DOI : 10.5958/2277-8934.2021.00003.5 *Vol 28 No 1, p 17-24*

COMPARATIVE TRANSCRIPTOME ANALYSIS PROVIDES POTENTIAL INSIGHTS INTO THE MECHANISM OF CAMEL MILK IN REGULATING ALCOHOLIC LIVER DISEASE IN MICE

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ABSTRACT

Alcoholic liver disease (ALD) is a general term used to refer to these alcohol-related liver damage. In this study, we investigated the hepatoprotective effect of camel milk (CM) in an ALD mouse model and its underlying mechanism at the transcriptome level. Male C57BL/6NCr were divided into 3 groups: normal diet (NC) ; normal diet, then ethanol (ET); and normal diet, then ethanol and camel milk (ET+CM). Comparative hepatic transcriptome analysis among the groups was performed by Illumina RNA sequencing. The result showed that a total of 526.76±19.87, 563.04±17.84, and 513.56±20.41 million clean reads were obtained for the NC, ET, and ET+CM groups, respectively. Compared with the Et group, 423 differentially expressed genes (DEGs) (including 160 upregulated and 263 downregulated genes) were identified in the NC group, and 186 differentially expressed genes (including 62 upregulated and 124 downregulated genes) were identified in the ET+CM group. The enrichment analyses revealed that the NOD-like receptor signaling pathway, the Toll-like receptor signaling pathway, the MAPK signaling pathway, and mTOR signaling pathway enriched the most differentially expressed genes. The findings of this study provide insights into the development of nutrition-related therapies for alcoholic liver disease (ALD) with camel milk.

Key words: Alcoholic liver disease, camel milk, mice transcriptome analyses

Alcoholic liver disease (ALD) is one of the main causes of chronic liver disease worldwide, including fatty liver, alcoholic hepatitis (AH) and cirrhosis and its complications (Singal *et al,* 2018). ALD can also be superimposed on other common liver diseases, including nonalcoholic liver disease (NAFLD) and hepatitis C virus (HCV) infection, accentuating their prevalence and severity (Dunn and Shah, 2016). Ethanol oxidative metabolism influences intracellular signaling pathways and deranges the transcriptional control of several genes, leading to fat accumulation, fibrogenesis and activation of innate and adaptive immunity (Ceni *et al,* 2014). The investigations have found that intracellular signal transduction pathways, transcription factors, innate immunity and chemokines participate in the pathogenesis of ALD (Gao and Bataller, 2011).

Camel milk has been reported to possess various human health benefits and used as a medicine to treat human diseases such as hepatitis, spleen problems etc. Camel milk is known to exhibit significant antioxidant effects as well as possess protective proteins which includes lysozyme, lactoperoxidase and lactoferrin (Krishnankutty *et al,* 2018). Studies have shown that the milk intake of camels may play an important role in reducing alcoholic liver damage (Ming *et al,* 2020 and Darwish *et al,* 2012). Therefore, CM can be used as a supplementary member in the treatment and management of ALD. Our previous study suggested that camel milk (CM) modulates liver inflammation and alleviates the intestinal microbial disorder caused by acute alcohol injury. In the present study, we investigated the hepatoprotective effects of CM and the underlying mechanism at the transcriptome levels in a mouse model of chronic alcoholic liver disease.

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

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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).

SPF male C57BL/6NCr mice (21±23 g) were obtained from Beijing Weitong Lihua Laboratory Animal Technology Co., Ltd. (China; license number SCXK 2016-0006). Mice were maintained in ventilated cages (3 per cage) under the following conditions: 22±2°C; 50 to 60% relative humidity; 12h light and dark cycle. Mice were given free access to food and water. All food, water, and experimental equipment were sterilised before use.

Camel milk administration

Bactrian camel milk 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 3,500 r/min 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

In this experiment, the NIAAA mouse model was established by referring to the previous research (Darwish *et al,* 2012 and Ming *et al,* 2020). After one week acclimation, a total of 9 mice were randomly divided into 3 groups: (1) the NC group (n = 3), given a ordinary maintenance feed for 10 weeks; (2) the ET group (n = 3), given a Lieber–DeCarli liquid diet for the first weeks, then an ethanol-containing Lieber–DeCarli liquid diet (i.e., 5% ethanol v/v accounted for 36% the total caloric intake) for another 9 weeks; (3) the ET+CM group (n = 3), given a Lieber–DeCarli liquid diet for the first weeks, then an ethanol-containing Lieber–DeCarli liquid diet and skimmed CM powder (3g/kg of BW; MOH, 2003) dissolved in 0.3mL of double-distilled water and fed for another 9 weeks.

