

# Regulatory role of *Lactobacillus acidophilus* on inflammation and gastric dysmotility in intestinal mucositis induced by 5-fluorouracil in mice

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## Abstract

**Purpose** *Lactobacillus acidophilus* is widely used for gastrointestinal disorders, but its role in inflammatory conditions like in chemotherapy-induced mucositis is unclear. Here, we report the effect of *L. acidophilus* on 5-fluorouracil-induced (5-FU) intestinal mucositis in mice.

**Methods** Mice weighing 25–30 g ( $n = 8$ ) were separated into three groups, saline, 5-FU, and 5-FU + *L. acidophilus* (5-FU-La) ( $16 \times 10^9$  CFU/kg). In the 5-FU-La group, *L. acidophilus* was administered concomitantly with 5-FU on the first day and alone for two additional days. Three days after the last administration of *L. acidophilus*, the animals were euthanized and the jejunum and ileum were removed for histopathological assessment and for evaluation of levels of myeloperoxidase activity, sulfhydryl groups, nitrite, and cytokines (TNF- $\alpha$ , IL-1 $\beta$ , CXCL-1, and IL-10). In addition, we investigated gastric emptying using spectrophotometry after feeding a 1.5-ml test meal by gavage and euthanasia. Data were submitted to ANOVA and Bonferroni's test, with the level of significance at  $p < 0.05$ .

**Results** Intestinal mucositis induced by 5-FU significantly ( $p < 0.05$ ) reduced the villus height–crypt depth ratio and GSH concentration and increased myeloperoxidase activity and the nitrite concentrations compared with the control

group. Furthermore, 5-FU significantly ( $p < 0.05$ ) increased cytokine (TNF- $\alpha$ , IL-1 $\beta$ , and CXCL-1) concentrations and decreased IL-10 concentrations compared with the control group. 5-FU also significantly ( $p < 0.05$ ) delayed gastric emptying and gastrointestinal transit compared with the control group. All of these changes were significantly ( $p < 0.05$ ) reversed by treatment with *L. acidophilus*.

**Conclusions** *Lactobacillus acidophilus* improves the inflammatory and functional aspects of intestinal mucositis induced by 5-FU.

**Keywords** Intestinal mucositis · 5-Fluorouracil · *Lactobacillus acidophilus* · Probiotics · Gastrointestinal dysmotility

## Introduction

Probiotics are “live microorganisms which, when administered in adequate amounts, confer benefits to the health of the host” [1]. Probiotics promote crypt cell proliferation, prevent cytokine-induced apoptosis [2], reduce pro-inflammatory cytokine production, and regulate the intestinal immune system [3]. Among probiotics, *Lactobacillus acidophilus* stands out for being a widely used, thermophilic, nonpathogenic bacteria [4]. *Lactobacillus acidophilus* is used for the treatment and prevention of gastrointestinal disorders associated with diarrhea of varying etiology [5].

Intestinal mucositis (IM) is inflammation associated with the cytotoxicity of chemotherapy and radiotherapy for cancer. Intestinal mucositis usually accompanies cell loss in the epithelial barrier of the lining of the gastrointestinal tract [6, 7]. Symptoms of IM include nausea, dyspepsia, dysphasia, vomiting, and diarrhea [8]. Intestinal mucositis development can be separated into three stages of increased epithelial

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dysfunction: an inflammatory stage, epithelial degradation stage, and an ulceration/bacterial stage. This dysfunction is rescued by restoration of the functional epithelia [9].

5-Fluorouracil (5-FU), widely used as chemotherapeutic for colorectal and breast cancer [10, 11], is known to induce intestinal damage through IM [12] and stem cell apoptosis [13]. Our group has shown that IM induced by 5-FU promotes infiltration of neutrophils, increases pro-inflammatory cytokine levels, and significantly delays gastric emptying [8]. Furthermore, IM induced by 5-FU promotes dysbiosis [14]. The effect of restoring the normal microbiota in IM has not been studied.

Therefore, the aim of the present study was to evaluate the effect of administration of *L. acidophilus* on the inflammatory and functional outcomes of 5-FU-induced IM in mice.

## Materials and methods

### Animals

Male Swiss mice weighing 25–30 g (supplied by the Department of Physiology and Pharmacology, UFC Medical School) were kept in a temperature-controlled room with ad libitum access to water and fasted for 24 h prior to all experiments. The study was previously approved by the local research ethics committee (protocol#34/10), and all procedures involving animals were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the US Department of Health and Human Services.

