Camel milk modulates ethanol-induced changes in the gut microbiome and transcriptome in a mouse model of acute alcoholic liver disease

Liang Ming,^{1*} XiangYu Qiao,^{1*} Li Yi,¹ Dalai Siren,^{1,2} Jing He,¹ Le Hai,¹ Fucheng Guo,¹ Yuchen Xiao,¹ and Rimutu Ji^{1,2†}

¹Key Laboratory of Dairy Biotechnology and Bioengineering, Ministry of Education, College of Food Science and Engineering, Inner Mongolia Agricultural University, Hohhot 010018, China

²Camel Research Institute of Inner Mongolia, Alashan 737300, China

ABSTRACT

Morbidity and mortality as a result of liver disease are major problems around the world, especially from alcoholic liver disease (ALD), which is characterized by hepatic inflammation and intestinal microbial imbalance. In this study, we investigated the hepatoprotective effects of camel milk (CM) in a mouse model of acute ALD and the underlying mechanism at the gut microbiota and transcriptome level. Male Institute of Cancer Research mice ($n = 24$; Beijing Weitong Lihua Experimental Animal Technology Co. Ltd., China) were divided into 3 groups: normal diet (NC); normal diet, then ethanol (ET); and normal diet and camel milk (CM), then ethanol (ET+CM). Analysis of serum biochemical indexes and histology revealed a reduction in hepatic inflammation in the ET+CM group. Sequencing of 16S rRNA showed that CM modulated the microbial communities, with an increased proportion of *Lactobacillus* and reduced *Bacteroides*, *Alistipes*, and *Rikenellaceae* RC9 gut group. Comparative hepatic transcriptome analysis revealed 315 differentially expressed genes (DEG) in the ET+CM and ET groups (150 upregulated and 165 downregulated). Enrichment analysis revealed that CM downregulated the expression of inflammation-related (*IL6* and *CXCL1*) genes in the IL-17 and tumor necrosis factor (TNF- α) pathways. We conclude that CM modulates liver inflammation and alleviates the intestinal microbial disorder caused by acute alcohol injury, indicating the potential of dietary CM in protection against alcohol-induced liver injury.

Key words: alcoholic liver disease, camel milk, gut microbiota disorder, hepatic inflammation, transcriptome

INTRODUCTION

Alcoholic liver disease (ALD) is a major cause of morbidity and mortality worldwide (Bertola et al., 2013). In Europe and the United States, ALD is one of the most common liver diseases (Osna et al., 2017), and approximately 300 million people are affected by hepatitis B virus, nonalcoholic fatty liver disease, and ALD in China (Wang et al., 2014). Alcoholic liver disease is characterized by a spectrum of morphological features including steatosis (fatty liver), hepatitis, and alcoholic cirrhosis (Smathers et al., 2011). Although alcohol-induced liver injury has been a research focus, the pathogenesis and transcriptomic consequences of ALD are not fully understood. However, oxidative stress (Wu and Cederbaum, 2009), inflammatory responses (Massey and Arteel, 2012), steatosis (Gao and Bataller, 2011), and gut microbiota disorders are currently proposed as the main causes of ALD (Woodhouse et al., 2018).

Currently, the choice of drugs available for the treatment of liver damage is limited, and most are associated with certain adverse side effects. Therefore, the use of nutritional agents for the treatment of ALD, such as those with anti-inflammatory and antioxidant properties, has attracted increasing attention (Gao and Bataller, 2011). The identification of inexpensive natural agents that effectively prevent ALD is a therapeutic challenge (Lee et al., 2007). Livestock milk has been reported to provide beneficial effects in the prevention and treatment of a variety of conditions, including ALD (Darwish et al., 2012; Zhong and Zhou, 2014), with camel milk (CM) being one of the best candidates. Similar to the milk of other ruminants, CM is rich in proteins, vitamins, and minerals, especially vitamin C and iron (Rao et al., 1970). Studies have shown that CM produces important beneficial effects on human health, as it contains low levels of β -lactoglobulin, making it suitable for consumption by lactose-intolerant individuals (Cardoso et al., 2010), in addition to high levels of insulin and IGF, which have anti-diabetic

Received July 11, 2019.

Accepted January 13, 2020.

*These authors contributed equally to this work.

†Corresponding author: yeluotuo1999@vip.163.com

properties (Abdulrahman et al., 2016; Ayoub et al., 2018), as well as a high level of unsaturated fatty acids, which enhance its overall nutritional quality (Karray et al., 2005; Konuspayeva et al., 2008). Additionally, CM contains whey protein, lactoferrin, immunoglobulins, lysozyme, and vitamin C, which may be related to antimicrobial activity, as well as protection against asthma, tuberculosis, and edema (Ali et al., 2019). Protection of the liver against the effects of alcohol (Darwish et al., 2012), carbon tetrachloride (Al-Fartosi et al., 2012; Althnaian et al., 2013; Hamed et al., 2019), paracetamol (Al-Fartosi et al., 2011), cadmium chloride (Al-Hashem et al., 2009), and aluminum chloride (Al-Hashem, 2009) is an area of growing interest, although the hepatoprotective effects of CM have not yet been investigated. Therefore, in this study, we investigated the hepatoprotective effects of CM and the underlying mechanism at the transcriptome and gut microbiota levels in a mouse model of acute ALD.

MATERIALS AND METHODS

Ethics Statement and Animals

This study was approved by the Review Committee for the Use of Human or Animal Subjects of the Food Science and Engineering College of Inner Mongolia Agricultural University (Hohhot, China). All procedures were conducted according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (Publication No.85-23, revised 1985).

Male Institute of Cancer Research mice (21 to 23 g) were obtained from Beijing Weitong Lihua Experimental Animal Technology Co. Ltd. (China; License Number SCXK 2016-0006). Mice were maintained in ventilated cages (5 per cage) under the following conditions: $22 \pm 2^\circ\text{C}$; 50 to 60% relative humidity; 12-h light and dark cycle. Mice were given free access to food and water. All food, water, and experimental equipment were sterilized before use.

Camel Milk Administration

Bactrian CM was collected from a private camel farm in Bayan Nur City, Inner Mongolia, China, and transported to the laboratory in cool boxes. Milk samples were centrifuged at $2,500 \times g$ at 4°C for 40 min to remove the milk fat, heated in a water bath at 65°C for 30 min, and freeze-dried under vacuum. Skimmed CM powder was stored at -20°C .

