Review

A medical and molecular approach to kefir as a therapeutic agent of human microbiota

A review

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Abstract: The imbalanced microbial composition called dysbiosis constitutes a tendency related to different kind of human diseases. To overcome the disadvantages of dysbiosis, the consumption of probiotics is an emerging and promising topic of the last decade. Kefir is a probiotic fermented beverage produced from the fermentation of kefir grains with changing varieties of milk and displays a symbiotic association of bacteria and yeast. The discovery of the concept that fermented foods/beverages such as kefir could modify gut microbiota in humans has widened the borders of precision medicine and now microbiome therapeutics can be considered as a significant part of this field. Kefir seems to have potential to guide and manipulate future replacement/complementary therapies with a variety of beneficial biological/medical properties it has. The aim of this review was a comprehensive recapitulation of probiotic beverage kefir's significant properties mainly focusing of antioxidative, immunomodulatory, apoptotic, antitumor, and neuroprotective properties. Apoptotic/antimetastatic effects are regulated at the molecular level by increases in TGF-β1, caspase-3, p53, Bax, Bax:Bcl-2 ratio, p21 and decreases in TGF-α, Bcl-2 and MMP polarization. Neuroprotective effects are revealed upon upregulation of SOD/catalase and anti-inflammatory Treg cells, decreases in repetitive behavior and modulation of apoptotic genes. Besides these significant features that may offer advantages in supplementary cancer therapies, the scope was also extended to recent emerging medical topics and also discussed and evaluated the concept of "psychobiotics". The therapeutic potential of psychobiotic effect is majorly attributed to the increased ratios of *Clostridium butyricum*, *Lactobacillus* and *Bifidobacterium*.

Keywords: kefir, probiotic, microbiota, apoptosis, antitumor, neuroprotection, psychobiotic

Introduction

The term probiotic (from the Latin word "pro" and the Greek word "bios," which means "for life" shows a modern historical process starting at the beginning of 1900s with the pioneering studies of a Russian scientist Elie Metchnikoff, who is now accepted as the father of microbiome therapeutics. Metchnikoff indicated the association between the regular consumption of fermented dairy products such as yogurt and the enhanced life expectancy of Bulgarian peasants. Extending this important phenomenon related to longevity, he also claimed that the aging process would stem from the toxins that originated from bacterial putrefaction in the large intestine, supportively Hippocrates who had 2000 years ago stated that "Bad digestion is the root of all evil" [1, 2]. The appropriate use of the term probiotic was proposed by a consensus document in 2014 and now we regard "probiotics" as live microorganisms which when administered in adequate amounts confer a health benefit on the host [3]. According to this report, foods from fermented milk such as yogurt were considered the first functional foods. To overcome the undesired effects of dysbiosis which can be defined as the imbalanced microbial state, the consumption of probiotics and/or some other beneficial microorganisms would be desirable to modify host gut microbiota composition in a balanced state [4]. The aim of this study was a comprehensive look at the potential implications of kefir in medicine, especially in cancer studies since there seems to be a requirement to extend the scope of the term "precision medicine" with the involvement of microbiome and thus pave the way for future microbiome therapeutics.

Microbiota/Microbiome and probiotic potential

Regarding "microbiome therapeutics" with the potential of one of the new and extended therapy regimens in the future,

it would be beneficial to remind the terms "microbiota" and "microbiome". While the term microbiota is related to the microbial population (composition), the microbiome refers to the genetic makeup of the microbial population [4]. In the last decades, probiotics have gained great attention because of their potential use in various medical fields and bridge the gap between the food industry and medicine. It is noteworthy to tell that more than 3500 different fermented foods and beverages are being produced all over the world [5]. Though nearly every civilization in the world took advantage of the fermentation process and fermented foods/beverages, kefir was strikingly characterized by probiotic potentials.

Kefir origin, content and nutritional value

Kefir originates from Balkan communities in Eastern Europe to Anatolia and Caucasian mountains and it has been especially preferred and consumed in Europe, Russia, and Southwest Asia as a probiotic fermented drink [6].

