 doi:10.20944/preprints202012.0041.v1

Review

The Role of the Gut Microbiome in Liver Fibrosis.

Na Young Lee [1], and Ki Tae Suk [1, *]

[1] Institute for Liver and Digestive Diseases, Hallym University College of Medicine, Chuncheon 24253, Republic of Korea; nylee@hallym.ac.kr (N.Y.L.);
* Correspondence: ktsuk@hallym.ac.kr; Tel.: +82-33-240-5826

Abstract: Liver cirrhosis is one of the most prevalent chronic liver diseases worldwide. In addition to viral hepatitis, genetic conditions such as steatohepatitis, autoimmune hepatitis, sclerosing cholangitis, and Wilson's disease can also lead to cirrhosis. Moreover, alcohol can cause cirrhosis on its own and exacerbate chronic liver disease from other causes. The treatment of cirrhosis can be divided into addressing the cause of cirrhosis and reversing liver fibrosis. To this date, there is still no clear consensus on the treatment of cirrhosis. Recently, there has been a lot of interest in potential treatments that modulate the gut microbiota and gut-liver axis for the treatment of cirrhosis. According to recent studies, modulation of the gut microbiome by probiotics ameliorates the progression of liver disease. The precise mechanism for relieving cirrhosis via gut microbial modulation has not been identified. This paper summarizes the role and effects of the gut microbiome in cirrhosis based on experimental and clinical studies on absorbable antibiotics, probiotics, prebiotics, and synbiotics. Moreover, it provides evidence of a relationship between the gut microbiome and liver cirrhosis.

Keywords: liver cirrhosis; liver fibrosis. gut microbiome; gut-liver axis

1. Introduction

Cirrhosis refers to scarring of tissue caused by long-term liver damage that prevents the liver from functioning properly. It is also called the end-stage of liver disease because it occurs after other stages of liver injury [1]. This can lead to serious, life-threatening complications. There is currently no cure for cirrhosis. The only way is to manage symptoms and complications, in addition to slowing the progression of cirrhosis. If the liver is severely damaged, the only treatment option may be a liver transplant. The cost burden of cirrhosis treatment ranges from $14 million to $2 billion, depending on the cause of the disease [2].

Recently, several diseases have been found to be influenced by processes in the gut microbiome. Gut microbiome has also been implicated in interactions with certain drugs, including some psychiatric medications. Many studies have been performed to slow the progression of liver disease due to the modulation of the gut microbiome in nonalcoholic fatty liver disease [3, 4]. These results showed that such changes in the gut microbial community can cause disorders in immune regulation which leads to disease.

Cirrhosis patients have altered gut-liver axis related to gut and systemic inflammation associated with changes in liver disease severity, damage to the gut barrier, and changes in the composition and function of gut microbiota [5]. Additionally, previous studies have reported that *Lachnospiraceae* and *Ruminococcaceae* are associated with the development of cirrhosis [6, 7]. In addition to these changes, it is said that alteration in the function of bacteria which includes increased release of endotoxin and decreased conversion of primary bile acids to secondary bile acids may lead to cirrhosis [8]. Therefore, it can be surmised that the modulation of the gut microbiome plays an important role in the progression of cirrhosis.

Research on the relationship between dysbiosis and cirrhosis may not only predict the onset of cirrhosis but may also lead to discovery of novel treatments. Previous studies have demonstrated that microbiota targeted biomarkers can be a useful tool for the diagnosis of various diseases which

 doi:10.20944/preprints202012.0041.v1

includes liver cirrhosis [9]. Based on this, recent studies using antibiotics, probiotics, prebiotics, and synbiotics are being performed to suppress the progression of liver fibrosis by the modulation of the gut microbiome.

2. Liver Cirrhosis

Liver cirrhosis is defined as the late stage of liver fibrosis caused by several forms of liver disease and conditions, including hepatitis and chronic alcoholism [1]. It results from excessive production of extracellular matrix under chronic injury [10]. The most common causes of cirrhosis are viral hepatitis, nonalcoholic steatohepatitis, and alcoholic liver disease [11]. Hepatic carcinoma (HCC) cases occur in the background of a cirrhotic liver [12]. However, there is still no clear consensus for cirrhosis treatment Therefore, thus far, the goal of cirrhosis treatment is management of symptoms and complications.

In the past, the liver damage resulting from illness, excessive drinking, or other cause was considered to be irreversible. Recently, however, many studies utilizing animal models provided evidence that cirrhosis may be reversible. In addition, some clinical studies have also shown the regression of cirrhosis on repeated biopsy samples [13]. Further research should be conducted regarding therapeutic agents that may play a role in reversing cirrhosis.

3. Gut Microbiome

The gut microbiota in the human digestive tract consists of bacteria, protozoa, fungi, archaea, and viruses [14]. The gut microbiota is a complex ecosystem with a total mass of about 1-2kg per person [15]. Most people have a population of bacteria in the gut that is about 10 times the number of cells in the body. The gut microbiota is responsible for removing invading pathogens, in addition to maintaining the balance of the immune system and preventing autoimmunity [16]. The gut microbiota is associated with essential health benefits, particularly regarding immune homeostasis.

Birth and breastfeeding help form an infant gut microbiota that gradually matures in childhood in response to environmental exposure, after which the gut microbiota is relatively stable until changes in immune function leads to diminishing diversity[17]. Humans have an interdependent relationship with the gut microbiota. More than 90% of human gut microbiota consists of four major divisions: *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria* [18]. However, in those with compromised immune systems or with a progressive disease, the proportion and diversity of the intestinal microorganisms are different when compared to those of healthy people. In regards to this subject, animal models and patients of various diseases had their microbiota analyzed for composition and diversity[19]. In our previous study, we have demonstrated that mice on westernized diet were associated with decrease in Bacteroidetes and increased Firmicutes in their gut when compared to normal mice [3]. According to other studies, gut microbiota plays an important role in the host, which includes host immunity, food digestion, intestinal endocrine regulation, drug action and metabolism, and toxin elimination [20].

4. Gut-Liver axis

The gut and liver communicate through tight bidirectional links through the biliary tract, portal vein, and systemic circulation [21]. The main primary bile acids are synthesized by the liver and are combined with taurine or glycine to be secreted into bile. Thereafter, the synthesized bile is stored in the gallbladder and delivered to the small intestine [22]. The close relationship between the gut and liver underlies the modulatory effect of gut microbiota on liver health [23]. Moreover, dysbiosis, which refers to quantitative and qualitative changes in gut microbiota and its overgrowth, may lead to an increase in intestinal permeability. As a result, endotoxins are transferred to the portal duct, leading to the activation of signaling pathways of various inflammatory cytokines in the liver [15]. Therefore, the close interaction between the gut and the liver can be a major factor in the pathogenesis of liver damage and liver disease progression.