### Model of IM induced by 5-FU

Twenty-four male Swiss mice were randomly divided into a saline group ( $n = 8$ ), a 5-FU group (saline + 5-FU 450 mg/kg, single dose, i.p.  $n = 8$ ), and a 5-FU-La group (5-FU 450 mg/kg, single dose, i.p. + *L. acidophilus*  $16 \times 10^9$  CFU/kg for 3 days,  $n = 8$ ). Saline served as control for the 5-FU group, and 5-FU served as a control for the 5-FU + *L. acidophilus* group. The animals in 5-FU + *L. acidophilus* received 5-FU and *L. acidophilus* simultaneously. The mice were euthanized three days after completing treatment with *L. acidophilus*. Blood samples were collected, and the jejunum and ileum were removed for morphological and histopathological analyses and for evaluation of myeloperoxidase activity (MPO), sulfhydryl groups, and levels of nitrite and cytokines (TNF- $\alpha$ , IL-1 $\beta$ , CXCL-1, and IL-10). The animals were weighed daily throughout the experiment.

### Intestinal morphometry and histopathology

Segments of jejunum (a 3-cm segment immediately distal to the ligament of Treitz) and distal ileum (a 6-cm segment

adjacent to the ileocecal valve) were collected, fixed, and stained with hematoxylin and eosin for the measurement of villus height and crypt depth. Ten intact and well-oriented villi and crypts were measured and averaged for each sample. The microscopy analysis was double-blinded. Mucosal inflammation was assessed using a modification of the histopathological scores described by [15].

### Intestinal MPO activity

Myeloperoxidase is found in azurophilic neutrophil granules and has been extensively used as a biochemical marker of granulocyte infiltration into various tissues, including the gastrointestinal tract. The extent of neutrophil accumulation in the intestinal mucosa was quantified using an MPO activity assay kit [16]. Briefly, intestinal tissue (50 mg/ml) was homogenized in HTAB buffer (Sigma-Aldrich, St Louis, MO). The homogenate was centrifuged at 4,500 rpm for 7 min at 4 °C. MPO activity in the resuspended pellet was assayed by measuring the change in absorbance at 450 nm using o-dianisidine dihydrochloride (Sigma-Aldrich, St Louis, MO) and 1 % hydrogen peroxide (Merck, Whitehouse Station, NJ). The results were expressed as MPO units/mg tissue. A unit of MPO was defined as the amount of enzyme required to convert 1  $\mu$ mol/min of hydrogen peroxide into water at 22 °C.

### Glutathione assay

Intestinal tissue levels of glutathione (GSH) were assessed with an assay for nonprotein sulfhydryl content [17]. In summary, 100 mg/ml frozen intestinal tissue was homogenized in 0.02 M EDTA. Aliquots of 400  $\mu$ l of homogenate were mixed with 320  $\mu$ l distilled water and 80  $\mu$ l 50 % trichloroacetic acid (TCA) to precipitate proteins. The material was centrifuged (3,000 rpm) for 15 min at 4 °C. Aliquots of 400  $\mu$ l of supernatant were mixed with 800  $\mu$ l 0.4 M Tris buffer (pH 8.9) and 20  $\mu$ l 5.5-dithiobis-(2-nitrobenzoic acid) (DTNB) (Fluka, St Louis, MO), followed by shaking for 3 min. Within 5 min of addition of DTNB, the absorbance was read at 412 nm against a blank reagent without homogenate. The results were expressed as  $\mu$ g GSH/mg tissue.

### Determination of nitrite levels

The production of NO was determined indirectly by measuring nitrite levels using the Griess reaction [18]. Briefly, 100  $\mu$ l intestinal tissue homogenate was incubated with 100  $\mu$ l Griess reagent (1 % sulfanilamide in 1 %  $H_3PO_4$ /0.1 % *N*-(1-naphthyl)ethylenediamine dihydrochloride/1 %  $H_3PO_4$ /distilled water, 1:1:1:1) at room

temperature for 10 min. A microplate reader measured the absorbance at 540 nm. Nitrite levels were determined from a standard nitrite curve generated using  $\text{NaNO}_2$ .

Detection of cytokines (TNF- $\alpha$ , IL-1 $\beta$ , CXCL-1, and IL-10)

Cytokine (TNF- $\alpha$ , IL-1 $\beta$ , CXCL-1, and IL-10) concentrations in jejunum and ileum samples were determined using enzyme-linked immunosorbent assay (ELISA) using protocols supplied by the manufacturer (R&D Systems, Minneapolis, USA). The results were expressed as pg/ml.