Experimental Groups and Treatment Protocol

After 1 wk of acclimatization, the mice were randomly divided into the following 3 groups: normal control

(**NC**, control group); ethanol (**ET**, model group); and ethanol plus CM (**ET+CM**, the CM treatment group; $n = 8$ per group). For 14 d, mice in the NC and ET groups were fed a normal diet and 0.3 mL of double-distilled water twice a day, whereas the ET+CM group were fed a normal diet and skimmed CM powder (6 g/kg of BW; MOH, 2003) dissolved in 0.3 mL of double-distilled water. On d 13, after 6 h of fasting, the ET and ET+CM groups received 50% (vol/vol) ethanol by oral gavage at a total cumulative dosage of 7.3 g/kg of BW in 3 equal doses administered at 1-h intervals. The mice in the NC group received the same volume of double-distilled water (Zhao et al., 2008; Ding et al., 2010; Ge et al., 2014). The experimental scheme used to establish the mouse model of acute ALD is presented in Supplemental Figure S1 (<https://doi.org/10.3168/jds.2019-17247>).

After modeling, the animals were fasted for 6 h and anesthetized with isoflurane gas. Aortic blood samples were collected and liver, kidney, and spleen tissues, and colonic contents were collected and stored at -80°C .

Histopathological Examination of the Liver

Staining and Histological Score. Liver specimens from the left lobe were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned (4- to 7- μm thickness), and stained with hematoxylin and eosin (HE) according to standard protocols. Sections were evaluated for morphological changes using a light microscope (Olympus BX 60, Tokyo, Japan). The liver histological score was evaluated under a microscope according to a previous study (Wang et al., 2016a).

Oil Red O Staining. Frozen liver sections (5- to 7- μm thickness) were fixed in 4% paraformaldehyde, washed with 60% isopropanol, and then stained with Oil Red O for 10 min. The background was cleared by washing the sections with 60% isopropanol and rinsing with water before counterstaining with a Mayer's hemalum solution (Ruisi Reagent, Chengdu, China). Sections were examined under a light microscope, and images were captured (Zhang et al., 2010).

TUNEL Staining. Liver cell apoptosis was analyzed using a terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining kit (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions. Sections were examined under a microscope (Nikon, Tokyo, Japan), and images were captured.

Determination of Serum/Liver Biochemical Indicators

Analyses of ALT, AST, and Serum Inflammatory Factors. Serum alanine transaminase (**ALT**) and

aspartate transaminase (**AST**) levels were analyzed as markers of liver injury (Wang et al., 2016b). Serum was isolated from aortic blood samples by centrifugation at $1,800 \times g$ for 20 min at 4°C, and levels of ALT and AST were determined using commercially available kits (MAK052 and MAK055, respectively; Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instructions. The levels of tumor necrosis factor- α (**TNF- α**), IL-6, and IL-1 β were detected using ELISA kits (ml002095, ml063159, and ml063132, respectively; ML Bio Solutions, Charlotte, NC) according to the manufacturer's protocol. Each sample was assayed in triplicate.

Analyses of MDA, SOD, GSH, and GSH-Px.

Liver samples (0.1 g) were homogenized for 15 s at 2.9 m/s in PBS (pH 7.4) and then centrifuged ($1,800 \times g$ at 4°C for 20 min). Supernatant was collected, and concentrations of malondialdehyde (**MDA**, nmol/g), superoxide dismutase (**SOD**, ng/g), glutathione (**GSH**, ng/g), and glutathione peroxidase (**GSH-Px**, ng/g) were determined using the relevant ELISA kits (Shanghai Enzyme-Linked Biotechnology Co. Ltd., Shanghai, China) according to the manufacturer's instructions. Each sample was assayed in triplicate.

Triglyceride and Total, High-Density Lipoprotein, and Low-Density Lipoprotein Cholesterol Analyses. Liver tissue supernatant was obtained according to the method described in the previous section. Concentrations of triglycerides (**TG**, nmol/g), total cholesterol (**TC**, μ mol/g), high-density lipoprotein cholesterol (mg/g), and low-density lipoprotein cholesterol (mmol/g) were determined using the relevant ELISA kits (Shanghai Enzyme-Linked Biotechnology Co. Ltd.) according to the manufacturer's instructions. Each sample was assayed in triplicate.

High-Throughput 16S rRNA Sequencing

To determine the composition of the microbiome and molecular ecology, 16S rRNA gene sequencing was performed for 18 colon content samples (NC group: NC1001, NC3101, NC3102, NC3105, NC3106, and NC3304; ET group: MC3202, MC3203, MC3206, MC3302, MC3305, and MC4105; ET+CM group: CM4205, CM4206, CM4301, CM4302, CM4305, and CM4306).

Metagenomic DNA was extracted with an MP FastDNA Spin Kit for soil (MP Biomedicals, Irvine, CA). We analyzed DNA purity and concentration with a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and DNA integrity via 1% agarose gel electrophoresis. The V3 to V4 regions of the 16S rRNA genes were amplified using general

primers (338F, 806R; Shanghai Majorbio Bio-Pharm Technology Co., Shanghai, China) and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA). Quantification and quality control were performed using QuantiFluor-ST (Promega, Madison, WI). The DNA library was constructed and purified amplicons were sequenced using the MiSeq system (MiSeq PE 300, Illumina, San Diego, CA) at the Shanghai Majorbio Bio-Pharm Technology Co. The raw sequencing data of the colon flora were submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) GenBank database under accession number PRJNA549421.

For quality control, low-quality data and chimeras were removed from the raw data using the QIIME pipeline (v. 1.7.0; Schmieder and Edwards, 2011). The operational taxonomic unit abundance of each sample was determined for each sample at the phylum and genus levels, with an identity cutoff of 97%, using UPARSE software (v. 7.0.1001, <http://drive5.com/uparse/>). α -Diversity, which reflects species richness (Chao), species diversity (Shannon index and Simpson index), and abundance-based coverage estimators (ACE) were calculated using QIIME software. Community structure analyses were based on phylum and genus taxonomy levels, and the Ribosomal Database Project (RDP) classifier (Wang et al., 2007) was used to classify each sample. Principal coordinate analysis was conducted using the Bray-Curtis dissimilarity.

mRNA Sequencing of Liver Tissue

To further clarify the mechanism responsible for the protective effect of CM on liver tissue, RNA sequencing was performed on 11 liver samples: NC1001, NC3102, and NC3304 from the NC group; MC3203, MC3206, MC3302, and MC4105 from the ET group; and CM4205, CM4301, CM4302, and CM4305 from the ET+CM group after intervention. High-throughput mRNA sequencing was performed at the Shanghai Majorbio Bio-Pharm Technology Co.

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA), and the transcriptome library was constructed using the TruSeq RNA Sample Preparation Kit (Illumina), according to the manufacturers' instructions. The libraries were sequenced on a HiSeq 4000 ultra-high-throughput sequencing system (Illumina), and all sequences were submitted to the NCBI SRA under accession no. PRJNA549342.