After modeling, the animals were fasted for 9h and anesthetised with isoflurane gas. Liver samples were collected and stored at -80°C.

mRNA sequencing of liver tissue

To clarify the mechanism responsible for the protective effect of CM on liver tissue, RNA sequencing was performed on 9 liver samples: NC0101, NC0112, and NC0113 from the NC group; ET0132, ET0202, and ET0212 from the ET group; and CE0221, CE0242, and CE0321 from the ET+CM group after intervention. High-throughput mRNA sequencing was performed at the Shanghai Ma-jorbio Bio-Pharm Technology Co.

Total RNA was extracted using TRIzol reagent (In-vitrogen, 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 (Illu-mina), and all sequences were submitted to the NCBI SRA under accession no. PRJNA680682.

After obtaining the raw data, the sequencing adapters, low-quality reads, and those 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.p6) using TopHat2 software (v. 2.1.1; Dong *et al,* 2019). Read counts for all mapped genes were calculated using RSEM (v.1.3.3; http://deweylab.biostat.wisc.edu/rsem/). Differentially expressed genes (DEGs) were identified using the edgeR package (v. 3.24.3; R Foundation for Statistical Computing, Vienna, Austria) based on $P < 0.05$ and $|\log2\text{-fold change}| \geq 2$. 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

Statistical significance in the DEG analyses was performed using the R statistical package. Values of $p < 0.05$ were considered statistically significant.

Results and Discussion

Overview of RNA sequencing analysis

After removing the low-quality reads and quality control, a total of 526.76±19.87, 563.04±17.84, and 513.56±20.41 million clean reads were obtained for the NC, ET, and ET+CM groups, respectively (Table 1). The clean GC content of each group ranged from 49.17 to 50.68%, the value of Q20 ranged from 98.92 to 99.05%, and the value of Q30 ranged from 96.29 to 96.71% (Table S1). To evaluate the quality of the RNA-Seq data, the total clean reads were mapped to the reference genome. A high proportion of the clean reads were mapped to the mouse

reference genome using TopHat2 (http://ccb.jhu.edu/software/tophat/index.shtml) ; that is, 86.80% from NC, 85.12% from ET, and 85.21% from ET+CM (Table 1). Through TopHat2 analysis, more than 94% of the reads of each group were mapped to known genes, and more than 83% of the reads were mapped to exons. These results indicated the reliability of the RNA-Seq data.

Table S1. Statistics of RNA-seq.

Sample	Reads Number	Bases (bp)	Q20 (%)	Q30 (%)	GC (%)
NC0101	53773458	8037719775	98.97	96.51	50.68
NC0112	53871652	7984617085	99.04	96.69	49.17
NC0113	50382590	7515918115	98.9	96.32	49.69
ET0132	56078612	8370264136	98.92	96.29	49.73
ET0202	58189636	8704281225	98.99	96.49	49.72
ET0212	54642576	8126486791	99.01	96.59	49.5
CE0221	53187818	7909360831	99.05	96.71	49.6
CE0242	49155490	7259103921	99.02	96.65	49.81
CE0321	51725642	7693583159	98.97	96.46	49.49

Table 1. Summary of RNA-sequencing data.

Sample	NC	ET	CE
Total reads ($\times 10^5$)	526.76± 19.87	563.04± 17.84	513.56± 20.41
Total mapped reads ($\times 10^5$)	500.90± 22.60	532.48± 18.80	485.01± 19.59
Mapped to reference genome (%)	86.80%	85.12%	85.21%
Mapped to gene (%)	95.08%	94.57%	94.44%
Mapped to exon (%)	83.46%	83.87%	83.28%
Mapped to intergene (%)	0.46%	0.42%	0.43%

Gene annotation and functional analysis

The genes were aligned with public databases, such as the RefSeq non-redundant proteins (NR), the Gene Ontology (GO) database, the Cluster of Orthologous Groups of proteins (COG), the Swiss-Port, the Kyoto Encyclopedia of Genes and Genomes (KEGG), and the Protein families (Pfam). As shown in table 2, most of the genes were annotated using the NR database (93.46%), followed by GO (85.83%), COG (84.81%), Swiss-Port (83.12%), KEGG (64.98%) and Pfam (64.59%).

GO is an international standardised gene functional classification system. In total, there were 22287 genes mapped in the GO database (Fig S1). The biological process group possessed more terms than the cellular component and molecular function groups and 65 terms were enriched in biological process (n = 21), cellular component (n = 15), and molecular function (n = 9; Fig S1). The highly enriched GO terms were in the binding (GO: 0005488), cell part (GO: 0044464), cellular process (GO: 0009987), biological regulation (GO: 0065007), organelle (GO: 0043226), and metabolic process (GO: 0008152) groups.