Kefir is an acidic, partially effervescent, slightly alcoholic, and viscous beverage that is easily digested [7] and produced from the fermentation of both traditional and commercial forms of kefir grains (2–10%, w/v) with changing varieties of semi-skimmed or skimmed pasteurized milk (goat, sheep, cow, camel, and buffalo). Kefir grains are rich in terms of a wide and varying microflora, including lactic acid bacteria (LAB), acetic acid bacteria, and yeasts [7, 8, 9, 10]. Kefir's nutritional content is quite rich since it contains at least 2.7% protein, 0.6% lactic acid, less than 10% fat, and also a huge diversity of vitamins, amino acids, and mineral compositions [7].

Antioxidative properties

The study conducted in rats proved that kefir could prevent the oxidative damage induced by lead and strengthen the antioxidant system dependent upon beneficial microorganisms and vitamin E content it has [11]. As an antioxidative property, kefir was demonstrated to display scavenging effects upon 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide radicals. It also inhibited linoleic-acid peroxidation [12]. Commercially produced flavored fermented milk samples consumed in Poland were analyzed for their antioxidant properties. Both yogurt and kefir displayed the highest antioxidant activity [13] in concordant with a previous study which proved kefir's and ayran's greater antioxidant capacities compared with milk on fecal water-induced DNA damage in human colon cells [14]. Recently, an innovative study reported the production of a functional food (milk kefir enriched with inulin-grafted seed extract from white wine pomace) and it was determined that the enriched polymer displayed a higher antioxidant property. Moreover, *in vitro* gastrointestinal digestion simulation analyses proved that 50 g of the kefir product would enable the delivery of antioxidants equivalent to 300 mg of ascorbic acid [15].

Immunomodulatory and antitumor properties

There are an increasing number of studies focusing on the modulation of the immune response, suppression of proliferation, apoptotic, and antitumor effects of kefir which are summarized with the molecular details in this section. Thus, kefir's anticancer effects via various cellular and molecular pathways seem to render it a favorable natural therapeutic ingredient. It may be used in the treatments of malignancies and a better comprehension of molecular pathways and microbial compounds can pave and enhance the way for future more individualized therapy regimens though the effects of kefir on tumorigenesis gained steam in the last decade. Kefir's anticancer properties were pronounced to be significantly affected via fermentation time, regardless of fermentation temperatures or kefir-to-milk ratio. Shorter fermentation time ranging between 24 and 48 h was reported to have superior anticancer profiles when compared with a longer period such as 72 h [16].

The beneficial antitumor effects of kefir were studied in a murine breast cancer model and it was shown that 2-d cyclic administration of kefir cell-free fraction (KF) in mice resulted in a significant increase in apoptotic cells and a decrease in Bcl-2 (+) cells in the mammary gland [17]. Another study evaluating kefir extracts *in vitro* at different doses with a comparable approach on human mammary cancer cells (MCF-7) and normal human mammary epithelial cells (HMECs) showed the inhibition of MCF-7 cell growth in a dose-dependent manner and 2.5% kefir dose resulted in a 56% decrease in tumor cell numbers [18]. A more recent study investigated the effects of kefir water and drew attention to its role as an antimetastasis and angiogenesis inhibitor agent on murine breast cancer cells (4T1) and *ex vivo* murine models, respectively [19].

Kefir displayed antiproliferative effect via downregulating TGF-α and upregulating TGF-β1 mRNA expression in both CEM and Jurkat cells which are human T-lymphotropic virus type I (HTLV-1)-negative malignant T-lymphocytes [20]. The therapeutic effect of kefir grain product was evaluated on multidrug-resistant myeloid leukemia and it was shown that Probiotics Fermentation Technology (PFT) in which *Lactobacillus kefiri* is the main constituent could induce apoptosis on cancer cells in a dose-dependent manner which

was maximal with 67.5% at 5 mg/ml concentration. The apoptotic effect was accomplished with activation of caspase 3, decreased Bcl-2 expression, and mitochondrial membrane potential (MMP) polarization [21].

