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Kefir reduces the incidence of pre-neoplastic lesions in an animal model for colorectal cancer

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ABSTRACT

This study aimed to evaluate the effect of regular consumption of milk kefir on the development of pre-neoplastic colonic lesions. Thirty Wistar rats received water (Control group) or milk (Milk group) or kefir (Kefir group) during five weeks. After that, colonic lesions were chemically induced and the treatments continued for more thirteen weeks. The regular consumption of kefir was able to reduce the incidence of aberrant crypt foci by 36%. Also, the consumption of kefir increased the cecal concentration of short chain fatty acids; reduced the lactulose/mannitol ratio; promoted an increase in the colonic concentration of TNF-α and IL-1β, and the enzyme catalase in comparison with the control group. Thus, kefir reduced the development of lesions, probably by increasing the production of short chain fatty acids; reduction of intestinal permeability; immunomodulation and improvement of colonic antioxidant activity.

1. Introduction

Colorectal cancer (CRC) affects colon and rectum, and is the third leading cause of death among different cancers around the world (WHO, 2014). Recently the regular consumption of probiotics has been associated with reduced risk for the development of this type of cancer (Reis et al., 2017). Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014).

In these sense, experimental studies have shown that the regular consumption of probiotics is able to inhibit the development of pre-neoplastic colonic lesions (Chang, Shim, Cha, Reaney, & Chee, 2012; Foo et al., 2011; Kumar et al., 2012; Mohania, Kansal, Sagwal, & Shah, 2013; Zhu et al., 2014). Some mechanisms have been pointed out as responsible for this effect such as: ability to modulate (quantitatively and qualitatively) the composition of the intestinal microbiota and its metabolic activity; binding and degradation of carcinogenic compounds present in the intestinal lumen; production of compounds with anticarcinogenic activity; immunomodulation; inhibition of proliferation; and induction of apoptosis of neoplastic cells (Reis et al., 2017).

Kefir is a potential probiotic fermented milk that has been gaining prominence in the scientific literature due to its therapeutic activity and ease of production at home (Rosa et al., 2014). The peculiarity of kefir compared to other fermented milks lies in the metabolic activity of a complex mixture of bacteria (lactic and acetic acid-producing) and yeast (lactose fermenters and non-lactose fermenters) that live in symbiotic association (Leite et al., 2013).

Regular consumption of kefir has been associated with a variety of therapeutic effects (Rosa, Dias, Grzes'kowiak, Reis, Conceição, & Peluzio, 2017), including antitumor activity (Hatmal, Nuirat, Zihlif, & Taha, 2018; Liu, Wang, Lin, & Lin, 2002; Rizk, Maalouf, & Baydoun, 2009). However, the effect of the regular consumption of kefir on the development of precancerous colonic lesions is unknown. Thus, this study aimed to evaluate the effect of regular consumption of this

Abbreviations: ACF, aberrant crypt foci; ACF > 3, plus three aberrant crypts; ACF ≤ 3, minus three aberrant crypts; CAT, catalase; CFU, colony forming units; CRC, colorectal cancer; DMH, 1,2-dimethylhydrazine; GST, glutathione-S-transferase; LAB, lactic acid bacteria; PAS, periodic acid Schiff; SCFA, short chain fatty acids; SOD, superoxide dismutase

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fermented milk on the development of the pre-neoplastic colonic lesions, chemically induced in an animal model, as well as the potential mechanisms involved.

2. Materials and methods

2.1 Production of kefir

Kefir grains were obtained from the Department of Nutrition and Health, Universidade Federal de Viçosa (Viçosa, Minas Gerais, Brazil). For the preparation of kefir (fermented milk), it was sought to reproduce home-made preparation conditions. Thus, 8 g of kefir grains were inoculated into 100 mL of pasteurized whole cow milk. This mixture was maintained at room temperature (22 ± 3 °C) for 24 h, without agitation, in pre-sterilized glass container with lid closed but not hermetically sealed.

After the fermentation period, the kefir grains were separated from the fermented milk by sieving. Subsequently, the grains were subjected to a new fermentation process and the fresh kefir obtained was used in the study (Rosa et al., 2016).

