Systematic Review

Kefir and Cancer: A Systematic Review of Literatures

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Abstract

Some studies have suggested chemopreventive effects of kefir, a fermented milk product, on carcinogenesis. The aim of this review study was to evaluate the scientific evidence for effects of kefir on cancer prevention and treatment. We systematically searched for all relevant studies published before June 2015, using PubMed, Google scholar, Cochrane and Science Direct, SID, MedLib and Srlst databases. Relevant studies were reviewed based on systematic review (PRISMA) guidelines. From a total of 2208 papers obtained at the initial database search, 11 publications including 7*in vitro* and 4 experimental studies were eligible. *In vitro* studies on breast, colon, skin and gastric cancers and leukemia cell lines and experimental studies on different sarcomas consistently showed beneficial effects of kefir on cancer prevention and treatment. The results of this systematic review suggest that kefir may be associated with cancer prevention and it also has beneficial effects in cancer treatment. This protection may be associated with kefir bioactive components including peptides, polysaccharides and sphingolipids.

Keywords: Cancer, fermented milk products, kefir, systematic review

Cite this article as: Rafie N, Golpour Hamedani S, Ghiasvand R, Miraghajani M. Kefir and Cancer: A Systematic Review of Literatures. *Arch Iran Med*. 2015; **18(12):** 852 – 857.

Introduction

Cancer, abnormal division and reproduction of cells that can spread throughout the body,[1] is a leading cause of death worldwide and the total number of cases is increasing globally.[2] A recent analysis estimated 13.2 million deaths and 20.3 million incident cases for cancer in 2030.[3] Thus preventive strategies are critical in every cancer control plan.[2] Among factors responsible for cancer, it has been estimated that diet accounts for about 20%–30% of all cases worldwide. Several studies support the idea that some dietary components, such as fruits and vegetables, are protective factors against cancer.[4] There is also evidence that a diet rich in fermented foods such as fermented milk products may have beneficial properties in reducing risk of some cancers.[5–7] Kefir is a type of fermented milk made from adding small cauliflower floret-like grains to pasteurized milk. The grains contain a mixture of yeast and bacteria living in a symbiotic community. This special symbiotic nature makes kefir different from other fermented dairy products such as yogurt. The alleged healthy capabilities of kefir include antifungal and antibacterial properties, benefits for digestive tract and beneficial effects on cholesterol. It has also been found to exert beneficial effects on preventing several types of cancer.[8] Anticancer properties of kefir have been linked to the presence of a number of bioactive components including peptides, polysaccharides[9] and sphingolipids which have significant roles in several types of signaling pathways and regulation of some cellular processes including cell proliferation, apoptosis and transformation.[10]

The present systematic review was conducted to summarize the results obtained from studies on the effects of kefir and kefir products on tumor prevention and treatment. So, we come to a conclusion regarding potential properties of this beverage in some cancer prevention and treatment programs.

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Accepted for publication: 9 September 2015

Materials and Methods

This systematic review methodology was performed based on the Preferred Reporting Item for Systematic Review and Meta-analysis (PRISMA) statement recommendation and all steps were reviewed independently by two investigators.

Search strategy

We searched the PubMed, Google scholar, Cochrane, Science Direct, EBSCO, MedLib, Srlst and SID databases for studies published up to June 2015 using the search terms ("kefir" [tiabs] OR, "cultured milk products" [tiabs] OR "fermented milk products" [tiabs]) AND ("cancer" [tiabs] OR "neoplasm" [tiabs] OR "neoplasms" [tiabs] OR "tumors" [tiabs]) to identify studies investigating the effects of kefir on cancer prevention and treatment. No restrictions were imposed. We also did a manual search on reference lists of retrieved relevant articles for additional primary studies.

Eligibility criteria

We included studies in this review if they were conducted on the effects of kefir on cancer prevention and treatment. The exclusion criteria were: 1) papers in which the main treatment or exposure was not kefir, 2) papers investigating conditions other than cancer, 3) papers with no report on the effects of kefir on cancer prevention and treatment, 4) papers not published in English or Persian.

Study selection

Briefly, 2208 papers were identified through the systematic search. In the first stage, an initial screen of identified abstracts or titles was established and 158 irrelevant papers were excluded.

For the next stage, 25 papers were screened based on full-text of articles to include studies reporting effects of kefir on cancer. Papers were excluded if they were irrelevant according to the exclusion criteria. Finally, a total of 11 papers were identified as eligible for this systematic review. A flow chart showing the study selection process is presented in Figure 1.

Data extraction

The information extracted from the included articles is as follows: name of the first author, publication year, culture media/model animal, type of treatment, dose of treatment, duration, events followed, observed effects and *P* value. The characteristics of the selected studies are presented in Table 1.