The pro-apoptotic effect of kefir via upregulation of Bax: Bcl-2 ratio and an increase in p53 independent-p21 expression were reported on colon adenocarcinoma cells [22]. The induced apoptosis with PFT which was associated with the decreases in MMP polarization and Bcl-2 expression was dose-dependent and peaked at 66.3% at 5.0 mg/ml in human gastric cancer cells (AGS) [23]. The apoptotic potential of kefir on acute erythroleukemia cell line (KG-1) was reported [24]. The aberrant crypt foci induced by azoxymethane in mice were attenuated in the kefir group compared with the controls and this finding indicated the potential of kefir treatment for the control of intestinal neoplastic cell growth [25]. Esener et al. [26] reported the apoptotic potential of kefir in Ehrlich ascites carcinoma (EAC) in mice. In a later study aiming to obtain a stronger pharmaceutical potential for tumor cell destruction, a combination of kefir/plant-derived compounds was tested in BALB/c mice; kefir-induced juglone and resveratrol fractions (JRK) treatment resulted in decreased anti-apoptotic Bcl-2 level and increased apoptotic Caspase-3 level [27]. Another study also aimed to determine the apoptotic and immunomodulatory effects of novel kefir product PFT for the inhibition of EAC in mice and highlighted molecular changes including p53 and Bax upregulation, Bcl-2 downregulation, and increased Bax/Bcl-2 ratio [28]. The authors emphasized that the apoptosis mechanism via PFT induction was mitochondrial-dependent since a significant decrease in mitochondrial polarization and an over two-fold increase in caspase-3 expression were observed [28].

Antitumor activity of kefir was recently reported in the U87 glioblastoma cancer cell line with a special emphasis on the potential utilization of this beverage in replacements/complementary cancer therapies [29]. Kefir may offer an advantage not only in the apoptotic process and management/inhibition of various tumor types, but it may also be beneficial in terms of controlling the potential side effects triggered by standard cancer therapy regimens; chemotherapy, and radiation. In the first study aiming to explore milk's and kefir's effects in the doxorubicin-treated rat model, 9-week milk or kefir consumption resulted in lower body size and higher heart mass, indicating health optimization before/during doxorubicin treatment [30]. This finding may be important in terms of preventing/decreasing chronic doxorubicin cardiotoxicity which was previously reported in mice receiving 30 mg/kg or higher doses [31]. A very recent study which may be significant for future radiation genotoxicity assessments suggested kefir's radioprotective effect alone or in combination with ascorbic acid against damage caused by X-irradiation in mice [32]. Furthermore, Smoak et al. [33] drew attention to the health benefits of 12-week kefir consumption post-exercise in a population of human cancer survivors.

Neuroprotective effects

Besides antitumor studies above-mentioned in detail, kefir may also have the potential to be used in neurodegenerative diseases (NDD). The anti-oxidative and neuroprotective effects via upregulation of SOD/catalase and the modulation of apoptotic genes Tp73, Bax, and Bcl-2 were reported in SH-SY5Y neuroblastoma cells [6]. Kefir supplementation also seems promising in order to improve life quality in autism spectrum disorder (ASD) subjects. Because, it decreased repetitive behavior and increased anti-inflammatory Treg cell levels in mesenteric lymph nodes in the mouse model [34].

Potential role of kefir in psychiatric diseases and the concept of "psychobiotics"

Mounting evidence reflects the role of gut microbiota along the gut-brain axis in modulating the host behavior, thus constituting a new concept termed "psychobiotics" which defines a subgroup of probiotics that may offer health benefits in psychiatric diseases [35, 36]. In one of the first studies evaluating the effects of two distinct kefir samples (Fr1 and UK4) in mice, Fr1 healed the stress-induced decrease in serotonergic signaling in the colon and reward-seeking behavior while UK4 ameliorated stress-induced deficits and resulted in a decreased repetitive behavior. Besides, both kefir samples increased GABA production of the gut microbiota as a result of increased *Lb. reuteri* prevalence, reflecting the significance and potential use of kefir as a dietary intervention to modulate mood [36]. As far as we know, this is the first study directly evaluating the effect of kefir on host behavior since other brain/behavior studies muchly focused on the use of a single psychobiotic strain instead of a multistrain probiotics approach.