After obtaining the raw data, the sequencing adapters, low-quality reads, and reads containing ploy-N were removed using in-house perl scripts. The Q20, Q30, GC content, reads, and bases were then calculated from

cleaned raw data. The clean reads were mapped to the reference genome of *Mus musculus* (GRCm38.p5) using TopHat2 software (v. 2.1.1; Kim et al., 2013). Read counts (mRNA abundance) for all mapped genes were calculated using RSEM (v. 1.1.17; <http://deweylab.github.io/RSEM/>). Differentially expressed genes (DEG) were identified using the edgeR package (v. 3.5; R Foundation for Statistical Computing, Vienna, Austria) based on $P < 0.05$ and $|\log_2\text{-fold change}| \geq 2$. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of DEG were performed using the relevant databases. The Benjamini–Hochberg approach was used to adjust P -values for controlling the false discovery rate (Benjamini and Hochberg, 1995), and a false discovery rate < 0.05 indicated significant enrichment.

Statistical Analysis

All data are presented as the mean $\pm$ standard error of the mean (SEM) using SPSS software (v. 17.0; SPSS Inc., Chicago, IL). Differences between groups were evaluated using one-way ANOVA, followed by Tukey's post-hoc multiple comparison tests using GraphPad Prism software (v. 7; GraphPad Software Inc., La Jolla, CA). Bioinformatic data analysis was presented using R software. A P -value < 0.05 was considered to indicate statistical significance.

RESULTS

Effects of CM on Histopathological Changes in the Mouse Model of Acute ALD

In the NC group, the liver lobule structure was clear and complete, with no edema or evident inflammatory cell infiltration. Hepatocytes exhibited an ordered arrangement, with round and centrally located nuclei (Figure 1A). In the ET group, hepatocytes around the central vein were enlarged, and marked inflammatory cell infiltration was apparent (Figure 1B, marked by arrows). Although the liver cells in the ET+CM group exhibited a disordered arrangement, the edema and inflammatory cell infiltration were reduced compared with the ET group. Compared with the ET group, the hepatic lobule structure of the ET+CM group returned to normal (Figure 1C). Oil Red O staining (Figure 1D–F) revealed mild steatosis in the livers of mice in the ET group but little in the ET+CM and NC groups. For livers in the NC group, the extent of liver injury was grade 0, and extensive liver damage was observed in the ET group (grades III or IV); however, the degree of liver inflammation and necrosis decreased, and the

extent of liver damage was reduced. In contrast, mice pretreated with camel milk revealed protective effects in the ET group ($P < 0.001$), with liver damage of grades 0, I, and II (Supplemental Table S1, <https://doi.org/10.3168/jds.2019-17247>). This result indicates that camel milk can ameliorate liver damage induced by ethanol. The TUNEL assays showed significantly more apoptotic cells in the ET group compared with those in the NC ($P < 0.01$) and ET+CM ($P < 0.05$) groups (Supplemental Figure S2, <https://doi.org/10.3168/jds.2019-17247>). These results indicate that CM alleviates the toxic effects of ethanol on hepatocytes and inhibits inflammatory infiltration, leading to recovery of hepatocyte structure.

Effects of CM on Serum ALT, AST, and Plasma Inflammatory Factors in the Mouse Model of Acute ALD

Alcohol treatment had marked effects on liver-specific enzymes, with a significant ($P < 0.05$) increase in serum ALT and AST activity observed in the ET group. Compared with the NC group, serum ALT levels in the ET group were 1.88 times higher than those in the NC group (Figure 2A), indicating liver cell damage. Serum levels of ALT and AST in the ET+CM group were significantly lower ($P < 0.01$ and $P < 0.05$, respectively; Figure 2A, B) than those in the ET group, indicating the protective effect of CM against acute alcohol-induced liver injury. Serum levels of TNF- α , IL-1 β , and IL-6 in the ET+CM group decreased by 14.62% ($P < 0.01$), 9.55% ($P < 0.01$), and 9.04% ($P < 0.01$), respectively, compared with levels in the ET group. We found no significant differences between the levels of these cytokines in the ET+CM and NC groups ($P > 0.05$; Figure 2C–E).

Effects of CM on Liver MDA, SOD, GSH, and GSH-Px Activity in the Mouse Model of Acute ALD

Activity of MDA in the liver tissues of the ET group was significantly higher than in the NC and ET+CM groups ($P < 0.01$, Table 1), and we observed no significant difference in MDA levels in the ET+CM and NC groups. However, compared with the NC group, the activities of SOD, GSH, and GSH-Px were lower in the ET group, although they did not reach the level of statistical significance ($P > 0.05$). The levels of SOD and GSH-Px in the liver of ET+CM group were significantly higher than those in the ET group ($P < 0.05$), with no significant difference between the ET+CM and NC groups ($P > 0.05$).

Effects of CM on Liver TG, TC, High-Density Lipoprotein, and Low-Density Lipoprotein in the Mouse Model of Acute ALD

As shown in Table 2, significantly increased levels of TG and TC were detected in the ET group (20.72%, $P < 0.01$, and 10.93%, $P < 0.01$, respectively) compared with those in the NC group; these findings were consistent with the liver Oil Red O staining results (Figure 1D–F). Levels of TG and TC in the ET+CM group were significantly decreased ($P < 0.01$) compared with those in the ET group, with no significant difference in the levels of TG between the ET+CM and NC groups. The high-density lipoprotein level in the ET+CM group was significantly higher (13.54%, $P < 0.01$) than in the ET group, with no significant difference in the low-density lipoprotein content between the ET+CM and NC groups ($P > 0.05$). These results indicate that CM effectively reduces TG and TC contents in the livers of mice with acute alcoholic liver injury and improves the transport capacity of cholesterol in the liver.

Effects of Acute Alcohol-Induced Liver Injury on the Gut Microbiota

The gut microbiota is recognized as a potential contributing factor to the development of ALD in mice. We performed a 16S rRNA comparative sequence analysis to investigate differences in the intestinal microbiota among the ET, NC, and ET+CM groups (Supplemental Table S2, <https://doi.org/10.3168/jds.2019-17247>). Notably, species α -diversity was significantly

higher ($P < 0.05$) in the ET group than in the NC and ET+CM groups, whereas species evenness was significantly lower ($P < 0.05$). In contrast, the ET+CM group exhibited increased colon microbial species richness compared with the other groups, due to higher α -diversity, although we identified no significant difference in species richness (Figure 3A–C). Bray–Curtis dissimilarity analysis of the population structure within the intestinal microbiome showed that NC and ET+CM groups were closer to each other compared with the ET group. These results showed a high degree of similarity between the microbial communities of the NC and ET+CM groups, indicating that dietary CM effectively reversed the microbial disorder caused by alcohol intake in mice (Figure 3D).

