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Original article

A comparative approach on the prophylactic impact of fermented beverages on acute ulcerative colitis in mouse model

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Abstract

Acute ulcerative colitis is an inflammatory disease of the colon that is becoming increasingly prevalent. Yet, a growing body of evidence supports the efficacy of dietary interventions in preventing acute ulcerative colitis. Fermented beverages have been the focus of research in humans and animals for several years due to their potential to influence overall health functions with an emphasis on gut health. This research comprehensively explores the preventive effect of three fermented beverages (water kefir, dairy kefir, and kombucha) on acute ulcerative colitis in a CD-1 mouse model. Histopathological evaluation of the colon samples indicated that consumption of kombucha led to increased alleviation of the gross and histopathological lesions. Oral administration of kombucha positively affected overall intestinal microecological homeostasis by decreasing the coliform counts in this group contrasting the water and milk kefir groups. Moreover, physicochemical evaluation of the fermented beverages was conducted covering key parameters such as pH, acidity, total solids, radical scavenging activity and total phenolic content (5.04 mg GAE/ mL), and total solids (0.70%), but the lowest pH (3.1) values.

The findings from this research offer valuable insights into the distinct contribution of different fermented beverages on prevention of acute ulcerative colitis. Kombucha unravels a promising natural prevention approach for acute colitis, opening new perspectives for future research.

Keywords: acute ulcerative colitis, fermented beverages, functional foods, probiotics, histopathology





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Introduction

Food harbors a suite of essential nutrients which collaborate to fuel metabolic processes in every cell of body (Chen et al. 2018). A substantial and expanding body of evidence indicates that consuming specific food categories has a beneficial impact on health and aids in the prevention of prevalent non-communicable diseases (Siddiqui et al. 2023). Food supplementation strategies have been leveraged to target development of functional products with enhanced nutritional properties (Dinçoğlu and Rugji 2021, Keyvan et al. 2021, Rugji et al. 2022, Rugji and Dinçoğlu 2022, Dinçoğlu et al. 2023). Lately, fermented foods have gained popularity due to their beneficial properties, particularly their remarkable effects on the gastrointestinal tract, both in humans and animals. Fermented feed can have several potential benefits for animal health such as improved digestibility, enhanced gut health, reduced digestive disorder, immune system support, reduction of antinutritional factors, enhanced palatability etc. (Yan et al. 2019). Several lactic acid bacteria (LAB) present in fermented dairy products have been found to have immunomodulatory effects, particularly in inflammatory bowel diseases (IBD-Crohn's disease and ulcerative colitis) (Klingberg et al. 2005, Marco et al. 2017). Increasing evidence suggests that regular consumption of fermented foods like kefir, water kefir, and kombucha may help ameliorate the proinflammatory effects associated with gut dysbiosis (Sniffen et al. 2018). Consumption of fermented products like kefir has been indicated as an approach to positively contribute to the treatment of specific diseases such as tuberculosis, cancer, and gastrointestinal disorders (Cevikbas et al. 1994).

Kombucha is a non-dairy, fermented beverage. It is typically prepared from sweetened green or black tea. A symbiotic amalgamation of bacteria and yeast, known as SCOBY, is responsible for the fermentation of the sweet tea mixture (Villarreal-Soto et al. 2018). The fermentation is typically conducted at room temperature and lasts between 7 and 21 days (Matei et al. 2018). The yeasts found in the SCOBY initially convert sucrose into ethanol, which is then metabolized by AAB into acetaldehyde and acetic acid. The presence of acetic acid leads to a decrease in pH, which has been reported to inhibit the growth of specific pathogenic bacteria like Helicobacter pylori, Escherichia coli, and Salmonella typhimurium, among others (Dimidi et al. 2019). Experimental animal studies have reported the effects of kombucha on blood glycaemia (Aloulou et al. 2012), oxidative stress (Dipti et al. 2003), diabetes (Morshedi et al. 2006), hypercholesterolemia (Yang et al. 2009), and indomethacin-induced gastric ulceration (Banerjee et al. 2010).

Kefir is a conventional fermented dairy product that is estimated to harbor more than 50 species of microorganisms including LAB, acetic acid bacteria (AAB) and yeasts (Kim et al. 2019). As stated in the Codex Standard for Fermented Milks, kefir is a fermented milk prepared from a unique starter culture containing a conglomerate of bacteria in an aggregate form known as kefir grain (Codex Alimentarius 2003). Some of the strains common in kefir have been identified to have probiotic properties (Bengoa et al. 2018). *Lactobacillus kefiri, Lactobacillus kefiranofaciens*, and *Lactobacillus acidophilus* cooperatively with the other LAB, AAC, and yeasts are responsible not only for the sensorial profile but also for the health benefits of kefir (Erdogan et al. 2018).

Water kefir is a non-dairy alternative to traditional kefir. The production of water kefir starts with combining a sucrose-containing fruit or extract with insoluble water kefir grains. Sucrose content plays a significant role in the production of exopolysaccharides (EPS), which are crucial for the structural integrity of kefir grains (Laureys et al. 2018). Kefir grains are a mixed aggregate of microorganisms that can ferment sucrose. The grains population is composed of LAB, AAB, *Bifidobacteria* and yeasts (Randazzo et al. 2016). These microorganisms are fixed together in a flexible, water-soluble branched galactoglucan matrix known as kefiran. Some of the bacteria in the kefir are probiotics (Fels et al. 2018).

