Modulating effects of the probiotic *Lactococcus lactis* on the hepatic fibrotic process induced by CCl_4 in Wistar rats

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Received January 9, 2020; Accepted December 4, 2020

DOI: 10.3892/etm.2021.9770

Abstract. Hepatic cirrhosis is a chronic disease that affects one fifth of the World's population and is the third leading cause of death in Mexico. Attempts have been made to develop treatments for this hepatic cirrhosis, which include manipulating the intestinal microbiota and thus decreasing the early inflammatory response. The microbiota is reportedly altered in patients with cirrhosis. Due to its immunomodulatory properties and its ability to survive in the gastrointestinal tract, *Lactococcus lactis* (*L. lactis*) has been used as a therapeutic measure in inflammatory disorders of the colon. The objective of the present study was to evaluate the efficacy of the *L. lactis* probiotic NZ9000 in preventing tetrachloromethane (CCl_4)-induced experimental hepatic fibrosis. The following 4 groups were included in the experimental stage (n=5): i) Control group; ii) *L. lactis* group; iii) CCl_4 group; and iv) *L. lactis*-CCl_4 group. For the first 2 weeks, *L. lactis* was orally administered to the *L. lactis* and *L. lactis*-CCl_4 groups; CCl_4 was then peritoneally administered to the lactis-CCl_4 group for a further 4 weeks (in addition to the probiotic), while the *L. lactis* group received the probiotic only. For the CCl_4 group, CCl_4 was administered for 4 weeks. The experimental groups were all compared with the control group and the *L. lactis* + CCl_4 group. Tissue samples were analyzed histologically and biochemically, and the gene expression levels of interleukin (IL)-1, IL-10 and forkhead box protein P3 (FoxP3) were determined. *L. lactis* decreased hepatic cirrhosis by preventing steatosis and fibrosis, and by reducing the levels of AST and ALT. Subchronic CCl_4 injury induced upregulation of the IL-1β gene in the liver, which was decreased by *L. lactis*. It was also found that the group treated with *L. lactis* showed increased expression of Foxp3 in the liver and IL-10 in the gut. These results suggested that oral administration of *L. lactis* may be a potential probiotic to prevent or protect against CCl_4-induced liver injury.

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Abbreviations: ECM, extracellular matrix; TGF-β, transforming growth factor β; MMP, matrix metalloproteinase; SMAD, small worm phenotype and mothers against decapentaplegic homologies; NF-κB, nuclear factor κ light-chain enhancer of activated B cells; FoxP3, forkhead box P3; IL-Iβ, interleukin 1β; IL-10, interleukin 10; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NK, natural killer; NKT, natural killer T cell; TNF-α, tumor necrosis factor α; IFN-γ, interferon γ; GALT, Gut-associated lymphoid tissue

Key words: Lactococcus lactis, interleukin 10, interleukin 1β, fibrosis, microbiota, probiotic

Introduction

Fibrosis is defined as an excessive component deposition of the extracellular matrix (ECM), collagen and peptidoglycans in organs and tissues as a result of the proliferation and activation of fibroblasts, stellate cells and myofibroblasts (1-3). Inflammatory reactions of both the innate and adaptive immune system contribute to the development of fibrosis; in the early stages of fibrosis, neutrophils, macrophages, natural killer (NK) cells and T lymphocytes promote pro-fibrotic processes, including hepatic stellate cell activation, increased

transforming growth factor (TGF)-β, platelet-derived growth factor, fibroblast growth factor, matrix metalloproteinase (MMP) 9 and metalloproteinase inhibitor-1/-2 expression, and a decrease in MMP13 expression (1-4). Activated macrophages produce tumor necrosis factor (TNF)-α and interleukin (IL)-1, which in turn activate hepatic stellate cells and fibroblasts to induce ECM overproduction. The signal transduction triggered by TNF-α leads to the expression of fibrogenic cytokines, primarily via the NF-κB and SMAD pathways (1,3). By contrast, interferon (IFN)-γ produced by activated NK cells (and a subsequent increase in IL-10) exerts antifibrotic effects (3). During cirrhosis, collagen types I and III are deposited in the hepatic stroma, creating fine or wide fibrous septa. Subsequently, new vascular channels are formed that facilitate communication between the portal region (hepatic arteries and portal veins) and the centrilobular veins, establishing an alternative circuit through which blood can bypass the sinusoids due to the increase of collagen fibers in the Dissé space (4,5).

Continuous collagen deposition in the Dissé space of the parenchyma is associated with the loss of sinusoidal endothelial cell fenestrae, in this process, the sinusoidal space takes on a capillary-like structure rather than a channel for the exchange of solutes between hepatocytes and the plasma (4). Collectively, this alters the secretion of hepatocellular proteins such as albumin, coagulation factors and lipoproteins (1,5). A number of therapeutic strategies have been developed to prevent this process and to subsequently decrease or reverse fibrosis/cirrhosis-associated liver damage (6); for example, the use of antioxidants (7), adrenoblockers (8), anti-inflammatory cytokines (9,10) and probiotics (11) has been suggested, but a complete cure for the disease has yet to be identified.