The relative abundance of bacterial species in the 3 groups at the phylum level are shown in Figure 4A. *Firmicutes*, *Bacteroidetes*, *Verrucomicrobia*, *Proteobacteria*, *Actinobacteria*, and *Deferribacteres* were detected, although *Firmicutes* and *Bacteroidetes* were the most abundant phyla among the groups (Figure 4A). The relative abundance of *Firmicutes* was significantly higher in the ET+CM group than in the ET and NC groups ($P < 0.05$), although the relative abundance of *Bacteroidetes* was significantly lower in the ET+CM group than in the ET and NC groups ($P < 0.05$; Supplemental Figure S3, <https://doi.org/10.3168/jds.2019-17247>). At the genus level, *Bacteroidales* and *Lactobacillus* were more abundant in the ET group than in the ET+CM and NC groups ($P < 0.05$; Figure 4A; Supplemental Figure S3); *Lachnospiraceae* was more abundant in the ET+CM group than in the ET and NC groups.

Figure 1. Pathological changes in liver tissue shown by staining with (A through C) hematoxylin and eosin (HE) and (D through F) Oil Red O. Arrows indicate inflammatory cell infiltration. NC = control group; ET = alcohol model group; ET+CM = alcohol and camel milk treatment group.

Figure 2. Effects of acute alcohol intake and camel milk on serum levels of liver enzymes and plasma inflammatory factors in mice: (A) alanine transaminase (ALT), (B) aspartate transaminase (AST), (C) tumor necrosis factor- α (TNF- α), (D) IL-1 β , and (E) IL-6. NC = control group; ET = alcohol model group; ET+CM = alcohol and camel milk treatment group. Significantly different from the control group at ** $P < 0.001$; significantly different from the model group at # $P < 0.05$ and ## $P < 0.001$. Data represent mean $\pm$ SEM (n = 8 mice).

The relative abundance of bacterial species at the genus level is depicted in Supplemental Figure S4 (<https://doi.org/10.3168/jds.2019-17247>). Compared with the ET group, the proportion of *Lactobacillus* was significantly increased ($P < 0.01$), whereas the proportions of *Bacteroides* and *Alistipes* were significantly decreased ($P < 0.05$) in the ET+CM group (Supplemental Figure S4A, <https://doi.org/10.3168/jds.2019-17247>). The abundance of *Alistipes*, *norank_f_Lachnospiraceae*, and *Blautia* were significantly increased in the ET group compared with the control group ($P < 0.01$), whereas *norank_f_Bacteroidales_S24-7_group* ($P < 0.05$), *Lactobacillus* ($P < 0.01$), and *Escherichia-Shigella* were significantly decreased ($P < 0.05$; Supplemental Figure S4B, <https://doi.org/10.3168/jds.2019-17247>). These results indicate that dietary CM can reverse the effects of acute alcohol intake on the composition of the gut microbiota.

Effects of Acute Alcohol-Induced Liver Injury on the Liver Transcriptome

We further investigated the mechanism underlying the hepatoprotective effects of CM against acute alcoholic liver injury by evaluating the DEG in the mouse liver transcriptome. We obtained more than 48,720,392 raw reads for each sample. After removing the low-quality reads and quality control, 48,271,992 clean reads, with more than 7 Gb of clean bases, remained for each sample. A high proportion (>93%) of the clean reads were mapped to the reference genome (version GRCm38.p5; Supplemental Table S3, <https://doi.org/10.3168/jds.2019-17247>). Several DEG were identified when comparing the NC, ET, and ET+CM groups (Supplemental Figure S5, <https://doi.org/10.3168/jds.2019-17247>). These results indicate the reliability of the RNA-Seq data.

Table 1. Effects of acute alcohol intake and camel milk on liver indexes¹

Group	MDA (nmol/g)	SOD (ng/g)	GSH (ng/g)	GSH-Px (ng/g)
NC	85.95 $\pm$ 5.65	60.38 $\pm$ 4.88	33.81 $\pm$ 2.34	366.12 $\pm$ 42.79
ET	102.87 $\pm$ 6.53**	55.37 $\pm$ 3.68	31.31 $\pm$ 1.27	330.73 $\pm$ 13.12
ET+CM	85.97 $\pm$ 3.64##	62.51 $\pm$ 4.78#	34.32 $\pm$ 3.50	396.44 $\pm$ 40.78#

¹Data represent the mean $\pm$ SEM (n = 8 mice). NC = control group; ET = alcohol model group; ET+CM = alcohol and camel milk treatment group; MDA = malondialdehyde; SOD = superoxide dismutase; GSH = glutathione; and GSH-Px = glutathione peroxidase.

**Significant difference from the control group at $P < 0.001$.

#Significant difference from the model group at $P < 0.05$ (#) or $P < 0.001$ (##).

Table 2. Effects of acute alcohol intake and camel milk on the liver lipid profile¹

Group	TG (nmol/g)	TC (μmol/g)	HDL-c (mg/g)	LDL-c (mmol/g)
NC	6.66 ± 0.44	38.99 ± 1.61	5.08 ± 0.14	39.13 ± 2.40
ET	8.04 ± 0.32**	43.25 ± 2.80**	4.43 ± 0.23**	39.12 ± 4.08
ET+CM	7.11 ± 0.45##	37.42 ± 1.67##	5.03 ± 0.10##	38.90 ± 4.12

¹Data represent the mean ± SEM (n = 8 mice). NC = control group; ET = alcohol model group; ET+CM = alcohol and camel milk treatment group. TG = triglyceride; TC = total cholesterol; HDL-c = high-density lipoprotein cholesterol; LDL-c = low-density lipoprotein cholesterol.

**Significant difference from the control group at $P < 0.001$.

##Significant difference from the model group at $P < 0.001$.

Gene expression in the NC, ET, and ET+CM groups is presented in the Venn diagram in Supplemental Figure S6A (<https://doi.org/10.3168/jds.2019-17247>), with DEG classified in a reads per kilobases per million (RPKM) ratio greater than 2. A total of 9,049, 9,101, and 9,228 DEG were identified in the NC, ET, and ET+CM groups, respectively. Among them, 8,455

DEG were commonly expressed in all 3 groups, whereas 302, 164, and 145 DEG were expressed only in the NC, ET, and ET+CM groups, respectively (Supplemental Figure S6A, <https://doi.org/10.3168/jds.2019-17247>).

Through analysis of DEG (Figure 5 and Supplemental Figure S6B, <https://doi.org/10.3168/jds.2019-17247>), we identified 315 DEG in the ET+CM and

Figure 3. Genus-level diversity of gut microbiota deduced by 16S rRNA sequencing: (A) bacterial diversity (Shannon index), (B) bacterial evenness (Simpson index), (C) bacterial richness (Chao1 index), and (D) principal coordinate (PC) analysis with Bray-Curtis dissimilarity analysis of the microbiomes of the 3 groups. NC = control group; ET = alcohol model group; ET+CM = alcohol and camel milk treatment group. Significantly different from the control group at $*P < 0.05$. Data represent mean ± SEM (n = 6 mice).