The administration of *Clostridium butyricum* MIYAIRI 588 could seem to be beneficial as adjuvant therapy for patients with mental disorders since CBM588 and the antidepressant combination was reported to be efficient and well-tolerated for treatment-resistant major depressive disorder (TRD) [37]. Augmentation of selective serotonin reuptake inhibitors (SSRI) with probiotic bacteria *Lb. plantarum* 299v was reported to increase cognitive performance and kynurenine concentration in patients suffering from major depression disorder (MDD) [38].

Table 1. Prominent health promoting properties of kefir

Organism/Cell line	Health benefit	Biological mechanism	Consequences	Reference
Rat (n=36)	Antioxidative	Prevention of oxidative damage induced by lead and strengthening of antioxidant system	After 6 weeks of treatment, blood levels of the glutathione (GSH) and malondialdehyde (MDA) became similar to control group. Kefir supplementation resulted with Vit E increase.	*Ozcan et al. (2009)* [11]
Biochemical study (cow-milk kefir and goat-milk kefir)	Antioxidative	Radical-scavenging effects, ferrous-ion chelating ability, reducing power and antioxidant activity	Compared to milk samples, kefirs displayed significantly greater scavenging effects upon 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide radicals, an inhibition effect upon linoleic-acid peroxidation, and more substantial reducing power	*Liu et al. (2005)* [12]
Biochemical study (milk kefir enriched with inulin-grafted seed extract from white wine pomace)	Antioxidative	Increase in total antioxidant capacity (TAC) values and free radical scavenging properties	An innovative functional food was produced by enriching milk kefir with inulin-grafted grape-seed extract from white wine pomace. The addition of antioxidant inulin conjugate (IN_PSS) to fermented milk improved its antioxidant performance. Freeze-dried milk kefir enriched with IN_PSS (KDIF) displayed a TAC value 4.5% higher than freeze-dried milk kefir enriched with Pecorello *cv* seeds extract (KDE). Besides with proven higher antioxidant capacity of this innovative food, it was also determined that these antioxidants were bioaccessible following gastrointestinal digestion.	*Carullo et al. (2022)* [15]
Biochemical study (commercial natural and flavoured fermented milks)	Antioxidative	Inhibition of the negative impact of free radicals	Among all analysed products, yoghurts and kefirs were characterised by the highest antioxidant activity. Ferric reducing antioxidant power (FRAP) and anti radical power (ARP) values were positively affected by the presence of probiotic *Lactobacillus casei.*	*Najgebauer-Lejko et al. (2015)* [13]
Human colon cells (HT-29 and Caco-2)	Antioxidative	Reduction of DNA damage determined with comet assay	DNA damage induced by fecal water samples was significantly decreased by kefir and ayran supernatants.	*Grishina et al. (2011)* [14]
Murine breast cancer model (25 to 30 mice)	Antitumor	Tumor growth inhibition, apoptosis induction and the number of apoptotic regulator Bcl-2(+) cells	Two-day cyclical administration of kefir and kefir cell-free fraction (KF) delayed tumor growth. A significant increase in apoptotic cells and a decrease in Bcl-2 (+) cells was also determined for KF.	*de Moreno de Leblanc et al. (2007)* [17]
Human mammary cancer cells (MCF-7)	Antitumor	Inhibition of cell growth in a dose-dependent manner	After 6 days of culture, extracts of kefir-fermented milk inhibited MCF-7 cell proliferation with a ratio of 29% at a concentration as low as 0.63% and at the 2.5% dose, this proliferation inhibition rate reached to 56%.	*Chen et al. (2007)* [18]
Murine breast cancer cells (4T1) and BALB/c mice injected with 4T1 cancer cells (n=21)	Antitumor	Apoptotic, immunomodulator of T helper cells and cytotoxic T cells, anti-inflammatory, antimetastatic, and antiangiogenesis inhibitor agent	Kefir water was cytotoxic toward 4T1 cells. Kefir water-treated mice displayed a significant reduction in tumor size, an increase in helper T cells (5-fold) and cytotoxic T cells (7-fold). Moreover, proinflammatory and proangiogenic markers were significantly reduced.	*Zamberi et al. (2016)* [19]
Human leukemia cell lines (CEM and Jurkat cells)	Antitumor	Inhibition of proliferation and induction of apoptosis	Kefir displayed antiproliferative effect via downregulating TGF-α and upregulating TGF-β1 mRNA expression.	*Maalouf et al. (2011)* [20]
Multidrug-resistant myeloid leukemia (HL60/AR) cells (Novel kefir product PFT (Probiotics Fermentation Technology) application)	Antitumor	Induction of apoptosis in a dose-dependent manner	PFT induced apoptosis in a dose-dependent manner which was maximal at 67.5% for 5 mg/ml. Induction of apoptosis was correlated with caspase 3 activation, decreased expression of Bcl-2 and decreased polarization of mitochondrial membrane potential (MMP).	*Ghoneum et al. (2014)* [21]