The kefir offered to the animals presented an average pH of 3.86 ± 0.1; titratable acidity of lactic acid was 0.88 ± 0.04 per mL of kefir; lactic acid bacteria (LAB) counts equal 10^9 colony forming units (CFU)/mL and yeasts colonies of 10^7 CFU/mL.

2.2. Animals

Thirty male Wistar rats, eight weeks old, from the Animal House at the Biological Sciences Center of Universidade Federal de Viçosa were used. The animals were kept in individual cages, with light/dark cycle of 12 h and mean temperature of 22 ± 2 °C. The animals received distilled water and standard food (Nuvilab®, São Paulo, Brazil) *ad libitum*, only during the sample collection for the intestinal permeability test the access to the diet was restricted.

This study was approved by the Ethics Committee on Animal Use of Universidade Federal de Viçosa, according to protocol number 84/2014 and it followed the principles established by the National Council on Animal Experimentation Control.

2.3. Experimental design

The animals were divided in three experimental groups containing 10 animals each, being: Control; Milk and Kefir. The first five experimental weeks were called the pre-induction phase. During this phase, the animals received, daily, via gavage 1 mL of distilled water (Control group); 1 mL of pasteurized whole milk (Milk group) and 1 mL of whole milk kefir (Kefir group).

After the pre-induction phase, all the experimental groups (Control, Milk and Kefir) were submitted to the induction of pre-neoplastic lesions with 1,2-dimethylhydrazine (DMH) (Sigma Aldrich®, St. Louis, USA). The animals received four subcutaneous injections of DMH at a dose of 40 mg/kg body weight, with two applications per week (induction phase) (Larangeira, Taha, Ferme, Lemos, & Papler, 1998). Thereby, the post-induction phase (13 weeks) began, where the animals continued to receive their respective interventions via gavage (water, milk or kefir).

After 20 weeks of experiment, the animals were euthanized. After 12 h of fasting, the animals were anesthetized with 3% isoflurane (Cristália®, Belo Horizonte, Brazil) and, then, submitted to total exsanguination. The samples were collected and properly stored for further analysis.

2.4. Aberrant crypt foci count

After euthanasia, the colon was washed with 50 mM phosphate buffer solution (PBS), opened longitudinally along the antimesenteric band, and placed in paraffin plates with the mucosa facing upwards. After fixation in Carson's formalin for 24 h, the tissue was measured and divided into three equidistant segments, which were identified as proximal, medial and distal in relation to the cecum.

In order to count the aberrant crypt foci (ACF), the colon segments were stained with 0.1% methylene blue solution for two minutes. The counting was performed with the aid of a light microscope (Olympus America InC., CBA model, Pennsylvania, USA) at 100× magnification by two double-blind trained observers. The categorization of ACF was performed based on the number of aberrant crypts per focus, being a focus defined as plus three (ACF > 3) or minus three aberrant crypts (ACF ≤ 3) (Bird, 1987).

2.5 Quantification of short chain fatty acids

Acetic, propionic and butyric SCFA were quantified in the cecal contents collected during euthanasia. The SCFAs were extracted with 25% metaphosphoric acid (Sigma Aldrich®, St. Louis, USA) solution, according to the methodology proposed by Smiricky-Tjardes, Grieshop, Flickinger, Bauer, and Fahey (2003). After centrifugation, the supernatant obtained was used for the quantification of SCFAs in a high performance liquid chromatograph (Shimadzu®, detector model: SPD-10A VP coupled to ultraviolet detector, Kyoto, Japan) using a wavelength of 210 nm. For the chromatographic separation of the samples, they were injected into a 250 cm × 4.6 mm diameter column (Macherey-Nagel®, EC 250/4.6 nucleosil 100-5 C18 model, Tokyo, Japan) with a flow rate of 0.6 mL/minute, 65 kgf pressure, and the mobile phase composed of 1% orthophosphoric acid in water.

2.6 Evaluation of intestinal permeability

In the last week of the post-induction phase was performed the intestinal permeability test. During this test, the animals had restricted access to diet but free access to water. A solution containing lactulose (200 mg, Daiichi Sankyo, Barueri, Brazil) and mannitol (100 mg, Synth®, Diadema, Brazil) was administered to the animals via gavage. From this moment onwards, all urine excreted in the subsequent 8 h was collected (Meddings, Jarand, Urbanski, Hardin, & Gall, 1999).