The pharmacological basis of a number of fibrosis treatments is the interaction between the intestinal microbiome and the host, which helps to maintain homeostasis (12-14). Haller *et al* (15) demonstrated that *Lactobacillus (L.) johnsonii* of an intestinal origin did not induce TNF-α or IL-1β release, but promoted that of TGF-β, presenting a global anti-inflammatory profile in a colitis model. In addition to *in vitro* studies, experimental animal models of colitis have demonstrated the usefulness of probiotics in the control of intestinal inflammation. In an acetic acid-induced rat colitis model, administration of *L. reuteri* R2LC immediately after induction prevented the development of colitis (16). Similarly, *L. plantarum* administration to rats decreased the severity of colitis in an intraperitoneal methotrexate-induced enterocolitis model (17,18).

L. lactis is a gram-positive, spherical, homolactic, non-sporulant and facultative anaerobic bacterium, with hundreds of strains and biovariants published to date (19). *L. lactis* is categorized into three subspecies: i) *L. lactis* ssp. *Lactis*; ii) *L. lactis* ssp. *Cremoris*; and iii) *L. lactis* ssp. *Hordniae* (19-21). Bajaj *et al* (11) demonstrated that *L. rhamnosus* GG induced a decrease in endotoxemia and systemic inflammation in patients with cirrhosis. Similar results were observed following the administration of lactulose, rifaximin and probiotics containing *Lactobacillus*, which partially reversed cirrhosis-associated enteric dysbiosis, together with improving the severity of encephalopathy (18). Due to its immunomodulatory properties (22-24) and ability to transit through the gastrointestinal tract, *L. lactis* does not colonize the intestine in the manner of other similar organisms, such as *Lactobacillus spp*. (24).

The primary beneficial effect reported for wild or recombinant strains of *L. lactis* is its anti-inflammatory potential, indicating its potential use as a therapeutic tool for chronic intestinal diseases. Cellular *in vitro* models, as well as mouse models of colitis, have been used to investigate the anti-inflammatory properties of *L. lactis*, where an increase in anti-inflammatory cytokines and a decrease in NF-κB have been reported (25-28). In the present study, the protective effects of oral administration of *L. lactis* were evaluated after tetrachloromethane (CCl_4)-induced fibrosis in Wistar rats.

Materials and methods

Bacterial strains and culture conditions. Pure stocks of *L. lactis* (10 μl) in 1 ml M17 medium (10% glucose and 30% glycerol) were donated by Dr Maria de Jesus Loera Arias of the Autonomous University of Nuevo León (Monterrey, Mexico). To reactivate the *L. lactis* strain, the cells were incubated overnight in 50 ml M17 (Difco) medium (supplemented with 10% glucose) at 30˚C without shaking. Subsequently, 1 ml culture was used to inoculate 50 ml M17 medium (10% glucose). The optical density (OD) was measured at 600 nm, and the cells were incubated again until they reached an OD of 0.8. The final bacterial concentration was $1x10^9$ cells/ml.

Animals. Male Wistar rats (age, 6-8 weeks; weight, 150-250 g) were obtained from the Laboratory Animal Service of the Autonomous University of Aguascalientes (Aguascalientes, Mexico). The animals were maintained on a light/dark cycle (12:12) with *ad libitum* access to Purina® Rodent Chow (Cargill, Inc.) and tap water. All animal experiments were approved by the Research Ethics Committee of the Autonomous University of Aguascalientes (approval no. A1-S-21375) and were conducted in accordance with institutional guidelines for caring for experimental animals and the national regulatory norm (NOM-062-Z00-1999). To prepare the intestinal environment, all animals were previously treated with neomycin sulfate and sulfadimethylpyrimidine for 7 days. For experimentation, the rats were divided into the following 4 groups (n=5 rats/group): i) Control group not treated with *L. lactis* or CCl_4; ii) *L. lactis* group administered *L. lactis*; iii) *L. lactis*-CCl_4 group orally treated with *L. lactis* and induced with CCl_4; and iv) CCl_4 group intraperitoneally administered CCl_4 for cirrhosis induction. All animals were sacrificed by overdose of sodium pentobarbital at 6 weeks, and different sections of the liver, the terminal region of the ileum (Peyer's Patches), the ascending portion of the large intestine and serum samples were obtained. The tissue sections were preserved in 10% formalin in PBS at room temperature and RNAlater (Invitrogen; Thermo Fisher Scientific, Inc.) at -80˚C for histological and molecular analysis, respectively, and the serum was used to conduct liver function tests.

Fibrotic induction. Fibrosis was induced in the *L. lactis*-CCl_4 and CCl_4 groups. Based on a pilot experiment in our laboratory, CCl_4 was diluted in petrolatum and intraperitoneally administered 3 times per week for 4 weeks as follows

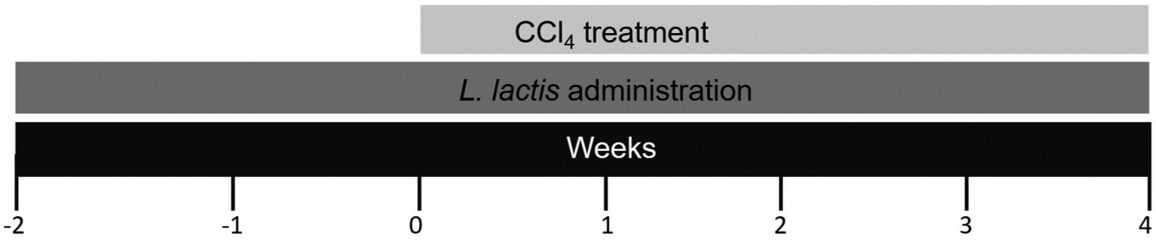

Figure 1. Experimental design. All treatments are represented along the timeline. The rats in the CCl_4 group received CCl_4 for 4 weeks; the rats in the *L. lactis*-CCl_4 group received oral administration of *L. lactis* for 2 weeks, followed by CCl_4 cirrhosis induction for 4 weeks. The rats in the *L. lactis* group received oral administration of *L. lactis* for 6 weeks. The control group did not receive any treatment. CCl_4, tetrachloromethane; *L.*, *Lactococcus*.