Gastric emptying and intestinal transit

Gastric emptying and intestinal transit were measured using the modified technique of Reynell and Spray [19]. Initially, the animals received a 300- $\mu\text{l}$  test meal by gavage, consisting of a nonabsorbable marker (0.75 mg/ml phenol red in 5 % glucose). After 20 min, the animals were euthanized by cervical dislocation. After laparotomy, the stomach and bowels were exposed and the esophageal–gastric, gastroduodenal, and ileocecal junctions were immediately isolated using ligatures. The specimens were removed and divided into stomach and proximal, medial and distal bowel. Each segment was placed in a graduated cylinder, and the total volume was measured by adding 10 ml of 0.1 N NaOH. Then, the samples were cut into small pieces and homogenized for 30 s. Twenty minutes later, 1 ml supernatant was removed and centrifuged for 10 min at 2,800 rpm. The proteins in the homogenate were precipitated by adding 20 % TCA and centrifuged again for 20 min at 2,800 rpm. Then, 150 ml of supernatant was collected and added to 200 ml 0.5 N NaOH. The absorbance of the samples was determined using spectrophotometry at 540 nm and expressed as optical density.

The fractional dye retention was expressed in percentage, according to the following equation: gastric dye retention = amount of phenol red recovered in stomach/total amount of phenol red recovered from two segments (stomach and small intestine). Intestinal transit was calculated for each bowel segment by dividing the amount of phenol red recovered from a given segment by the amount of phenol red recovered from all three segments and is expressed as a percentage.

Statistical analysis

The results were reported as the mean values  $\pm$  standard error of the mean (SEM) for each group. The data were submitted to analysis of variance (ANOVA) followed by Bonferroni's test. The level of statistical significance was set at  $p < 0.05$ .

## Results

Effect of *L. acidophilus* on the weight of mice with IM induced by 5-FU

The average weight of the animals in the saline group increased throughout the study period ( $6.13 \pm 1.22$  %), with the highest weight registered on the last day. In contrast, weight in the 5-FU group decreased considerably ( $-14.54 \pm 2.03$  %) by day 3 after 5-FU administration in relation to that of the saline group. The weight of the 5-FU + *L. acidophilus* group decreased  $-5.48 \pm 0.66$  % in relation to that of the 5-FU group.

Effect of *L. acidophilus* on histopathological changes in the intestinal mucosa

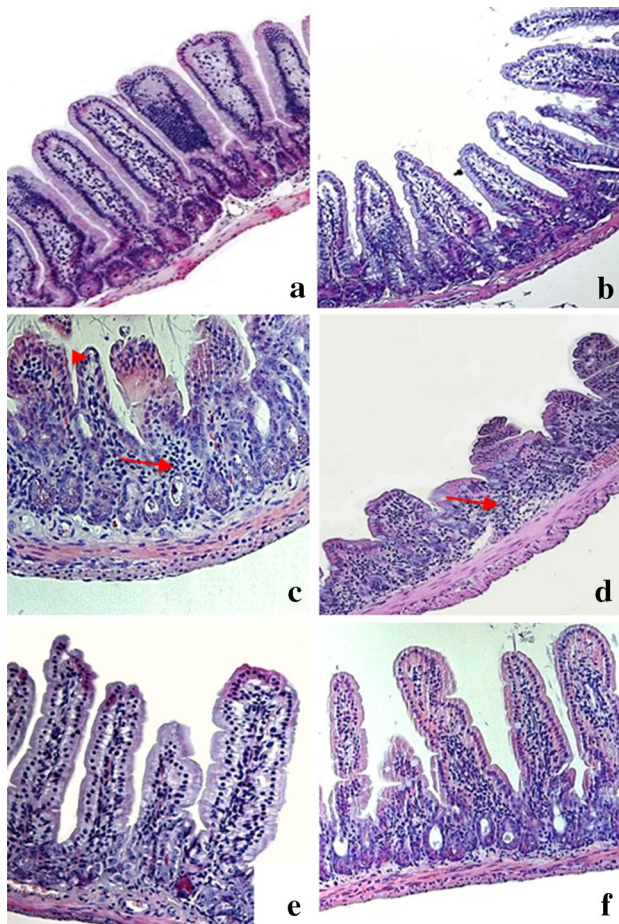
The animals in the 5-FU group presented the following histopathological changes in the jejunum and ileum: mucosa with shortened villi with vacuolated cells, intense inflammatory infiltrate and vacuolization (Fig. 1c, d) compared to those in the control group (Fig. 1a, b). The 5-FU + *L. acidophilus* group experienced a significant improvement of histopathological changes, as shown by photomicrographs (Fig. 1e, f).

The 5-FU group showed a significant decrease in villus height (Fig. 2a), an increase in crypt depth (Fig. 2b), and a decrease in the villus/crypt ratio (Fig. 2c) of the jejunum and ileum, compared to the control group. Panel b, Fig. 2, shows that treatment with *L. acidophilus* significantly ( $p < 0.05$ ) reversed the 5-FU-induced increase in crypt depth in both segments. Likewise, the decrease in the villus/crypt ratio observed in the 5-FU group was significantly reversed in the 5-FU + *L. acidophilus* group (Fig. 2c).