Figure 4. Relative abundance of bacterial species in the gut microbiota of mice in the 3 treatment groups (NC = control group; ET = alcohol model group; ET+CM = alcohol and camel milk treatment group) at the (A) phylum and (B) genus levels.

ET groups (150 upregulated and 165 downregulated; Figure 5A) and 1,764 DEG in the ET and NC groups (1,001 upregulated and 763 downregulated; Figure 5B).

Gene Annotation and Functional Enrichment Analysis

Genes were annotated using the following publicly available databases: GO, KEGG, Clusters of Orthologous Groups, Non-Redundant, Swiss-Prot, and Pfam. A total of 51,912 potential genes were detected, and 40,106 were annotated (Table 3). In total, 19,805 genes were detected in the GO database, and 65 terms were enriched in biological process ($n = 27$), cellular component ($n = 19$), and molecular function ($n = 19$; Figure 6). Annotation and classification of the genes using the KEGG database revealed that genes were highly enriched in the pathways of human diseases ($n = 9,616$), organismal systems ($n = 7,555$), genetic information processing ($n = 4,779$), environmental information processing ($n = 4,116$), metabolism ($n = 3,884$), and cellular processes ($n = 3,314$; Figure 7).

To gain insight into the potential mechanisms responsible for the protective effects of CM against ALD, we performed KEGG enrichment analysis of DEG in the liver identified by comparisons of the ET and NC groups and the ET+CM and ET groups. Three KEGG pathways (IL-17 and TNF- α signaling pathways and retinol metabolism) were significantly enriched (false discovery rate < 0.05) from the ET+CM and ET groups (Supplemental Table S4 and Supplemental Figure S7, <https://doi.org/10.3168/jds.2019-17247>). We identified

8 DEG (upregulated: *Gm20257*; downregulated: *Il1b*, *S100a8*, *S100a*, *Cebpb*, *Mmp9*, *Cxcl1*, and *Map3k8*) in the IL-17 and TNF- α signaling pathways, and 6 DEG (upregulated: *Cyp2b9*, *Cyp2b10*, *Cyp2c38*, and *Cyp2c39*; downregulated: *Dhrs9* and *Adh6-ps1*) in the retinol metabolism pathway. In our previous study, we found that the DEG induced by CM protection intervention in mice with acute liver injury induced by carbon tetrachloride were also significantly enriched in the retinol metabolic pathway (Zhang et al., 2018); therefore, further research is needed to reveal the specific role of CM in liver protection. In the ET and ET+CM groups, the top 3 significantly enriched pathways were the mitogen-activated protein kinase signaling pathway, arachidonic acid metabolism, and the inflammatory mediator regulation of transient receptor potential channels (Supplemental Table S5 and Supplemental Figure S8, <https://doi.org/10.3168/jds.2019-17247>).

DISCUSSION

Oxidative stress is one of the key mechanisms responsible for the development of ALD. Metabolism of ethanol in the liver can increase the content of radicals (mainly reactive oxygen species, **ROS**) and induce oxidative stress (Wu and Cederbaum, 2009). The end product of lipid oxidation, MDA is used as a marker of lipid peroxidation; SOD and GSH-Px are 2 important antioxidant enzymes involved in the elimination of ROS, whereas GSH is an important non-enzymatic cellular antioxidant (Dey and Cederbaum, 2006). Acute alcoholic liver injury leads to decreased SOD, GSH,

Table 3. Functional annotation based on 6 publicly available databases

Database ¹	Transcripts	Annotated genes	Transcripts (%)	Genes (%)
GO	63,429	19,805	0.4928	0.3815
KEGG	72,591	22,737	0.564	0.438
COG	103,023	32,997	0.8004	0.6356
NR	114,028	39,895	0.8859	0.7685
Swiss-Prot	102,218	32,636	0.7941	0.6287
Pfam	51,311	11,609	0.3986	0.2236
Total_anno	114,334	40,106	0.8883	0.7726
Total	128,717	51,912	1	1

¹GO = Gene Ontology (<http://www.geneontology.org/>); KEGG = Kyoto Encyclopedia of Genes and Genomes (<http://www.genome.jp/kegg/>); COG = Clusters of Orthologous Groups (<http://www.ncbi.nlm.nih.gov/COG/>); NR = nonredundant (<ftp://ftp.ncbi.nlm.nih.gov/blast/db/>); Swiss-Prot (http://web.expasy.org/docs/swiss-prot_guideline.html); Pfam (<http://pfam.xfam.org/>). Total_anno = total annotated.

and GSH-Px activity in the livers of mice (Zhao et al., 2008). In our study, acute alcohol administration caused liver injury, as evidenced by the increase in serum ALT and AST, liver tissue TG and TC, degeneration of hepatocytes, and swelling, indicative of the early biochemical and pathological changes in alcoholic liver disease. After administration of a large amount of alcohol in mice, we showed that the MDA content in the liver of ALD model mice increased by 19.69% compared with that in the NC group ($P < 0.01$), indicating severe lipid peroxidation and preventing a strong inflammatory response, whereas the contents of SOD, GSH, and GSH-Px were reduced to varying degrees, indicating

the ability of CM to restore and preserve the activity of these 2 enzymes. In contrast, we found the opposite results in the ET+CM group, indicating that CM increased not only lipid peroxidation but also the content of antioxidants such as SOD, GSH, and GSH-Px in the liver. The anti-oxidative stress effects of CM have been reported in several diseases (Wang et al., 2017): for example, in type-1 diabetic rats (Meena et al., 2016) and patients with autism spectrum disorder (Al-Ayadhi and Elamin, 2013). Histopathological changes induced by alcohol were also significantly improved by CM treatment. In general, the serum biochemical indexes and liver histology test results indicated that CM has

Figure 5. Differentially expressed genes (DEG; FC = fold change): downregulated and upregulated DEG of (A) ET+CM (alcohol and camel milk treatment group) compared with ET (alcohol model group), and (B) ET compared with NC (control group). Blue dots = no change; red dots = upregulated; green dots = downregulated.

Figure 6. Gene Ontology (GO) functional classification.

a protective effect against acute alcoholic liver injury in mice, which was achieved by inhibiting lipid peroxidation, enhancing the antioxidative defense system, and suppressing cell apoptosis and the inflammatory response of the liver.