(CCl_4:petrolatum by volume): Weeks 1, 1:6; 2, 1:5; 3, 1:4; 4, 1:3. These proportions were prepared according to the number of experimental animals (n=5 for each group) and the number of applications per week (3 per week).

Administration of L. lactis. For 6 weeks, 1 ml *L. lactis* ($1x10^9$ cells), was orally administered to the *L. lactis* and *L. lactis*-CCl_4 groups on a daily basis. In the *L. lactis*-CCl_4 group, the probiotic was administered two weeks prior to CCl_4, and was subsequently continued for an additional 4 weeks together with CCl_4. For the *L. lactis* group, the probiotic was administered alone for 6 weeks as a control (Fig. 1).

Histological analysis. Liver damage and Peyer's patches were evaluated histologically by light microscopy. Sirius red staining (with polarized light microscopy) was used to identify collagen fiber deposits (type I, red; type III, green). For histopathological analysis of Peyer's patches. The tissue sections were preserved in 10% formalin in PBS at room temperature for 24 h, and transverse cuts of 5-μm thickness were made in the terminal portion of the ileum to reveal clusters of lymphatic tissue (lymph nodes) that cover the lamina propria, which were then stained with hematoxylin and eosin. The histological preparations were visualized under a Axioskop 40/40 FL light polarized microscope (Carl Zeiss AG) and analyzed using Image-Pro Plus Software 4.5.1 (Media Cybernetics, Inc.). The percentage of fibrosis was determined as the ratio of the fibrotic area to the total tissue area.

Markers of liver damage. To determine the degree of liver damage, serum levels of glucose (cat. no. BSIS19-P), albumin (cat. no. BSIS02-E), bilirubin (cat. no. BSIS92-I) total protein (cat. no. BSIS30-E), urea (cat. no. BSIS35-I), alanine aminotransferase (ALT; cat. no. BEIS11-E) and aspartate transaminase (AST; cat. no. BEIS09-E) were quantified. Kits for all biochemical tests were obtained from Spinreact SAU. Each test was performed according to the manufacturer's instructions. The samples were read on a BTS-350 semi-automatic spectrophotometric analyzer (BioSystems S.A.).

Total RNA isolation and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from 100 mg liver tissues using the SV Total RNA Isolation System (Promega Corporation) according to the manufacturer's protocol. The RNA was quantified using NanoDrop-2000 (NanoDrop Technologies; Thermo Fisher Scientific, Inc.) and stored at -80˚C until required. Reverse transcription was performed with 1 μg total RNA using the GoScript™ Reverse Transcription System (Promega Corporation) according to the manufacturer's instructions. Subsequently, qPCR was performed using the qPCR GreenMaster with UNG-clear (Jena Bioscience GmbH) using StepOne™ equipment (Applied Biosystems; Thermo Fisher Scientific, Inc.) with the following thermocycling conditions: 50˚C for 2 min and 95˚C for 45 sec, followed by 40 cycles of 95˚C for 45 sec and 60˚C for 45 sec. The oligonucleotide primers are displayed in Table I. Relative expression levels were normalized to those of β-actin, and the differences were determined using the $2^{-\Delta\Delta Cq}$ method (29).

Statistical analysis. GraphPad Prism 5.00 (GraphPad Software, Inc.) was used for statistical analysis and figures. Data are presented as the mean ± standard error of the mean for each group. Significant differences between mean values were assessed using one-way ANOVA followed by Tukey's test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Macroscopic and histopathological analysis of the livers of control and treated animals. At a macroscopic level, the livers of the control and *L. lactis* groups exhibited a smooth surface and the classic dark brown color of a healthy liver (Fig. 2A and D). By contrast, the liver tissues of the CCl_4 group were rough and irregular, with a lighter brown color (Fig. 2G). The livers of the rats om the *L. lactis*-CCl_4 presented with a similar coloration and texture to those of the control group (Fig. 2J). At the microscopic level (magnification, x10 and x40), the control and *L. lactis* groups displayed classic liver lobules, with hepatocytes and normal hepatic sinusoids (yellow arrows) that did not affect the liver histology (Figs. 2C and F). In the CCl_4 groups, steatosis (black arrows) and pyknotic nuclei (black asterisk) were observed in zone I of the liver acini (Fig. 2I). In the *L. lactis*-CCl_4 group (magnification, x10), a small number of hepatocytes in zone II presented with CCl_4-induced damage (to a lesser degree compared with that in the CCl_4 group) and a smaller area of steatosis, described as a microvesicular type (black arrowheads) compared with the CCl_4 group. Additionally, at x40 magnification, acidophilic cells were observed with a larger cytoplasm; it was therefore speculated that these cells exhibited a degree of incipient damage in the CCl_4 and *L. lactis*-CCl_4 groups (yellow asterisk; Fig. 2I and L).

The liver sections stained with Sirius Red and analyzed under a polarized light microscope exhibited normal

Table I. Primers used for quantitative PCR.

