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Intestinal anti-inflammatory activity of goat milk and goat yoghurt in the acetic acid model of rat colitis

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ABSTRACT

The intestinal anti-inflammatory effect of goat milk and goat yoghurt with addition of *Lactobacillus acidophilus*, with or without native bee honey, was evaluated in rats with 10% acetic acid-induced colitis. The pre-treatment with goat milk, goat yoghurt or sulfasalazine significantly improved the myeloperoxidase activity, levels of leukotriene B_4 (LTB_4), interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α). It also promoted a significant reduction in oxidative stress that could be seen by the reduction in malondialdehyde and the increase in glutathione. The benefit of the pre-treatments was also demonstrated in the preservation of colonic cytoarchitecture and the decreased expression of cyclooxygenase-2 and inducible nitric oxide synthase. These results suggest that goat milk and goat yoghurt exert protective effects similar to those of sulfasalazine on intestinal damage induced by acetic acid and that goat milk and goat yoghurt may act as functional foods in inflammatory bowel disease.

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1. Introduction

Inflammatory bowel disease (IBD), which comprises Crohn's disease (CD) and ulcerative colitis (UC), is a debilitating and immunologically-mediated disease characterised by excessive inflammatory and effector mucosal responses; these responses lead to tissue destruction in the gastrointestinal tract (Viladomiu, Hontecillas, Yuan, Lu, & Bassaganya-Riera, 2013). The aetiology of IBD is unknown, but alterations in the intestinal immune system contribute to inflammation (Gálvez et al., 2001). This results in an increased synthesis and release of various pro-inflammatory mediators, including reactive oxygen species (ROS), nitrogen metabolites, eicosanoids, cytokines and chemokines, all of which contribute to the perpetuation of the inflammatory response in the intestine (Strober & Fuss, 2011). In addition, expression of inflammatory proteins such as cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS), which are capable of producing pro-inflammatory mediators such as prostaglandin E2 (PGE_2) and nitric oxide (NO), respectively, play an important role in IBD (Dong et al., 2003).

Milk and dairy products can provide nutritional support for patients with intestinal inflammation because their components confer benefits to gastrointestinal health and may be useful as part of the diet (Russ et al., 2010). Goat milk is a nutritional and therapeutic food with unique beneficial features that are superior to those of bovine milk (Slačanac et al., 2010). It can be consumed as an alternative to cows' milk because it is less allergenic and has better digestibility (García, Rovina, Boutoial, & López, 2014). Goat milk is an excellent matrix for development of functional foods (Silanikove, Leitner, Merin, & Prosser, 2010), and the nutritional benefits of goat dairy products can be improved by enriching them

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with strains of probiotics (Mukdsi, Haro, González, & Medina, 2013).

Lactobacillus acidophilus, Bifidobacterium spp., and *Lactobacillus casei* are lactic acid bacteria commonly used in fermented dairy products (Asharaf, Vasiljevic, Day, Smith, & Donkor, 2014). The desirable number of viable probiotic bacteria has not been firmly established; levels between 10^6 to 10^9 colony forming units (cfu) g^{-1} have been suggested (Abadía-García et al., 2013). Probiotic foods provide health benefits because they help maintain a good balance and composition of intestinal flora and increase resistance against the invasion of pathogens (Tripathi & Giri, 2014). Yoghurt is a fermented dairy product that contains viable bacteria with health-promoting effects (Morelli, 2014). Studies in experimental models of IBD have demonstrated that yoghurt is effective at reducing inflammation (Gobbato, Rachid, & Perdigón, 2008; LeBlanc, Chaves, & Perdigón, 2009). Although yoghurt from cows' milk is largely consumed (Ranadheera, Evans, Adams, & Baines, 2012), there is a high demand for alternatives to cows' milk due to problems associated with food allergy and gastrointestinal disorder and a desire for new dairy products (Haenlein, 2004; Ranadheera et al., 2012). Goat yoghurt constitutes an appropriate matrix for the inclusion of ingredients such as candied fruit, jam, honey, and nuts that are well liked by consumers (García et al., 2014).

Research on the nutritional and therapeutic importance of goat milk, as well as the functional properties of probiotics, has led to the development of goat yoghurt with honey. Therefore, this study aimed to evaluate the intestinal anti-inflammatory effect of goat milk and goat yoghurt with addition of *L. acidophilus,* with or without native bee (*Melipona scutellaris*) honey, in rats with induced colitis.

2. Materials and methods

2.1. Goat milk and goat yoghurt

Goat milk “in natura” was obtained from the Cooperativa dos Produtores Rurais de Monteiro Ltda – CAPRIBOM® – Brazil. Alpina Francesa goats were selected with 38.1 ± 2.6 kg of live weight, milk yield of 1.8 L day^{-1} and maintained at an intensive system. The diets were prepared according to NRC (2007) guidelines to meet the milk production requirements of 2.0 kg day^{-1}, with 4% of fat.