To the best of our knowledge, no previous research has been conducted to compare the effects of milk kefir, water kefir, and kombucha on acetic acid-induced acute ulcerative colitis.

Therefore, this study provides valuable insights into how various fermented beverages distinctly contribute to preventing acute ulcerative colitis.

Materials and Methods

Beverage production

Commercial kefir grains (*Danem, Süt ve* Ürünleri *Ltd.* Şti.) were used to produce kefir. Standardized UHT milk containing 3% fat was heated to 25°C. After the addition of 3% kefir starter culture, the lids of the jars were closed and incubated at 37°C for 12 hr. The final product was put into a glass container and stored at 4°C. Water kefir was produced with water-kefir grains (*Danem, Süt ve* Ürünleri *Ltd.* Şti.). Preparation was done in accordance with the method described by Laureys and De Vuyst (2014) with few modifications. Black tea kombucha was prepared according to Ardheniati et al. (2009). A commercial starter (*SCOBY, Kombucha*) was used in this investigation.

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Table 1. Mice beverage groups.

Group	Kombucha	Milk kefir	SBW	Water kefir
K	+	-	-	-
MK	-	+	-	-
С	-	-	+	-
WK	-	-	-	+

K-kombucha, MK-milk kefir, C-control, WK-water kefir, SBW-saline buffered water

Approximately 10 g of the black tea was extracted in 500 mL of boiling water for 10 min prior to filtering and sweetening (10% sugar). The sugared tea extract was put into a glass container and cooled to room temperature before adding 10% kombucha starter aseptically. The jar was then covered with a sterile cheesecloth and incubated at ambient temperature for 10 days. Beverages were prepared once over the course of study.

Proximate analysis

The pH values of the samples were determined by a digital pH meter (704 pH Meter, Metrohm) at $25^{\circ}C\pm2^{\circ}C$. The acidity and total solids were evaluated according to AOAC standards (Williams 1984). The proximate composition was evaluated on the first and last day of the shelf life of the beverages (D1 and D14).

Radical scavenging activity of the beverages

The radical scavenging activity (%) of each sample was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) Free Radical Test. The analysis was conducted in accordance with Kahraman et al. (2021). At first a methanolic DPPH solution with a concentration of 200 μ M was prepared. After that, we added 150 μ L of the DPPH solution to each well of the 96-well plate and then 50 μ L of two concentrations (10% and Neat) from each fermented beverage (made in distilled water). The absorbance was measured at 517 nm after 30 minutes of incubation at room temperature in the dark using a microplate reader (Multiskan Go, Thermo Scientific). The blank was made up of pure methanol. The following formula was used to determine the relative amount of radical scavenging activity (RSA):

DPPH scavenging activity (%) = (Ac-As)/Ac x 100 Ac: Absorbance of control [DPPH + Methanol without sample]

As: Absorbance of sample [DPPH + Sample]

Total phenolic content of the beverages (TPC)

The Folin-Ciocalteu method was used to examine the TPC content in a 96-well microplate with certain modifications, as described by Yırtıcı et al. in (2022). First, 12.5 μ L of diluted Folin-Ciocalteu reagent (1:9 dilution) was combined with 25 μ L of the beverages and 187.5 μ L of ultrapure water in each well of the microplate. To this mixture, 25 μ L of 20% sodium carbonate solution (w/v) was added. The absorbance at 760 nm was measured using a microplate reader. The results were reported as milligrams of gallic acid equivalents per mL of beverage (mg GAE/mL).

Mice and experimental protocol

This research received approval from the Burdur Mehmet Akif Ersoy University Animal Research Local Ethics Committee on November 18, 2020, under protocol number 687. A total of 40 male CD-1 mice (ages 6-8 weeks) weighing 30-35 g were obtained from the Experimental Animal Production and Experimental Research Center of Burdur Mehmet Akif Ersoy University, Türkiye. Male mice were chosen as they are less protected against chemically induced colitis when compared to females (Bábíčková et al. 2006). After a week of acclimatization, the animals were randomly assigned into four groups (n=10 animals per group). They were housed in a room under standard conditions of humidity (50-60%) and temperature ($23\pm2^{\circ}$ C) with a 12-h light/dark cycle.

A diet with standard laboratory pellet and water ad libitum was provided. After the acclimation period, the groups were given their designated fermented beverages for seven consecutive days. The control group (C) was given saline buffered water (SBW), whereas the other groups were provided with milk kefir (MK), water kefir (WK), and kombucha (K) (Table 1). The administration of the fermented products was done in accordance with the method described by Erdogan et al. (2018) with immaterial modification. Each day, 0.2 mL of fermented beverage was given to the mice by intragastric gavage to each animal.

Acetic acid induced colitis

All groups were fasted for 12 hours after feeding on the 7th day (D7). After the 12 h fasting period, 1 mL of 5 % acetic acid (AA) solution was administered intrarectally with a soft 22 G and 1.2 mm diameter (Intechlabs, USA) feeding tube to induce acute colitis.