The quantification of the aforementioned sugars in the urine collected was performed by high performance liquid chromatography (Shimadzu®, detector model: RID 10A coupled to ultraviolet detector, Kyoto, Japan) using a wavelength of 220 nm. For chromatographic separation, the samples were injected into a 300 mm × 7.8 mm diameter column (Aminex®, HPX-87H model, Dublin, Ireland), with a flow rate of 1 mL/minute, a 54 kgf pressure, and a mobile phase composed of 0.05 mol/L sulfuric acid in water.

2.7. Histological analysis

Colon fragments were fixed in Carson's formalin and later dehydrated in increasing ethanol concentrations, diaphonized with xylol and embedded in paraplast (Sigma Aldrich®, St. Louis, USA). Cross sections of 5 μm thickness were obtained on a rotating microtome (Olympus America InC., CUT 4055 model, Pennsylvania, USA).

In order to quantify the goblet cells that produce acidic mucopolysaccharide, neutral mucopolysaccharide and both types, histological sections were submitted to alcian blue and/or periodic acid Schiff (PAS) stains. To capture the images, a 200X magnification was used, and 20 images of each coloration per animal were captured directly from the light microscope (Zeiss®, Primo Star model, Oberkochen, Germany) through a photographic camera (Zeiss®, Aixo ERc5s model, Oberkochen, Germany).

The goblet cell counts was performed using an image analysis software (Image Pro Plus 4.5, Media Cybernetcs Inc, Rockville, USA). The overlap of a square lattice grid (35 points × 35 points) in the fields photographed was standardized, and only the cells coincident with the

intersections were counted.

2.8. Quantification of pro-inflammatory cytokines

The pro-inflammatory cytokines IL-1β, IL12p70, IFN-ɣ and TNF-α concentration in the colonic tissue was determined. For this, the samples were homogenized with a buffer (10 mM PBS, 0.05% Tween-20, 5% aprotinin, pH 7.4) and subsequently centrifuged so that the supernatant could be used for the quantification of the cytokines.

The concentration was determined simultaneously using the MILLIPLEX ®MAP Rat Cytokine/Chemokine Magnetic Panel (#RECYTMAG-65K-04, Linco Research, Millipore, Massachusetts, USA), using the LuminexCorporation'sxMAP™ technology (Chicago, USA).

2.9 Determination of antioxidant enzyme concentration

The concentration of the antioxidant enzymes was evaluated in the colon homogenate. The concentration of catalase (CAT) was determined according to Aebi (1984). Superoxide dismutase (SOD) concentration was determined based on the ability of this enzyme to reduce the auto-oxidation of pyrogallol (Dieterich, Bieligk, Beulich, Hasenfuss, & Prestle, 2000). And, the concentration of glutathione-S-transferase (GST) was determined according to Habig, Pabst, and Jakoby (2002), which is based on the ability of GST to metabolize 1-chloro-2,4-dinitrobenzene (Sigma Aldrich®, St. Louis, USA) through the conjugation of reduced glutathione (Sigma Aldrich®, St. Louis, USA). All readings were performed in a spectrophotometer (Thermo Scientific®, Multiskan GO model, Vantaa, Finland), and the data were expressed in unit (U)/mg protein.

2.10 Protein determination

The concentration of proteins present in the colonic tissue used in the CAT, SOD and GST analyses was performed according to Lowry, Rosebrough, Farr, and Randall (1951). The readings were carried out in a spectrophotometer (Thermo Scientific®, Multiskan GO model, Vantaa, Finland) at a wavelength of 700 nm

2.11 Statistical analyses

The normality of the variables was determined by the Kolmogorov-Smirnov test. As all variables of the study showed normal distribution, the comparisons between the groups were performed by variance analysis (ANOVA one way) followed by the Tukey *post hoc* test. The Pearson correlation test was used to evaluate the presence of correlation between the variables of interest. A 5% α was considered significant and the results were expressed as mean ± standard error (SEM). Statistical analysis was performed using SPSS software (20 version, IBM® SPSS, Chicago, USA).