Effect of *L. acidophilus* on MPO activity and GSH and nitrite concentrations

Following administration of 5-FU, the animals experienced a significant ( $p < 0.05$ ) increase in neutrophil infiltration in the jejunum ( $7.84 \pm 2.28$  UMPO/mg) and ileum ( $8.31 \pm 2.40$  UMPO/mg) compared to the saline group (jejunum:  $2.11 \pm 0.71$  UMPO/mg; ileum:  $1.64 \pm 0.81$  UMPO/mg). Treatment with *L. acidophilus* significantly ( $p < 0.05$ ) reduced neutrophil infiltration in the jejunum ( $0.90 \pm 0.46$  UMPO/mg) and in the ileum ( $0.97 \pm 0.42$  UMPO/mg) compared to those of the 5-FU group (Fig. 3a).

5-FU significantly ( $p < 0.05$ ) reduced GSH concentrations in the jejunum ( $376.30 \pm 16.79$   $\mu\text{g}/\text{mg}$ ) and in the ileum ( $322.30 \pm 37.01$   $\mu\text{g}/\text{mg}$ ) compared to those in the saline group (jejunum:  $779.30 \pm 94.60$   $\mu\text{g}/\text{mg}$ ; ileum:  $543.40 \pm 64.02$   $\mu\text{g}/\text{mg}$ ). Treatment with



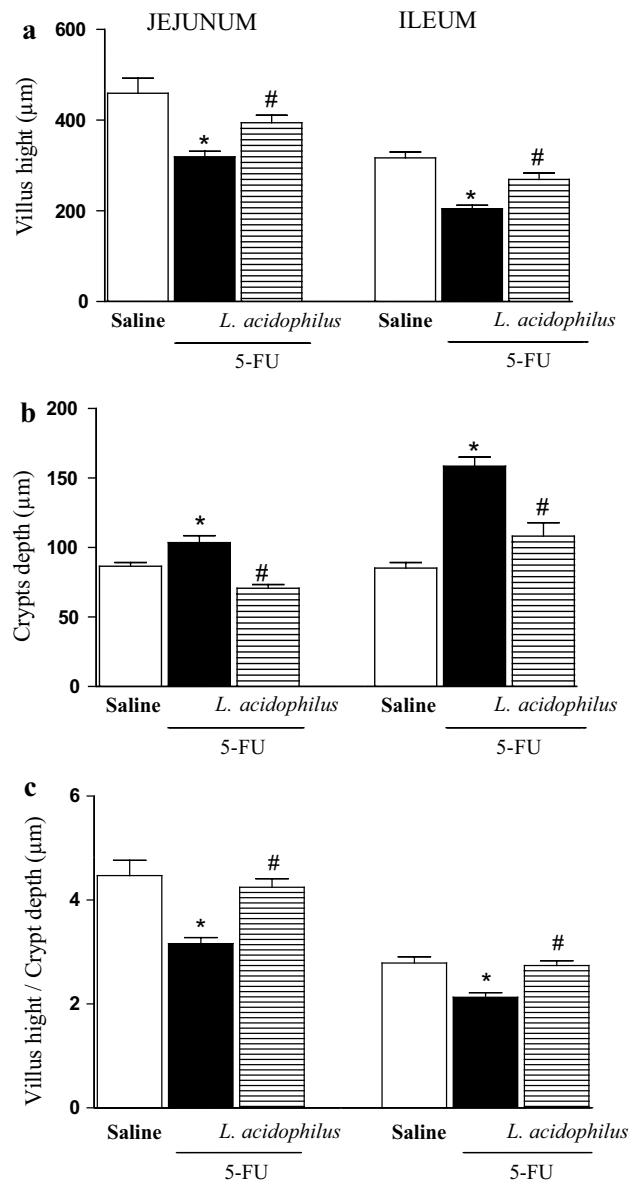
**Fig. 1** Administration of *Lactobacillus acidophilus* reduced intestinal damage in mice caused by exposure to 5-fluorouracil. Photomicrographs ( $\times 200$ ) of the jejunum and ileum of mice treated with saline (**a, b**), 5-FU + saline (**c, d**), and 5-FU + *L. acidophilus* for 3 days (**e, f**). Note that treatment with *L. acidophilus* reversed 5-FU-induced shortening of the villi (**c** arrowhead) and reduced the intensity of inflammatory infiltrates (**c, d** arrow)

*L. acidophilus* significantly ( $p < 0.05$ ) reversed GSH reductions in the jejunum ( $693.00 \pm 100.05 \mu\text{g/g}$ ) and in the ileum ( $514.80 \pm 51.91 \mu\text{g/g}$ ) (Fig. 3b).