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Table 2. Gross and histopatholo	gical scoring	g criterion of the mouse colon.		
	0	1	2	
Gross lesion scores of colons	No lesion	Inflammation and ulceration at 1 or 2 foci	Inflammation and ulceration more than 3 foci	Diffuse of

Table 2. Gross and histopathological scoring criterion of the mouse colon.

Gross lesion scores of colons	No lesion	Inflammation and ulceration at 1 or 2 foci	Inflammation and ulceration more than 3 foci	Diffuse damage in colon
Histopathological scores	No lesion	Few scattered inflammatory cells and ulcerations not exceeding lamina Muscularis mucosae	Distributed inflammatory cell infiltrations and ulcerations not exceeding submucosa	Severe inflammatory cell infiltrations and ulcerations exceeding submucosa
Table 3. Proximate composition	of beverage	es.		
		Group	D1	D14
		К	3.2 ± 0.01	3.1 ± 0.01
pH values		MK	4.4 ± 0.01	4.1 ± 0.01
		WK	5.8 ± 0.01	6.7 ± 0.01
		К	0.90 ± 0.01	0.99 ± 0.01
Titratable acidity (% LA)		MK	0.76 ± 0.01	0.81 ± 0.01
		WK	<0.1	<0.1
		К	0.63 ± 0.01	0.70 ± 0.01
Total solids (%)		MK	0.50 ± 0.01	0.46 ± 0.01
		WK	0.08 ± 0.01	0.09 ± 0.01

D1 - day 1, D14 - day 14 (beverage shelf life), K - kombucha, MK - milk kefir, WK - water kefir

Immediately, mice were held in the supine Trendelenburg position for 30 seconds to prevent leakage (Sun et al. 2019). Then, the beverages continued to be given to the groups for 7 more days. Following 14 days (D14) of treatments, all mice were euthanized. The colons were excised and sectioned for further analysis.

Fecal microbial analysis

Prior to the administration of the fermented beverages (D0), stool samples were taken, and the genera of the fecal samples were analyzed (D0-before product administration). The genera of the fecal microbiota were also analyzed after 7 days of product ingestion (D7) and after 7 days of colitis induction (D14). The fecal microbial analyses were performed by media-dependent assay and the following bacterial populations were investigated: total aerobic mesophilic counts (TAMC), *Lactobacillus* spp., *Lactococcus* spp., and coliform counts. The mediums and incubation times were like those in our previous study (Rugji et al. 2022).

Colon histopathology

During the necropsy, colon specimens were collected and fixed in 10% neutral formalin solution. After fixation, tissue samples were taken with an automatic tissue processor (*Leica ASP300S, Wetzlar, Germany*) and embedded in paraffin. Paraffin blocks were prepared and cut in sections of 5 µm thickness by a rotary microtome (Leica RM2155, Leica Microsystems, Wetzlar, Germany). Then, the sections were stained with hematoxylin-eosin (HE), mounted with a coverslip, and examined under a light microscope. The severity of the acute inflammatory reaction and the degree of the spread of inflammation in the gut was graded using the macroscopic and histological scoring criteria (Table 2), which were modified from a previous study (Qin et al. 2012). Five random fields were selected on each slide. Morphometric analyses and microphotography were performed using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Co., Tokyo, Japan). The results were saved and statistically analyzed.

Statistical analysis

The statistical analysis of histopathological scores were compared between the groups. A one-way ANOVA Duncan test was used with a SPSS-22.00 package program.

Results

Proximate analysis

Fermented beverages (water kefir, milk kefir, and kombucha) were evaluated in terms of pH and total solids as seen in Table 3. Kombucha tea had the lowest pH values both on D1 and D14 (p<0.05). Water kefir

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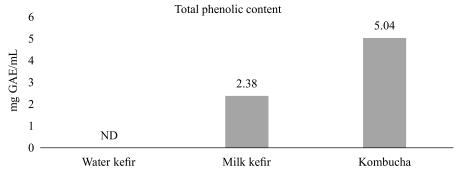


Fig. 1. Total phenolic content of the fermented beverages.

Group	100%
К	85.61 ± 0.01
МК	57.54 ± 0.01
WK	7.58 ± 0.01

K - kombucha, MK - milk kefir, WK - water kefir, DPPH - 2,2-diphenyl-1-picrylhydrazyl

had the highest pH values on D1 and D14. The pH values of the water kefir were pH 5.8 (D1) and pH 6.7 (D14). The milk kefir showed a slight decrease in pH values from pH 4.4 on D1 to pH 4.1 on D14. The initial titratable acidity of the water kefir, milk kefir, and kombucha was <0.1, 0.76 and 0.90% respectively. The titratable acidity of the milk kefir and the kombucha gradually increased during 14 days of storage. On D14, titration acidity values for the milk kefir and kombucha were 0.81 and 0.99 respectively. Variations were seen in the total solids content of all samples on D1 and D14. The total solids (%) on D1 were 0.079 for water kefir, 0.50 for milk kefir, and 0.09 for kombucha. On D14 the values were 0.70, 0.49 and 0.09 for kombucha, milk kefir and water kefir, respectively.