3. Results

3.1. Aberrant crypt foci count

In the proximal segment of colon, the Kefir group had a lower ACF count ≤ 3 compared to the other experimental groups. In the medial segment, the ACF count ≤ 3 was lower in the Milk and Kefir groups compared to Control. And, in the distal segment, the ACF count ≤ 3 was similar among the experimental groups (Table 1).

Regarding the ACF > 3, the Kefir and Milk groups presented a lower count than the Control group in the proximal, medial and distal segments (Table 1).

Comparing the total ACF count per colon segment, the Kefir group presented lower counts in the proximal segment compared to the other groups. And, in the medial and distal segments, the Kefir and Milk groups presented lower counts than Control. Considering the total colonic ACF, the Milk and Kefir groups presented a reduction of 27 and 36% in relation to the Control group, respectively (Table 1).

3.2. Short chain fatty acids

The total concentration of SCFA was higher ($p = 0.046$) in the Milk and Kefir groups compared to Control. And, the concentration of propionic acid was higher ($p = 0.011$) in the Kefir group compared to Control (Fig. 1).

3.3. Intestinal permeability

The percentage of mannitol excreted as well as lactulose/mannitol ratio ($p = 0.020$) was lower in the Kefir group compared to Control (Fig. 2). Furthermore, by the Pearson correlation test, a strong positive correlation ($r = 0.884$) was observed between the lactulose/mannitol ratio and the total ACF count in the colon of the Kefir group.

3.4 Histological evaluation

The percentages of goblet cells that produce acidic, neutral and both types of mucopolysaccharides did not differ among the experimental groups ($p > 0.05$; data not shown).

3.5 Pro-inflammatory cytokines

The colonic concentration of the proinflammatory cytokines IL12p70 and IFN-ɣ was similar among the experimental groups ($p > 0.05$; data not shown). However, TNF-α ($p = 0.038$) and IL-1β ($p = 0.017$) concentration was higher in the colon of the Kefir group compared to the other groups (Fig. 3).

3.6. Antioxidant enzymes

In the present study, the concentration of CAT was higher in the colon of Milk and Kefir groups compared to Control. On the other hand, the concentration of SOD was similar among the experimental groups, and the GST concentration was higher in the Milk group compared to the other groups (Table 2).

4 Discussion

CRC has a multifactorial cause, and most of its environmental etiological factors can be modulated in an attempt to reduce the risk for the development of this disease. In the case of the intestinal microbiota, it can be modulated by the regular consumption of probiotics (Serban, 2014). The regular consumption of probiotics would present a more effective anticarcinogenic effect in the early stages of carcinogenesis (Capurso, Marignani, & Fave, 2006; LeBlanc & Perdigón, 2004). In the case of CRC, its initial development phase is characterized by the appearance of ACF (pre-neoplastic lesions). ACF are in a constant state of transformation, which allows them to evolve or regress according to the stimulus they receive. For this reason, the ACF count is an important marker, since the higher the incidence of ACFs, the greater the chance of progressing to a tumor in the future (Bird & Good, 2000).

Considering the total colonic ACF count, the Kefir group presented a reduction of 36% in comparison to the Control group. Similar results have been reported in the scientific literature regarding the regular consumption of probiotics and the progression of ACF development (Verma & Shukla, 2013; Walia, Kamal, Kanwar, & Dhawan, 2015; Zhu et al., 2014).

The regular consumption of milk also led to a reduction in the incidence of ACF by 27% in comparison to Control. Some components of milk, such as calcium, casein, vitamin D and lactose are the potential responsible for the anticarcinogenic activity of this food. These nutrients are capable of binding to carcinogenic compounds present in the

Table 1
Aberrant crypt foci count per colon segment.