For yoghurt preparation, goat milk “in natura” was pasteurised (65 °C, 30 min) and cooled, sugar was then added and a heat treatment (90 °C, 15 min) applied. After cooling to 45 °C the starter culture (YF-L903, Christian Hansen®, Valinhos, Minas Gerais, Brazil) comprising *Streptococcus salivarius* subsp. *thermophillus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (0.4 g L^{-1}) and probiotic culture (La-05, Christian Hansen®, Valinhos, Minas Gerais, Brazil) comprising *L. acidophilus* (0.1 g L^{-1}) were added. The yoghurt was fermented (4 h), cooled, and honey (10%) was added. The yoghurt was packed and stored under refrigeration. The final product had count of 107 cfu mL^{-1}.

2.2. Reagents

All the reagents were obtained from Sigma Chemicals (São Paulo, SP, Brazil). Acetic acid and ethanol were obtained from Nova Chemicals. Nitric oxide synthase and cyclooxygenase antibodies were obtained from Santa Cruz Biotechnology, Inc. (Dallas, Texas, USA), and the kits for leukotriene B_4 (LTB_4) and cytokines tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were obtained from R&D Systems (Minneapolis, MN, USA).

2.3. Animals and experimental design

This study was approved by the Ethics Committee on Animal Use (CEUA/UFPB, protocol n° 0109/13). Sixty female Wistar rats ages 30 to 32d were obtained from the Biotechnology Center—Cbiotec/UFPB. The animals remained in cages for growth with free access to water and food (Purina®) and maintained at a temperature of 22 °C ± 2 °C under a 12 h/12 h light/dark cycle with. After growth, animals (190–240 g) were randomised into six groups (n = 10): non-colitic, colitic, goat milk, goat yoghurt (without honey), goat yoghurt with honey (10%), and sulfasalazine (250 mg kg^{-1}). All the groups were orally administered (by gavage) 1 mL of their respective product daily for fourteen days before the colitis induction and 24 h after the induction.

2.4. Colitis induction

Colitis was induced by the method originally described by MacPherson and Pfeiffer (1978) and subsequently modified by Millar et al. (1996) with minor adjustments. The animals were fasted 24 h and then anaesthetised with ketamine/xylazine. They were rectally administered acetic acid (0.5 mL 10%, v/v, in 0.9% saline) by 2 mm diameter cannula 8 cm deep. After the administration, the animals were kept in a head-down position for 30 s and then returned to their cages to recover from anaesthesia. The rats in the non-colitic group received 0.5 mL saline intracolonically. Two days later, the animals were sacrificed, under ketamine (Venil, São Paulo, Brazil)/xylazine (Calmium, São Paulo, Brazil) anaesthesia, and their colons were removed to assess the macroscopic damage, histological and biochemical parameters.

2.5. Macroscopic assessment of colonic damage

The colons of the animals were removed, placed on an ice-cold plate, cleaned of fat and mesentery, weighed, and the lengths were measured. The colon was opened longitudinally and evaluated for the extent and severity of macroscopic damage on a scale of 0–10 according to the model described by Bell, Gall, and Wallace (1995). Then, the colon was divided longitudinally into four sections and frozen at −80 °C until biochemical analysis. The fragment for the determination of glutathione was weighed and frozen at −80 °C with 1 mL of 5% (w/v) trichloroacetic acid.

2.6. Biochemical evaluation of colonic damage

The determination of myeloperoxidase (MPO) in the colonic mucosa was performed by the method described by Krawisz, Sharon, and Stenson (1984). The results were expressed as U g^{-1} of wet tissue, and one unit of myeloperoxidase activity was defined as that degrading 1 nmol min^{-1} hydrogen peroxide at 25 °C. Colonic malondialdehyde (MDA) content was evaluated according to the method proposed by Esterbauer and Cheeseman (1990) and expressed as nmol g^{-1} wet tissue. The total glutathione content determination was performed by the method described by Anderson (1985), and the results were expressed as nmol g-1 tissue. Levels of LTB4 and pro-inflammatory cytokines, TNF-α and IL-1β, were quantified by enzyme-linked immunosorbent assay (ELISA) using an ELISA kit (R&D Systems) according to the manufacturer's protocol. The results were expressed as ng g^{-1} wet tissue. Each colonic tissue was processed using tissue homogeniser (Lab 1000, LM-D160/1) for analysis of MPO, MDA, glutathione content, LTB_4 and cytokines.

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