Radical scavenging activity

Results of the DPPH assessment of the fermented beverages is presented in Table 4. DPPH radical scavenging activity for all samples was 85.61, 57.54 and 7.58%, for kombucha, milk kefir and water kefir, respectively.

TPC of the beverages

Figure 1 displays the TPC of all samples. The results were reported as gallic acid equivalents (mg GAE) per mL of material. The results highlighted that the kombucha exhibited the highest total phenolic content, followed by milk kefir. In contrast, the total phenolic content in water kefir was below the detectable level. The total phenolic content in kombucha was 5.04 while the milk kefir was 2.38 mg GAE/ mL.

Fecal microbial analysis

The data presented in Table 5 reveals the results of the bacterial populations present in the feces from all experimental groups throughout the study. The assessed bacterial populations demonstrated consistent variation in magnitude. TAMC were found to be at the level of 7 log₁₀CFU for all groups in the initial evaluation (D0). The highest counts were found on D14 in group MK (8.27 \log_{10} CFU). At the end of the study, the population of *Lactobacillus* spp. increased in all groups compared to the initial levels and was determined to be at the level of 8 \log_{10} CFU. The *Lactococcus* spp. population was at the level of 7 log₁₀CFU for all groups in the initial evaluation, but changes were noted on D7 and D14. The Lactococcus spp. counts increased to 8 log₁₀CFU in all groups on the D14. The coliform count varied across all groups throughout the study. The highest counts were found in the MK group (6.64 \log_{10} CFU), while the lowest counts were found in the K group (2.16 \log_{10} CFU). The groups that received kombucha exhibited the lowest coliform counts overall on the last day of the experiment.

Colon histopathology

During the study, 6/10 mice from the milk kefir, 5/10 mice from the control group, and 4/10 mice from the water kefir group died. No death was observed in the kombucha group. During necropsy, an inflammatory reaction was observed in all mice, with varied severity. Some of the mice had marked and diffuse inflammation while some of them had only slight reactions. The most severe lesions were noticed in group C. In the kombucha group, lesions were slight compared

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	Group	D0	D7	D14
- TAMC -	К	7.20 ± 0.36	7.49 ± 0.06	$\boldsymbol{6.70\pm0.03}$
	MK	7.15 ± 0.22	$\boldsymbol{6.95\pm0.16}$	8.27 ± 0.01
	WK	7.25 ± 0.07	7.02 ± 0.36	7.90 ± 0.56
	С	7.84 ± 0.14	7.67 ± 0.04	8.16 ± 0.12
	К	8.00 ± 0.20	8.05 ± 0.07	8.69 ± 0.11
I actobacillus com	МК	7.70 ± 0.01	7.38 ± 0.09	8.59 ± 0.06
Lactobacillus spp. —	WK	8.05 ± 0.19	7.64 ± 0.20	8.86 ± 0.20
_	С	8.13 ± 0.16	8.17 ± 0.23	8.47 ± 0.06
	K	7.21 ± 0.28	7.49 ± 0.11	8.40 ± 0.08
	MK	7.13 ± 0.29	6.97 ± 0.19	8.54 ± 0.30
Lactococcus spp. —	WK	7.19 ± 0.07	7.22 ± 0.44	8.31 ± 0.07
_	С	7.74 ± 0.19	7.56 ± 0.11	8.27 ± 0.04
	K	4.61 ± 0.65	4.93 ± 0.20	2.16 ± 0.12
– Coliform – –	MK	4.85 ± 0.40	4.83 ± 0.29	6.64 ± 0.10
	WK	3.88 ± 0.14	4.26 ± 0.31	4.72 ± 0.05
	С	4.89 ± 0.01	5.35 ± 0.07	4.47 ± 0.45

Table 5. Fecal microbial analysis.

D0 - day 0, D7 - day 7, D14 - day 14 (feces sampling intervals), K - kombucha, MK - milk kefir, WK - water kefir, TAMC - total aerobic mesophilic counts

to the other groups. Microscopical evaluation of the colons revealed parallel findings with gross lesions. In the control group, severe infiltrations extended the tunica muscularis. Most mice had severe necrosis in the mucosa. The number and severity of lesions in the mice that were dosed with any of the fermented beverages were lower than those in the control group. In the water kefir, milk kefir, and kombucha groups relatively normal areas were also noticed (Fig. 2). Statistical analysis results of the gross and histopathological findings are illustrated in Fig. 3. Acetic acid induced colitis is a confirmed experimental model like human ulcerative colitis. AA (5%) increases the intestinal permeability by altering the epithelium structure and loss of crypts. This is followed by the entrance of luminal bacteria into the mucosa and induction of intestinal inflammation mediated by the local proinflammatory cytokines.