Results

Among 11 eligible articles, 7 and 4 studies were performed using *in vitro* human cancer cell lines and *in vivo* animal models, respectively. Considering the main aspects of the total included papers and based on the location of the cancer, the results were as follows:

Breast cancer

Two studies were found that examined the effects of kefir on breast cancer cells.[9,11] The first study by Chujian Chen, *et al*.[9] evaluated *in vitro* antiproliferative effects of kefir extracts on human mammary cancer cells compared to normal human mammary epithelial cells at different doses which were expressed based on cell numbers. Four kefir products were used in this study (K1_K4). Milk was incubated with kefir grains (K1) and fermented for 24 hr to prepare the mother culture (K2). The resulting mother culture was added (1%–3%) to pasteurized milk (K3) and by fermenting this product for approximately 24 hour, the final product (K4) was prepared. A pasteurized milk sample and different yogurt extracts were used for comparison. After 6 days of cell culture with kefir products, the fermented mother culture (K2) and the final kefir product (K4) showed significant ($P < 0.01$) dose-dependent suppressive effects on malignant cells proliferation with no inhibitory effects on normal cells. In comparison to the controls, the malignant cells treated with K1 product showed significant growth at all doses above 0.31%. The K3 product showed no effect on malignant cell proliferation relative to controls. As kefir extracts used in this study were produced with different degrees of fermentation, they possibly contained different profiles of bioactive components including peptides which could explain the differences observed between products.

Another study by de Moreno de Leblanc, *et al*.[11] in 2010 studied the effects of 2 or 7 days of cyclical consumption of kefir and a kefir cell free fraction (KF), prepared by centrifuging kefir at 4ºC for 20 min and then drying and refrigerating the resultant supernatant until tested, on hormone dependent breast cancer prevention in mice. In four groups of mice, after 2 or 7 consecutive days of