ACF/colon segment	Control	Milk	Kefir	*p*
Proximal				
ACF ≤ 3	50.20 ± 1.69[a]	49.00 ± 1.68[a]	32.40 ± 1,02[b]	0.007
ACF > 3	15.00 ± 1.07[a]	6.02 ± 1.05[b]	3.04 ± 1.4[b]	< 0.001
Total of the proximal segment	65.20 ± 1.28[a]	55.20 ± 1.40[a]	35.80 ± 1.07[b]	< 0.001
Middle				
ACF ≤ 3	118.20 ± 2.17[a]	87.80 ± 1.87[b]	82.60 ± 2.21[b]	< 0.001
ACF > 3	78.20 ± 1.57[a]	51.20 ± 1.82[b]	47.80 ± 2.09[b]	< 0.001
Total of the middle segment	196.40 ± 1.64[a]	139.00 ± 1.85[b]	130.40 ± 1.72[b]	< 0.001
Distal				
ACF ≤ 3	65.00 ± 2.65	55.60 ± 3.25	50.10 ± 3.33	0.175
ACF > 3	31.00 ± 1.03[a]	11.20 ± 1.01[b]	10.06 ± 0.94[b]	< 0.001
Total of the distal segment	96.00 ± 2.16[a]	66.80 ± 2.68[b]	60.70 ± 3.04[b]	0.001
Total (all segments)	357.60 ± 1.92[a]	261.00 ± 2.25[b]	226.90 ± 2.26[b]	< 0.001

Values are expressed as means ± SEM (n = 10). Data within a row with unlike superscript letters were significantly different ($p < 0.05$), according to Tukey's *post hoc* ANOVA statistical analysis. ACF: aberrant crypt foci.

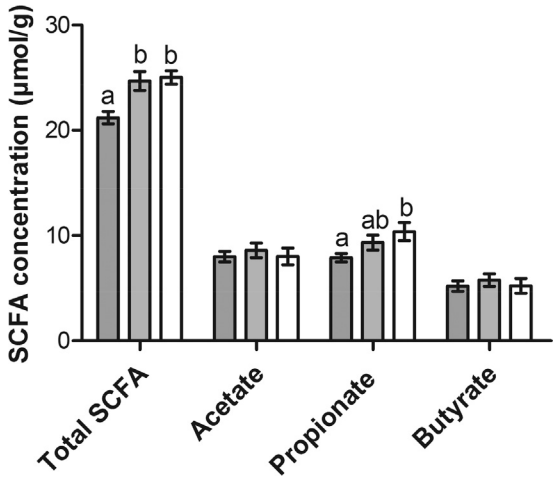

Fig. 1. SCFA concentration (acetic, propionic and butyric) in the cecal content. Values are expressed as means ± SEM (n = 10). Data with different superscript letters are significantly different at $p < 0.05$, according to Tukey's *post hoc* ANOVA statistical analysis. Dark gray blocks represent the Control group, light gray blocks the Milk group and the white blocks the Kefir group. SCFA: short chain fatty acids.

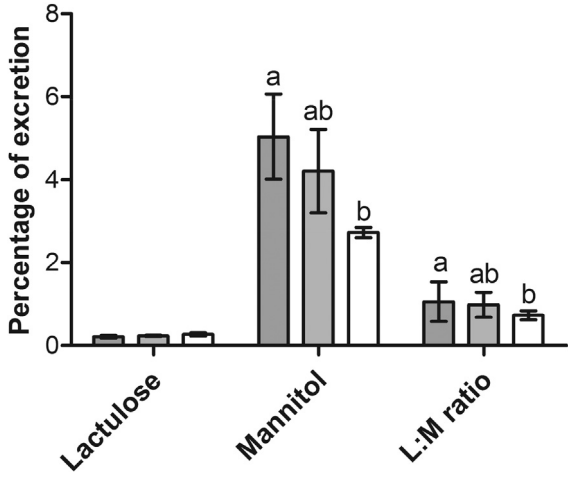

Fig. 2. Intestinal permeability. Values are expressed as means ± SEM (n = 8). Data with different superscript letters are significantly different at $p < 0.05$, according to Tukey's *post hoc* ANOVA statistical analysis. Dark gray blocks represent the Control group, light gray blocks the Milk group and the white blocks the Kefir group. L: lactulose, M: mannitol.

intestinal lumen, preventing them from interacting with the colonocytes. Furthermore, these nutrients are capable of influencing the occurrence of cell differentiation, apoptosis and proliferation, in addition to exhibiting antioxidant and immunomodulatory activities (Davoodi, Esmaeili, & Mortazavian, 2013).