Discussion

During fermentation, the acidity of the kombucha beverage intensified due to organic acid production (Watawana et al. 2015). The reduction in pH and increase in acidity observed in milk kefir is caused by the key products of fermentation: lactic acid, ethanol, and CO_2 . These components contribute to the characteristic viscosity, acidity, and low alcohol content of milk kefir. In addition, minor constituents such as diacetyl, acetaldehyde, ethyl, and amino acids are in

part responsible for the flavor profile associated with milk kefir (de Oliveira Leite et al. 2013). By contrast, the elevated pH levels in water kefir are attributed to the presence of low initial nutrient concentrations, which can cause sluggish fermentation and thus reduced metabolite concentrations which ultimately result in higher pH values (Lynch et al. 2021).

The DPPH evaluation is widely recognized as a reliable method for measuring antioxidant properties due to the capacity of the components to scavenge free radicals and donate hydrogen or electrons (Baliyan et al. 2022). The kombucha beverage exhibited higher DPPH radical scavenging activity compared to milk and water kefir. During the fermentation of kombucha, numerous compounds with radical scavenging abilities are released from the tea leaves themselves (Malbaša et al. 2011). The main group of these compounds found in tea, belonging to the flavanol group, are polyphenols and catechins. Polyphenols possess significant broad--spectrum antioxidant properties due to their capacity to neutralize free radicals and reactive oxygen species (ROS). Polyphenols make up around 30% of the total dry weight of fresh tea leaves, with epigallocatechin, epigallocatechin-3-gallate, epicatechin-3-gallate, and epicatechin being the most prominent types of polyphenols present in tea leaves (Watawana et al. 2015).

Kombucha exhibited the highest TPC, followed by milk kefir. In contrast, the total phenolic content in water kefir was below the detectable level. The TPC in kombucha was 5.04 while the milk kefir was 2.38 mg GAE/ mL. The findings of this study align with



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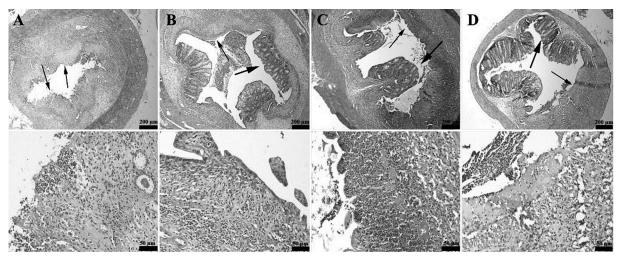


Fig. 2. Histopathological appearance between the mice groups. (A) Severe inflammatory reaction and ulcers (thin arrows) in a mouse from the control group. (B) Mild inflammation and ulcers (thin arrow and relatively normal gut mucosa (thick arrow) in water kefir group. (C) Ulcers (thin arrow) and gut mucosa (thick arrow) in milk kefir group. (D) Relatively slight ulcers (thin arrow) and gut mucosa (thick arrow) in kombucha group. Higher magnification of the lesions (below row). Scale bars=200 µm for upper row and 50 µm for below row.

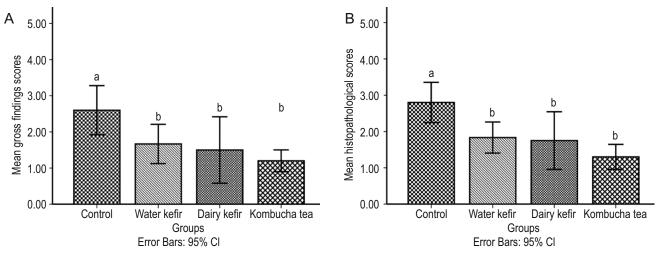


Fig. 3. Statistical analysis results of gross and histopathological scores between the mice groups. The differences between the means of groups carrying different letters are statistically significant. p<0.001. Data standard deviation (SD). One-Way Anova Duncan test.

previous reports (Javabalan et al. 2007, Chakravorty et al. 2016, Özyurt et al. 2020). De Filippis et al. (2018) also investigated the TPC of kombucha, focusing on the fermentation process using either ceylon black or bancha green tea. The process of microbial fermentation in kombucha leads to the metabolic transformation of tea components through enzymatic activity, which potentially contributes to the enhanced antioxidant activity of kombucha compared to unfermented tea. Moreover, the numerous health benefits associated with kombucha, including alleviating inflammation and arthritis, cancer prevention, and immune system enhancement, may be attributed to its antioxidant properties. These properties may be explained by the presence of polyphenols as well as certain organic acids that are produced during the fermentation process (Ahmed et al. 2020).

Various clinical and experimental studies utilizing diverse models have emphasized the importance of understanding the composition of the intestinal microbiota to gain deeper insights into the origins of colitis. While various fermented foods have been associated with enhanced human health, the effects of these foods on the composition of the gut microbiome have not been adequately characterized (Rettedal et al. 2019). Bedani et al. (2010) reported that mice who were fed a soy product fermented with E. faecium CRL 183 and L. helveticus 416, or a pure culture of E. faecium CRL 183, exhibited an elevation in the Enterococcus spp. population. Kombucha is renowned for its notable antimicrobial properties, effectively targeting a wide spectrum of microorganisms, including both Gram-positive and Gram-negative types. The antimicrobial effect exhibited by the broth is primarily

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attributed to its low pH, with acetic acid playing a significant role alongside various other organic acids and catechins present in the beverage (Watawana et al. 2015). Similarly, Erdogan et al. (2018) in their study on the effect of kefir produced from natural kefir grains on the intestinal microbial populations and antioxidant capacities of CD-1 mice found an increase in *Lactobacillus* spp. and yeast counts in the fecal microbiota. Unlike the present study, no significant changes were observed in the *Enterobacteriaceae* counts.