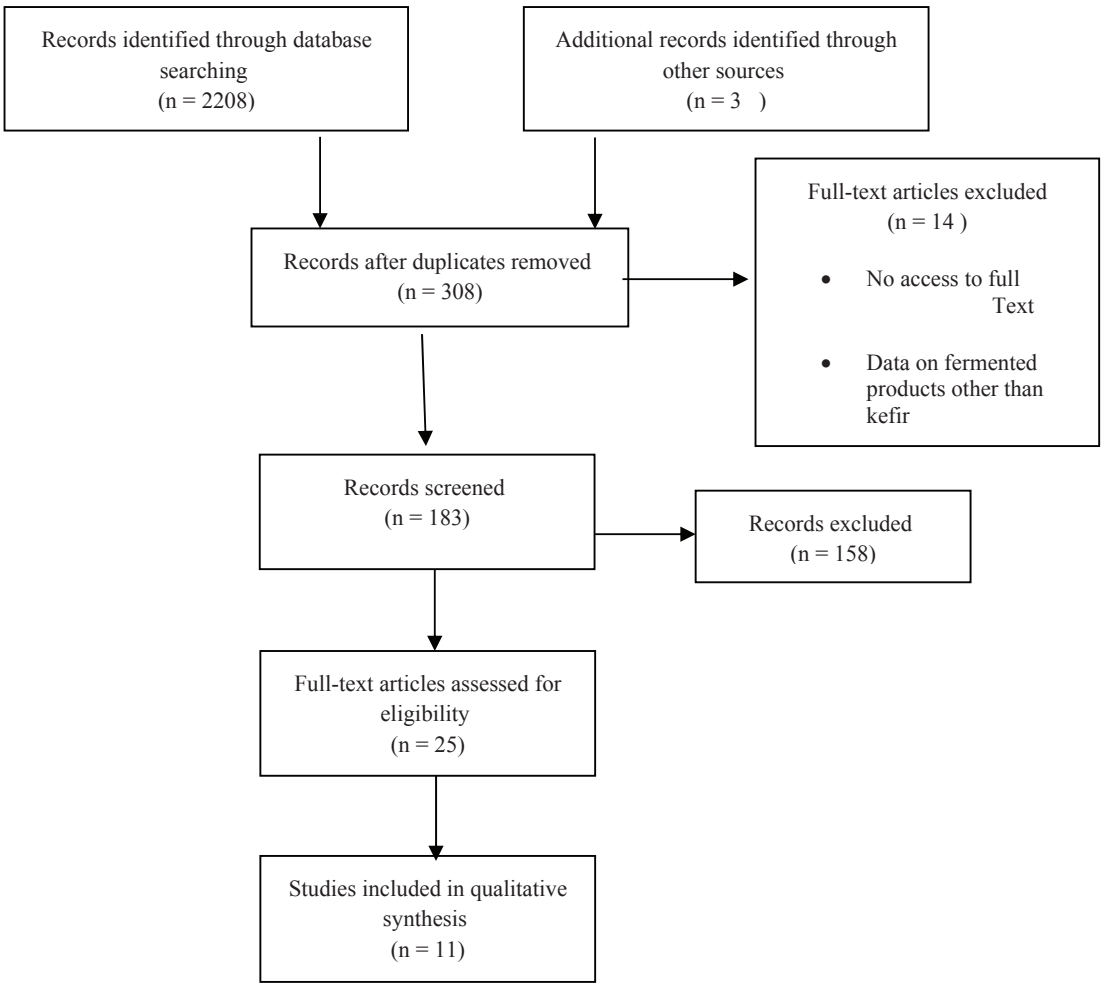

Figure1. Study flow diagram of the search process.

Table 1. Characteristics of the included articles.

First author, year of publication	Study design	Culture media/ animal model	Type of treatment	Dose of treatment	Duration of exposure	Events followed	Effects observed
Chen[9], 2006	*In vitro*	Human breast cancer cell line	Four kefir products (K1- K4)	0.31%, 0.63%, 1.25%, 2.5%, 5%, 10%	6 days	Cell proliferation	Antiproliferation effects on cancer cells and no effects on normal cells ($P < 0.05$)
Grishina[14], 2009	*In vitro*	Human colon adenocarcinoma cells	Kefir supernatant	20, 50, 100, 200µL/mL	30 min	DNA damage	Reduced DNA damage ($P < 0.001$)
JieGao[17], 2013	*In vitro*	Human gastric cancer cell line	cell free fraction of TK	0, 2, 4, 6, 8, 10 mg/mL	24 hours	Cell proliferation	Dose dependent anti proliferative effects ($P < 0.05$)
Tsutomu Nagira[18], 2002	*In vitro*	Human melanoma cells	Aqueous extract of kefir	9.6 mg/mL	5 hours	DNA damage	Suppression of DNA damage ($P < 0.05$)
Katia Maalouf[12], 2011	*In vitro*	Human leukemia cell line	Cell free fraction of kefir	0, 20, 40, 60, 80 mg/mL	6, 24 and 48 hours	Cell proliferation/ cytotoxicity / apoptosis	Reduced proliferation/ proapoptotic effects/ little cytotoxicity ($P < 0.05$)
Sandra Rizk[13], 2009	*In vitro*	Human leukemia cell line	Cell free fraction of kefir	0, 20, 40, 60, 80 mg/mL	6, 24 and 48 hours	Cell proliferation/ cytotoxicity / apoptosis	Reduced proliferation/ proapoptotic effects/ little cytotoxicity ($P < 0.05$)
Nathalie Khoury[15], 2014	*In vitro*	Human colorectal adenocarcinoma cell lines	Cell free fraction of kefir	5% 10% 15% 20%	24hr 48hr 72hr	Cell proliferation/ apoptosis	Reduced proliferation/ proapoptotic effects ($P < 0.05$)
Je- Ruei Liu[20], 2009	Experimental	Sarcoma tumor cells	Oral administration of milk and soy milk kefirs or distilled water	5 mL	0- 30 days after administration	Tumor growth	Inhibition of tumor growth ($P < 0.05$)
AdileCevikbas[19], 1994	Experimental	Sarcoma tumor cells	Intraperitoneal administration of kefir or saline	0.5 mL/d	20 days	Tumor size	Significant decrease in tumor size ($P < 0.05$)
Shiomi[21], 1982	Experimental	Sarcoma tumor cells	Oral/ intraperitoneal administration of KGF- C	0.02%, 0.1% , 0.3%	From day -7 or day 0 to the day of tumor excision	Tumor growth/ cytotoxicity	Inhibition of growth/ no or little cytotoxicity ($P < 0.05$)
de Moreno de Leblanc[11], 2006	Experimental	Murine breast cancer	Oral administration of kefir and KF	KF 200 µL/d and kefir diluted 1/100 in sterile water	2 or 7 days of cyclical consumption from the day 4 post-injection	Tumor growth	Diminished tumor growth ($P < 0.05$)

feeding with kefir (diluted 1/100 in sterile water substituted for drinking water) or KF (200 μL/d), tumor cells were injected in the mammary gland. After four days of injection, the mice received kefir or KF cyclically until day 27 post-injection. In the fifth group, the mice were injected with tumor cells without receiving any of the products. All groups also received a balanced diet and water ad libitum. Tumor length and width were measured with acaliper to evaluate the tumor growth. The result of this study showed that 2 days cyclical administration of both products diminished tumor growth with the most significant effect shown for KF. Seven days cyclical feeding did not show significant effects in tumor volume as compared to the control group.