These conclusions were confirmed by transcriptome research. The GO enrichment analysis showed significant upregulation of neuroglobin (*Ngb*) gene expression in the ET+CM group. Neuroglobin is associated with clearance of ROS (Fordel et al., 2007) and plays a role in reducing the production of inflammatory factors and apoptosis (Amri et al., 2017). Our transcriptomics analysis revealed increased *Ngb* in the liver in the ET+CM group, which might have enhanced the clearance of liver ROS and improved oxidation; however, these findings remain to be verified. Overall, our results indicate that dietary CM alleviates the oxidative damage to the liver caused by the intake of large amounts of ethanol. This effect may be related to the protection of the antioxidant defense system and improvement of the ROS scavenging ability in the liver.

Abnormal cytokine metabolism is another major feature of ALD (Tilg et al., 2016); TNF- α , IL-1 β , and IL-6 are proinflammatory cytokines, and TNF- α and IL-1 β play an important role in ALD. Khan et al. (2011) showed that ethanol-induced acute liver injury can lead to nuclear factor (NF)- κ B activation and an increase

in TNF- α levels in the liver. In accordance with previous reports, we observed significant increases in serum TNF- α , IL-1 β , and IL-6 levels in the ET group, indicating that inflammatory factors are involved in early ALD (Khan et al., 2011). The inflammatory response caused by acute alcoholic liver injury can be alleviated by intervention with plant extracts (Khan et al., 2011) and drugs (Zhao et al., 2008). Our results showed that CM reduces the levels of inflammatory factors in the serum of mice with acute alcoholic liver injury. Analysis via KEGG enrichment showed that the DEG associated with dietary CM were significantly enriched in inflammation-related signaling pathways. This suggests that CM inhibits the expression of inflammation-related cytokines, such as IL-1 β and CXCL1, in the liver of mice with acute alcoholic liver injury, causing a decrease in the levels of inflammatory factors in the liver, thereby protecting the liver against alcohol-induced injury.

Oxidative stress is a key factor in development of steatosis, which is an early response of the liver to the intake of large quantities of ethanol (Gao and Bataller, 2011; Louvet and Mathurin, 2015). Acetaldehyde and TNF- α also increase expression of sterol regulatory element-binding protein (SREBP)-1c, which causes steatosis. In this study, we observed a marked accumulation of lipid droplets and TG in the liver in the ET group. Inhibition of peroxisome proliferator-activated

receptors (PPAR)- α has been shown to result in lipid accumulation in the liver (Gao and Bataller, 2011). Our analysis of the liver transcriptome showed that the DEG in the ET group were significantly enriched in the PPAR signaling pathway, and PPAR- α was significantly downregulated. Overall, our findings indicate that dietary CM alleviates steatosis by reducing lipid droplet accumulation and decreasing TC and TG levels in the liver. Intestinal microecological disorders and bacterial translocation play an important role in ALD (Louvet and Mathurin, 2015), and ethanol increases intestinal permeability (Zhou and Zhong, 2017). Lipopolysaccharide (LPS) produced by microbial metabolism enters the liver in large quantities and activates inflammatory responses through pathogen-associated molecular patterns (PAMP). Intake of large amounts of alcohol can also lead to bacterial overgrowth and colonic microecological disorders (Zhou and Zhong, 2017) as well as decreased *Bacteroidetes* abundance and increased *Proteobacteria* abundance. This microecological dysregulation is associated with increased LPS in vivo (Mutlu et al., 2009; Zhou and Zhong, 2017). Studies showed that probiotics (Bull-Otterson et al., 2013) and the antioxidant astaxanthin (Liu et al., 2019) can influence intestinal microecological disorders and limit the development of ALD.

In this study, acute alcohol intake caused a significant decrease in the relative abundance of *Lactobacillus* in

the colon of mice ($P < 0.01$), whereas the relative abundance of *Bacteroides*, *Akkermansia*, *Alisipes*, *Lachnospiraceae* bacteria, such as *Lachnospiraceae_NK4A136_group* and *norank_f_Lachnospiraceae*, increased. The CM intervention significantly increased the relative abundance of *Lactobacillus* ($P < 0.01$) and decreased the relative abundance of *Bacteroides* ($P < 0.05$), which is consistent with the report by Bull-Otterson et al. (2013). Intestinal *Lachnospiraceae* is known to be a harmful bacteria, associated with inflammatory bowel disease (Berry and Reinisch, 2013). Excessive *Akkermansia* results in mucosal barrier permeability and induces an inflammatory response (Swidsinski et al., 2011). *Lactobacillus* is a probiotic that participates in the regulation of the mucous layer of the colon and promotes the proliferation of intestinal epithelial cells (Swanson et al., 2011). In this study, dietary CM improved the relative abundance of probiotic genera in the intestinal tract of mice, thus alleviating the damage caused by alcohol. We also found that CM reduces the relative abundance of pathogenic genera to improve the intestinal microecological balance. We found that intake of CM could prevent an imbalance in gut microbiota by promoting probiotics and reducing harmful bacteria in the gut microbiota. Whether the effects of CM on the intestinal flora are mediated by modulating the relative abundance of a particular flora or the susceptibility of the flora to alcohol requires further investigation.

Figure 7. Kyoto Encyclopedia of Genes and Genomes (KEGG) classification.

ACKNOWLEDGMENTS

This work was supported by grants from the High-Level Talents Introduction to Scientific Research Start-Up Project (NDYB2017-28), the Inner Mongolia Natural Science Foundation Project (2018BS03017), Inner Mongolia Autonomous Region Science and Technology Innovation Guide Project (KCMS2018048), and double-class discipline innovation team building (NDSC2018-14), Inner Mongolia, China. The authors have not stated any conflicts of interest.