Figure 3c shows that administration of 5-FU significantly ( $p < 0.05$ ) increased nitrite concentrations in the jejunum ( $86.43 \pm 10.93 \mu\text{M}$ ) and in the ileum ( $44.66 \pm 5.46 \mu\text{M}$ ) compared to those in the saline group ( $37.00 \pm 2.93$  and  $25.08 \pm 1.38 \mu\text{M}$ , respectively). Treatment with *L. acidophilus* significantly ( $p < 0.05$ ) reduced nitrite concentrations in both the jejunum and ileum ( $39.13 \pm 3.91$  and  $31.65 \pm 1.67 \mu\text{M}$ , respectively) compared to those in the 5-FU group.

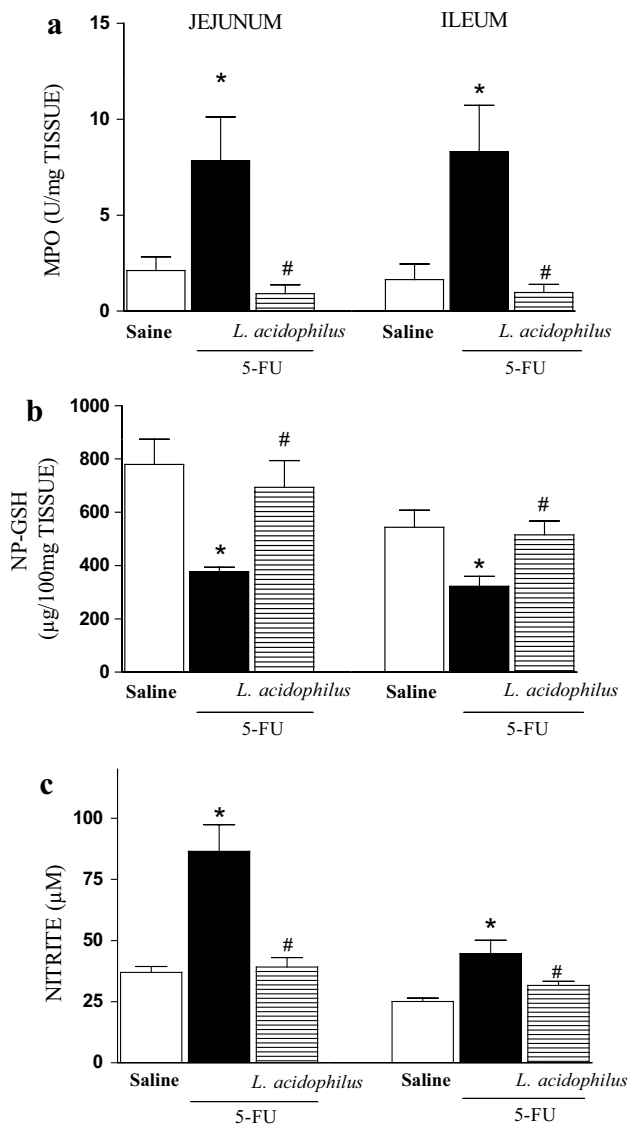
Effect of *L. acidophilus* on TNF- $\alpha$ , IL-1 $\beta$ , CXCL-1, and IL-10 production

Figure 4 shows that the animals with IM present significantly ( $p < 0.05$ ) increased levels ( $p < 0.05$ ) of



**Fig. 2** Administration of *Lactobacillus acidophilus* decreased 5-fluorouracil-induced intestinal morphometric changes in mice. Segments of the jejunum and ileum were processed to measure the height of the villi (**a**), crypt depth (**b**), and the villus/crypt ratio (**c**). Values are expressed as the mean  $\pm$  SEM. The data were submitted to ANOVA followed by Bonferroni's test. \* $p < 0.05$  compared with Group I (saline only). # $p < 0.05$  compared with Group II (5-FU + saline)

TNF- $\alpha$ , IL-1 $\beta$ , and CXCL-1 in the jejunum (56.3, 350.6, and 163.5 %, respectively) and in the ileum (195.4, 77.6, and 535.7 %, respectively) compared to those of the controls (panel a, b, and c). IL-10 showed significant ( $p < 0.05$ ) reductions in the jejunum and ileum (39.1 and 52.2 %, respectively). In contrast, these increases in TNF- $\alpha$ , IL-1 $\beta$ , and CXCL-1 and decreases in IL-10 concentrations were significantly ( $p < 0.05$ ) reversed by treatment with *L. acidophilus* (panel d).