Leukemia

To our knowledge, there are two studies which have investigated the antitumor effects of kefir on leukemia cancers.[12,13] In one study by Maalouf, *et al*.,[12] the effects of cell free fraction of kefir was demonstrated on proliferation, cell cycle arrest and apoptosis of two types of human leukemia cell lines. It was shown that, in a dose- and time-dependent manner, kefir effectively inhibits the proliferation of leukemia cells [maximum activity of 80 μg of kefir dissolved in μL of media (RPMI 1640) after 48hr]. Also, it had proapoptotic effects in cancerous cells without any significant necrotic effect on normal cells. The antiproliferatory effect of kefir was assessed based on the amount of metabolically active cells detected.

In the other research,[13] aimed to examine the beneficial effects of different concentrations of kefir in another type of human leukemia cell line, although a significant dose- and time-dependent growth suppressive effect on the cancerous cell lines was demonstrated at all concentrations used, a maximum cytotoxicity effect was shown at 80 μg/μL concentration after 48 hr. Moreover, the results indicated that kefir was not toxic to normal cells.

Colon cancer

Two studies[14,15] evaluated the anticancer capacity of kefir supernatant using human colon adenocarcinoma cells. As there is evidence that compounds such as acetate can reduce the activity of specific agents in feces which are indicated in DNA damage in colon cells,[16] authors in a study[14] examined the properties of kefir supernatant, containing high amounts of acetic and lactic acid on adenocarcinoma cells. The supernatant was prepared by centrifuging kefir for 20 min at 4ºC and neutralized to pH 7.0 DNA strand breaks in cells were measured to determine the protective effect of kefir supernatant against DNA damage and carcinogenesis. The ability of kefir supernatant to exert protective effects on DNA damage induced by carcinogen agents was shown at all the concentrations used in this study (with the maximum activity seen at 200 μL/mL at 37ºC after 30 min treatment). Since DNA damage is a crucial event in carcinogenesis, kefir supernatant can possibly reduce the risk of colon cancer.

Khoury, *et al*.[15] also reported that kefir significantly reduced proliferation of human colorectal adenocarcinoma cells in a time- and dose-dependent manner based on the activity of mitochondrial dehydrogenase (with the maximum effects observed at 20% concentration after 72 hours). Also, kefir could induce apoptosis, as seen by cell death ELISA (with maximum effect at 15% dose after 48 hours).

Gastric cancer

In one study,[17] the antiproliferative and apoptotic effects of a cell free fraction of Tibetan kefir (TK), which is fermented by Tibetan kefir grain with the same effects of kefir, on a specific type of human gastric cancer cell line after 24 hr was investigated. This type of kefir is used in Tibet and is made by Tibetan kefir grains which are similar to the kefir grains used in other areas over the world. The cell free fraction was prepared by removing yeast and bacteria from kefir samples and then the samples were centrifuged and finally the supernatant was filtered. Metabolically active cells were measured to explore the antiproliferatory effects of kefir. The results showed a dose-dependent antiproliferative effect by cell cycle arrest which was distinct at doses above 8 mg/mL. Also, the study indicated that cell-free fraction of TK could cause early apoptosis of cancer cells.

Skin cancer

One study examined the effect of kefir on skin cancer cells[18] and evaluated the DNA-repairing effect of a commercial kefir extract powder on the morphological changes of different types of human melanoma cells induced by UVC irradiation and normal fibroblast cells. The amount of reactive oxygen species (ROS), unscheduled DNA synthesis and morphological changes of cells were measured as an indicator of kefir effects on UV damage. The results suggest that the kefir extract, at 9.6 and 19.2 mg/mL doses used in this study, has the ability to protect cells from UV damage, after 5 and 24 hr, respectively. Also, it was shown that these effects are related to active substances of kefir other than antioxidant agents.

Sarcoma

Three studies were identified on the antitumor effects of kefir in different types of sarcoma cells.[19–21] Liu, *et al*.[19] investigated the antitumor effects of oral administration of soy milk kefir, prepared by inoculation of soy milk with kefir grains, and milk kefir on female mice bearing sarcoma tumor cells. One week after tumor inoculation, with oral administration of 5mL/kg/day of different treatments for 30 days, tumor growth was inhibited 64.8% and 70.9% in the tumor-bearing group with milk and soy milk kefirs respectively, compared with controls. Tumor volume was measured to assess antitumor activities of kefir.