The increase in the fecal concentration of SCFA has been considered a potential mechanism by which probiotics would exert its anticarcinogenic activity (Reis et al., 2017). In this sense, it was observed that the consumption of kefir increased the total concentration of SCFA, as well as propionic acid, in comparison to the control group. The increase in the total concentration of SCFA is an important result, since these fatty acids act synergistically to exert their beneficial effects (Kilner et al., 2012). With regard to propionic acid, it has been suggested that this acid is capable of stimulating apoptosis and inhibiting the proliferation of neoplastic cells. In addition, propionic acid would be equivalent to butyric acid with regard to anti-inflammatory activity, which exerts a great influence on the progression of tumor development (Hosseini, Grootaert, Verstraete, & de Wiele, 2011; Tedelind, Westberg, Kjerrulf, & Vidal, 2007).

The effect of butyric acid on CRC has been extensively studied due to its ability to regulate colonocyte proliferation, differentiation and apoptosis (Serban, 2014). LAB does not produce butyric acid (Hijova & Chmelarova, 2007), therefore, if we consider that the kefir used in this study presented an expressive count of these bacteria (10^9 CFU mL^{-1}) in its composition, then the results found for this SCFA are justified, since its concentration was similar between the experimental groups.

Another positive effect of kefir consumption observed in this study is the reduction in the excretion percentage of mannitol and lactulose/mannitol ratio in comparison to the control group. Lactulose and mannitol sugar are used as markers of intestinal permeability. While the monosaccharide mannitol is a transcellular pathway marker, the disaccharide lactulose marks the paracellular pathway (Teixeira, Moreira, Souza, Frias, & Peluzio, 2014). Thus, a high lactulose/mannitol ratio indicates an increase in the intestinal permeability, and evidences the occurrence of intestinal barrier dysfunction.

Intestinal barrier dysfunction can increase the passage of antigens from the lumen to the intestinal mucosa. This process may initiate a chronic inflammatory response, which has been consistently related to increase the risk for the development of CRC (Schwabe & Jobin, 2013). Perhaps it is because of this that a strong positive correlation (r = 0.884) was observed in the present study between the lactulose/mannitol excretion ratio and the total ACF count in the colon of the animals that consumed kefir. Thus, the reduction of intestinal permeability would constitute another mechanism used by kefir to exert its anticarcinogenic activity.

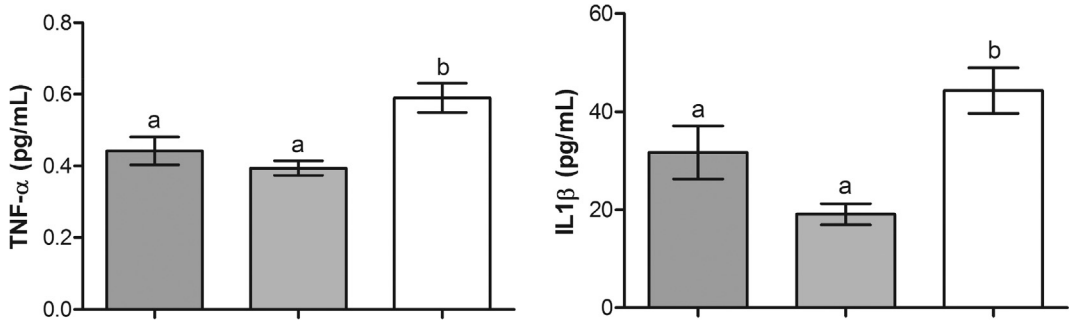

Fig. 3. Colonic concentration of the pro-inflammatory cytokines. A: TNF-α concentration. B: IL-1β concentration. Values are expressed as means ± SEM (n = 6). Data with different superscript letters are significantly different at $p < 0.05$, according to Tukey's *post hoc* ANOVA statistical analysis. Dark gray blocks represent the Control group, light gray blocks the Milk group and the white blocks the Kefir group.

One of the mechanisms by which the regular consumption of probiotics would improve the function of the intestinal barrier is related to the increase in the mucus production by the goblet cells (Kahouli, Tomaro-Duchesneau, & Prakash, 2013). This effect would occur through an increase in the number of goblet cells and/or the amount of mucus produced by these cells (Carasi et al., 2014). Thus, mucus production may increase without altering the number of goblet cells, which may be the case of the present study, since the regular consumption of kefir did not alter the number of goblet cells that produces acidic mucins, neutral ones, and both types.