**Fig. 3** Administration of *Lactobacillus acidophilus* improved inflammatory parameters (MPO, GSH, and nitrite) of 5-fluorouracil-induced intestinal mucositis in mice. Note the increased levels of MPO activity (a), GSH consumption (b), and nitrite (c) due to exposure to 5-fluorouracil. Treatment with *L. acidophilus* (a–c) improved inflammatory parameters. The results are presented as the mean  $\pm$  SEM. The data were submitted to ANOVA followed by Bonferroni's test. \* $p < 0.05$  compared with Group I (saline only). # $p < 0.05$  compared with Group II (5-FU + saline)

#### Effect of *L. acidophilus* on gastric emptying and gastrointestinal transit

Gastric retention was significantly ( $p < 0.05$ ) higher in the 5-FU group ( $49.06 \pm 5.42$  %) than in the saline group ( $23.78 \pm 2.73$  %) (Fig. 5a). This was reversed by treatment with *L. acidophilus* ( $33.58 \pm 1.85$  %). Panel b shows that gastrointestinal transit was significantly slower in the 5-FU group than in the saline group (median geometric center:

$2.02 \pm 0.11$  vs.  $2.43 \pm 0.08$ ) ( $p < 0.05$ ), but was normalized by treatment with *L. acidophilus* ( $2.34 \pm 0.06$ ).

Likewise, a significant level of retention in the proximal, medial, and distal bowel segments (5-FU vs. saline) was associated with diarrhea. Treatment with *L. acidophilus* significantly ( $p < 0.05$ ) reversed these changes (Fig. 5c).

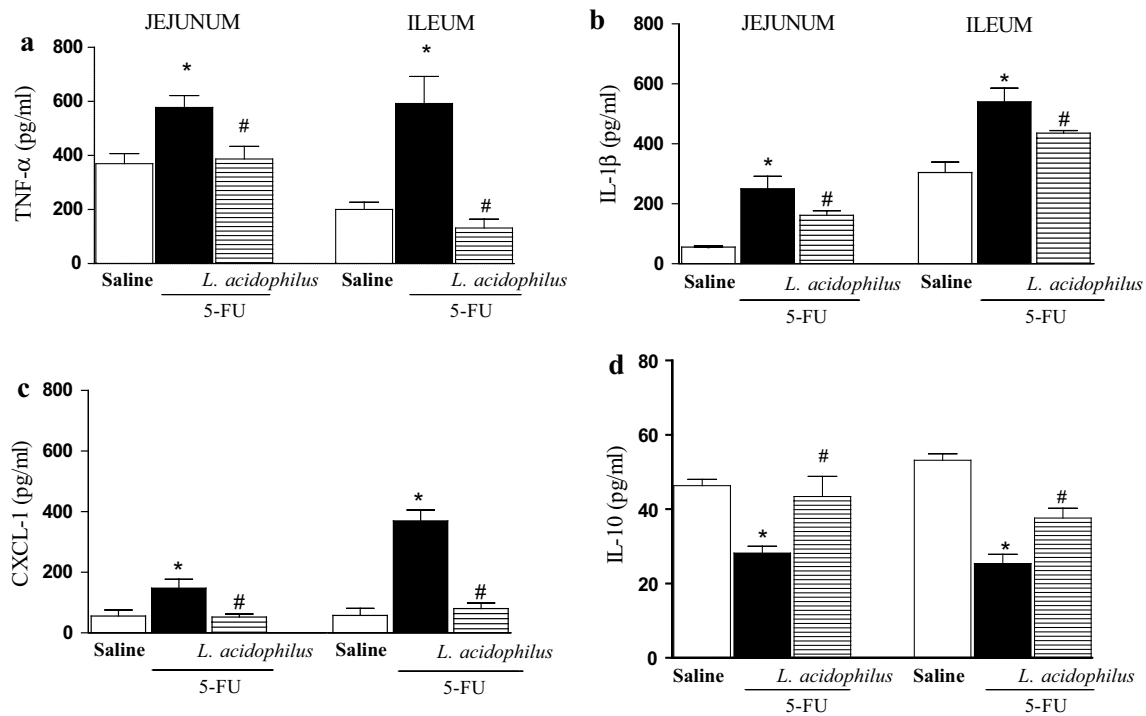
#### Discussion

Treatment with *L. acidophilus* improved the histopathological changes in murine IM induced by 5-FU, possibly due to reductions in inflammatory markers (levels of nitrite, GSH, cytokines, and neutrophil infiltration). In addition, *L. acidophilus* reversed the gastrointestinal dysmotility induced by 5-FU.