5. Dysbiosis

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 December 2020 doi:10.20944/preprints202012.0041.v1

Dysbiosis is a term for a microbial imbalance or maladjustment inside the body. The imbalance of normal gut microbiome is associated with a wide range of systemic symptoms of gastrointestinal diseases such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), obesity, type 2 diabetes, and atopy [24]. As such, the imbalance of the microbiota can serve as an indicator for pathological state.

Dysbiosis is associated with gut barrier dysfunction and immunity since the microbiota and its products modulate barrier function by affecting the epithelial inflammatory response and mucosal repair function [25]. Previous studies have shown that cirrhosis is associated with altered immune responses that potentially allows dysbiosis or altered microbiota in the stool, intestinal mucosa, ascites, liver, serum, and saliva [26]. For this reason, many studies are being performed to investigate the possibility of alleviating cirrhosis by modulating the gut microbiome.

6. Treatment for Cirrhosis

6-1. Cirrhosis and Antibiotics

People with cirrhosis, especially those with decompensated cirrhosis, have an increased risk of bacterial infection, which can further promote other hepatic decompensation, including liver failure [27]. In theory, antibiotics may eliminate deleterious bacteria and their efficacy in treating liver disease has been proven in research [28]. For this reason, many studies are being performed to investigate antibiotics treatment in the context of cirrhosis (Table 1).

Rifaximin is a gastrointestinal selective antibiotic with a wide range of antimicrobial activity, minimal drug interactions and negligible effect on the overall gut microbiome [29] (Table 1). In a study in EtOH-induced liver injury in obese mice, treatment with rifaximin increased proportion of the *Bacteroidales* and decreased ALT and TG levels via modulation of small intestine [30].

Most treatments for HE patients rely on manipulation of the intestinal environment, so antibiotics acting on the gut form the main treatment strategy [31]. In previous research, rifaximin treatment effects were shown to decrease the risk of recurrent encephalopathy [32]. Patients with cirrhosis who developed candidemia also were shown with a lower rate of candidemia when treated with rifaximin [33]. Cirrhotic patients with refractory ascites when given rifaximin were associated with mitigated ascites and increased survival [34]. As such, rifaximin may be effective but how it affects these therapeutic outcomes remains unknown. It is not yet clear to what extent antibiotics can control the composition and diversity of gut microbiota in a variety of clinical settings. Another study showed that rifaximin-α treatment had no effects on macrophage activation and disruption of fibrosis [35]. Another previous study has shown that in cirrhotic patients, treatment by rifaximin reduced *Veillonellaceae* and secondary/primary BA ratios [36]. It suggests that cirrhosis is associated with a reduced conversion of primary to secondary Bas, which is associated with the abundance of major gut microbiota. In a randomized controlled trial of patients with advanced cirrhosis, treatment with norfloxacin did not reduce mortality, but significantly reduced the incidence of Gram-negative bacterial infections without increasing infections due to multiple resistant bacteria [37].

Antibiotics have a significant direct or indirect effect on the intestinal microbiota and some changes disappear immediately after stopping antibiotic treatment, but others remain indefinitely [38]. Antibiotics for cirrhosis prevent bacterial infection and other cirrhosis complications such as recurrent varicose bleeding and death. However, their widespread use has led to development of antibiotic resistance, which makes standard empirical antibiotics for suspected infections ineffective and perhaps reduces the effectiveness of antibiotic prophylaxis. To prevent the occurrence of antibiotic resistance, an empirical antibiotic strategy, step-down rules, and antibiotic pharmacokinetics, and pharmacodynamic administration strategies should be formulated.

Moreover, antibiotics affect the bacteria that cause infections as well as the resident microbiota [39]. It may result in the decrease of taxonomic richness, diversity, and evenness of the community. Although this side effect has long been appreciated, advances in sequencing technologies enable a detailed study of how antibiotics alter the gut microbiome. Direct effects on the immune system, reproducibility in terms of duration and frequency of antibiotic exposure, antibiotic resistance, and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 December 2020 doi:10.20944/preprints202012.0041.v1

individualized response to the same treatment all influence the outcome of antibiotic studies. It is necessary to develop strategies to mitigate the effects of antibiotics on the immune system.

Table 1. Animal and human studies using antibiotics.

	Conditions	Treatment	Main Results	Ref
Animal	EtOH-induced liver injury in obese mice	Rifaximin	(↓): ALT, TG (↑): Proportion of the *Bacteroidales*	[30]
Human	HE	Rifaximin	(↓): Recurrent encephalopathy	[32]
	Cirrhosis developing candidemia	Rifaximin	(↓): Rate of candidemia	[33]
	Cirrhotic patients with refractory ascites	Rifaximin	(↑): Mitigates ascites, survival of cirrhotic patients	[34]
	Cirrhosis	Rifaximin-α	No effects on macrophage activation and disruption of fibrosis	[35]
	Cirrhotic	Rifaximin	(↓): *Veillonellaceae*, secondary/primary BA ratios	[36]
	Advanced cirrhosis	Norfloxacin	(↓): Incidence of Gram-negative bacterial infection (↑): Survival of patients with low ascites protein concentration	[37]

↑ indicates an increase in condition, ↓ indicates a decrease in condition, ALT, alanine aminotransferase; BA, bile acids; HE, hepatic encephalopathy; TG, triglycerides;

6-2. Cirrhosis and Probiotics

Probiotics are defined as live microorganisms such as bacteria or yeasts of human origin that provide health benefits when consumed [40]. Many studies have been conducted on patients using different types of probiotics in different settings [41, 42] (Table 2). Current studies have shown that probiotics regulates the gut microbiota by promoting the growth of beneficial bacteria and reducing harmful bacteria in the gut [43].

Bile duct ligation (BDL) is a surgical method that is used to induce liver fibrosis. It leads to acute progress to cirrhosis with portal fibrosis [44]. In the BDL model, BA de novo synthesis was decreased after the administration of *L. rhamnosus GG*. In addition, the administration resulted in the reduction of AST, ALT, ALP, and TBIL serum levels and α-SMA, Col1, Col3, and TGF-β mRNA levels. [45]. Carbon tetrachloride (CCl_4) is typically used to create models of liver fibrosis and cirrhosis [46]. In a study using CCl_4 injection to induce liver cirrhosis in mice, Mixture of *S. cerevisiae* and *L. acidophilus* protected mice from inflammation, hepatic oxidative stress by reducing MAPK signaling and increasing SIRT1 signaling [47]. In another study, *L. fermentum* and *L. plantarum* administration was associated with reduced AST, ALT, MDA, SOD, GSH, and IL-1β levels; in contrast Bcl-2 was increased[48]. In rats CCl_4 injection and treatment with *L. salivarius LI01*, and *P. pentosaceus LI05* were associated with reduction of the Col1a, Timp1, and TGF-β when compared with the control group. Moreover, these strains increased the expression of tight junction protein Zo-1 [49]. In the EtOH-induced model, combination with *L. fermentum* resulted in reduced AST, ALT, iNOS, and Hsp60 [50]. This study suggests probiotics are associated with therapeutic potential in alcoholic liver disease. Mixture of *L. paracasei*, *L. casei*, and *W. confuse* treatment significantly lowered serum enzyme levels, less inflammation, and less fibrosis on TAA-induced liver fibrosis in rats [51]. In this experiment, rats were fed 10^9 CFU/mL microbial cells daily by oral gavage.