Immunomodulation provided by the regular consumption of probiotics is another mechanism responsible for the anticarcinogenic activity of these microorganisms (Reis et al., 2017). In this study, regular consumption of kefir provided an increase in the concentration of the proinflammatory cytokines TNF-α and IL-1β in the colonic tissue. These cytokines are defined as "alarm cytokines", since they stimulate the expression of pro-inflammatory genes, which allow the immune system to remain in a state of vigilance, being ready to act and eliminate the cause of inflammation, for example, the pre-neoplastic cells (Voronov & Apte, 2015). In a study conducted by Vinderola et al. (2006), it was observed that macrophages extracted from Peyer's patches of healthy BALB/c mice that regularly consumed kefir had an increased production of TNF-α. In addition, these authors found an increase in the phagocytic activity of the peritoneal macrophages without causing damage to adjacent tissues. Thus, the immunomodulation promoted by kefir may have contributed to the reduction of the development of ACF, since it would place the immune cells in a state of vigilance.

The colon is constantly exposed to the activity of free radicals. These radicals would be naturally produced by some microorganisms present in the intestinal microbiota (Irrazábal, Belcheva, Girardin, Martin, & Philpott, 2014). In addition, DMH used for the induction of the pre-neoplastic lesions elevates the production of free radicals in the colon (Sengottuvelan, Senthilkumar, & Nalini, 2006). Thus, considering the relationship between oxidative stress and cancer, an efficient antioxidant system is indispensable for combating the development of this disease.

In the present study, an increased concentration of the antioxidant enzyme CAT was observed in the colon of the animals that consumed kefir. Similar results have been found in studies that evaluated antioxidant response provided by the consumption of probiotics in animal models with chemically induced pre-neoplastic lesions (Kumar et al., 2012; Mohania et al., 2013). Additionally, in these studies, the reduction in the number of ACF was related to the improvement in the antioxidant response induced by the regular consumption of probiotics.

In practice, if we consider an adult individual (~70 kg of body weight) and the kefir dose used in this study (1 mL/day), this represents a daily recommended intake of approximately 140 mL of kefir, an amount that can be easily incorporated into a diet. Moreover, it should be noted that the consumption of a probiotic food is more advantageous when compared to the isolated supplements of probiotics, since the food matrix increases the chances of survival of the microorganisms during the passage through the gastrointestinal tract (Martins, Deliza, Walter, Martins, Rosenthal, 2018). Also, the food matrix itself can be used as a substrate for the production of bioactive compounds (Champagne, da Cruz, & Daga, 2018). Thus, kefir can be considered a probiotic and probioactive food.

5 Conclusion

Regular consumption of kefir was able to suppress the development of chemically induced ACF in an animal model. In order to exert this effect, kefir increased the production of total SCFAs, mainly propionic acid; reduced intestinal permeability; promoted immunomodulation and improved local antioxidant response.

Kefir is a safe potential probiotic food that can be easily made at home. Considering that dysbiosis (local or disseminated alteration of the colon microbiota composition) can negatively affect host health and is also associated with an increased risk for the development of several chronic diseases, such as CRC, kefir consumption should be encouraged.

6. Declarations of interest

None.

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Table 2
Concentration of the antioxidant enzymes CAT, SOD and GST in the colonic tissue.

Antioxidants enzymes	Control	Milk	Kefir	*p*
CAT (U/mg of protein)	2.86 ± 0.22[a]	9.02 ± 0.77[b]	7.04 ± 0.50[b]	< 0.001
SOD (U/mg of protein)	27.42 ± 1.08	29.31 ± 2.51	23.33 ± 1.75	0.391
GST (nmol/min/mg of protein)	9.96 ± 0.98[a]	24.52 ± 2.08[b]	15.04 ± 1.02[a]	0.003

Values are expressed as means ± SEM (n = 10). Data within a row with unlike superscript letters were significantly different ($p < 0.05$), according to Tukey's *post hoc* ANOVA statistical analysis. CAT: catalase; GST: glutathione S-transferase; SOD: superoxide dismutase.

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