Currently, some probiotics have achieved success in the treatment of mucositis induced by 5-FU. *Streptococcus thermophilus* TH-4 improved the mitotic count, cryptal fissions, and histological deficits caused by 5-FU [20]. *Lactobacillus fermentum* BR11 showed a partial rescue of histological pathology while being ineffective in combination with prebiotics [21]. Supernatant of *Escherichia coli* Nissle 1917 partially reduced the damage caused by 5-FU [22]. However, *L. rhamnosus* GG, *Bifidobacterium lactis* BB12, and skim milk were totally ineffective in rescuing the mucositis caused by 5-FU [23]. Several studies have shown epithelial damage and neutrophil infiltration in the mucosa during the inflammatory stage of the mucositis induced by antineoplastic drugs [24]. Edens et al. [25] reported changes in the permeability of the intestinal epithelium caused by migration of neutrophils to epithelial cells. This finding can explain the dyspepsia in animals with 5-FU-induced IM. Lima et al. [26] found that pentoxifylline and thalidomide inhibited the lesions and myeloperoxidase activity in 5-FU-induced oral mucositis. Another study using methotrexate reported a significant increase in villous atrophy in the rat intestinal mucosa [27]. Intestinal injury may include changes in brush border hydrolase activity, blunted villus height, deepening and increased cell apoptosis in the crypt, and decreased proliferation [28, 29].

Few studies have assessed the effects of *L. acidophilus* on inflammation. One of these studies found lower levels of leukocyte migration in animals treated with *L. acidophilus* in a model of IM induced by irinotecan [30]. Several studies have reported reduced inflammatory effects using other probiotic species.

Probiotics derived from *E. coli* and *L. fermentum* lowered the inflammation caused by 5-FU in the rat jejunum [22]. Moreover, *L. brevis* reduced nitrite and nitrate levels in patients with chronic periodontitis [31]. Matsumoto et al. [32] evaluated the effect of *L. casei* strain Shirota on murine chronic inflammatory bowel disease and reported a



**Fig. 4** Administration of *Lactobacillus acidophilus* reversed increased levels of TNF- $\alpha$ , IL-1 $\beta$ , CXCL-1, and IL-10 in 5-fluorouracil-induced intestinal mucositis in mice. Note the increased levels of TNF- $\alpha$  (a), IL-1 $\beta$  (b), and CXCL-1 (c) and decreased levels of IL-10 (d) due to exposure to 5-fluorouracil. Treatment with *L. acidophilus*

reversed normalized levels of cytokines. The results are presented as the mean  $\pm$  SEM. The data were submitted to ANOVA followed by Bonferroni's test. \* $p < 0.05$  compared with Group I (saline only). # $p < 0.05$  compared with Group II (5-FU + saline)

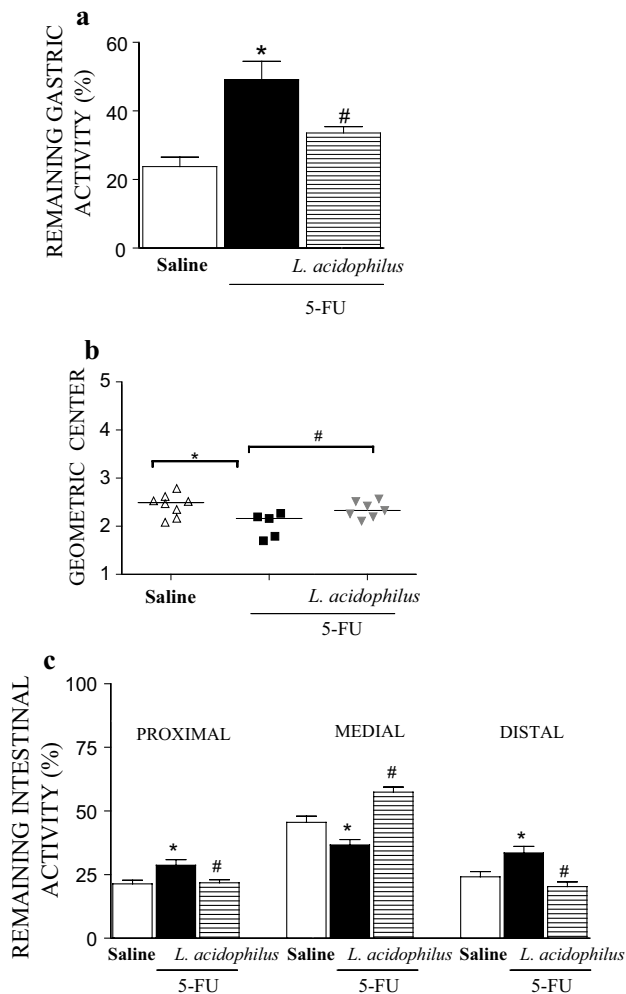
reduction in body weight loss, diarrhea, and occult blood. In an earlier study, we reported that *Saccharomyces boulardii* lowered pro-inflammatory cytokine levels (TNF- $\alpha$ , IL-1 $\beta$ , and CXCL-1) in the rat jejunum and ileum induced by 5-FU [33]. *Saccharomyces boulardii* may have a protective effect against diarrheal pathogens by reducing the pro-inflammatory response [34]. These authors reported lower secretion of pro-inflammatory cytokines (IL-1 $\beta$ ) and higher levels of anti-inflammatory cytokines (IL-4 and IL-10) in animals treated with *S. boulardii*. The mechanisms of *S. boulardii* may be similar to the mechanisms of *L. acidophilus* action in the model of IM induced by 5-FU used in our study.