Primer	Sequence (5'→3')
FoxP3	F: CGGGAGAGTTTCTCAAGCAC
	R: CACAGGTGGAGCTTTTGTCA
IL-1β	F: CTGTGACTCGTGGGATGATG
	R: GGGATTTTGTCGTTGCTTGT
IL-10	F: TGGCTCAGCACTGCTATGTT
	R: TTGTCCAGCTGGTCCTTCTT
β-actin	F: GTCGTACCACTGGCATTGTG
	R: GCTGTGGTGGTGAAGCTGTA

IL, interleukin; FoxP3, forkhead box protein P3.

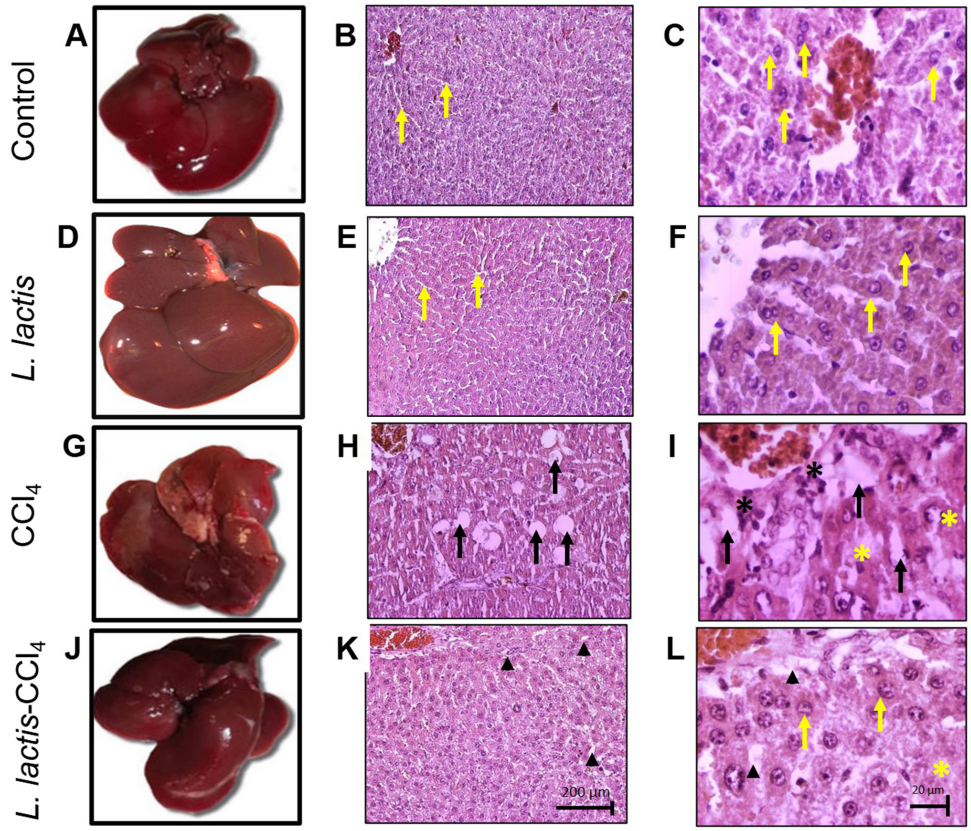

Figure 2. Oral administration of L. lactis prevents hepatic damage. At the macroscopic level, the livers of the (A) control and (D) L. lactis groups possessed a smooth surface and brown color. The livers of the (G) CCl4 group exhibited a rough and irregular surface, and those of the (J) L. lactis-CCl4 group presented with normal coloration and texture. At the microscopic level, in the (B and C) control and (E and F) L. lactis groups (magnification, x10 and x40), hepatocytes and normal hepatic sinusoids were observed (yellow arrows); (H) In the CCl4 group (magnification, x10), wide area with microvesicular steatosis (black arrows); (I) in the CCl4 group (magnification, x40), steatotic cells (black arrows) and pyknotic nuclei (black asterisk) were observed. (K) In the L. lactis-CCl4 group (magnification, x10), a small number steatotic cells of the incipient microvesicular type were apparent (black arrowhead); (L) at x40 magnification, cells exhibited a greater proportion of acidophilus cells with larger cytoplasm (yellow asterisks), suggesting a degree of incipient damage in the CCl4 andL. lactis-CCl4 groups.

histological architecture in the control and *L. lactis* groups, and type III collagen was observed (green arrow; Fig. 3A-a and b). In the CCl_4 group, an increase in type I collagen (red) was evident around blood vessels (red arrows; Fig. 3A-c) along with a low level of type III collagen (green arrow), indicating a fibrotic lesion. By contrast, a significant decrease in type I collagen fibers was observed in the *L. lactis*-CCl_4 group (red arrow; Fig. 3A-d). To confirm the degree of fibrosis, a morphometric analysis of the hepatic parenchyma was performed; an increase in collagen fibers was evident in the CCl_4 group compared with that in the control group ($P<0.001$; Fig. 3B). In addition, the percentage of total collagen was lower in the *L. lactis*-CCl_4 group compared with the CCl_4 group.