Table 1. Prominent health promoting properties of kefir (Continued)

Organism/Cell line	Health benefit	Biological mechanism	Consequences	Reference
Human colon adenocarcinoma cells (Caco-2 and HT-29)	Antitumor	Induction of cell cycle arrest at the G1 phase and induction of apoptosis	Pro-apoptotic effect was displayed via upregulation of Bax:Bcl-2 ratio and increase in p53 independent-p21 expression.	*Khoury et al. (2014)* [22]
Human gastric cancer cells (AGS) (Novel kefir product PFT (Probiotics Fermentation Technology) application)	Antitumor	Induction of apoptosis in a dose-dependent manner.	Apoptosis was detected at a concentration of 0.3 mg/ml (20.8%) and reached to peak (66.3%) at 5.0 mg/ml. Induction of apoptosis was achieved via decreasing mitochondrial membrane potential (MMP) polarization and Bcl-2 expression.	*Ghoneum and Felo (2015)* [23]
Human acute erythroleukemia cell line (KG-1)	Antitumor	Induction of apoptosis and necrosis	MTT results displayed a significant decrease in proliferation of KG-1 cell line treated with kefir. Flow cytometry analysis showed that kefir induced apoptosis and necrosis in a dose- and time-dependent manner; highest rate of apoptosis was observed with a dose of 300 μl and 72 h incubation time while milk had no remarkable effect.	*Jalali et al. (2016)* [24]
BALB-c mice (n=18)	Antitumor	Prevention and control of the growth of intestinal neoplastic cells	Aberrant crypt foci were induced in BALB-c mice via 2 subcutaneous injections of azoxymethane (15 mg/kg) and kefir was administered by daily gavage for 8 weeks (5 ml/kg). The aberrant crypt foci were attenuated by approximately 43% (height) and 20% (width) in the kefir group.	*Melo et al. (2018)*[25]
Mouse model of Ehrlich ascites carcinoma (EAC) (n=34)	Antitumor	Induction of apoptosis and suppression of proliferation	Adult male Swiss albino mice were divided into groups and tap water, donkey milk and donkey milk kefir were administered by gavage for 10 days. Donkey milk kefir induced apoptosis, suppressed proliferation and decreased co-expression of iNOS and eNOS. Surprisingly, donkey milk promoted development of the tumors.	*Esener et al. (2018)* [26]
Mouse model of Ehrlich ascites carcinoma (EAC) (Novel kefir product PFT (Probiotics Fermentation Technology) application) (n=69)	Antitumor	Arrest of the tumor cell cycle in the sub-G0/G1 phase and induction of apoptosis via mitochondrial-dependent pathway	PFT was administered to mice orally (2 g/kg/day) 6 days/week, either 2 days before tumor cell inoculation or 9 days after tumor inoculation. Apoptotic /immunomodulatory effects were observed via p53 and Bax upregulation, Bcl-2 downregulation, increased Bax/Bcl-2 ratio, decrease in the polarization of MMP, and caspase-3 activation.	*Badr El-Din et al. (2020)* [28]
Human primary glioblastoma cell line (U87)	Antitumor	Reduction of the growth rate in a dose-dependent manner	According to the results of the MTT test, treatment of the cells with the fermented drink for 48-hour demonstrated the most cell cytotoxicity compared with the control group. Toxicity effect was dose-dependent: The most cell viability: 90% at 1.5 mg/ml concentration and 44% at 20 mg/ml concentration.	*Fatahi et al. (2021)* [29]
Doxorubicin-treated rat model (n=72)	Antitumor (Replacements/ complementary cancer therapies)	Protective effects against DOX-induced cardiac damage	Sprague Dawley rats were assigned to control (n=24), milk (n=24), or kefir (n=24) groups with equivalent macronutrient profiles during 9-wk and the results of doxorubicin (15 mg/kg) and saline injections were tested. Nine week of milk or kefir ingestion resulted in health optimization (lower body size and higher heart mass) after DOX treatment.	*Stewart et al. (2019)* [30]