REFERENCES

- Abdulrahman, A. O., M. A. Ismael, K. Al-Hosaini, C. Rame, A. M. Al-Senaidy, J. Dupont, and M. A. Ayoub. 2016. Differential effects of camel milk on insulin receptor signaling—Toward understanding the insulin-like properties of camel milk. *Front. Endocrinol. (Lausanne)* 7:4. <https://doi.org/10.3389/fendo.2016.00004>.
- Al-Ayadhi, L. Y., and N. E. Elamin. 2013. Camel milk as a potential therapy as an antioxidant in autism spectrum disorder (ASD). *Evid. Based Complement. Alternat. Med.* 2013:602834. <https://doi.org/10.1155/2013/602834>.
- Al-Fartosi, K. G., O. S. Khuon, and H. I. Al-Tae. 2011. Protective role of camel's milk against paracetamol induced hepatotoxicity in male rats. *Int. J. Res. Pharm. Biomed. Sci.* 2:1795–1799.
- Al-Fartosi, K. G., A. Majid, M. A. Auda, and M. H. Hussein. 2012. The role of camel's milk against some oxidant-antioxidant markers of male rats treated with CCl₄. *Int. J. Res. Pharm. Biomed. Sci.* 3:385–389.
- Al-Hashem, F. 2009. Camel's milk protects against aluminum chloride-induced toxicity in the liver and kidney of white albino rats. *Am. J. Biochem. Biotechnol.* 5:98–108. <https://doi.org/10.3844/ajbbsp.2009.98.108>.
- Al-Hashem, F., M. Dallak, N. Bashir, M. Abbas, R. Eleassa, M. Khalil, and M. Al-Khateeb. 2009. Camel's milk protects against cadmium chloride induced toxicity in white albino rats. *Am. J. Pharmacol. Toxicol.* 4:107–117. <https://doi.org/10.3844/ajptsp.2009.107.117>.
- Ali, A., B. Baby, and R. Vijayan. 2019. From desert to medicine: A review of camel genomics and therapeutic products. *Front. Genet.* 10:17. <https://doi.org/10.3389/fgene.2019.00017>.
- Althnaian, T., I. Albokhadaim, and S. M. El-Bahr. 2013. Biochemical and histopathological study in rats intoxicated with carbontetrachloride and treated with camel milk. *Springerplus* 2:57. <https://doi.org/10.1186/2193-1801-2-57>.
- Amri, F., I. Ghoulil, M. Amri, A. Carrier, and O. Masmoudi-Kouki. 2017. Neuroglobin protects astroglial cells from hydrogen peroxide-induced oxidative stress and apoptotic cell death. *J. Neurochem.* 140:151–169. <https://doi.org/10.1111/jnc.13876>.
- Ayoub, M. A., A. R. Palakkott, A. Ashraf, and R. Iratni. 2018. The molecular basis of the anti-diabetic properties of camel milk. *Diabetes Res. Clin. Pract.* 146:305–312. <https://doi.org/10.1016/j.diabres.2018.11.006>.
- Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* 57:289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>.
- Berry, D., and W. Reinisch. 2013. Intestinal microbiota: A source of novel biomarkers in inflammatory bowel diseases? *Best Pract. Res. Clin. Gastroenterol.* 27:47–58. <https://doi.org/10.1016/j.bpg.2013.03.005>.
- Bertola, A., S. Mathews, S. H. Ki, H. Wang, and B. Gao. 2013. Mouse model of chronic and binge ethanol feeding (the NIAAA model). *Nat. Protoc.* 8:627–637. <https://doi.org/10.1038/nprot.2013.032>.
- Bull-Otterson, L., W. Feng, I. Kirpich, Y. Wang, X. Qin, Y. Liu, L. Gobejishvili, S. Joshi-Barve, T. Ayvaz, J. Petrosino, M. Kong, D. Barker, C. McClain, and S. Barve. 2013. Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of *Lactobacillus rhamnosus* GG treatment. *PLoS One* 8:e53028. <https://doi.org/10.1371/journal.pone.0053028>.
- Cardoso, R. R., R. Santos, C. Cardoso, and M. Carvalho. 2010. Consumption of camel's milk by patients intolerant to lactose. A preliminary study. *Rev. Alerg. Mex.* 57:26–32.
- Darwish, H. A., N. R. Abd Raboh, and A. Mahdy. 2012. Camel's milk alleviates alcohol-induced liver injury in rats. *Food Chem. Toxicol.* 50:1377–1383. <https://doi.org/10.1016/j.fct.2012.01.016>.
- Dey, A., and A. I. Cederbaum. 2006. Alcohol and oxidative liver injury. *Hepatology* 43(Suppl 1):S63–S74. <https://doi.org/10.1002/hep.20957>.
- Ding, W. X., M. Li, X. Chen, H. M. Ni, C. W. Lin, W. Gao, B. Lu, D. B. Stolz, D. L. Clemens, and X. M. Yin. 2010. Autophagy reduces acute ethanol-induced hepatotoxicity and steatosis in mice. *Gastroenterology* 139:1740–1752. <https://doi.org/10.1053/j.gastro.2010.07.041>.
- Fordel, E., L. Thijs, L. Moens, and S. Dewilde. 2007. Neuroglobin and cytoglobin expression in mice: Evidence for a correlation with reactive oxygen species scavenging. *FEBS J.* 274:1312–1317. <https://doi.org/10.1111/j.1742-4658.2007.05679.x>.
- Gao, B., and R. Bataller. 2011. Alcoholic liver disease: Pathogenesis and new therapeutic targets. *Gastroenterology* 141:1572–1585. <https://doi.org/10.1053/j.gastro.2011.09.002>.
- Ge, X., T. M. Leung, E. Arriazu, Y. Lu, R. Urtasun, B. Christensen, M. I. Fiel, S. Mochida, E. S. Sørensen, and N. Nieto. 2014. Osteopontin binding to lipopolysaccharide lowers tumor necrosis factor- α and prevents early alcohol-induced liver injury in mice. *Hepatology* 59:1600–1616. <https://doi.org/10.1002/hep.26931>.
- Hamed, H., K. Bellassoued, A. E. Feki, and A. Gargouri. 2019. Evaluation of the hepatoprotective effect of combination between fermented camel milk and *Rosmarinus officinalis* leaves extract against CCl₄ induced liver toxicity in mice. *J. Food Sci. Technol.* 56:824–834.
- Karray, N., C. Lopez, M. Ollivon, and H. Attia. 2005. La matière grasse du lait de dromadaire: Composition, microstructure et polymorphisme. Une revue. *Ol. Corps Gras Lipides* 12:439–446. <https://doi.org/10.1051/ocl.2005.0439>.
- Khan, A. Q., S. Nafees, and S. Sultana. 2011. Perillyl alcohol protects against ethanol induced acute liver injury in Wistar rats by inhibiting oxidative stress, NF- κ B activation and proinflammatory cytokine production. *Toxicology* 279:108–114. <https://doi.org/10.1016/j.tox.2010.09.017>.
- Kim, D., G. Pertea, C. Trapnell, H. Pimentel, R. Kelley, and S. L. Salzberg. 2013. TopHat2: Accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol.* 14:R36. <https://doi.org/10.1186/gb-2013-14-4-r36>.
- Konuspayeva, G., É. Lemarie, B. Faye, G. Loiseau, and D. Montet. 2008. Fatty acid and cholesterol composition of camel's (*Camelus bactrianus*, *Camelus dromedarius* and hybrids) milk in Kazakhstan. *Dairy Sci. Technol.* 88:327–340. <https://doi.org/10.1051/dst:2008005>.
- Lee, C.-H., S.-W. Park, Y. S. Kim, S. S. Kang, J. A. Kim, S. H. Lee, and S.-M. Lee. 2007. Protective mechanism of glycyrrhizin on acute liver injury induced by carbon tetrachloride in mice. *Biol. Pharm. Bull.* 30:1898–1904. <https://doi.org/10.1248/bpb.30.1898>.
- Liu, H., H. Liu, L. Zhu, Z. Zhang, X. Zheng, J. Liu, and X. Fu. 2019. Comparative transcriptome analyses provide potential insights into the molecular mechanisms of astaxanthin in the protection against alcoholic liver disease in mice. *Mar. Drugs* 17:181. <https://doi.org/10.3390/md17030181>.
- Louvet, A., and P. Mathurin. 2015. Alcoholic liver disease: Mechanisms of injury and targeted treatment. *Nat. Rev. Gastroenterol. Hepatol.* 12:231–242. <https://doi.org/10.1038/nrgastro.2015.35>.
- Massey, V. L., and G. E. Arteel. 2012. Acute alcohol-induced liver injury. *Front. Physiol.* 3:193.
- Meena, S., Y. S. Rajput, A. K. Pandey, R. Sharma, and R. Singh. 2016. Camel milk ameliorates hyperglycaemia and oxidative damage in type-1 diabetic experimental rats. *J. Dairy Res.* 83:412–419. <https://doi.org/10.1017/S002202991600042X>.