Liver Function. Liver function was evaluated by the quantification of albumin, glucose, bilirubin and total

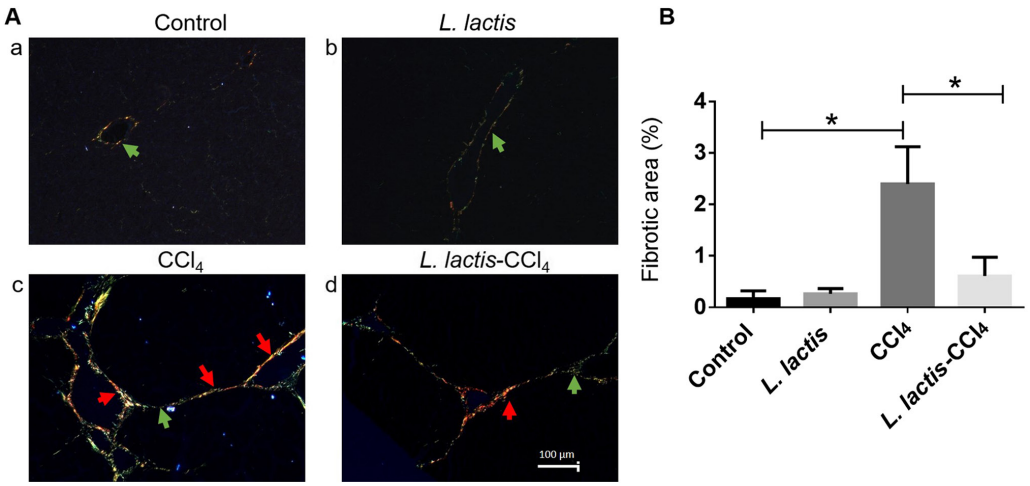

Figure 3. *L. lactis* decreases the number of collagen fibers in the portal area. (A) In the *L. lactis*-CCl_4 group, type III collagen fibers (green) were observed in the parenchyma, with reduced accumulation of type I collagen (red) around the blood vessels compared with the CCl_4 group. The CCl_4 group presented with an incremental increase in type I collagen deposition in the blood vessels and liver parenchyma. (B) In the *L. lactis* group, the percentage of total collagen was decreased to a similar level to that in the control group, which was lower compared with that in the CCl_4 group. *P<0.001. *L.*, *Lactococcus*; CCl_4, tetrachloromethane.

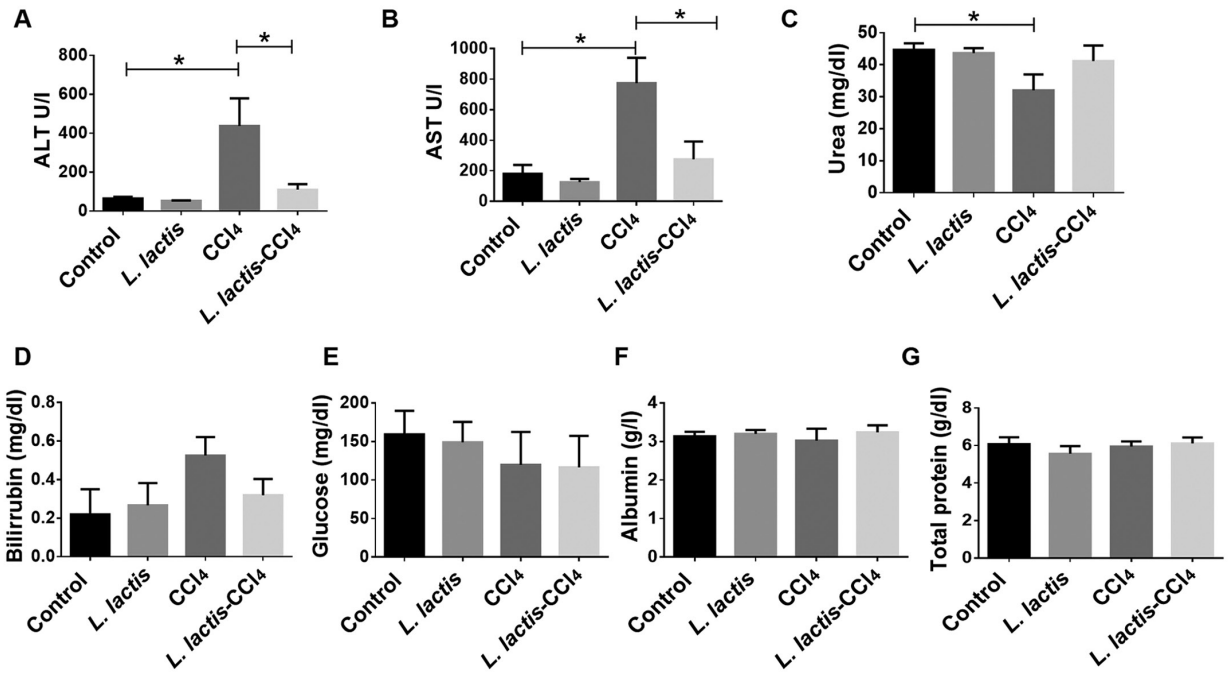

Figure 4. Detection of serum biochemical markers demonstrates that *L. lactis* prevents liver damage. The CCl_4 group exhibited increased levels of (A) ALT and (B) AST and a reduction in (C) urea levels corresponding with liver damage when compared with the control group; these levels were restored following *L. lactis* administration. No significant differences were observed in the serum levels of (D) bilirubin, (E) glucose, (F) albumin and (G) total protein among the groups. *P<0.001. *L.*, *Lactococcus*; CCl_4, tetrachloromethane; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