Table 1. Prominent health promoting properties of kefir (Continued)

Organism/Cell line	Health benefit	Biological mechanism	Consequences	Reference
Mice exposed to X-irradiation	Antitumor (Replacements/ complementary cancer therapies)	Protection of lymphocyte blood cells from radiation-induced DNA injury and genotoxicity	Total Comet Score (TCS) value was 1.39 and 1.5 fold less in the kefir and ascorbic acid groups, respectively compared to irradiated mice only. Both ascorbic acid and kefir displayed strong free radical scavenging capacity.	*Koohian et al. (2021)* [32]
Human cancer survivors (n=24)	Antitumor (Replacements/ complementary cancer therapies)	Improvements in lean body mass, depression, fatigue, gastric distress, and gut dysbiosis.	All participants were enrolled in a structured exercise training program and divided into kefir treatment or control groups. Kefir consumption (12 weeks) was associated with 6.3% improvements in lean body mass, as well as 51.4%, 39.3% and 64.7% improvements in measures of depression, fatigue, and gastric distress, respectively. Moreover, kefir improved overall and classical monocyte numbers.	*Smoak et al. (2021)* [33]
Human neuroblastoma cell line (SH-SY5Y)	Neuroprotective	Upregulation of SOD and catalase and the modulation of apoptotic genes Tp73, Bax, and Bcl-2	The antioxidant and apoptotic potentials of 6 different kefir water samples were analysed. From the analyzed samples (A-F), kefir B revealed an efficient neuroprotection ability in differentiated SH-SY5Y cells and showed the most potency in eradicating intercellular ROS.	*Kumar et al. (2021a)* [6]
Mouse model of autism spectrum disorder (n=20)	Neuroprotective	Modulation of the microbiota and decrease in repetitive behaviour which is one of the hallmarks of autism spectrum disorder (ASD)	Adult mice were administered either kefir (UK4) or a milk control for three weeks, after which they were assessed for their behavioural phenotypes. Decreased repetitive behavior and increased anti-inflammatory Treg cell levels in mesenteric lymph nodes were observed. Kefir also increased the relative abundance of Lachnospiraceae bacterium A2, which was associated with reduced repetitive behaviour and increased Treg cells in mesenteric lymph nodes (MLNs).	*van de Wouw et al. (2021)* [34]
Mice (n=48)	Potential "psychobiotics" * (Mood modulation)	Modulation of specific aspects of the microbiota-gut-brain axis and host behaviour	Two distinct kefirs (Fr1 and UK4) or unfermented milk control were administered to mice and their behavioural phenotypes were characterized. Shotgun metagenomic sequencing of ileal, caecal and faecal matter and systemic immunity measures and gut serotonin levels were determined. Healings in stress-induced deficits and reward-seeking behavior, decrease in repetitive behavior were observed. Analysis of the gut microbiota revealed that both kefirs significantly changed the composition and functional capacity of the host microbiota.	*van de Wouw et al. (2020)* [36]