In a human study, randomized patients were given VSL#3 probiotics for 6 months. Patients who received probiotics were associated with decreased HE incidence, and CTP and MELD scores were also reduced [52]. In another study, *B. breve, L. acidophilus, L. plantarum, L. paracasei, L. bulgarius* and S. *thermophilus* treatment of patients with HE improved CTP score and psychometric hepatic encephalopathy scores [53]. In clinical trials, the effects of treatment with the probiotic *L. rhamnosus GG* in patients with cirrhosis were evaluated [54]. This study showed that ingestion of *L. rhamnosus*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 December 2020 doi:10.20944/preprints202012.0041.v1

GG decreased *Enterobacteriaceae*, endotoxemia and TNF-α. The fecal microbiome composition of *Lachnospiraceae* was increased and harmful bacteria was reduced after ingestion of *L. rahmnosus GG*. The study concluded that*L. rhamnosus GG* modulates the gut microbiome, metabolome and endotoxemia in cirrhosis patients. In another study, treatment by *C. butyricum* combined with *B. infantis* in minimal MHE in HBV-induced cirrhosis patients were associated with decreased *Enterococcus*, *Enterobacteriaceae*, and ammonia levels [55]. Moreover, their cognitive ability was improved.

Subsequently, various studies with patients and animal models of liver fibrosis are aiming to investigate the improvement of liver fibrosis and cirrhosis following the ingestion of probiotics. However, well-designed long-term clinical trials with probiotics are required to assess the probiotics' effects on the progression of liver disease and regression of liver fibrosis. Further research to elucidate the mechanism underlying the role of probiotics in modulating the gut microbiome study is required.

Table 2. Animal and human studies using probiotics.

Conditions		Treatment	Main Results	Ref
Animal	BDL	*Lactobacillus rhamnosus GG*	(↓): BA de novo synthesis, ALT, AST, ALP, TBIL, a-SMA, Col1, Col3, TGF-β, Timp1, Mmp2, F4/80, TNF-α, IL-6, IL-1B (↑): FGF-15, BA excretion	[45]
	CCl_4	Mixture of *Saccharomyces cerevisiae* + *Lactobacillus acidophilus*	(↓): hepatic oxidative stress, ER stress, inflammation, MAPK signaling, AST, ALT, Col1, α-SMA (↑): SIRT1 signaling	[47]
		Lactobacillus fermentum	(↓): Inflammation, AST, ALT, MDA, SOD, GSH, IL-1β, Bax, TNF-α, Caspase 3 ↓, NF-κB, p65 (↑): Bcl-2	[48]
		Lactobacillus plantarum	(↓): ALT, AST, MDA, SOD, GSH, IL-1β, TNF-α, Bax, NF-κB p65, Caspase (↑): Bcl-2	
		Lactobacillus salivarius LI01	(↓): AST, ALT, GGT, TLR2,4,5,9, intestinal barrier integrity, Col1a, Timp1, TGF-B (↑): Zo-1	[49]
		Pediococcus pentosaceus LI05	(↓): AST, ALT, GGT, TLR2,4,5,9, Col1a, Timp1, TGF-β (↑): Zo-1	
	EtOH	*Lactobacillus fermentum*	(↓): steatosis score, iNOS, Hsp60, AST, ALT	[50]
	TAA	Mixture of *Lactobacillus paracasei* + *Lactobacillus casei* + *Weissella confusa*	(↓): serum enzyme levels, inflammation, fibrosis	[51]
Human	Cirrhosis with HE	VSL #3: *Lactobacillus* (*acidophilus* + *delbrueckii subspbulgaricus* + *casei* + *plantarum*) + *Bifidobaceria* (*breve* + *longum* + *infantis*) + *Streptococcus salivarius subspthermophilus*	(↓): CTP score, MELD score, IL-1β, IL-6, TNF-α, Indole, Renin, Aldosterone, Brain-type natriuretic peptide	[52]
	Cirrhosis without overt HE	*Bifidobacterium breve*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*,	(↓): CTP score, psychometric hepatic encephalopathy scores	[53]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 December 2020 doi:10.20944/preprints202012.0041.v1

	Lactobacillus bulgaricus, Streptococcus thermophilus		
Cirrhotic with MHE	*Lactobacillus rhamnosus GG*	(↓): *Enterobacteriaceae*, endotoxemia, TNF-a (↑): *Clostridiales*, *Lachnospiraceae* relative abundance	[54]
Minimal MHE in HBV-induced Cirrhosis	*Clostridium butyricum* + *Bifidobacterium infantis*	(↓): *Enterococcus, Enterobacteriaceae*, ammonia level (↑): Cognitive ability	[55]

↑ indicates an increase in condition, ↓ indicates a decrease in condition, ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; α-SMA, alpha-smooth muscle actin; BA, bile acids; Bax, Bcl-2-associated X protein; BCL-2, b-cell lymphoma 2; BDL, bile duct ligation; CCl4, carbon tetrachloride; Col; Collagen, type; CTP, Child-Turcotte-Pugh; EtOH, ethylalcohol; GGT, gamma glutamyl peptidase; GSH, Glutathione; HBV, hepatitis B virus; HE, hepatic encephalopathy; HSP, heat shock proteins; IL, interleukin; iNOS, inducible nitric oxide synthase; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; MELD, model for end-stage liver disease; MHE, minimal hepatic encephalopathy; Mmp, matrix metallopeptidases; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; SIRT, selective internal radiation therapy; SOD, superoxide dismutase; TAA, thioacetamide; TG, triglycerides; TBIL, total bilirubin; Timp, tissue inhibitor of metallopeptidase; TLR, toll-like receptor; TNF- α, tumor necrosis factor alpha; Zo-1, zonula occludenes-1;

6-3. Cirrhosis and Prebiotics

Prebiotics were first identified and defined as an indigestible food ingredient that has a beneficial effect on improving host health by selectively stimulating the growth or activity of bacteria by Marcel Roberfroid and Glenn Gibson in 1995 [56]. Namely, probiotics are food ingredients that induce the growth or activity of beneficial microorganisms in the gut. The food ingredients can feed the gut microflora, and the products of their breakdown such as SCFAs are released into the blood circulation, which affect not only the gastrointestinal tract, but also other distant organs [57]. Sources of prebiotics include breast milk, soybeans, inulin, raw oats, unrefined wheat, unrefined barley, yacon, undigestible carbohydrates, and undigestible oligosaccharides [58].