The kombucha group had the most dramatic lesion reduction when compared to all other groups. According to the histological results of previous studies, fermented beverages have been shown to be effective on IBD. Filtered kombucha tea showed a beneficial effect in healing colitis in mice by reducing neutrophil infiltration, epithelial defect, mucosal disruption, edema, and other pathological manifestations such as apoptosis (Pakravan et al. 2019). In a study investigating the healing activity of black tea and black tea fermented separately with Candida parapsilosis and kombucha culture in mice, it was observed that black tea fermented with kombucha culture was histologically effectively healed compared to the other two products (Banerjee et al. 2010). Another study investigated the effect of kefir treatment on dextran sulfate sodium--induced colitis in rats and revealed that kefir was able to significantly reduce the histologic colitis scores (Senol et al. 2015). Similarly, Celiberto et al. (2017) outlined those mice fed with fermented soy beverages exhibited a lower degree of inflammation and ulceration in their colon. The greater healing effect of kombucha tea compared to other fermented beverages may be due to its increased total phenol compound (Banerjee et al. 2010). The beneficial impact of kombucha on ulcerative colitis may result from several mechanisms. Recently it has been reported that kombucha polysaccharides can decrease intestinal permeability, enhance the expression of tight junction proteins, support the maintenance of goblet cell numbers, and stimulate mucus secretion. Moreover, adding kombucha polysaccharides to the diet boosts the diversity of the gut microbiota and alters its composition (Ji et al. 2024). Veterinary medicine is crucial in medical research, particularly in developing treatments and functional foods for both animals and humans. This cross-species research strengthens the understanding of diseases and enhances medical innovations for both fields. In this sense, the current study has provided important findings by helping to compare different fermented products in preventing acute colitis in experimental animals.

Conclusion

A growing body of clinical trials have reported the therapeutic effect of fermented beverages on gut health. Nevertheless, the current study aims to contribute to the comprehension of the prophylactic effect of fermented beverages on acute ulcerative colitis. Obtained data from the oral administration of the beverages demonstrates that intake of kombucha possesses greater preventive capacity of acetic acid induced acute colitis in mice compared to milk and water kefir. These findings suggest that incorporating kombucha into a daily regimen presents a promising natural preventative measure for acute colitis, paving the way for further exploration in future research endeavors. This study provides useful information on the impact of different fermented beverages on various health metrics. However, it has some limitations, such as mouse mortality and a restricted number of fecal samples because of induced colitis. Integrating research on animal studies, fermented foods, and veterinary public health fosters a comprehensive approach to health, benefiting animals, people, and the environment. This approach ensures that practices are based on solid evidence, resulting in better health outcomes and more effective policies. Howbeit, to enhance our understanding, future research should explore the measurement of bacterial metabolites and evaluate microbial gene functions using techniques like RNA--sequencing.

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References

- Ahmed RF, Hikal MS, Abou-Taleb KA (2020) Biological, chemical and antioxidant activities of different types Kombucha. Ann Agric Sci 65: 35-41.
- Aloulou A, Hamden K, Elloumi D, Ali MB, Hargafi K, Jaouadi B, Ayadi F, Elfeki A, Ammar E (2012) Hypoglycemic and antilipidemic properties of kombucha tea in alloxaninduced diabetic rats. BMC Complement Altern Med 12: 63.
- Ardheniati M, Andriani MA, Amanto BS (2009) Fermentation kinetics in kombucha tea with tea kind variation based on its processing. Asian J Nat Prod Biochem 7: 48-55.
- Bábíčková J, Tóthová Ľ, Lengyelová E, Bartoňová A, Hodosy J, Gardlík R, Celec P (2015) Sex Differences in Experimentally Induced Colitis in Mice: a Role for Estrogens. Inflammation 38: 1996-2006.
- Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM (2022) Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. Molecules 27: 1326.

A comparative approach on the prophylactic impact of fermented ...