All the findings presented in the table are statistically significant at the $P<0.05$ level. *A new medical concept termed "psychobiotics" may offer potential health benefits in the complementary psychiatric disease therapies. Though the mentioned study is the first one directly evaluating kefir's effect (multistrain probiotics approach) on host behavior, ongoing studies focus on the use of a single psychobiotic strain in mice/rats and also in humans. Accordingly, these studies which may contain kefir microorganisms were also comprehensively discussed in the article since they reflect promising supportive ways for the treatments of mood disorders in future.

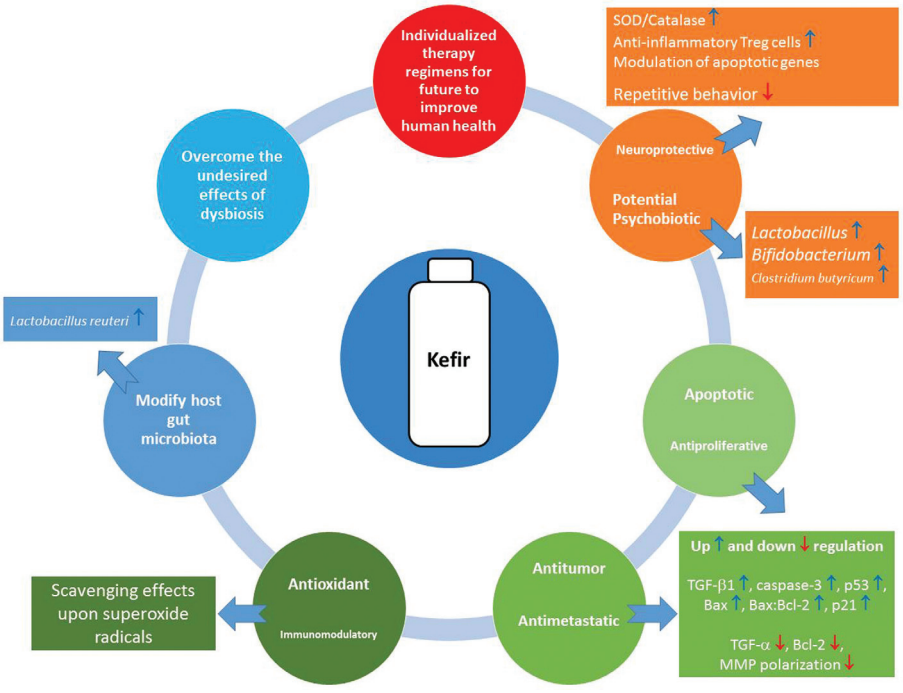

Figure 1. Potential medical applications of kefir.

The combination of a gluten-free diet and probiotic supplementation (*Lb. helveticus* R0052 and *Bifidobacterium longum* R0175) was found to inhibit immune-inflammatory cascade in the MDD patients and it healed psychiatric and gut barrier-associated traits via restoring gut eubiosis, conformably with the macrophage theory of depression [39].