Garlic consumption is known to be beneficial in various liver diseases [59]. In one study, ingestion of garlic polysaccharides reduced the ratio of AST, ALT, TGF-B1, and TNF-a in the ALF model [60]. In addition, garlic polysaccharides affected the gut microbiota, resulting in an increase in *Lachnospiraceae* and *Lactobacillus* and a decrease in *Facklamia* and *Firmicutes*. With the CCl4-injected mice, which is a widely known liver cirrhosis model, studies on intake of various prebiotics have been performed. Ingestion of polysaccharides from *Grifola frondosa* decreased AST, ALT, TBIL, MDA, TNF-α, IL-1β, and IL-6, and increased SOD and GSH-Px [61]. In this study, it was reported that polysaccharides from *Grifola frondosa* inhibited oxidative stress and inflammatory reactions to regulate the TGF-β1 / Smad signaling pathway and slow the progression of liver fibrosis. In addition, the ingestion of *Dendrobium officinale* polysaccharide was shown to alleviate fibrosis tissue and reduce intestinal mucosa damage [62]. Because the expression of Bax and caspase-3 proteins was downregulated, the expression of occludin, claudin-1, ZO-1 and Bcl-2 proteins was upregulated. In another study, olive oil combined with *Lycium barbarum* polysaccharide improved hepatocellular death, inflammation, and fibrosis markers in liver cirrhosis induced rat model [63]. It was shown that TGF-β1, TNF-α, and Timp-1 were decreased, and IL-10, IL-10/TNF-α were increased. Inulin is an indigestible storage polysaccharide that is found in many vegetables [64]. In the ALD animal model, inulin was shown to increase intestinal content of propionic acid, butyric acid and valeric acid [65]. SCFAs with a small number of carbon atoms, such as propionic acid and butyric acid, are partially absorbed and are reported to inhibit cholesterol synthesis in the liver and promote the decomposition of low density lipoprotein cholesterol [66].

Non-absorbable disaccharides are recommended as the main treatment for HE since their beneficial effects involve the reduction of the intestinal production and absorption of ammonia [67]. Lactitol, one of the non-absorbable disaccharide, is a crystalline powder sweetener similar in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 December 2020 doi:10.20944/preprints202012.0041.v1

sweetness to sugar. [68]. In a previous study, a randomized clinical trial was performed in which people with cirrhosis and hepatic encephalopathy were given lactitol [69]. Although there were no statistical differences between randomized clinical trials when evaluating HE, lactitol intake was associated with beneficial effects on the quality of life. It also had beneficial effects on mortality versus placebo.

A number of studies continue the investigation of new polysaccharides for the development of effective treatments for liver damage and liver disease [70]. Further research on the mechanism by which probiotics can play a role as a therapeutic agent for liver cirrhosis by modulating the gut microbiome is further needed.

Table 3. Animal and human studies using prebiotics.

Conditions		Treatment	Main Results	Ref
Animal	ALF	Garlic polysaccharide	(↓): AST, ALT, MDA, TC, TG, TGF-β1, TNF-α, *Lachnospiraceae, Lactobacillus* (↑): SOD, GSH-Px, GSH, *Firmicutes, Facklamia*	[60]
	CCl_4	Polysaccharides from *Grifola frondosa*	(↓): AST, ALT, TBIL, MDA, TNF-α, IL-1β, IL-6 (↑): SOD, GSH-Px	[61]
		Dendrobium officinale polysaccharide	(↓): Bax, caspase 3, TNF-α, α-SMA (↑): occludin, claudin-1, ZO-1, Bcl-2 TEER, IL-10	[62]
		Olive oil combined with *Lycium barbarum* polysaccharides	(↓): TGF- β1, TNF-α, Timp-1 (↑): IL-10, IL-10/TNF-α	[63]
	ALD	Inulin	(↓): iNOS, inflammation, TNF-α (↑): propionate, butyrate, valeric, IL-10	[65]
Human	Cirrhosis with HE	Lactitol	(↓): Mortality	[69]

↑ indicates an increase in condition, ↓ indicates a decrease in condition, ALD, alcoholic liver disease; ALF, alcoholic liver fibrosis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; α-SMA, alpha-smooth muscle actin; Bax, Bcl-2-associated X protein; CCl_4, carbon tetrachloride; GSH, glutathione; HE, hepatic encephalopathy; IL, interleukin; MDA, malondialdehyde; SOD, superoxide dismutase; TC, total cholesterol; TG, triglycerides; TBIL, total bilirubin; TNF-α, tumor necrosis factor-alpha

6-4. Cirrhosis and Synbiotics

Synergistic combinations of probiotics and prebiotics are defined as synbiotics [58]. Synbiotics were developed to overcome possible survival difficulties for probiotics. As well as promoting the growth of probiotics and bacteria, synbiotics contribute to a more efficient homeostasis in the gut and maintenance of a healthy body [71].

In a previous study, HE patients were treated with a mixture of four probiotics (*L. paracasei* + *L. plantarum* + *L. mesenteriodes* + *P. pentosaceus*) in combination with four fibers (oat bran, pectin, resistant starch, and inulin) [72] (Table 3). There were no significant differences between randomized groups at baseline. In cirrhosis patients with MHE and not overt HE, treatment of synbiotics which consisted of mixture of four probiotics (*P. pentoseceus* + *L. mesenteroides* + *L. paracasei* + *L. plantarum*) with three fibers (beta glucan + pectin + resistant starch) was associated with decreased TBIL levels in serum and increased ALB levels [73].

Table 4. Human studies using synbiotics.

Conditions	Treatment	Main Results	Ref

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 December 2020 doi:10.20944/preprints202012.0041.v1

Human	HE	Mixture of 4 probiotics (*Lactobacillus paracasei* + *Lactobacillus plantarum* + *Leuconostoc mesenteriodes* + *Pediococcus pentosaceus*) with 4 fibers (oat bran, pectin, resistant starch, and inulin)	No change in cognitive function	[72]
	Cirrhosis with MHE	Mixture of 4 probiotics (*Pediacoccus pentoseceus* + *Leuconostoc mesenteroides* + *Lactobacillus paraacasei* + *Lactobacillus plantarum*) with 3 fibers (beta glucan + pectin + resistant starch)	(↓): TBIL (↑): ALB	[73]

↑ indicates an increase in condition, ↓ indicates a decrease in condition, ALB, albumin; HE, hepatic encephalopathy; MHE, minimal hepatic encephalopathy; TBIL, total bilirubin;

7. Cirrhosis and Gut Microbiome

The liver is the organ that is in closest contact with the gut tract and is exposed to a substantial number of bacterial components and metabolites. Previous studies have proposed that microbiota-based biomarkers may be a tool to diagnose cirrhosis [9]. For example, cirrhosis patients have increased bacteremia, increased levels of serum lipid polysaccharides (LPS), and increased intestinal permeability [74].