Furthermore, the genes were annotated and classified using the KEGG database. As shown in Fig S2, genes assigned to human diseases (48) occupied the maximum proportion, followed by those assigned to environmental information processing (34), organismal systems (33), metabolism (22), cellular processes (19) and genetic information processing (4).

Table 2. Functional annotation of transcriptome data in three public databases.

Data base	Annotated	Per cent
NR	24269	93.46
GO	22287	85.83
COG	22022	84.81
Swiss-Port	21583	83.12
KEGG	16873	64.98
Pfam	16772	64.59

Analysis of differentially expressed genes (DEGs)

Gene expression levels of NC, ET, and ET+CM were quantified and compared (Fig 1). The genes with a reads per kilobases per million (RPKM) ratio greater than two fold were defined as DEGs. As shown in Fig 1A, a total of 11,817, 11,092, and 10,987 DEGs were identified in the NC, ET, and ET+CM groups, respectively. Among these DEGs, there were 853, 212, and 180 DEGs uniquely expressed in NC, ET, and ET+CM, respectively. Moreover, 10,489 DEGs were commonly expressed in all the groups.

Significant DEGs, including upregulated or downregulated genes, were identified by DEGseq (Fig 1B). Compared with the Et group, 423 DEGs (including 160 upregulated and 263 downregulated genes) were identified in the NC group; 186 DEGs (including 62 upregulated and 124 downregulated genes) were identified in the ET+CM group.

KEGG enrichment analyses of DEGs

To gain insight into the potential mechanisms responsible for the protective effects of camel milk 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. Table S2 and Table 3 summarise the KEGG pathways that were significantly enriched in each comparison group.

Through analysis, we found compared with NC group, DEGs related to NOD-like receptor signaling pathway (map04621) and Toll-like receptor signaling pathway (map04620) were significantly down-regulated in ET group (Table S2). Based on this result, it is speculated that ethanol may destroy the NOD-like receptor pathway and activate the Toll-like receptor pathway, and aggravate alcoholic liver damage in mice.

As shown in table 3, the DEGs related to the MAPK signaling pathway (map04010) and the mTOR signaling pathway (map04150) in the ET+CM group were significantly down-regulated compared with the ET group. Therefore, targeting the MAPKs signaling pathway and mTOR signaling pathway may be an effective treatment strategy to inhibit the deterioration of liver injury.

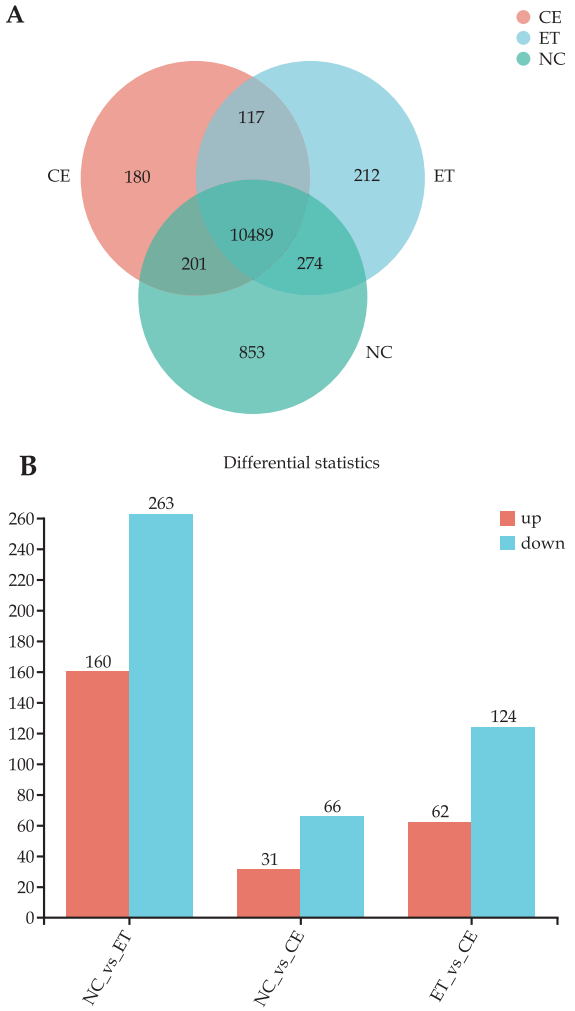

Fig 1. Statistical analysis of the gene expression detected by RNA-sequencing (RNA-Seq). **(A)** Venn diagram of gene counts expressed in the NC, ET and ET+CM groups. **(B)** Number of total differentially expressed genes (DEGs) and down or upregulated DEGs, respectively.

Table 3. Statistics on the KEGG pathway enrichment of DEGs between ET+CM and ET.