Although the above-mentioned studies mainly involve *Lactobacillus*, *Bifidobacterium* seems to be the other significant psychobiotic candidate. *Bifidobacterium* supplementation has the potential for future management of neurological and psychiatric diseases. Both the phylum and genus level differences in bacterial abundance displayed decreased Firmicutes, increased Bacteroidetes, and declined *Bifidobacterium* at the genus level in the microbiome of Alzheimer's disease (AD) participants [40]. Providing new insight into the pathophysiology of psychiatric disorders, lower *Bifidobacterium* and/or *Lactobacillus* counts were reported in patients with MDD compared to the healthy controls [41]. This study was supported by a subsequent study conducted in mice and reported that *Bifidobacterium* E41 and M2CF22M7 had an antidepressant effect in mice partly in a 5-hydroxytryptophan (5-HTP) dependent and microbiota-regulating manner [42]. The potential effects of *B. longum* 1714 on stress modulation and neurocognition were investigated in healthy volunteers and consumption of this putative psychobiotic strain was associated with reduced stress and improved memory [43]. *B. longum* mitigated the depressive-like symptoms which were assessed by three different behavior tests and reduced caspase-3 activity in the limbic system in rats [44]. Though *Bifidobacterium*/*Lactobacillus* counts did not play a major role in the pathophysiology of bipolar disorder (BD), a negative correlation between *Lactobacillus* counts and sleep and that between *Bifidobacterium* counts and serum cortisol levels reflected the possible roles of these bacteria both in sleep and stress response [45] and absolutely necessitate more studies conducted in BD. In a study conducted with drug-naive, first-episode schizophrenia patients, patients were characterized with significantly lower numbers of fecal *Bifidobacterium* spp., *Escherichia coli*, and *Lactobacillus* spp. compared with the healthy controls and a 24-week risperidone treatment led to significant changes in certain fecal bacteria [46]. Though the concept of the changes in the microbiota in the pathophysiology of neurological and/or psychiatric studies is still in its infancy, the above-mentioned studies clearly emphasize the potential role of bacteria composition, especially *Bifidobacterium*, and thus may become an emerging supportive way for mood disorders in future.

The prominent health promoting effects of kefir were summarized in Table 1 and shown in Figure 1.

It is quite noteworthy to remind that, except for very limited neurological and psychiatric studies investigating the potential effects of kefir, most of these studies practiced a single strain approach as in the case of *Lactobacillus*/*Bifidobacterium* in adjuvant therapy approaches contrary

to the cancer cell line studies conducted directly with kefir extracts. On the other hand, we can never rule out the potential strong possibility that kefir already comprises these bacterial strains at varying levels. That is why the purpose of this review was extended to include both kefir and potential bacterial strains it can involve. That would be found a bit imaginary in the last decade when studies conducted were mainly culture-dependent. Nevertheless, the development of culture-independent powerful techniques such as metagenomics allowed us to investigate the microbial composition of probiotics/prebiotics in foods/beverages and overcome the limitations of the last decade. Thus, the microbial composition of different kefir beverages both commercial and noncommercial can be determined and can also be enriched for the desired bacterial strains if it is planned to be used as a therapeutic supplementary agent. An increasing number of comprehensive metagenomic studies from 2013 to our age have identified the microbial composition of different kefir grains/beverages consumed in different populations and revealed both predominant and quite varying abundances of bacterial compositions [47, 48, 49, 50, 51, 52, 53, 54, 55]. Thus, it now seems quite feasible to strengthen potential applications of kefir as a supportive ingredient in a quite wide range of medical fields.

Conclusion

After the pioneering studies of Metchnikoff, focused investigations on probiotic foods have gained great significance step by step and now reached to a highly promising perspective including microbiome therapeutics as a valuable tool in precision medicine. The regular consumption of probiotic beverage kefir seems to be quite important because of the potential health benefits it can offer. Besides its highest antioxidant activity, it is remarkable in terms of its antitumor effects as comprehensively recapitulated in this review. Kefir's promising potential effects as a supplementary ingredient both in antitumorigenesis and also controlling the potential side effects triggered by standard cancer therapy regimens may pave and enhance the way for future anticancer study designs and cancer microbiome therapeutics. Though the concept of the changes in the microbiota in the pathophysiology of neurological and/or psychiatric studies is still in its infancy, the recapitulated studies in this review clearly emphasize the potential role of bacteria composition and recommend further studies conducted with kefir's microbial composition via taking advantage of metagenomic analysis. Thus, its potential role as a psychobiotic can also be better evaluated as an emerging supportive way for mood disorders in the future.

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