proteins, and no significant differences were observed (Fig. 4). However, increased plasma ALT and AST (indicators of liver damage) levels were observed following induction with CCl_4. The *L. lactis*-CCl_4 group exhibited a significant decrease in ALT and AST levels compared with those in the CCl_4 group (P<0.001; Fig. 4A and B), indicating that *L. lactis* improved liver function. Urea is primarily formed in the liver as an end product of protein metabolism (6,11); a significant decrease in the urea level was observed in the CCl_4 group compared with that in the control group (P<0.05; Fig. 4C), whereas the *L. lactis*-CCl_4 group presented with similar levels to those of the control group, which suggested that the liver was functionally transforming ammonium to urea for excretion. The recovery of liver functional enzymes may be associated with the histological improvement presented in Fig. 2. No changes in hepatic function were observed in the *L. lactis* group compared with the control group.

Histopathological analysis of Peyer's Patches. The *L. lactis*-CCl_4 group induced ~8 well-defined nodules in a single tissue portion (black arrows; Fig. 5A); however, the control and CCl_4 groups possessed a mean of 3 nodules

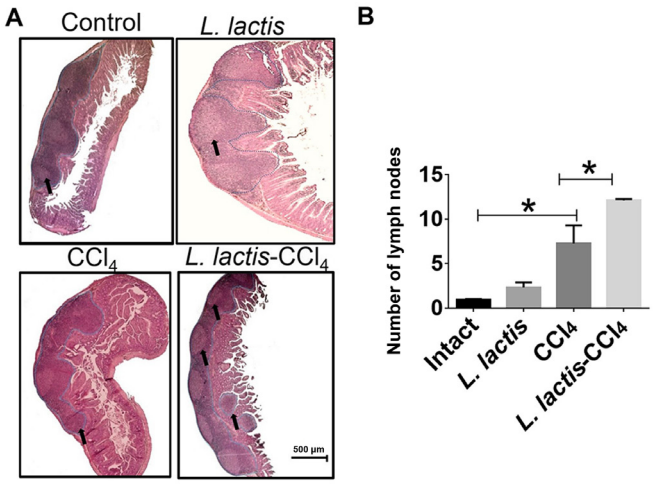

Figure 5. *Lactococcus lactis* administration increases the number of lymph nodes in the intestinal Peyer's patches. (A) Histopathological analysis of the Peyer's patches, where well-defined lymphoid nodules were observed in each group (black arrow). (B) The mean of lymphoid nodules for each group, where the *L. lactis*-CCl4 group showed an increased number of nodules compared with the other experimental groups. *P<0.05. L., *Lactococcus*; CCl_4, tetrachloromethane.

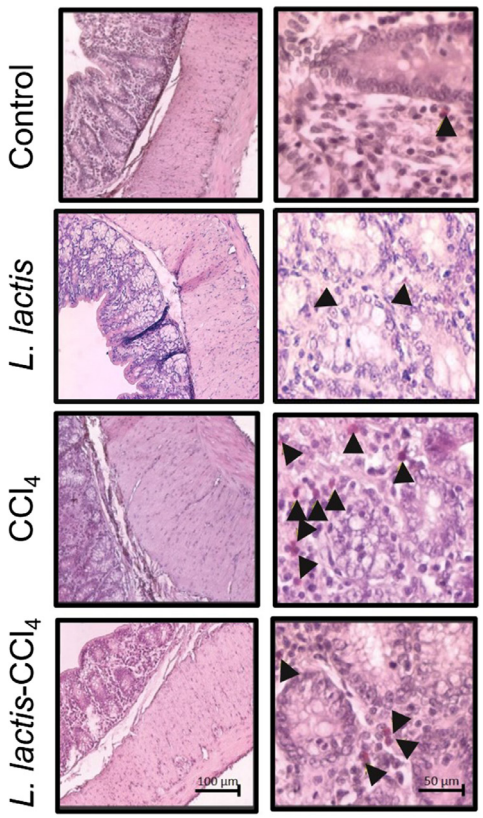

Figure 6. *L. lactis* attenuates the inflammatory response and protects the intestinal architecture. Normal histology was observed in the control group; however, the colonic tissue of the CCl_4 group presented with cellular infiltrate (black arrowheads). This infiltrate was diminished in the groups treated with *L. lactis*. L., *Lactococcus*; CCl_4, tetrachloromethane.

(P<0.01), which were smaller in size. Although few lymphoid nodules were observed in the *L. lactis* group, these were larger than those in the control group (Fig. 5A and B). To corroborate these size variations, a morphometric analysis was performed, and no significant differences were apparent between any of the groups. However, the *L. lactis*-CCl_4 group exhibited the largest area of these nodules (Fig. 5B).

Histopathological analysis of the large intestine (cecum). Transverse cuts of the cecum were made in animals from each of the study groups (Fig. 6). Normal histology was observed in the control group; however, the colonic tissue of the CCl_4 group presented with cellular infiltrate (black arrowheads) in the region of the mucosa layer. This infiltrate was diminished in the CCl_4 group treated with *L. lactis*.