Pathway ID	Pathway	ET up	ET+CM up	P Value
map04710	Circadian rhythm	2	1	4.47×10^{-4}
map04350	TGF-beta signaling pathway	2	3	2.54×10^{-4}
map05206	MicroRNAs in cancer	4	2	9.89×10^{-4}
map04978	Mineral absorption	3	0	9.32×10^{-3}
map04550	Signaling pathways regulating pluripotency of stem cells	2	2	8.23×10^{-3}
map04931	Insulin resistance	2	1	1.88×10^{-2}
map04390	Hippo signaling pathway	2	3	1.52×10^{-2}
map00590	Arachidonic acid metabolism	2	1	1.81×10^{-2}
map04933	AGE-RAGE signaling pathway in diabetic complications	1	2	2.33×10^{-2}
map04010	MAPK signaling pathway	3	2	2.70×10^{-2}
map04150	mTOR signaling pathway	5	0	3.35×10^{-2}
map04152	AMPK signaling pathway	3	0	3.69×10^{-2}
map04068	FoxO signaling pathway	2	1	4.72×10^{-2}
map00140	Steroid hormone biosynthesis	1	2	3.34×10^{-2}
map04210	Apoptosis	2	2	4.68×10^{-2}
map04920	Adipocytokine signaling pathway	1	1	4.62×10^{-2}

ET up: the DEGs which were up-regulated in ethanol group, ET+CM up: the DEGs which were up-regulated in ethanol plus astaxanthin group.

The development of alcoholic liver disease (ALD) is a complex process. The increase of oxidative stress and the activation of the innate immune system are essential elements in the pathophysiology of ALD. The oxidative stress of exposure to ethanol is due to the increased production of reactive oxygen species. The antioxidant activity of liver cells is reduced, and

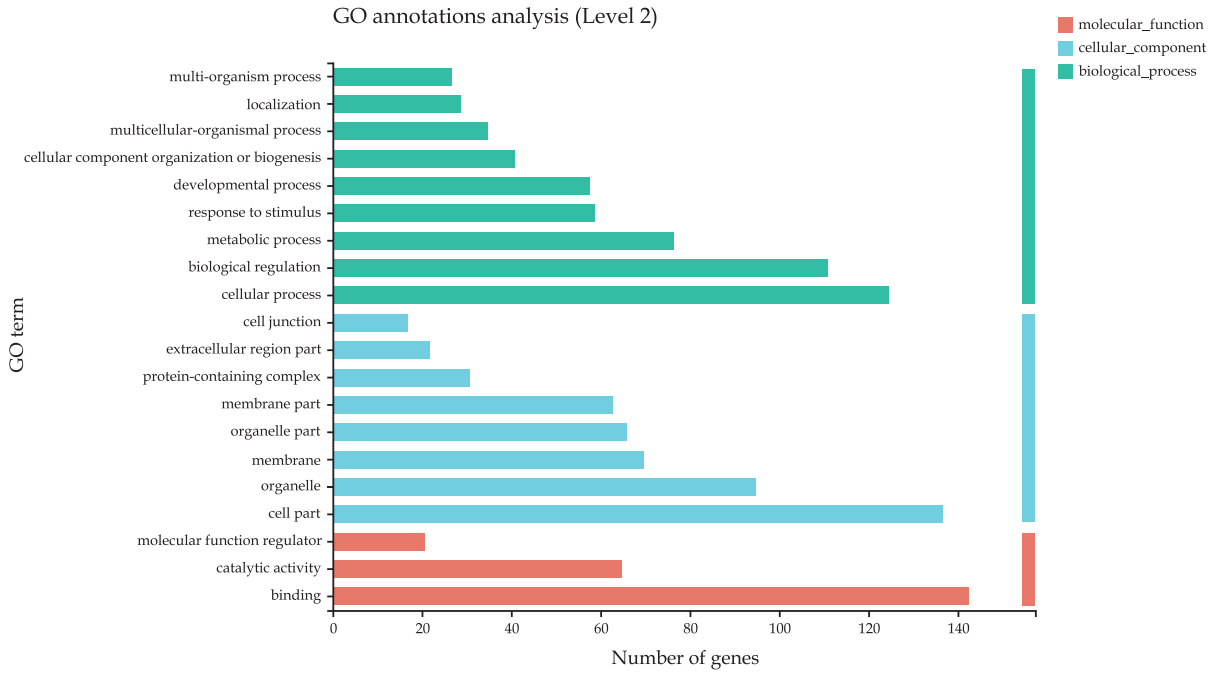

Fig S1. Histogram presentation of gene distribution in Gene Ontology (GO) functional classification. The x-axis represents level to GO terms; the left y-axis represents gene numbers in each GO term. Genes were further classified into sub-groups in biological process, cellular component and molecular function.