ALD is caused by various factors which includes genetics, immune system, dietary components, and the gut microbiota. In a previous study, a subgroup of ALD patients exhibited dysbiosis with lower median abundances of *Bacteroidetes* and higher levels of *Proteobacteria* [75]. Certain microorganisms can induce ALD, while others can exert beneficial effects and have protective effects. In addition, in cirrhosis patients, *Bacteroidetes* were shown to be decreased significantly, while *Proteobacteria* and *Fusobacteria* were increased significantly when compared to healthy people [6]. These findings suggest the important role of gut microbiome in patients with cirrhosis.

The major role of the gut microbiota in liver disease is also supported by various studies showing that several complications of serious liver disease, such as HE are efficiently treated by the modulation of gut microbiome via use of probiotics, prebiotics and antibiotics. The pathogenesis of cirrhosis and the precise function of gut microbiome are not yet clear, but these findings highlight the importance of modulation of the gut microbiome, suggesting novel approaches for therapeutic strategies for liver fibrosis.

8. Conclusion

The liver is the organ that metabolizes and detoxifies various compounds. Therefore, toxicity from the most common and serious drug should be considered. Therefore, recent studies are trying to find a treatment for liver disease using pharmabiotics.

A recent review revealed that the modulation of gut microbiota with a healthy diet, multi-biotics based supplements, and transplantation of a healthy fecal microbiome to promote the growth of "good" microbiota may ameliorate dysbiosis in patients and improve their prognosis [76]. Many studies have shown that the gut-liver axis plays an important role in the pathogenesis of liver diseases, including liver fibrosis and cirrhosis. Therefore, it is necessary to evaluate the manipulation of the intestinal microbiota in the context of liver cirrhosis. Traditional antibiotics may not be effective in controlling microbiota due to side effects and the emergence of antibiotic resistance. Nevertheless, treatment with rifaximin has shown promising results in relieving cirrhosis while modulating gut microbiota. Furthermore, ingestion of probiotics can ameliorate cirrhosis of the liver, in addition to many immune effects involving various cytokines such as IL-6, TNF-a, and IL-1B.

Consequently, a comprehensive understanding of the pathologic biology of liver cirrhosis is important for improving clinical outcomes, as integrated signaling pathways appear to play an important role in pathogenesis of liver cirrhosis. Further studies are needed to study the interaction between gut microbes and the host immune system in order to elucidate the pathogenesis of liver fibrosis and open new opportunities in immunity or gut microbiome-based treatments.

Author Contributions: Conceptualization and Writing-original draft preparation, N.Y.L. and K.T.S.

Funding: This research was funded by Hallym University Research Fund (HURF-2018-67), and Korea National Research Foundation (NRF-2020R1A6A1A03043026).

Conflicts of Interest: The authors have no potential conflicts of interest.

Abbreviations

ALB	albumin
ALD	alcoholic liver disease
ALF	alcoholic liver fibrosis
ALT	alanine aminotransferase
ALP	alkaline phosphatase
AST	aspartate aminotransferase
α-SMA	alpha-smooth muscle actin
BA	bile acid
Bax	Bcl-2-associated X protein
BCL-2	b-cell lymphoma 2
BDL	bile duct ligation
CCl_4	carbon tetrachloride
Col	collagen type
CTP	Child-Turcotte-Pugh
EtOH	ethyl alcohol
GGT	gamma glutamyl peptidase
GSH	glutathione
HCC	hepatocellular carcinoma
HE	hepatic encephalopathy
HSP	heat shock proteins
IL	interleukin
iNOS	inducible nitric oxide synthase
MAPK	mitogen-activated protein kinase
MDA	malondialdehyde
MHE	minimal hepatic encephalopathy
MELD	model for end-stage liver disease
Mmp	matrix metallopeptidases
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
SIRT	selective internal radiation therapy
SCFAs	short chain fatty acids
SOD	superoxide dismutase
TAA	thioacetamide
TEER	transepithelial electrical resistance
TG	triglycerides
TBIL	total bilirubin
Timp	tissue inhibitor of metallopeptidase
TLR	toll-like receptor
TNF-α	tumor necrosis factor alpha
Zo-1	zonula occludenes-1

References

1. Schuppan, D.; Afdhal, N. H., Liver cirrhosis. *Lancet* **2008,** 371, (9615), 838-51.
2. Prinja, S.; Bahuguna, P.; Duseja, A.; Kaur, M.; Chawla, Y. K., Cost of Intensive Care Treatment for Liver Disorders at Tertiary Care Level in India. *Pharmacoecon Open* **2018,** 2, (2), 179-190.
3. Lee, N. Y.; Yoon, S. J.; Han, D. H.; Gupta, H.; Youn, G. S.; Shin, M. J.; Ham, Y. L.; Kwak, M. J.; Kim, B. Y.; Yu, J. S.; Lee, D. Y.; Park, T. S.; Park, S. H.; Kim, B. K.; Joung, H. C.; Choi, I. S.; Hong, J. T.; Kim, D.

J.; Han, S. H.; Suk, K. T., Lactobacillus and Pediococcus ameliorate progression of non-alcoholic fatty liver disease through modulation of the gut microbiome. *Gut Microbes* **2020,** 11, (4), 882-899.