Evaluation of inflammatory markers in the liver. L. lactis is known to have an immunomodulatory effect due to its association with IL-10, a potent anti-inflammatory cytokine that represses the expression of inflammatory cytokines such as TNF-α, IL-6 and IL-1β produced by macrophages activated during liver injury (30). To analyze the possible effect of intestinal *L. lactis* on CCl_4-induced liver damage, the levels of specific cytokines were assessed in the liver tissue, such as pro-inflammatory IL-1β, the anti-inflammatory IL-10 and a T-cell regulatory transcription factor forkhead box protein P3 (FoxP3) (Fig. 7A-C); IL-10 expression was also assessed in intestinal tissues; in the *L. lactis*-CCl_4 group, intestinal IL-10 expression was increased compared with the control group (P<0.001), and CCl_4 groups (P<0.001), (Fig. 7D). No significant differences in IL-10 expression were observed among any of the experimental groups, although there was a non-significant tendency towards higher expression in the *L. lactis*-CCl4 group. IL-1β expression was decreased in the liver tissues (P<0.05). FoxP3 is the primary regulator of the development and function of regulatory T cells, and its expression was increased in the *L. lactis*-CCl_4 group compared with that in the CCl_4 group (P<0.05). Collectively, these results demonstrated the immunoregulatory effects of intestinal *L. lactis* on hepatic pathology.

Discussion

In the present study, the inhibitory effect of *L. lactis* NZ9000 on CCl_4-induced hepatic fibrosis was analyzed. Oral administration of *L. lactis* induced a physiological change and modified the development of CCl_4-induced fibrosis in the liver tissue in the following manners: i) Reducing structural liver damage; ii) reducing the area of fibrosis; iii) increasing the number of lymph nodes in the Peyer's patches; iv) decreasing ALT and AST expression; v) increasing the mRNA expression of IL-10 in small intestine samples; vi) increasing FoxP3 levels in liver samples; and vii) decreasing the expression of IL-1β in the liver.

The aim of the present study was to investigate a protective strategy to reverse liver damage using the physiological interconnection between the digestive tract and the liver via the hepatic portal system (31). The human microbiome is defined as the collective genome of >1,000 different types of microorganisms that exist in association with the human body, the vast majority of which reside in the distal intestine (32,33). This ecological system interacts with internal and external organs, factors that help to maintain the overall health of the

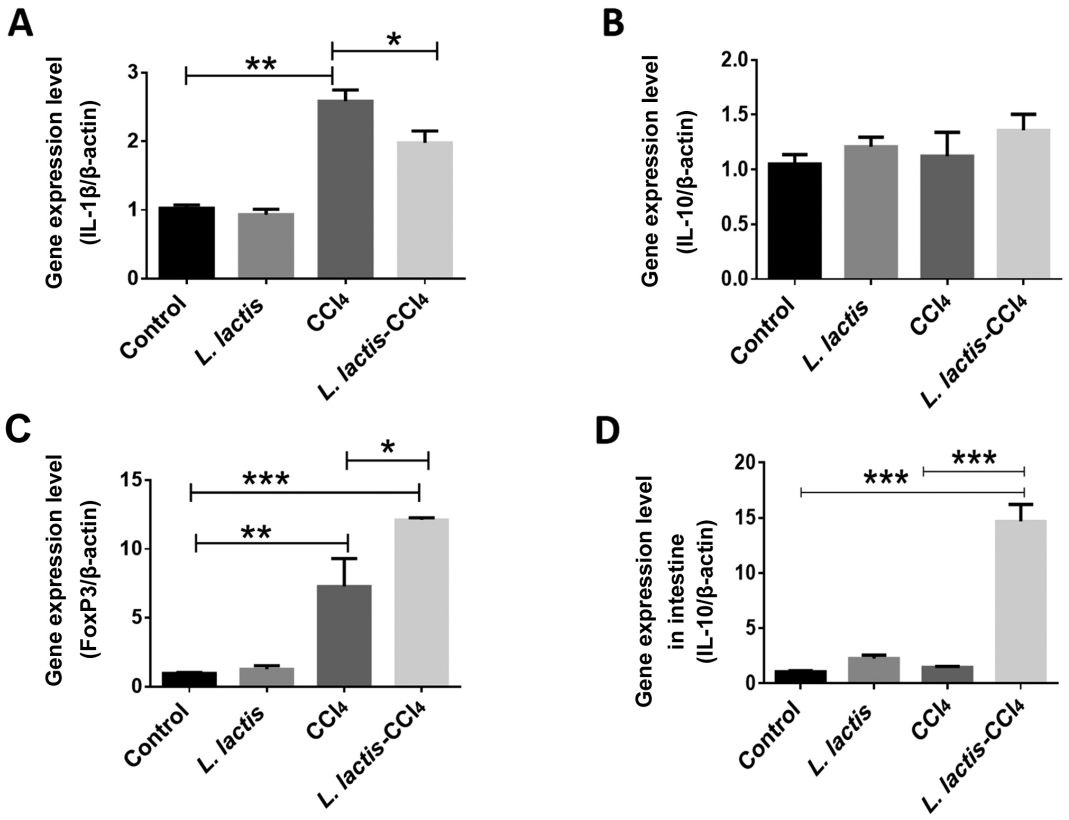

Figure 7. Liver inflammation is reduced by L. lactis administration. (A) In the liver, the CCl4 group exhibited increased expression of IL-1β compared with that in the control group, which was reduced in the L. lactis-CCl4 groups. (B) No significant differences in IL-10 expression were observed among any of the experimental groups. (C) The expression of FoxP3 in the liver was increased in the L. lactis-CCl4 group compared with those in the control and CCl4 groups. (D) At the intestinal level, the expression of IL-10 in the L. lactis-CCl4 groups was higher compared with that in the CCl4 groups. *P<0.05, **P<0.01 and ***P<0.001. *L.*, *Lactococcus*; CCl_4, tetrachloromethane; IL, interleukin; FoxP3, forkhead box protein P3.

individual (12). Taking advantage of this physiological association, anatomical-functional communication was investigated with the aim to induce an immunoregulatory response in the intestine, with ultimate effects on liver inflammation.

