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Exploring the influence of culture conditions on kefir's anticancer properties

Ma'mon M. Hatmal,* Abeer Nuirat,* Malek A. Zihlif,† and Mutasem O. Taha‡[1]
*Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, The Hashemite University, Zarqa 13133, Jordan
†Department of Biochemistry, Faculty of Medicine, The University of Jordan, Amman 11942, Jordan
‡Drug Discovery Unit, Department of Pharmaceutical Sciences, Faculty of Pharmacy, The University of Jordan, Amman 11942, Jordan

ABSTRACT

Cancer is a major health problem in many parts of the world. Conventional anticancer treatments are painful, expensive, and unsafe. Therefore, demand is increasing for cancer treatments preferentially in the form of functional foods or nutritional supplements. Kefir, a traditional fermented milk dairy product, has significant antimutagenic and antitumor properties. This research addresses the hypothesis that kefir's anticancer properties are affected by fermentation conditions. Initially, kefir extracts prepared under standard conditions were screened against 7 cancer cell lines using the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay. Colon cancer and chronic myelogenous leukemia cells were found to be most susceptible to kefir extracts. Subsequently, a factorial design was implemented to assess the effects of 3 fermentation times (24, 48, and 72 h), 3 kefir-to-milk ratios (2, 5, and 10% wt/vol), and 3 fermentation temperatures (4, 25, and 40°C) on kefir's anticancer properties. Remarkably, exploration of the fermentation conditions allowed the anticancer properties of kefir to be enhanced by 5- to 8-fold against susceptible cell lines. Overall, these results demonstrate the possibility of optimizing the anticancer properties of kefir as a functional food in cancer therapy.
Key words: kefir, fermentation conditions, factorial design, cancer cell lines

INTRODUCTION

Cancer is a significant health problem in many parts of the world. The number of cancer patients is expected to reach 11.5 million in 2030 worldwide (Farmer et al., 2010). Current conventional cancer treatments (e.g., chemotherapy and radiation) are painful, expensive, and unsafe (Fulda, 2004; Fulda and Debatin, 2006; Ghoneum, 2014). Hence, there is great current interest in the development of new cancer therapies.

Kefir grains are composed of a complex symbiotic mixture of bacteria and yeast (Fulda, 2004; Fulda and Debatin, 2006; Gao et al., 2013). Kefir is a dairy product produced by fermentation of milk using kefir grains (Liu et al., 2002; de Moreno de LeBlanc et al., 2007; Ghoneum, 2014). Kefir exhibits significant health benefits including pronounced anticancer properties (Liu et al., 2002; Chen et al., 2006; de Moreno de LeBlanc et al., 2007). The main products of kefir fermentation are lactic acid, ethanol, CO_2, and many aromatic compounds (Farnworth, 2005; Guzel-Seydim et al., 2011; Altay et al., 2013). As in yogurt, the lactose content is reduced in kefir; however, kefir is rich in free AA and peptides (Wang et al., 2008; Ahmed et al., 2013). The beneficial health characteristics of kefir are attributed to protein, vitamins, antioxidants, minerals, and certain biogenic compounds present in the grains (Farnworth, 2003; Ahmed et al., 2013; de Oliveira Leite et al., 2013). Traditionally, kefir beverage is prepared by adding kefir grains (2–10% wt/vol) to pasteurized milk. After a period of fermentation lasting over approximately 24 h (Hallé et al., 1994) and under mesophilic conditions (Motaghi et al., 1997), the grains are removed by filtration. The resulting filtrate is ready for consumption. The grains can be reused in subsequent fermentation (Hallé et al., 1994).

Many studies have established the antitumor properties of kefir against breast cancer (de Moreno de LeBlanc et al., 2006; Chen et al., 2007; de Moreno de LeBlanc et al., 2007), colorectal cancer (Farnworth and Mainville, 2003; Khoury et al., 2014), malignant T lymphocytes (Maalouf et al., 2011), and lung carcinoma (Furukawa et al., 2000). The cell-free fraction of kefir was found to inhibit cancer cell proliferation by cell cycle arrest and induction of apoptosis through upregulating bax and downregulating bcl-2 (Gao et al., 2013). Apparently, the main kefir polysaccharide, kefiran, is responsible for the antitumor properties of kefir (Sharifi et al., 2017). Additionally, kefir contains unique sphingomyelins that promote the secretion of antiproliferative cytokines (particularly IFN-β) in human osteosarcoma cells (Osada et al., 1993; Sharifi et al., 2017).