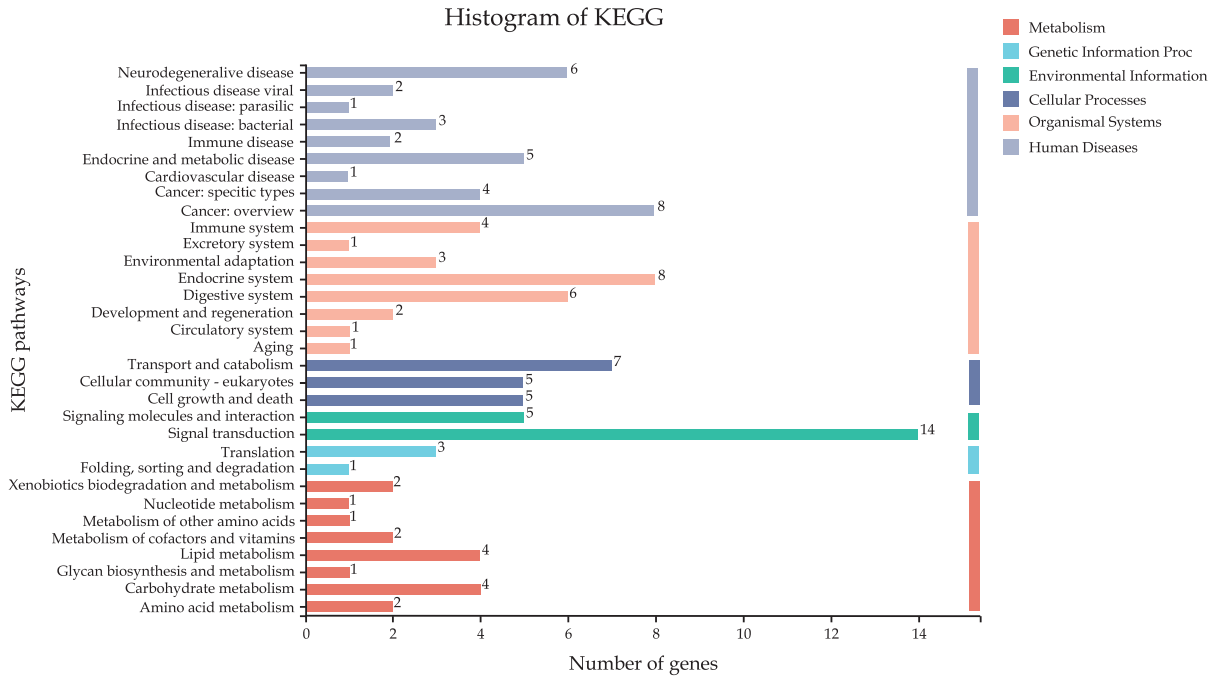

Fig S2. Histogram presentation of gene distribution in KEGG classification. The x-axis represents level to KEGG terms; the left y-axis represents gene numbers in each term. Genes were further classified into sub-groups in metabolism, signal transduction, human diseases and cell process.

the cells and circulating components of the innate immune system are activated by exposure to ethanol, thereby exacerbating ethanol-induced liver damage (Cohen *et al*, 2011). Previous studies have shown that camel milk can enhance the body's immune system (Khan, 2017), reduce the risk of cancer (Badawy *et al,* 2018) and lower blood sugar and anti-thrombotic effects (Korish *et al,* 2020). In addition, camel milk also has a potential protective effect on liver injury, by inhibiting lipid peroxidation, enhancing the

Table S2. Statistics on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of DEGs between NC and ET.