4. Duseja, A.; Acharya, S. K.; Mehta, M.; Chhabra, S.; Shalimar; Rana, S.; Das, A.; Dattagupta, S.; Dhiman, R. K.; Chawla, Y. K., High potency multistrain probiotic improves liver histology in non-alcoholic fatty liver disease (NAFLD): a randomised, double-blind, proof of concept study. *BMJ Open Gastroenterol* **2019,** 6, (1), e000315.
5. Bajaj, J. S., Altered Microbiota in Cirrhosis and Its Relationship to the Development of Infection. *Clin Liver Dis (Hoboken)* **2019,** 14, (3), 107-111.
6. Chen, Y.; Yang, F.; Lu, H.; Wang, B.; Chen, Y.; Lei, D.; Wang, Y.; Zhu, B.; Li, L., Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology* **2011,** 54, (2), 562-72.
7. Bajaj, J. S.; Heuman, D. M.; Hylemon, P. B.; Sanyal, A. J.; White, M. B.; Monteith, P.; Noble, N. A.; Unser, A. B.; Daita, K.; Fisher, A. R.; Sikaroodi, M.; Gillevet, P. M., Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* **2014,** 60, (5), 940-7.
8. Acharya, C.; Bajaj, J. S., Altered Microbiome in Patients With Cirrhosis and Complications. *Clin Gastroenterol Hepatol* **2019,** 17, (2), 307-321.
9. Qin, N.; Yang, F.; Li, A.; Prifti, E.; Chen, Y.; Shao, L.; Guo, J.; Le Chatelier, E.; Yao, J.; Wu, L.; Zhou, J.; Ni, S.; Liu, L.; Pons, N.; Batto, J. M.; Kennedy, S. P.; Leonard, P.; Yuan, C.; Ding, W.; Chen, Y.; Hu, X.; Zheng, B.; Qian, G.; Xu, W.; Ehrlich, S. D.; Zheng, S.; Li, L., Alterations of the human gut microbiome in liver cirrhosis. *Nature* **2014,** 513, (7516), 59-64.
10. Iredale, J. P.; Thompson, A.; Henderson, N. C., Extracellular matrix degradation in liver fibrosis: Biochemistry and regulation. *Biochim Biophys Acta* **2013,** 1832, (7), 876-83.
11. Smith, A.; Baumgartner, K.; Positis, C., Cirrhosis: Diagnosis and Management. *Am Fam Physician* **2019,** 100, (12), 759-770.
12. Desai, A.; Sandhu, S.; Lai, J. P.; Sandhu, D. S., Hepatocellular carcinoma in non-cirrhotic liver: A comprehensive review. *World J Hepatol* **2019,** 11, (1), 1-18.
13. Jung, Y. K.; Yim, H. J., Reversal of liver cirrhosis: current evidence and expectations. *Korean J Intern Med* **2017,** 32, (2), 213-228.
14. Cho, I.; Blaser, M. J., The human microbiome: at the interface of health and disease. *Nat Rev Genet* **2012,** 13, (4), 260-70.
15. Suk, K. T.; Kim, D. J., Gut microbiota: novel therapeutic target for nonalcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol* **2019,** 13, (3), 193-204.
16. Wu, H. J.; Wu, E., The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes* **2012,** 3, (1), 4-14.
17. Fan, Y.; Pedersen, O., Gut microbiota in human metabolic health and disease. *Nature Reviews Microbiology* **2020**.
18. Rinninella, E.; Raoul, P.; Cintoni, M.; Franceschi, F.; Miggiano, G. A. D.; Gasbarrini, A.; Mele, M. C., What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. *Microorganisms* **2019,** 7, (1).
19. Durack, J.; Lynch, S. V., The gut microbiome: Relationships with disease and opportunities for therapy. *J Exp Med* **2019,** 216, (1), 20-40.
20. Lynch, S. V.; Pedersen, O., The Human Intestinal Microbiome in Health and Disease. *N Engl J Med* **2016,** 375, (24), 2369-2379.
21. Tripathi, A.; Debelius, J.; Brenner, D. A.; Karin, M.; Loomba, R.; Schnabl, B.; Knight, R., The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* **2018,** 15, (7), 397-411.

22. Chiang, J. Y., Bile acid metabolism and signaling. *Compr Physiol* **2013,** 3, (3), 1191-212.
23. Ji, Y.; Yin, Y.; Sun, L.; Zhang, W., The Molecular and Mechanistic Insights Based on Gut-Liver Axis: Nutritional Target for Non-Alcoholic Fatty Liver Disease (NAFLD) Improvement. *Int J Mol Sci* **2020,** 21, (9).
24. Bull, M. J.; Plummer, N. T., Part 1: The Human Gut Microbiome in Health and Disease. *Integr Med (Encinitas)* **2014,** 13, (6), 17-22.
25. Fukui, H., Role of Gut Dysbiosis in Liver Diseases: What Have We Learned So Far? *Diseases* **2019,** 7, (4).
26. Albillos, A.; Lario, M.; Alvarez-Mon, M., Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. *J Hepatol* **2014,** 61, (6), 1385-96.
27. Fernandez, J.; Tandon, P.; Mensa, J.; Garcia-Tsao, G., Antibiotic prophylaxis in cirrhosis: Good and bad. *Hepatology* **2016,** 63, (6), 2019-31.
28. Ponziani, F. R.; Scaldaferri, F.; Petito, V.; Paroni Sterbini, F.; Pecere, S.; Lopetuso, L. R.; Palladini, A.; Gerardi, V.; Masucci, L.; Pompili, M.; Cammarota, G.; Sanguinetti, M.; Gasbarrini, A., The Role of Antibiotics in Gut Microbiota Modulation: The Eubiotic Effects of Rifaximin. *Dig Dis* **2016,** 34, (3), 269-78.
29. Koo, H. L.; DuPont, H. L., Rifaximin: a unique gastrointestinal-selective antibiotic for enteric diseases. *Curr Opin Gastroenterol* **2010,** 26, (1), 17-25.
30. Kitagawa, R.; Kon, K.; Uchiyama, A.; Arai, K.; Yamashina, S.; Kuwahara-Arai, K.; Kirikae, T.; Ueno, T.; Ikejima, K., Rifaximin prevents ethanol-induced liver injury in obese KK-A(y) mice through modulation of small intestinal microbiota signature. *Am J Physiol Gastrointest Liver Physiol* **2019,** 317, (5), G707-G715.
31. Patidar, K. R.; Bajaj, J. S., Antibiotics for the treatment of hepatic encephalopathy. *Metab Brain Dis* **2013,** 28, (2), 307-12.
32. Flamm, S. L., Rifaximin treatment for reduction of risk of overt hepatic encephalopathy recurrence. *Therap Adv Gastroenterol* **2011,** 4, (3), 199-206.
33. Bartoletti, M.; Rinaldi, M.; Pasquini, Z.; Scudeller, L.; Piano, S.; Giacobbe, D. R.; Maraolo, A. E.; Bussini, L.; Del Puente, F.; Incicco, S.; Angeli, P.; Giannella, M.; Baldassarre, M.; Caraceni, P.; Campoli, C.; Morelli, M. C.; Cricca, M.; Ambretti, S.; Gentile, I.; Bassetti, M.; Viale, P., Risk factors for candidaemia in hospitalized patients with liver cirrhosis: a multicentre case-control-control study. *Clin Microbiol Infect* **2020**.
34. Vadell, A. K. E.; Barebring, L.; Hulander, E.; Gjertsson, I.; Lindqvist, H. M.; Winkvist, A., Anti-inflammatory Diet In Rheumatoid Arthritis (ADIRA)-a randomized, controlled crossover trial indicating effects on disease activity. *Am J Clin Nutr* **2020,** 111, (6), 1203-1213.
35. Kimer, N.; Gudmann, N. S.; Pedersen, J. S.; Moller, S.; Nielsen, M. J.; Leeming, D. J.; Karsdal, M. A.; Moller, H. J.; Bendtsen, F.; Gronbaek, H., No effect of rifaximin on soluble CD163, mannose receptor or type III and IV neoepitope collagen markers in decompensated cirrhosis: Results from a randomized, placebo controlled trial. *PLoS One* **2018,** 13, (9), e0203200.
36. Kakiyama, G.; Pandak, W. M.; Gillevet, P. M.; Hylemon, P. B.; Heuman, D. M.; Daita, K.; Takei, H.; Muto, A.; Nittono, H.; Ridlon, J. M.; White, M. B.; Noble, N. A.; Monteith, P.; Fuchs, M.; Thacker, L. R.; Sikaroodi, M.; Bajaj, J. S., Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. *J Hepatol* **2013,** 58, (5), 949-55.
37. Moreau, R.; Elkrief, L.; Bureau, C.; Perarnau, J. M.; Thevenot, T.; Saliba, F.; Louvet, A.; Nahon, P.; Lannes, A.; Anty, R.; Hillaire, S.; Pasquet, B.; Ozenne, V.; Rudler, M.; Ollivier-Hourmand, I.; Robic, M. A.; d'Alteroche, L.; Di Martino, V.; Ripault, M. P.; Pauwels, A.; Grange, J. D.; Carbonell, N.; Bronowicki,