In another survey on a different form of sarcoma cells in mice, the beneficial therapeutic effects of kefir to decrease tumor size were established by Cevikbas, *et al*.[20] Following 20 days of treatment with 0.5mLkefir, compared with the saline administered to the controls, reduction was seen in tumor size based on size of tumors after treatment. Furthermore, tumor disappearance was observed in those mice receiving kefir treatments.

Shiomi, *et al*.[21] studied the effect of a water-soluble polysaccharide (KGF-C) isolated from kefir grain on the growth of two types of sarcoma tumor cells inoculated in male mice. To prepare the polysaccharide, kefir grains were washed with distilled water followed by homogenation and centrifugation. To the supernatant, ethanol was added to obtain precipitate (crude KGF-C). It was given to the mice ad libitum in drinking water in different concentrations. Oral administration of KGF-C, compared to the controls, inhibited the growth of both tumor cells which was measured by the difference between tumor weight in the intervention and control groups. Also, *in vitro* direct cytotoxicity of KGF-C on tumor cells was investigated. There was no or little direct cytotoxicity against the tumor cell based on the ratio of the dead cells to the total cells.

Discussion

A consistent body of evidence suggests a beneficial effect of kefir on cancer prevention and treatment but current research findings are not sufficient to validate these effects. So, the aim of this review was to create a full picture of these evidences. Since no systematic review has been done in this regard in the past, this paper is the first to focus on possible anticancer effects of this fermented milk beverage and the mechanisms involved.

Papers used in this review had either an *in vitro* design (7 papers) or used animal models (4 papers). While the studies consistently showed beneficial effects of kefir on cancer prevention and treatment, since existing animal - *in vitro* tumors have biological characteristics which are very different from those of human cancers, application of these results to human subjects is limited.

In general, the antitum orogenic effects of kefir have been attributed to special constituents produced by the fermentation process including peptides and sphingolipids.[9] Although the mechanisms of action of these components are not clear yet, several possible pathways have been indicated. First, biologically active peptides are released during kefir production, which induce apoptosis possibly through a pathway mediated by production of intracellular Reactive oxygen species (ROS), and activation of Ca-/Mg -dependent endonucleases that are responsible for the cleavage of DNA during apoptosis. In addition, ROS can induce a mitochondrial pathway of apoptosis through cytochrome release from mitochondria that leads to caspase cascade activation and cell death through apoptosis. The cationic nature of these peptides allows the peptide to target negatively charged cancer cells, whereas healthy untransformed cells are spared.[22]

Second, the polysaccharides of the kefir grains can possibly exert their effects through activation of macrophages, natural killer cells which are a component of innate immune system and some T lymphocyte subpopulations which can secrete tumor necrosis factor.[23] This factor can induce apoptotic and necrotic pathways.[11]

Moreover, kefir can induce apoptosis pathways by down-regulation of TGF- α, a cytokine that induces the proliferation of cells, and up-regulation of TGF-β mRNA expression which is a pro-apoptotic transforming growth factor.[12] Furthermore, it may have an important role in up-regulation of bax and down-regulation of bcl-2 genes which accelerate the process of programmed cell death.[17]

In addition, kefir contains unique sphingomyelins which can enhance the secretion of interferon beta, an antiproliferative cytokine.[24]

It was also shown that kefir has the potential to decrease DNA damage and carcinogenesis process via several mechanisms including directly scavenging intracellular ROS or stimulating scavenging enzymes such as superoxide dismutase and catalase. Also, it has been shown that kefir might protect cells from UV damage by cutting UV.[18] Although, it is not clear which components of kefir are involved in this process.

It also has been demonstrated that the antiproliferative effect of kefir, especially on estrogen dependent cancer cells, is exerted through the decreases of interleukin 6 (IL- 6) which is a proinflammatory cytokine involved in estrogen synthesis. Thus, the decrease of estrogen level is followed by a decrease in tumor growth.[9] Kefir also has high amounts of short chain fatty acids including lactate and acetate which can protect cells from DNA damage associated with carcinogenesis via promoting the immune system.[14] Short chain fatty acids, by stimulating production of T cells, antibodies and cytokines, have critical roles in immune protection. Also, they improve the barrier properties of the colonic mucosal layer, by inhibiting adhesion irritants, which contributes to immune functions.[25]

In conclusion, evidence suggests a beneficial effect of kefir on cancer prevention and treatment. Further studies are warranted in human subjects to determine whether such protective effects are exerted *in vivo*, with implications for reduction in cancer risk and to identify the bioactive components in kefir and the mechanisms by which these beneficial effects are exerted.

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