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A novel beer fermented by kefir enhances anti-inflammatory and anti-ulcerogenic activities found isolated in its constituents

Kamila Leite Rodrigues [a], *Tomaz Henrique Araújo* [b], *José Maurício Schneedorf* [c], *Cláudia de Souza Ferreira* [a], *Gabriel de Oliveira Isac Moraes* [c], *Rita Sinigaglia Coimbra* [d], *Maria Rita Rodrigues* [a,*]

[a] *Department of Toxicological and Clinical Analysis, Farmaceutic Sciences Faculty, Federal University of Alfenas, UNIFAL-MG, Alfenas, MG, Brazil*

[b] *Department of Histology, Biomedical Sciences Institute, UNIFAL MG, Alfenas, MG, Brazil*

[c] *Department of Biochemistry, Biomedical Sciences Institute, UNIFAL-MG, Alfenas, MG, Brazil*

[d] *Electron Microscopy Center, Federal University of São Paulo, UNIFESP, São Paulo, SP, Brazil*

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ABSTRACT

Kefir grown in molasses was activated to ferment malt for the production of beer. As kefir and beer are known to exhibit some beneficial health properties, anti-inflammatory and anti-ulcerogenic activities of a beer made with kefir were assessed using rat paw edema and ethanol-induced gastric ulcer model, respectively. The polyphenol content presented in the beers was evaluated by HPLC, whereas serum cholesterol, triacylglycerol, HDL cholesterol, ALT, AST, catalase and glutathione peroxidase of treated animals were determined by molecular absorption. The results revealed similar levels of beer polyphenols and serum markers among all treated animals, but marked decreases in inflammatory and ulcerogenic responses in the group treated with kefir beer, and with a control beer modified with aqueous kefiran. Scanning electron microscopy showed a coating layer on the stomach of animals treated only with kefir-based samples. These results suggest a functionality of a specialty beer made with kefir as single fermenter.

1. Introduction

Of all alcoholic beverages, beer is one of the oldest and most commonly consumed on a regular basis by many people around the world (Campbell-Platt, 1994). Beer is rich in nutrients as well as non-nutrient components, including carbohydrates, amino acids, minerals, vitamin B complex, citric acid, ascorbic acid, silicic acid and phenolic compounds (Guido et al., 2007). Phenolic constituents of beer are derived from malt (70–80%) and hops (20–30%). These molecules may include coumarins and catechins, proanthocyanidins, flavonoids and chalcones, among others. Beta, alpha and iso-alpha acids are also found in moist hops (Gerhäuser, 2005). Malt derived from barley for

* *Corresponding author.* Departamento de Análises Clínicas e Toxicológicas, FCF, Universidade Federal de Alfenas – UNIFAL-MG, Rua Gabriel Monteiro da Silva, 700, Alfenas, MG CEP-37130-000, Brazil. Tel.: +55 35 3299 1221; fax: +55 35 3299 1112.
E-mail address: rodrigues.mrita@gmail.com (M.R. Rodrigues).
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the manufacture of beer also contains several phenolic compounds that result from the malting process (Maillard reaction products). These compounds can play a significant role in malting and brewing via their antioxidant properties (Maillard, Soum, Boivin, & Berset, 1996). Malting contributes to both the total phenolic content and the antioxidant properties of beer (Lu et al., 2007). Food intake, including that of beer, may contribute to the total antioxidant capacity of plasma, which is an important strategy for inhibiting or delaying oxidative stress. Phenolic compounds such as tannin, flavonoids and diterpenes exhibit significant antioxidant activity (Ghiselli et al., 2000; Serafini, Maiani, & Ferro-Luzzi, 1998). As a consequence, several studies suggest that consuming one to two alcoholic beverages daily lowers the risk of coronary heart disease in middle-aged and older adults (Dietary Guidelines Advisory Committee, 2010; Rabi et al., 2011). Previous research also suggest that the additive or synergistic effects of alcohol and non-alcoholic compounds found in beer (e.g., antioxidants) inhibit all phases of the atherosclerotic process (Mann & Folts, 2004). Some studies have also demonstrated the anti-mutagenic effects of beer (Yoshikawa et al., 2002), beer-induced reduction of oxidative stress levels and products of lipid peroxidation in cerebral tissue (Gonzalez-Munoz et al., 2008), beer-induced protection from obesity, beer-induced regulation of type 2 diabetes and lipid metabolism (Kondo, 2004) and beer-induced reduction of central GABAergic activity in normotensive and hypertensive rats without any effect on cardiovascular function (Jastrzebski et al., 2007). Beer is commonly made using *Saccharomyces cerevisiae* as primary biofermenter, although it can also be manufactured using additional strains, such as *Brettanomyces* sp. In this work, we used kefir, a consortium of a dozen yeasts and lactic acid bacteria, including *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Torula*, *Kluyveromyces* and *Saccharomyces*, to ferment barley malt for the production of a kefir beer. Kefir is a fermented beverage originating from the Caucasian regions, and several health benefits have been claimed to be associated with both its probiotic and prebiotic content (Schneedorf, 2012). Kefir and its related products, e.g., kefiran, an exopolysaccharide produced by some strains, have been reported to have anti-inflammatory, antimicrobial, anti-neoplasic and antioxidant activities (Chen et al., 2015; Rodrigues, Caputo, Carvalho, Evangelista, & Schneedorf, 2005a). The present work was undertaken to produce a kefir-based beer to evaluate its plausible anti-inflammatory and anti-ulcerogenic activities to further the development of a potential candidate alcoholic functional food for the human diet.