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[1]Corresponding author: mutasem@ju.edu.jo

Table 1. The concentration of the extract that inhibits cell growth by 50% (IC_{50}) values (mg/mL) of kefir extracts prepared under standard conditions (24 h, 5% wt/vol, and at 25°C) and doxorubicin hydrochloride (positive control) against different cancer cell lines

	Kefir extract		Doxorubicin	
Cancer cells[1]	IC_{50} (mg/mL)	SD	IC_{50} (mg/mL)	SD
K562	11.36	1.338	2.84×10^{-4}	0.019
HCT116	17.39	2.157	0.21×10^{-4}	0.002
SKOV3	26.5	5.429	2.78×10^{-4}	0.085
MCF-7	43.07	4.343	0.42×10^{-4}	0.010
PANC1	42.39	3.740	2.43×10^{-4}	0.039
A549	35.27	0.919	0.61×10^{-4}	0.006
PC3	32.74	3.426	4.17×10^{-4}	0.144
Fibroblasts (control)	72.38	4.156	29.69×10^{-3}	12.380

[1]K562 = chronic myelogenous leukemia; HCT116 = colon cancer; SKOV3 = ovarian cancer; MCF-7 = breast cancer; PANC1 = pancreas cancer; A549 = lung carcinoma; PC3 = prostate cancer.

Interestingly, despite extensive studies on the antitumor properties of kefir, the literature lacks any attempt to explore the effects of fermentation conditions on kefir's anticancer properties. Accordingly, the current research addresses the hypothesis that kefir's anticancer properties are significantly influenced by fermentation conditions.

The current study commenced by scanning the anticancer activities of kefir extracts against breast cancer (**MCF-7**), chronic myelogenous leukemia (**K562**), lung carcinoma (**A549**), pancreas cancer (**PANC1**), prostate cancer (**PC3**), ovarian cancer (**SKOV3**), and colorectal cancer (**HCT116**), as in Table 1.

Subsequently, we implemented factorial design using 3 factors, namely, fermentation times (24, 48, and 72 h), fermentation temperatures (4, 25, and 40°C), and kefir-to-milk ratios (2, 5, and 10% wt/vol), to explore the effects of fermentation conditions on the anticancer properties of kefir against sensitive cancer cells. The investigated fermentation conditions were selected in such a way to mimic routine conditions used by communities in different geographical areas. For example, in its Tibet home region, kefir is normally brewed at low temperatures over few days, whereas in hot geographical zones it is usually fermented more rapidly.

MATERIALS AND METHODS

Materials

The following materials were used in the project and were purchased from the corresponding companies. Fetal bovine serum (HyClone, Logan, UT), HEPES buffer (HyClone), McCoy's 5a Medium Modified (HyClone), Dulbecco's modified Eagle medium (**DMEM**) high glucose medium with L-glutamine (Capricorn, Ebsdorfergrund, Germany), DMEM Hams F12 medium with L-glutamine (Capricorn), RPMI 1640 medium with L-glutamine (Capricorn), Matrix 96-Well Tissue Culture Treated Plates (Sigma-Aldrich, St. Louis, MO), L-glutamine (Capricorn), sterile syringe filter pore 0.22 μm (Millipore, Billerica, MA), doxorubicin HCl (Sigma-Aldrich), PBS (Capricorn), the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (**MTT**) kit (Promega, Madison, WI), and dimethyl sulfoxide (Gibco, Waltham, MA).

The tested cell lines and their ATCC numbers (in parentheses) are as follows: HCT116 (CCL247), MCF-7 (HTB22), K562 (CCL243), A549 (CCL185), PANC1 (CRL1469), PC3 (CRL1435), and SKOV3 (HTB77).

Kefir Production and Preparation of Kefir Extracts

Kefir grains were cultured in pasteurized full-fat cow milk (200 mL, composed of 3.1 g of fat, 3.1 g of protein, 4.7 g of carbohydrate, 100 mg of calcium, and 80 IU of vitamin D_3, Al-Marai Dairy Company, Jordan) in screw-capped glass containers without stirring, and at different kefir grain-to-milk ratios (2, 5, and 10% wt/vol), fermentation times (24, 48, and 72 h), and fermentation temperatures (4, 25, and 40°C), as in Table 2.

Afterward, kefir grains were removed by filtration using plastic colander, then the extracts were centrifuged at 3,000 × *g* for 20 min at 4°C. The supernatant fractions were lyophilized overnight at −50°C (Freeze Dry System, Telstar, Spain) then stored at −80°C. Before testing against cell lines, the lyophilized supernatant samples were serially diluted in corresponding culture medium (see Antiproliferation Assay below), adjusted to pH 7.0 using NH_4OH (10 *M*, Sigma-Aldrich), and passed through a 0.22-μm Millipore filter.

Antiproliferation Assay

In the initial screening step (Table 1), cancer cells were seeded into 96-well plates at the following cellular densities (in parentheses): K562 (3.5×10^4 diluted in RPMI 1640 medium), HCT116 (5×10^3 diluted in DMEM high glucose medium), SKOV-3 (7×10^3 diluted in McCoy's 5a medium), MCF-7 (8×10^3 diluted in RPMI 1640 medium), PANC1 (8×10^3 diluted in DMEM high glucose medium), A549 (5×10^3 diluted in DMEM high glucose medium), PC3 (5×10^3 diluted in DMEM high glucose medium), and fibroblasts (control, 5×10^3 diluted in DMEM high glucose medium). Subsequently, cells were incubated for 24 h at 37°C in 5% CO_2 humidified atmosphere using a NuAire incubator (Plymouth, MN). Thereafter, cells were treated with