J. P.; Payance, A.; Rautou, P. E.; Valla, D.; Gault, N.; Lebrec, D.; Investigators, N. T., Effects of Long-term Norfloxacin Therapy in Patients With Advanced Cirrhosis. *Gastroenterology* **2018,** 155, (6), 1816-1827 e9.

38. Willing, B. P.; Russell, S. L.; Finlay, B. B., Shifting the balance: antibiotic effects on host-microbiota mutualism. *Nat Rev Microbiol* **2011,** 9, (4), 233-43.
39. Kim, S.; Covington, A.; Pamer, E. G., The intestinal microbiota: Antibiotics, colonization resistance, and enteric pathogens. *Immunol Rev* **2017,** 279, (1), 90-105.
40. Hill, C.; Guarner, F.; Reid, G.; Gibson, G. R.; Merenstein, D. J.; Pot, B.; Morelli, L.; Canani, R. B.; Flint, H. J.; Salminen, S.; Calder, P. C.; Sanders, M. E., Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* **2014,** 11, (8), 506-14.
41. Egresi, A.; Drexler, D.; Hagymasi, K.; Blazovics, A.; Jakab, Z.; Kocsis, I.; Dako, S.; Bacsardi, A.; Lengyel, G., The potential role of organic and conventional yoghurt consumption in the treatment of non-alcoholic fatty liver disease. *Orv Hetil* **2020,** 161, (35), 1466-1474.
42. Andresen, V.; Gschossmann, J.; Layer, P., Heat-inactivated Bifidobacterium bifidum MIMBb75 (SYN-HI-001) in the treatment of irritable bowel syndrome: a multicentre, randomised, double-blind, placebo-controlled clinical trial. *Lancet Gastroenterol Hepatol* **2020,** 5, (7), 658-666.
43. Pessione, E., Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows. *Front Cell Infect Microbiol* **2012,** 2, 86.
44. Tag, C. G.; Sauer-Lehnen, S.; Weiskirchen, S.; Borkham-Kamphorst, E.; Tolba, R. H.; Tacke, F.; Weiskirchen, R., Bile duct ligation in mice: induction of inflammatory liver injury and fibrosis by obstructive cholestasis. *J Vis Exp* **2015**, (96).
45. Liu, Y.; Chen, K.; Li, F.; Gu, Z.; Liu, Q.; He, L.; Shao, T.; Song, Q.; Zhu, F.; Zhang, L.; Jiang, M.; Zhou, Y.; Barve, S.; Zhang, X.; McClain, C. J.; Feng, W., Probiotic Lactobacillus rhamnosus GG Prevents Liver Fibrosis Through Inhibiting Hepatic Bile Acid Synthesis and Enhancing Bile Acid Excretion in Mice. *Hepatology* **2020,** 71, (6), 2050-2066.
46. Dong, S.; Chen, Q. L.; Song, Y. N.; Sun, Y.; Wei, B.; Li, X. Y.; Hu, Y. Y.; Liu, P.; Su, S. B., Mechanisms of CCl4-induced liver fibrosis with combined transcriptomic and proteomic analysis. *J Toxicol Sci* **2016,** 41, (4), 561-72.
47. Liu, Y.; Liu, Q.; Hesketh, J.; Huang, D.; Gan, F.; Hao, S.; Tang, S.; Guo, Y.; Huang, K., Protective effects of selenium-glutathione-enriched probiotics on CCl4-induced liver fibrosis. *J Nutr Biochem* **2018,** 58, 138-149.
48. Chen, X.; Zhang, J.; Yi, R.; Mu, J.; Zhao, X.; Yang, Z., Hepatoprotective Effects of Lactobacillus on Carbon Tetrachloride-Induced Acute Liver Injury in Mice. *Int J Mol Sci* **2018,** 19, (8).
49. Shi, D.; Lv, L.; Fang, D.; Wu, W.; Hu, C.; Xu, L.; Chen, Y.; Guo, J.; Hu, X.; Li, A.; Guo, F.; Ye, J.; Li, Y.; Andayani, D.; Li, L., Administration of Lactobacillus salivarius LI01 or Pediococcus pentosaceus LI05 prevents CCl4-induced liver cirrhosis by protecting the intestinal barrier in rats. *Sci Rep* **2017,** 7, (1), 6927.
50. Barone, R.; Rappa, F.; Macaluso, F.; Caruso Bavisotto, C.; Sangiorgi, C.; Di Paola, G.; Tomasello, G.; Di Felice, V.; Marciano, V.; Farina, F.; Zummo, G.; Conway de Macario, E.; Macario, A. J.; Cocchi, M.; Cappello, F.; Marino Gammazza, A., Alcoholic Liver Disease: A Mouse Model Reveals Protection by Lactobacillus fermentum. *Clin Transl Gastroenterol* **2016,** 7, e138.