Pathway ID	Pathway	NC up	ET up	P Value
map00830	Retinol metabolism	4	14	1.31×10^{-13}
map00140	Steroid hormone biosynthesis	1	15	9.86×10^{-11}
map05204	Chemical carcinogenesis	2	13	2.76×10^{-9}
map04640	Hematopoietic cell lineage	18	0	1.76×10^{-8}
map05140	Leishmaniasis	16	1	1.63×10^{-7}
map05169	Epstein-Barr virus infection	22	3	1.13×10^{-6}
map00590	Arachidonic acid metabolism	1	9	2.83×10^{-6}
map05310	Asthma	14	1	1.24×10^{-5}
map05152	Tuberculosis	18	0	1.13×10^{-5}
map05323	Rheumatoid arthritis	16	1	1.67×10^{-5}
map04621	NOD-like receptor signaling pathway	14	1	2.61×10^{-5}
map00591	Linoleic acid metabolism	0	7	2.57×10^{-5}
map04659	Th17 cell differentiation	10	3	2.48×10^{-5}
map04145	Phagosome	18	1	3.80×10^{-5}
map04672	Intestinal immune network for IgA production	14	0	7.85×10^{-5}
map05330	Allograft rejection	15	0	8.61×10^{-5}
map04650	Natural killer cell mediated cytotoxicity	11	2	1.25×10^{-4}
map05320	Autoimmune thyroid disease	15	0	1.39×10^{-4}
map04750	Inflammatory mediator regulation of TRP channels	3	6	1.80×10^{-4}
map00100	Steroid biosynthesis	4	0	1.73×10^{-4}
map05164	Influenza A	13	1	2.08×10^{-4}
map04662	B cell receptor signaling pathway	10	2	2.24×10^{-4}
map05146	Amoebiasis	11	2	2.54×10^{-4}
map05416	Viral myocarditis	16	0	2.93×10^{-4}
map04658	Th1 and Th2 cell differentiation	9	1	3.45×10^{-4}
map05321	Inflammatory bowel disease (IBD)	7	2	3.63×10^{-4}
map00982	Drug metabolism - cytochrome P450	2	5	4.19×10^{-4}
map04664	Fc epsilon RI signaling pathway	10	1	4.38×10^{-4}
map04620	Toll-like receptor signaling pathway	5	2	5.08×10^{-4}
map04623	Cytosolic DNA-sensing pathway	5	1	5.57×10^{-4}
map05150	*Staphylococcus aureus* infection	15	0	5.79×10^{-4}
map04666	Fc gamma R-mediated phagocytosis	11	1	8.99×10^{-4}
map05340	Primary immunodeficiency	11	0	9.71×10^{-4}
map00980	Metabolism of xenobiotics by cytochrome P450	2	5	1.11×10^{-3}
map05322	Systemic lupus erythematosus	15	1	1.74×10^{-3}
map05143	African trypanosomosis	9	0	1.72×10^{-3}
map04060	Cytokine-cytokine receptor interaction	10	1	2.13×10^{-3}
map04010	MAPK signaling pathway	6	6	2.29×10^{-3}
map04380	Osteoclast differentiation	4	3	2.25×10^{-3}
map04062	Chemokine signaling pathway	8	1	2.97×10^{-3}
map05166	Human T-cell leukemia virus 1 infection	10	4	2.90×10^{-3}
map05414	Dilated cardiomyopathy (DCM)	11	0	3.23×10^{-3}
map00030	Pentose phosphate pathway	0	4	3.73×10^{-3}
map04931	Insulin resistance	0	6	4.33×10^{-3}
map04072	Phospholipase D signaling pathway	10	1	4.91×10^{-3}
map00983	Drug metabolism - other enzymes	2	5	5.05×10^{-3}
map05145	Toxoplasmosis	7	0	5.49×10^{-3}
map04933	AGE-RAGE signaling pathway in diabetic complications	3	3	6.45×10^{-3}
map05160	Hepatitis C	7	2	7.10×10^{-3}
map04726	Serotonergic synapse	0	7	7.75×10^{-3}
map04710	Circadian rhythm	1	2	7.63×10^{-3}
map04668	TNF signaling pathway	5	1	8.46×10^{-3}
map00072	Synthesis and degradation of ketone bodies	2	0	9.93×10^{-3}

NC up: the DEGs which were up-regulated in control group, ET up: the DEGs which were up-regulated in ethanol group.

antioxidant defence system, inhibiting cell apoptosis and liver inflammation, and protecting the liver from ethanol-induced liver injury (Hamed *et al,* 2019 and Ming *et al,* 2020). This study mainly discussed the *in vivo* experiment of feeding the mouse model of chronic alcoholic liver injury with camel milk, and performed the transcriptomics analysis on the liver samples of ALD mice. Based on the selected DEGs, many significantly changed GO functions and KEGG pathway enrichment were found. At the same time, it was found that most of the enriched KEGG pathway is related to the immune system and oxidative stress. The study further confirmed that camel milk can effectively prevent liver damage caused by ethanol.

Alcoholic liver disease (ALD) caused by alcohol abuse is the main cause of acute and chronic liver damage (Lamas-Paz *et al,* 2018). In previous studies, it was found that alcohol can damage the immune system-related NOD-like receptor signaling pathway (Liu *et al,* 2019) and Toll-like receptor signaling pathway (Saikia *et al,* 2017) and cause alcoholic liver damage. The NOD-like receptor signaling pathway is involved in the occurrence of inflammatory diseases. Ethanol can destroy the NOD-like receptor signaling pathway in the body and significantly aggravate liver steatosis, inflammation and fibrosis. At the same time, the levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in mice also increase (Ji *et al,* 2020). Improper stimulation of Toll-like receptor signaling pathway plays a key role in inflammation and autoimmunity (Chen and Sun, 2011). Data showed that alcoholic steatohepatitis is not only caused by liver cell damage and reactive oxygen stress, but also by increased binding of specific ligands to their receptors, includes lipopolysaccharide bound to toll-like receptors. Therefore, Toll-like receptor signaling pathway is closely related to alcoholic liver injury (Byun *et al,* 2013). The study found that, the DEGs related to NOD-like receptor signaling pathway and Toll-like receptor signaling pathway were significantly down-regulated in the ET group compared with the NC group. This result may be due to alcohol destroying the NOD-like receptor signaling pathway and Toll-like receptor signaling pathway causing alcoholic liver damage in mice.