2. Materials and methods

2.1. Kefir culture

Dried starter grains (50 g) stored at –20 °C in our lab (UNIFAL-MG, Brazil) were thawed and continuously cultured in 50 g/L of molasses solution until 30 g/L was obtained for mash fermentation. The grains were placed in polyethylene vessels containing nutrient medium and allowed to grow for up to 48 h. The suspensions were withdrawn from the containers, and the grains were gently washed in distilled water and settled again with a fresh nutrient preparation. The microbial profile of the starter grains was previously identified (Bergmann et al., 2010).

2.2. Beer production

Unfiltered and unpasteurized beers were made with kefir grains (kefir beer) or *Saccharomyces cerevisiae* (control beer). Kefir and control beers were produced using similar approaches. Briefly, 150 g/L of pilsen and vienna malt were ground to a fine powder to extract the fermentable sugars, followed by mashing at 62 °C for 40 min and 72 °C for 20 min. During this process, the malt enzymes are activated, converting the starch of the grains to dextrins, and then to fermentable sugars, such as maltose. After which, the mixture was heated to 75 °C to inactivate the enzymes. The mash was then separated for gravity filtering and pumped to separate the wort from the grain husks. The wort was brought to a controlled boil for 60 min, followed by the addition of Northern Brewer hops (1.5 g/L), whirlpool and chilling processes. The latter was carried out by a counterflow plate heat exchanger that refrigerated the wort for 30 min at 25 °C. To start the fermentation, *Saccharomyces cerevisiae* (10 mL/L, 1×10^6 viable cells/mL) or kefir grains (30 g/L) were added to fermenter chambers and kept for 7 days at 18 °C. After this souring period, the beers were transferred to another container for maturation for 10 days. Finally, the beers were bottled with 5 g/L of sucrose to provide a second fermentation and carbonation. The wort was evaluated with a hydrometer to estimate alcohol by volume, and the samples containing $4.0 \pm 0.2\%$ (v/v) ethanol were selected after 7 days of fermentation and 10 days of maturation. The bottled beers were stored at 20 °C in the dark for 10 days prior to beginning the experiments.

2.3. Determination of phenolic compounds in beers

Liquid chromatography (HPLC) profiles were obtained using Shimadzu LC-100 equipment with a UV/VIS detector at 280 nm, an automatic injector and LC solution software (Shimadzu, Kyoto, Japan). The HPLC apparatus was equipped with a C18 column (Shimadzu CLC-ODS; 4.6 mm × 250 mm; 5 μm). The mobile phase consisted of 0.5 mM aqueous acetic acid (A) and acetonitrile (B) with a flow rate of 0.7 mL/min. The following elution profile was used: 0–5 min 95:5 (v/v; A:B) (isocratic); 5–13 min 87:13 (v/v; linear); 13–27 min 87:13 (v/v; isocratic) and 27–30 min 95:5 (v/v; linear). An equilibration period of 10 min was included between runs. Phenolic standards used for the runs were quercetin, catechin, gallic, caffeic, coumaric and chlorogenic acids (Nardini, Natella, Scaccini, & Ghiselli, 2006).

Phenolic compounds present in the beer preparations were identified at 0, 10, 20, 30, 60 and 90 days after bottling, by comparing their retention times and spectral parameters with those of the standards. The concentrations of individual phenolic compounds in beer were calculated using calibration lines prepared by plotting peak area as a function of concentration for a series of standards (0.01–1 mg/mL). The results are expressed as mg/L.

2.4. Animals

Male Wistar rats weighing 250–300 g were randomly separated and fed a standard chow diet and received water *ad*