In cases of colitis, the *L. reuteri* R2LC strain has been demonstrated to exert an anti-inflammatory effect in the large intestine (13,14,16,17). Different experimental animal models (predominantly of colitis) have demonstrated the benefits of probiotics in controlling intestinal inflammation. In an acetic acid-induced rat colitis model, the administration of *L. reuteri* R2LC immediately after induction prevented the establishment of colitis (16). Previous reports have demonstrated the effect of probiotics on the gut microbiota under inflammatory process. The oral administration of *L. plantarum* attenuated inflammatory bowel disease in a mouse model; *L. plantarum* also affected the proportion of *Firmicutes* and *Bacteroides*, which may be associated with the inflammation of the mouse gut (34). The intestinal microbiota has been reported to serve a fundamental role in homeostatic maintenance of the systemic immune system; for example, *L. johnsonii* of an intestinal origin did not induce the release of TNF-α or IL-1β following downregulation of the transcription factor NF-κB, whereas TGF-β expression was increased, resulting in a global anti-inflammatory profile (15). Specific recognition of commensal microorganisms occurs in the mesenteric lymph nodes. Most antigens or infectious agents pass into the venous system or tissues through mucous membranes, which includes the lining of the gastrointestinal, respiratory and genitourinary tracts. At these mucosal surfaces, the mucus represents the first barrier against the entry of microorganisms, while gut-associated lymphoid tissues (GALT), which include intestinal Peyer's patches, is critical for efficient protective immune response, making the GALT an attractive portion of the small intestine to study (35). In the present study, the probiotic *L. lactis* generated an anti-inflammatory environment in the small intestine by increasing the expression of IL-10, as well as the number of lymph nodes in the Peyer's patches; these findings suggested a stimulus that may potentially increase the number of regulatory, anti-inflammatory lymphoid cells. However, one of the limitations of the present study was not determining whether *L. lactis* may influence the proportions of various taxonomic and functional groups of the gut microbiota, which may increase our knowledge about the mechanisms of action of probiotics.

IL-10 decreases and regulates dendritic cell- and macrophage-associated inflammatory responses by activating STAT-3 (14). IL-10 also suppresses the adaptive immune response by inhibiting NF-κB secretion by $CD4^+$ T cells and the production of IL-1 and TNF-α by macrophages (14). The results of the present study revealed a notable decrease in hepatic IL-1β expression in the *L. lactis*-CCL_4 group compared with the CCl_4 group; this was potentially due to an increase in intestinal IL-10 as a result of CCl_4-induced damage, which was subsequently transported to the liver via the portal-hepatic

system. Additionally, an increase in the expression of Foxp3 mRNA was observed in the liver, which supports the increase in intestinal IL-10 in the *L. lactis*-CCL_4 group, and may been affected by the downregulation of NF-κB (36,37). This supports the existence of an immunoregulatory process induced by *L. lactis* in the intestine, which has an inhibitory effect on CCl_4-associated fibrosis; thus, *L. lactis* may exert an anti-fibrotic effect in the early stages of inflammation, which potentially modifies the adhesion properties of epithelial cells, altering the local host immune response (1,38). For this reason, potential new therapies for liver fibrosis may target the recovery of the microbiota to reduce the possible adverse effects associated with pharmacological treatment (18). *L. lactis* may therefore be an optional co-treatment for decreasing inflammation in the early stages of cirrhosis.

In conclusion, *L. lactis* prevented liver damage in an animal model of CCl_4-induced liver fibrosis. The results of the present study suggested that oral administration of *L. lactis* in its native form may be a potential means to prevent and protect against CCl_4-induced liver damage.

Acknowledgements

We acknowledge to MVZ Karen Estefany Sánchez Hernández, Central Bioterium, Autonomous University of Aguascalientes, for providing and caring for the animals for this study; and LAQB Cintya Esquivel-Dueñas and LAQB Mariana Perez-Villalobos, Department of Chemistry, Autonomous University of Aguascalientes, for their excellent technical assistance.

Funding

The present study was supported by CONACYT (grant nos. 241312 and A1-S-21375) and the Autonomous University of Aguascalientes (grant no. PIBB18-8). CSDV gratefully acknowledges the financial fellowship granted by CONACYT (grant no. 617146) as part of the master's program in Toxicology Sciences of the Autonomous University of Aguascalientes.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SLMH contributed to the analysis and interpretation of the histological data. CSDV developed the histological technique for the intestine and liver tissues. DCG performed reverse transcription-quantitative PCR analysis. RMDOL and MDJLA donated the *L. lactis* cultures and analyzed the quantitative PCR data. MGMM developed the liver function study. JVJ and MHMO contributed to study conception and design, and the writing and revision of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were approved by the Research Ethics Committee of the Autonomous University of Aguascalientes (approval no. A1-S-21375) and were conducted in accordance with institutional guidelines for caring for experimental animals and the national regulatory norm (NOM-062-Z00-1999).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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