Previous studies have also shown that alcohol activates the MAPK signaling pathway (Cui *et al,* 2019) and the mTOR signaling pathway (Chen *et al,* 2018) causes liver damage. Mitogen activated protein kinase (MAPK) pathways are the main signal transduction pathway that controlled cell life and death. The activation of MAPKs is related to oxidative stress in the liver of mice. MAPKs play significant role in a myriad of pathophysiological pathways (Sadek *et al,* 2018). In recent years, a large number of reports have shown that the MAPKs signaling pathway plays a vital role in liver injury-related diseases (Li *et al,* 2017 and Morio *et al,* 2013). This study proved that camel milk can down-regulate the activation of the MAPK pathway and effectively inhibit the activation of MAPKs in a mouse model of alcoholic liver injury. The mTOR signaling pathway is the key to the regulation and treatment of liver injury (Wang *et al,* 2019). More studies have found that inhibition of mTOR signaling pathway can reduce liver fibrosis (Zhang *et al,* 2019), prevent alcoholic liver disease (Tedesco *et al,* 2018) and treat liver damage (Wang *et al,* 2019). The results of the study showed that compared with ET mice, DEGs related to mTOR signaling pathway were significantly down-regulated in ET+CM mice. This result might be because camel milk effectively inhibited the mTOR signaling pathway and interfered with liver damage caused by alcohol intake in mice.

According to this study, we can infer that in ALD mice or patients, camel milk can prevent further liver damage caused by long-term alcohol intake by inhibiting MAPK signaling pathway and mTOR signaling pathway.

Acknowledgements

This work was supported by grants from the Science and Technology Project of the College of Food Science and Engineering (SPKJ201901), and the Inner Mongolia Natural Science Foundation Project (2018BS03017).

References

Badawy AA, EL-Magd MA and AlSadrah SA (2018). Therapeutic effect of camel milk and its exosomes on MCF7 Cells *In Vitro* and *In Vivo*. Integrative Cancer Therapies 4:1235-1246.

Benjamini Y and Hochberg Y (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Methodological) 1:289-300.

Byun JS, Suh YG, Yi HS, Lee YS and Jeong WI (2013). Activation of toll-like receptor 3 attenuates alcoholic liver injuryby stimulating Kupffer cells and stellate cells to produceinterleukin-10 in mice. Journal of Hepatology 58:342-349.

Ceni E, Mello T and Galli A (2014). Pathogenesis of alcoholic liver disease: role of oxidative metabolism. World Journal of Gastroenterology 47:17756-17772.

Chen H, Shen F, Sherban A, Nocon A, Li Y, Wang H, Xu MJ, Rui X, Han J, Jiang H, Lee D, Li N, Nejad FK *et*

al (2018). DEP domain–containing mtor–interacting protein suppresses lipogenesis and ameliorates hepatic steatosis and acute-on-chronic liver injury in alcoholic liver disease. Hepatology 2:496-514.

Chen Y and Sun R (2011). Toll-like receptors in acute liver injury and regeneration. International Immunopharmacology. 11:1433-1441.

Cohen JI, Chen X and Nagy LE (2011). Redox signaling and the innate immune system in alcoholic liver disease. Antioxid Redox Signal 2:523-534.

Cui Y, Jiang L, Shao Y, Mei L and Tao Y (2019). Anti-alcohol liver disease effect of Gentianae macrophyllae extract through MAPK/JNK/p38 pathway. Journal of Pharmacy and Pharmacology 71:240-250.

Darwish HA, Raboh NR and Mahdy A (2012). Camel's milk alleviates alcohol-induced liver injury in rats. Food and Chemical Toxicology 50:1377-1383.

Dong Y, Zhang T, Li X, Yu F and Guo Y (2019). Comprehensive analysis of coexpressed long noncoding RNAs and genes in breast cancer. The Journal of Obstetrics and Gynaecology Research 2:428-437.

Dunn W and Shah VH (2016). Pathogenesis of alcoholic liver disease. Clinical Liver Disease 3:445-456.

Gao B and Bataller R (2011). Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology 5:1572-1585.