51. Jantararussamee, C.; Rodniem, S.; Taweechotipatr, M.; Showpittapornchai, U.; Pradidarcheep, W., Hepatoprotective Effect of Probiotic Lactic Acid Bacteria on Thioacetamide-Induced Liver Fibrosis in Rats. *Probiotics Antimicrob Proteins* **2020**.
52. Dhiman, R. K.; Rana, B.; Agrawal, S.; Garg, A.; Chopra, M.; Thumburu, K. K.; Khattri, A.; Malhotra, S.; Duseja, A.; Chawla, Y. K., Probiotic VSL#3 reduces liver disease severity and hospitalization in patients with cirrhosis: a randomized, controlled trial. *Gastroenterology* **2014,** 147, (6), 1327-37 e3.
53. Lunia, M. K.; Sharma, B. C.; Sharma, P.; Sachdeva, S.; Srivastava, S., Probiotics prevent hepatic encephalopathy in patients with cirrhosis: a randomized controlled trial. *Clin Gastroenterol Hepatol* **2014,** 12, (6), 1003-8 e1.
54. Bajaj, J. S.; Heuman, D. M.; Hylemon, P. B.; Sanyal, A. J.; Puri, P.; Sterling, R. K.; Luketic, V.; Stravitz, R. T.; Siddiqui, M. S.; Fuchs, M.; Thacker, L. R.; Wade, J. B.; Daita, K.; Sistrun, S.; White, M. B.; Noble, N. A.; Thorpe, C.; Kakiyama, G.; Pandak, W. M.; Sikaroodi, M.; Gillevet, P. M., Randomised clinical trial: Lactobacillus GG modulates gut microbiome, metabolome and endotoxemia in patients with cirrhosis. *Aliment Pharmacol Ther* **2014,** 39, (10), 1113-25.
55. Xia, X.; Chen, J.; Xia, J.; Wang, B.; Liu, H.; Yang, L.; Wang, Y.; Ling, Z., Role of probiotics in the treatment of minimal hepatic encephalopathy in patients with HBV-induced liver cirrhosis. *J Int Med Res* **2018,** 46, (9), 3596-3604.
56. Hutkins, R. W.; Krumbeck, J. A.; Bindels, L. B.; Cani, P. D.; Fahey, G., Jr.; Goh, Y. J.; Hamaker, B.; Martens, E. C.; Mills, D. A.; Rastal, R. A.; Vaughan, E.; Sanders, M. E., Prebiotics: why definitions matter. *Curr Opin Biotechnol* **2016,** 37, 1-7.
57. Davani-Davari, D.; Negahdaripour, M.; Karimzadeh, I.; Seifan, M.; Mohkam, M.; Masoumi, S. J.; Berenjian, A.; Ghasemi, Y., Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications. *Foods* **2019,** 8, (3).
58. Pandey, K. R.; Naik, S. R.; Vakil, B. V., Probiotics, prebiotics and synbiotics- a review. *J Food Sci Technol* **2015,** 52, (12), 7577-87.
59. Zou L., Z. R., Gao H., Xiao J., Tipoe G.L., *The Liver: Oxidative Stress and Dietary Antioxidants*

Elsevier: Chapter 28-Garlic and Liver disease, 2018; p 337-347.

60. Wang, Y.; Guan, M.; Zhao, X.; Li, X., Effects of garlic polysaccharide on alcoholic liver fibrosis and intestinal microflora in mice. *Pharm Biol* **2018,** 56, (1), 325-332.
61. Li, C.; Meng, M.; Guo, M.; Wang, M.; Ju, A.; Wang, C., The polysaccharides from Grifola frondosa attenuate CCl4-induced hepatic fibrosis in rats via the TGF-β/Smad signaling pathway. *RSC Advances* **2019,** 9, (58), 33684-33692.
62. Wang, K.; Yang, X.; Wu, Z.; Wang, H.; Li, Q.; Mei, H.; You, R.; Zhang, Y., Dendrobium officinale Polysaccharide Protected CCl4-Induced Liver Fibrosis Through Intestinal Homeostasis and the LPS-TLR4-NF-kappaB Signaling Pathway. *Front Pharmacol* **2020,** 11, 240.
63. Yun Y.C., J. C. C., Olive oil combined with Lycium barbarum polysaccharides attenuates liver apoptosis and inflammation induced by carbon tetrachloride in rats. *Journal of Functional Foods* **2018,** 48, 329-336.
64. Chikkerur, J.; Samanta, A. K.; Kolte, A. P.; Dhali, A.; Roy, S., Production of Short Chain Fructo-oligosaccharides from Inulin of Chicory Root Using Fungal Endoinulinase. *Applied Biochemistry and Biotechnology* **2020,** 191, (2), 695-715.
65. Wang, Z.; Zhang, X.; Zhu, L.; Yang, X.; He, F.; Wang, T.; Bao, T.; Lu, H.; Wang, H.; Yang, S., Inulin alleviates inflammation of alcoholic liver disease via SCFAs-inducing suppression of M1 and facilitation of M2 macrophages in mice. *Int Immunopharmacol* **2020,** 78, 106062.

66. Zhao, Y.; Liu, J.; Hao, W.; Zhu, H.; Liang, N.; He, Z.; Ma, K. Y.; Chen, Z. Y., Structure-Specific Effects of Short-Chain Fatty Acids on Plasma Cholesterol Concentration in Male Syrian Hamsters. *J Agric Food Chem* **2017,** 65, (50), 10984-10992.
67. Morgan, M. Y., Current state of knowledge of hepatic encephalopathy (part III): non-absorbable disaccharides. *Metab Brain Dis* **2016,** 31, (6), 1361-1364.
68. Ruiz-Ojeda, F. J.; Plaza-Diaz, J.; Saez-Lara, M. J.; Gil, A., Effects of Sweeteners on the Gut Microbiota: A Review of Experimental Studies and Clinical Trials. *Adv Nutr* **2019,** 10, (suppl_1), S31-S48.
69. Gluud, L. L.; Vilstrup, H.; Morgan, M. Y., Non-absorbable disaccharides versus placebo/no intervention and lactulose versus lactitol for the prevention and treatment of hepatic encephalopathy in people with cirrhosis. *Cochrane Database Syst Rev* **2016**, (5), CD003044.
70. Yuan, Y.; Che, L.; Qi, C.; Meng, Z., Protective effects of polysaccharides on hepatic injury: A review. *Int J Biol Macromol* **2019,** 141, 822-830.
71. Pena, A. S., [Intestinal flora, probiotics, prebiotics, symbiotics and novel foods]. *Rev Esp Enferm Dig* **2007,** 99, (11), 653-8.
72. Vidot, H.; Cvejic, E.; Finegan, L. J.; Shores, E. A.; Bowen, D. G.; Strasser, S. I.; McCaughan, G. W.; Carey, S.; Allman-Farinelli, M.; Shackel, N. A., Supplementation with Synbiotics and/or Branched Chain Amino Acids in Hepatic Encephalopathy: A Pilot Randomised Placebo-Controlled Clinical Study. *Nutrients* **2019,** 11, (8).
73. Liu, Q.; Duan, Z. P.; Ha, D. K.; Bengmark, S.; Kurtovic, J.; Riordan, S. M., Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. *Hepatology* **2004,** 39, (5), 1441-9.
74. Brenner, D. A.; Paik, Y. H.; Schnabl, B., Role of Gut Microbiota in Liver Disease. *J Clin Gastroenterol* **2015,** 49 Suppl 1, S25-7.
75. Tilg, H.; Cani, P. D.; Mayer, E. A., Gut microbiome and liver diseases. *Gut* **2016,** 65, (12), 2035-2044.
76. Bakhshimoghaddam, F.; Alizadeh, M., Modulation of the gut microbiota represents a new management for non-alcoholic fatty liver disease. *Hepatobiliary Surg Nutr* **2020,** 9, (2), 223-226.