In a clinical trial, *L. acidophilus* associated with *B. bifidum* was satisfactory for diarrhea prophylaxis during pelvic radiation therapy with concomitant cisplatin. Acute inflammatory changes might play an important role in the pathogenesis of these symptoms [35]. Furthermore, *L. casei* strain Shirota may be a useful probiotic to manage inflammatory bowel disease. *L. casei* improves murine chronic inflammatory bowel disease and is associated with a down-regulation of pro-inflammatory cytokines such as IL-6 [32]. Strains of *L. acidophilus*, such as CBA4P, decrease leptin-immunostimulated activity by lowering levels of macrophage IL-1 $\beta$  and TNF- $\alpha$  in mice [36].

Other pathways to explain the *L. acidophilus* action that we observed include the stimulation of apical Cl<sup>-</sup>/OH<sup>-</sup> exchange activity, corresponding to increased surface expression of DRA in Caco-2 cells via the PI-3 kinase-mediated pathway. Thus, *L. acidophilus* may be useful in treating diarrhea and other intestinal inflammatory disorders involving impairment of electrolyte absorption [37].

Moreover, 5-FU-induced IM is associated with delayed gastric emptying/intestinal transit of liquids [38]. Hypercontractility of the deep muscles of the stomach and duodenum in both the inflammatory and post-inflammatory phase has also been reported. Patients receiving anticancer therapy experience gastrointestinal symptoms such as dyspepsia, dysphagia, and diarrhea [39], referred to as cancer-associated dyspepsia syndrome (CADS).

In this study, *L. acidophilus* reversed 5-FU-induced changes in gastrointestinal motility, enhancing intestinal transit and gastric emptying and decreasing retention in the distal bowel segment. This may partially account for the observed improvement in diarrhea and reduced weight loss. Thus, we hypothesize that treatment with *L. acidophilus* normalizes bowel function by reducing inflammation associated with 5-FU-induced IM.



**Fig. 5** Administration of *Lactobacillus acidophilus* reversed delayed gastric emptying and gastrointestinal transit associated with 5-FU-induced intestinal mucositis. Note delayed gastric emptying (a), displacement of the geometric center (b), and intestinal transit (c) due to exposure to 5-fluorouracil. Treatment with *L. acidophilus* (a–c) improved gastrointestinal symptoms. The results are presented as the mean  $\pm$  SEM. The data were submitted to ANOVA followed by Bonferroni's test. \* $p < 0.05$  compared with Group I (saline only). # $p < 0.05$  compared with Group II (5-FU + saline)

Few studies have evaluated the effects of probiotic bacteria on intestinal motility. One study, [40], suggested that *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* mediate relaxation in colonic motility. This relaxation could explain the observed improvement in diarrhea by reducing stool frequency and restoration of the microflora. Czerucka and Rampal [41] demonstrated that *S. boulardii* restored luminal electrolyte transport in cholera toxin-induced diarrhea in the rabbit jejunum and suggested a mechanism involving cAMP-dependent chloride secretion. Budriesi et al. [42] used a mixture containing *Castanea sativa* and *S. boulardii* to induce antispasmodic and spasmolytic effects in segments of intestinal smooth muscle contracted by carbachol,

histamine, KCl, and BaCl<sub>2</sub>. These effects may be due to the inhibition of voltage-dependent Ca<sup>2+</sup> channels.

Dysmotility accompanies inflammatory bowel. Cells present in inflammatory conditions, such as macrophages, produce nitric oxide and prostaglandins, which lead to dysmotility [43]. Inflammatory factors such as IL-1 $\beta$  and IL-6 [44], monocyte chemoattractant protein-1, and CCL-2 [45] also induce dysmotility. These data corroborate the fact that *L. acidophilus* reduces inflammation and thus improves intestinal motility.

In summary, this study showed, for the first time, the anti-inflammatory effects of *L. acidophilus* and its regulatory role in the dysmotility associated with IM induced by 5-FU. We hope that our findings will contribute to the discovery of new probiotic-based treatments of gastrointestinal toxicity associated with antineoplastic therapy.

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