- Banerjee D, Hassarajani SA, Maity B, Narayan G, Bandyopadhyay SK, Chattopadhyay S (2010) Comparative healing property of kombucha tea and black tea against indomethacin-induced gastric ulceration in mice: possible mechanism of action. Food Funct 1: 284-293.
- Bedani R, Pauly-Silveira ND, Roselino MN, de Valdez GF, Rossi EA (2010) Effect of fermented soy product on the fecal microbiota of rats fed on a beef-based animal diet. J Sci Food Agric 90: 233-238.
- Bengoa AA, Llamas MG, Iraporda C, Dueñas MT, Abraham AG, Garrote GL (2018) Impact of growth temperature on exopolysaccharide production and probiotic properties of Lactobacillus paracasei strains isolated from kefir grains. Food Microbiol 69: 212-218.
- Celiberto LS, Bedani R, Dejani NN, Ivo de Medeiros A, Sampaio Zuanon JA, Spolidorio LC, Tallarico Adorno MA, Amâncio Varesche MB, Carrilho Galvão F, Valentini SR, Font de Valdez G, Rossi EA, Cavallini DC (2017) Effect of a probiotic beverage consumption (Enterococcus faecium CRL 183 and Bifidobacterium longum ATCC 15707) in rats with chemically induced colitis. PloS One 12: e0175935.
- Cevikbas A, Yemni E, Ezzedenn FW, Yardimici T, Cevikbas U, Stohs SJ (1994) Antitumoural antibacterial and antifungal activities of kefir and kefir grain. Phytother Res 8: 78-82.
- Chakravorty S, Bhattacharya S, Chatzinotas A, Chakraborty W, Bhattacharya D, Gachhui R (2016) Kombucha tea fermentation: Microbial and biochemical dynamics. Int J Food Microbiol 220: 63-72.
- Chen Y, Michalak M, Agellon LB (2018) Importance of nutrients and nutrient metabolism on human health. Yale J Biol Med 91: 95.
- Codex Alimentarius (2003) Codex-Stan CXS 243-2003; Codex Standard for Fermented Milks. Retrieved from http://www. fao.org/fao-who-codexalimentarius/en/. Accessed October 10, 2018.
- De Filippis F, Troise AD, Vitaglione P, Ercolini D (2018) Different temperatures select distinctive acetic acid bacteria species and promotes organic acids production during Kombucha tea fermentation. Food Microbiol 73: 11-16.
- de Oliveira Leite AM, Miguel MA, Peixoto RS, Rosado AS, Silva JT, Paschoalin VM **(2013)** Microbiological, technological and therapeutic properties of kefir: a natural probiotic beverage. Braz J Microbiol 44: 341-349.
- Dimidi E, Cox SR, Rossi M, Whelan K (2019) Fermented Foods: Definitions and Characteristics, Impact on the Gut Microbiota and Effects on Gastrointestinal Health and Disease. Nutrients 11: 1806.
- Dinçoğlu AH, Ileri A, Rugji J (2023) Determination of bioactive properties of Capparis spinosa fruits and use in production of Tulum cheese. Emir J Food Agric 11: 1-12.
- Dinçoğlu AH, Rugji J (2021) Use of rose oil in probiotic fermented whey as a functional food. J Food Sci Technol 58: 2705-2713.
- Dipti P, Yogesh B, Kain AK, Pauline T, Anju B, Sairam M, Singh B, Mongia SS, Kumar GI, Selvamurthy W (2003) Lead induced oxidative stress: beneficial effects of Kombucha tea. Biomed Environ Sci 16: 276-282.
- Erdogan FS, Ozarslan S, Guzel-Seydim ZB, Taş TK (2019) The effect of kefir produced from natural kefir grains on the intestinal microbial populations and antioxidant capacities of Balb/c mice. Food Res Int 115: 408-413.

- Fels L, Jakob F, Vogel RF, Wefers D (2018) Structural characterization of the exopolysaccharides from water kefir. Carbohydr Polym 189: 296-303.
- Green N, Miller T, Suskind D, Lee D (2019) A Review of Dietary Therapy for IBD and a Vision for the Future. Nutrients 11: 947.
- Ji ZH, Xie WY, Zhao PS, Ren WZ, Jin HJ, Yuan B (2024) Kombucha polysaccharide alleviates DSS-induced colitis in mice by modulating the gut microbiota and remodeling metabolism pathways. Front Microbiomes, 3: 1341824.
- Jayabalan R, Marimuthu S, Swaminathan K (2007) Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. Food Chem 102: 392-398.
- Kahraman HA, Tutun H, Kaya MM, Tutun S, Usluer MS, Rugji J, Yurdakul O (2021) Total phenolic content, antiradical, antimicrobial and antibiofilm properties of grape and apple vinegar. J VetBio Sci Tech, 6: 150-158.
- Keyvan E, Rugji J, Dinçoğlu AH (2021) Probiotic Starter Cultures in Food Products. In: Mortazavian AM, Khorshidian N, Gomes da Crus (eds) In Vitro Functionality of Probiotics in Foods Edition:1. Chapter: 2 Publisher: Nova Science Publishers, pp 15-54.
- Kim DH, Jeong D, Kang IB, Lim HW, Cho Y, Seo KH (2019) Modulation of the intestinal microbiota of dogs by kefir as a functional dairy product. J Dairy Sci 102: 3903-3911.
- Klingberg TD, Axelsson L, Naterstad K, Elsser D, Budde BB (2005) Identification of potential probiotic starter cultures for Scandinavian-type fermented sausages. Int J Food Microbiol 105: 419-431.
- Laureys D, De Vuyst L (2014) Microbial species diversity, community dynamics, and metabolite kinetics of water kefir fermentation. Appl Environ Microbiol 80: 2564-2572.
- Laureys D, Aerts M, Vandamme P, De Vuyst L (2018) Oxygen and diverse nutrients influence the water kefir fermentation process. Food Microbiol 73: 351-361.
- Lynch KM, Wilkinson S, Daenen L, Arendt EK (2021) An update on water kefir: Microbiology, composition and production. Int J Food Microbiol 345: 109128.
- Malbaša RV, Lončar ES, Vitas JS, Čanadanović-Brunet JM (2011) Influence of starter cultures on the antioxidant activity of kombucha beverage. Food Chem 127: 1727-1731.
- Marco ML, Heeney D, Binda S, Cifelli CJ, Cotter PD, Foligné B, Gänzle M, Kort R, Pasin G, Pihlanto A, Smid EJ, Hutkins R (2017) Health benefits of fermented foods: microbiota and beyond. Curr Opin Biotechnol 44: 94-102.
- Matei B, Salzat J, Diguta CF, Cornea CP, Luta G, Utoiu ER, Matei F (2018) Lactic acid bacteria strains isolated from Kombucha with potential probiotic effect. Rom Biotechnol Lett 23: 13592-13598.
- Morshedi A, Dashti MH, Rafati A, Mosaddegh MH, Salami AS (2006) The chronic effect of Kombucha Tea consumption on weight loss in diabetic rats. J Med Plants 5: 17-22.
- Özyurt H (2020) Changes in the content of total polyphenols and the antioxidant activity of different beverages obtained by Kombucha 'tea fungus'. Int J Agric Environ Food Sci 4: 255-261.
- Pakravan N, Kermanian F, Mahmoudi E (2019) Filtered Kombucha tea ameliorates the leaky gut syndrome in young and old mice model of colitis. Iran J Basic Med Sci 22: 1158-1165.
- Qin HY, Xiao HT, Wu JC, Berman BM, Sung JJ, Bian ZX (2012) Key factors in developing the trinitrobenzene sulfonic



