of a study conducted by Jabber et al. have indicated that beta-glycan, extracted from *S. cerevisiae*, induced an inhibitory effect on the growth of the AMN3 cell line in dose- and time-dependent manners. They have reported that the greatest inhibitory effect of beta-glycan was detected at the concentration of 500 μg/ml 48 h post-treatment (Jabber et al. 2011). Chenet et al. have reported that *S. boulardii* prevented the induction of epidermal growth factor (EGF)-induced proliferation, and reduced cell colony formation and promoted apoptosis in the human colonic cancer cells, namely HT-29 and SW480 (Chen et al. 2009). Sougioultzis et al. have found that *S. cerevisiae* produced low molecular weight peptides (< 1 kDa) which blocked NF-kB activation by downregulating the NF-kB-mediated *IL-8* gene expression in the intestinal epithelial cells and monocytes (Papatheodoridis et al. 2006). Hence, Kühle et al. have shown that *S. cerevisiae* decreased

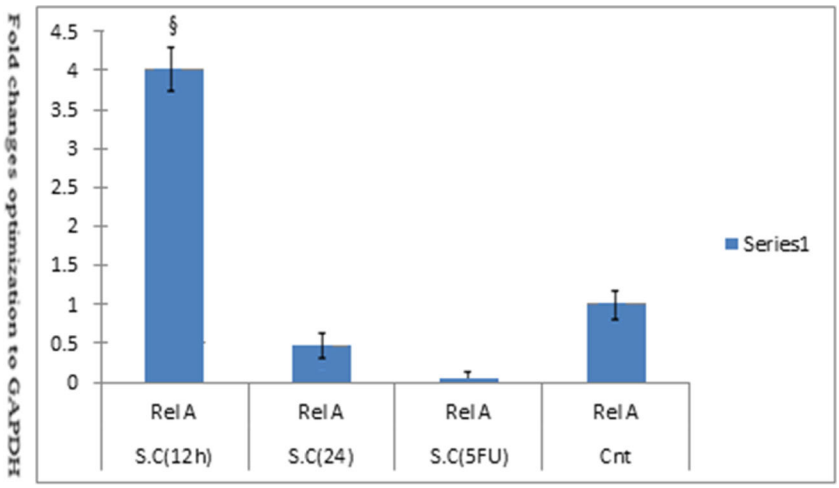

Fig. 8 The expression levels of *RelA* gene in the *Saccharomyces cerevisiae*-treated HT-29 cells. The data are expressed as fold changes. *S.C* (12 h), HT-29 cells treated with *Saccharomyces cerevisiae* for 12 h; *S.C* (24 h), HT-29 cells treated with *Saccharomyces cerevisiae* for 24 h; *S.C* (5FU), HT-29 cells treated with 5FU and Cnt, untreated HT-29 cells (used as the negative control). All the experiments were performed, at least, in triplicate. All the data are presented as mean ($n = 3$) ± 1 standard deviation ($P < 0.05$). 5FU 5-fluorouracil, HT-29 human colorectal adenocarcinoma cell line

the expression levels of pro-inflammatory cytokine *IL-1a*, in the porcine intestinal epithelial cell lines (Van der Aa Kühle et al. 2005; Jacobson 2015). Orland et al. have studied the effects of heat-killed *Bacillus ramenus* (*B. ramenus*) *GG* and *Bacillus paracoxus IMPC 2.1* on the DLD-1 colon cancer cell line and found that they inhibited cell growth or proliferation (Madempudi and Kalle 2017). Fortin et al. have examined the effects of β-glucan, extracted from the wall of *S. cerevisiae*, on the CRC cells and found that it inhibited the proliferation of the target cells (Fortin et al. 2018). Vahed et al. have treated the HT-29 cells with the conditioned medium, containing *Lactobacillus mesenteroides*, and studied apoptosis using DAPI staining, flow cytometry, DNA ladder assays, as well as real-time q-PCR and found that *L. mesenteroides* induced apoptosis via upregulation of the expression levels of CASP3 as well as downregulation of the expression levels of AKT, NF-κB, and *Bcl-XL* in the HT-29 cell line (Vahed et al. 2017). The present study investigated the effect of the *S. cerevisiae* supernatant on the HT-29 cells 12, 24, 48, and 72 h post-treatment. We found that the *S. cerevisiae* supernatant reduced both the proliferation and migration rates and increased apoptosis in the HT-29 cells.

Conclusion

The results of the present study indicated that the supernatant obtained from *Saccharomyces cerevisiae* significantly inhibited cell growth and induced apoptosis in the HT-29 cells. *Saccharomyces cerevisiae* is present in some of the world's most popular foods, and studies have indicated that it can effectively inhibit the growth of cancer cells; therefore, we suggest performing further studies on other cancer cell lines.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent This article does not need any informed consent.

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