Hamed H, Bellassoued K, Feki AE and Gargouri A (2019). Evaluation of the hepatoprotective effect of combination between fermented camel milk and *Rosmarinus officinalis* leaves extract against CCl 4 induced liver toxicity in mice. Journal of Food Science and Technology 2:824-834.

Ji X, Li L, Lu P, Li X, Tian D and Liu M (2020). NLRP6 exerts a protective role via NF-kB with involvement of CCL20 in a mouse model of alcoholic hepatitis. Biochemical and Biophysical Research Communications 528:485-492.

Khan M (2017). Immune potentiating and antitoxic effects of camel milk against cyclophosphamide-induced toxicity in BALB/C mice. International Journal of Health Sciences 4:18-22.

Korish AA, Gader AG MA and Alhaider AA (2020). Comparison of the hypoglycemic and antithrombotic (anticoagulant) actions of whole bovine and camel milk in streptozotocin-induced diabetes mellitus in rats. Journal of Dairy Science 1:30-41.

Krishnankutty R, Iskandarani A, Therachiyil L, Uddin S, Azizi F, Kulinski M, Bhat AA and Mohammad RM (2018). Anticancer activity of camel milk via induction of autophagic death in human colorectal and breast cancer cells. Asian Pacific Journal of Cancer Prevention 12:3501-3509.

Lamas-Paz A, Hao F, Nelson LJ, Vazquez MT, Canals S, Moral MGd, Martinez-Naves E, Nenzorova YA and Cubero FJ (2018). Alcoholic liver disease: utility of animal models. World Journal of Gastroenterology 45:5063-5075.

Li C, Li L, Yang C, Zhong Y, Wu D, Shi L, Chen L and Li Y (2017). Hepatoprotective effects of methyl ferulic acid on alcohol-induce dliver oxidative injury in mice by inhibiting the NOX4/ROS-MAPKpathway. Biochemical and Biophysical Research Communications. 493:277-285.

Liu H, Liu H, Zhu L, Zhang Z, Zheng X, Liu J and Fu X (2019). Comparative T ranscriptome analyses provide potential insights into the molecular mechanisms of astaxanthin in the protection against alcoholic liver disease in mice. Marine Drugs 17:181.

Ming L, Qiao XY, Yi L, Siren D, He J, Guo F, Xiao Y and Ji R (2020). Camel milk modulates ethanol-induced changes in the gut microbiome and transcriptome in a mouse model of acute alcoholic liver disease. Journal of Dairy Science 5:3937-3949.

MOH (Ministry of Health of the People's Republic of China). (2003). Technical Specifications for Inspection and Evaluation of Health Foods. MOH, Beijing, China.

Morio Y, Tsuji M, Inagaki M, Nakagawa M, Asaka Y, Oyamada H, Furuya K and Oguchi K (2013). Ethanol-induced apoptosis in human liver adenocarcinoma cells (SK-Hep1) : Fas- and mitochondria-mediated pathways and interaction with MAPK signaling system. Toxicology *In Vitro* 6:1820-1829.

Sadek KM, Lebda MA, Abouzed TK, Nasr SM and EL-Sayed Y (2018). The molecular and biochemical insight view of lycopene in ameliorating tramadol-induced liver toxicity in a rat model: implication of oxidative stress, apoptosis, and MAPK signaling pathways. Environmental Science and Pollution Research 25: 33119-33130.

Saikia P, Bellos D, McMullen RM, Pollard AK, Motte ldC, Nagy EL (2017). MicroRNA 181b-3p and its target importin a5 regulate toll-like receptor 4 signaling in kupffer cells and liver injury in mice in response to ethanol. Hepatology 2:602-615.

Singal AK, Bataller R, Ahn J, Kamath PS and Shah VH (2018). ACG clinical guideline: alcoholic liver disease. The American Journal of Gastroenterology 2:175-194.

Tedesco L, Corsetti G, Ruocco C, Ragni M, Rossi F, Carruba MO, Valerio A and Nisoli E (2018). A specific amino acid formula prevents alcoholic liver disease in rodents. American Journal of Physiology-Gastrointestinal and Liver Physiology 5:G566-G582.

Wang, H, Liu Y, Wang D, Xu Y, Dong R, Yang Y, Lv Q, Chen X and Zhang Z (2019). The upstream pathway of mTOR-mediated autophagy in liver diseases. Cells 12:1597.

Zhang Z, Wen H, Weng J, Feng L, Liu H, Hu X and Zeng F (2019). Silencing of EPCAM suppresses hepatic fibrosis and hepatic stellate cell proliferation in mice with alcoholic hepatitis via the PI3K/Akt/mTOR signaling pathway. Cell Cycle 18:2239-2254.