acid-induced post-inflammatory irritable bowel syndrome model in rats. World J Gastroenterol 18: 2481-2492.

- Randazzo W, Corona O, Guarcello R, Francesca N, Germanà MA, Erten H, Moschetti G, Settanni L (2016) Development of new non-dairy beverages from Mediterranean fruit juices fermented with water kefir microorganisms. Food Microbiol 54: 40-51.
- Rettedal EA, Altermann E, Roy NC, Dalziel JE (2019) The Effects of Unfermented and Fermented Cow and Sheep Milk on the Gut Microbiota. Front Microbiol 10: 458.
- Rugji J, Çalışkan Z, Dinçoğlu AH, Özgür M, Erol Z, Özgür EB (2022) Prebiotic effect of D-allulose and β-glucan on whey beverage with Bifidobacterium animalis and investigation of some health effects of this functional beverage on rats. Food Sci Technol 42: e07022.
- Rugji J, Dinçoğlu AH (2022) Biocontrol of Listeria monocytogenes by Bacillus coagulans GBI-30, 6086 in a synbiotic white brined cheese: An in vitro model study. LWT 169: 113982.
- Senol A, Isler M, Sutcu R, Akin M, Cakir E, Ceyhan BM, Kockar MC (2015) Kefir treatment ameliorates dextran sulfate sodium-induced colitis in rats. World J Gastroenterol 21: 13020-13029.
- Siddiqui SA, Erol Z, Rugji J, Taşçı F, Kahraman HA, Toppi V, Musa L, Di Giacinto G, Bahmid NA, Mehdizadeh M, Castro-Muñoz R (2023) An overview of fermentation in the food industry-looking back from a new perspective. Bioresour Bioprocess 10: 85.

Sniffen JC, McFarland LV, Evans CT, Goldstein EJ (2018)

Choosing an appropriate probiotic product for your patient: An evidence-based practical guide. PloS One 13: e0209205.

- Sun HB, Jing XS, Zhang GQ, Hai Y, Liu YZ, Wang DC (2019) Preliminary Study of Obese Patients with Chronic Obstructive Pulmonary Disease Suffering from Painful Osteoporotic Vertebral Compression Fracture Treated by Percutaneous Vertebroplasty in Improved Prone Position and Right Lateral Position. World Neurosurg, 130: e933-e940.
- Villarreal-Soto SA, Beaufort S, Bouajila J, Souchard JP, Taillandier P (2018) Understanding Kombucha Tea Fermentation: A Review. J Food Sci 83: 580-588.
- Watawana MI, Jayawardena N, Gunawardhana CB, Waisundara VY (2015) Health, wellness, and safety aspects of the consumption of kombucha. J Chem 2015: 591869.
- Williams S (1984) Official Methods of Analysis. Association of Official Analytical Chemists. 14th Edition, AOAC, Arlington.
- Yan J, Zhou B, Xi Y, Huan H, Li M, Yu J, Zhu H, Dai Z, Ying S, Zhou W, Shi Z (2019) Fermented feed regulates growth performance and the cecal microbiota community in geese. Poult Sci 98: 4673-4684.
- Yang ZW, Ji BP, Zhou F, Li B, Luo Y, Yang L, Li T (2009) Hypocholesterolaemic and antioxidant effects of kombucha tea in high-cholesterol fed mice. J Sci Food Agric 89: 150-156.
- Yırtıcı Ü, Ergene A, Atalar MN, Adem Ş (2022) Phytochemical composition, antioxidant, enzyme inhibition, antimicrobial effects, and molecular docking studies of Centaurea sivasica. S Afr J Bot 144: 58-71.