- MOH (Ministry of Health of the People's Republic of China). 2003. Technical Specifications for Inspection and Evaluation of Health Foods. MOH, Beijing, China.
- Mutlu, E., A. Keshavarzian, P. Engen, C. B. Forsyth, M. Sikaroodi, and P. Gillevet. 2009. Intestinal dysbiosis: A possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. *Alcohol. Clin. Exp. Res.* 33:1836–1846. <https://doi.org/10.1111/j.1530-0277.2009.01022.x>.
- Osna, N. A., T. M. Donohue Jr., and K. K. Kharbanda. 2017. Alcoholic liver disease: Pathogenesis and current management. *Alcohol Res.* 38:147.
- Rao, M., R. Gupta, and N. Dastur. 1970. Camels' milk and milk products. *Indian J. Dairy Sci.* 23:71–78.
- Schmieder, R., and R. Edwards. 2011. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 27:863–864. <https://doi.org/10.1093/bioinformatics/btr026>.
- Smathers, R. L., J. J. Galligan, B. J. Stewart, and D. R. Petersen. 2011. Overview of lipid peroxidation products and hepatic protein modification in alcoholic liver disease. *Chem. Biol. Interact.* 192:107–112. <https://doi.org/10.1016/j.cbi.2011.02.021>.
- Swanson, P. A., A. Kumar, S. Samarin, M. Vijay-Kumar, K. Kundu, N. Murthy, J. Hansen, A. Nusrat, and A. S. Neish. 2011. Enteric commensal bacteria potentiate epithelial restitution via reactive oxygen species-mediated inactivation of focal adhesion kinase phosphatases. *Proc. Natl. Acad. Sci. USA* 108:8803–8808. <https://doi.org/10.1073/pnas.1010042108>.
- Swidsinski, A., Y. Dörffel, V. Loening-Baucke, F. Theissig, J. C. Rückert, M. Ismail, W. A. Rau, D. Gaschler, M. Weizenegger, S. Kühn, J. Schilling, and W. V. Dörffel. 2011. Acute appendicitis is characterised by local invasion with *Fusobacterium nucleatum/necrophorum*. *Gut* 60:34–40. <https://doi.org/10.1136/gut.2009.191320>.
- Tilg, H., A. R. Moschen, and G. Szabo. 2016. Interleukin-1 and inflammasomes in alcoholic liver disease/acute alcoholic hepatitis and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Hepatology* 64:955–965. <https://doi.org/10.1002/hep.28456>.
- Wang, F., Y. Xue, J. Yang, F. Lin, Y. Sun, T. Li, and C. Wu. 2016a. Hepatoprotective effect of apple polyphenols against concanavalin A-induced immunological liver injury in mice. *Chem. Biol. Interact.* 258:159–165. <https://doi.org/10.1016/j.cbi.2016.08.018>.
- Wang, F. S., J. G. Fan, Z. Zhang, B. Gao, and H. Y. Wang. 2014. The global burden of liver disease: The major impact of China. *Hepatology* 60:2099–2108. <https://doi.org/10.1002/hep.27406>.
- Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73:5261–5267. <https://doi.org/10.1128/AEM.00062-07>.
- Wang, Y., Y. Wan, G. Ye, P. Wang, X. Xue, G. Wu, and B. Ye. 2016b. Hepatoprotective effects of AdipoRon against d-galactosamine-induced liver injury in mice. *Eur. J. Pharm. Sci.* 93:123–131. <https://doi.org/10.1016/j.ejps.2016.08.017>.
- Wang, Z. X., X. Y. Qiao, S. N. Hao, and R. Ji. 2017. Demonstration of hepatoprotective action of camel milk through improving anti-oxidant activity and regulating gene expression in mice. *J. Camel Pract. Res.* 24:169. <https://doi.org/10.5958/2277-8934.2017.00026.1>.
- Woodhouse, C. A., V. C. Patel, A. Singanayagam, and D. L. Shawcross. 2018. Review article: The gut microbiome as a therapeutic target in the pathogenesis and treatment of chronic liver disease. *Aliment. Pharmacol. Ther.* 47:192–202. <https://doi.org/10.1111/apt.14397>.
- Wu, D., and A. I. Cederbaum. 2009. Oxidative stress and alcoholic liver disease. Pages 141–154 in *Proc. Seminars in Liver Disease*. Thieme Medical Publishers, New York, NY.
- Zhang, J., Z. Wang, D. Huo, and Y. Shao. 2018. Consumption of goats' milk protects mice from carbon tetrachloride-induced acute hepatic injury and improves the associated gut microbiota imbalance. *Front. Immunol.* 9:1034. <https://doi.org/10.3389/fimmu.2018.01034>.
- Zhang, W., H. Kudo, K. Kawai, S. Fujisaka, I. Usui, T. Sugiyama, K. Tsukada, N. Chen, and T. Takahara. 2010. Tumor necrosis factor- α accelerates apoptosis of steatotic hepatocytes from a murine model of non-alcoholic fatty liver disease. *Biochem. Biophys. Res. Commun.* 391:1731–1736. <https://doi.org/10.1016/j.bbrc.2009.12.144>.
- Zhao, J., H. Chen, and Y. Li. 2008. Protective effect of bicyclol on acute alcohol-induced liver injury in mice. *Eur. J. Pharmacol.* 586:322–331. <https://doi.org/10.1016/j.ejphar.2008.02.059>.
- Zhong, W., and Z. Zhou. 2014. Alterations of the gut microbiome and metabolome in alcoholic liver disease. *World J. Gastrointest. Pathophysiol.* 5:514. <https://doi.org/10.4291/wjgp.v5.i4.514>.
- Zhou, Z., and W. Zhong. 2017. Targeting the gut barrier for the treatment of alcoholic liver disease. *Liver Research* 1:197–207.

ORCIDS

Liang Ming <https://orcid.org/0000-0002-5020-9529>