Protective Effect of Probiotic in Alcohol-Induced Liver Disorders

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Role of Probiotics in Alcohol-Induced Liver Disorders

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Abstract

The major key culprit which produces burden on liver is concerned with abnormal dietary habits include high fat, high fructose rich products, and alcohol beverages. Thus, on the basis of dietary culprits, liver disorders are classified into two broad categories which include non-alcoholic fatty liver disease and alcoholic fatty liver disease. The alcohol consumption not only alter the physiological function of the liver but also affect the gut microbiota. The gut microbiota includes bacteria, fungi, and archaea which co-evolved to live in the human gut which helps in the regulation of various physiological activities and they together play a vigorous role in the management of numerous metabolic disorders. Alcohol produces deleterious effect on the natural gut microbiota which leads to microbial dysbiosis resulting into increased gut permeability to bacterial endotoxins. The chronic disruption of normal gut microbiota due to alcohol consumption produces various pathological effects like oxidative stress, inflammation and interferes with fasting-induced adipose factor (FIAF) and modulates lipid metabolism ultimately causes fatty liver, fibrosis, cirrhosis, and HCC. The therapeutic trends have now shifted towards the probiotics treatment which contains live microbial preparations that modify or restore the gut microflora and help in the treatment of alcohol-induced liver disorders.

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8.1 Introduction

Alcoholic liver disease (ALD) is a canopy term covering a variety of disorders progressing from steatosis, steatohepatitis, fibrosis, and cirrhosis to hepatocellular cancer (Meroni et al. 2019). Most of the fatalities due to chronic liver disease are of alcohol-related aetiology. Despite intensive research in this field, there is currently no specific therapy or treatment to ameliorate ALD progression. Numerous studies have highlighted the critical role of the gut–liver axis and gut microbiome in ALD pathogenesis. It has been shown that alcohol induces change in gut microbiota composition, i.e. intestinal dysbiosis along with the increased intestinal permeability (Hartmann et al. 2015). The surge in the harmful bacteria results in increased levels of microbial products, i.e. bacterial translocation from the gut to the liver and elevated pathogen-associated molecular patterns, for example, lipopolysaccharide (LPS), which acts as inflammatory signals, produces inflammation observed in ALD by activating toll-like receptor-4 on the Kupffer cells (Ceccarelli et al. 2014). Previous reports reveal how compositional and functional changes in the intestinal microbiome are observed in patients with alcohol misuse along with increased intestinal permeability and eminent levels of gut-derived microbial products present systematically (Bajaj 2019). Therefore, pathological bacterial translocation and disturbed intestinal homeostasis appear vital for the alcoholic liver disease pathogenesis, thus suppressing cellular responses to microbial products, steadying the mucosal gut barrier and maintaining eubiosis, and protecting from alcoholic liver disease (Cassard and Ciocan 2018).

The gut–liver axis and related dysbiosis being established as important controllers in the pathophysiology of ALD might include new therapeutic tactics such as prebiotics, probiotics symbiotics, faecal microbiota transplantation (FMT), and bile acid regulation for gut microbiota modulation (Imani Fooladi et al. 2013; Sarin et al. 2019). Recently, role of probiotics has been recognized in alleviating and averting the advancement of ALD (Hong et al. 2019). A probable mechanism is that the probiotics restore gut eubiosis by altering the composition of pathogenic intestinal microbiota, leading to reduced bacterial translocation, intestinal permeability, endotoxemia, and thus hampering the ALD expansion and its progression to a stage of an irreversible damage.

Of late many preclinical studies and clinical trials have demonstrated that probiotics retreated hepatic steatosis and inflammation induced by alcohol and helped improve liver enzymes in animal models and in patients (Liu et al. 2020). Probiotics lessen the levels of pro-inflammatory cytokines and oxidative stress by reducing reactive oxygen species (ROS) production and augment immune responses induced by alcohol in both intestine and liver. Thus, for combating alcohol-induced

hepatic steatosis, probiotics tend to reduce lipogenesis and increase fatty acid β-oxidation (Hong et al. 2019).

Although alteration of gut microbiota through probiotics seems to be a favourable therapeutic approach for the management of intestinal barrier dysfunction, there is a paucity of research that focuses on role of probiotics from the ALD prospective. Although potential therapeutics are being identified against ALD in the form of a growing quantity of probiotic strains and their related products, the defined mechanisms stating the significance of probiotics in regulating gut microbiota, gut–brain axis, intestinal barrier function, and the pathogenesis of ALD still needs to be elucidated. Therefore, it will be worthwhile to investigate the mechanisms of probiotic action on alcohol-induced liver injury. This kind of study will have a major impact on the development of a probiotics-based new therapeutic strategy for the inhibition and treatment of alcoholic liver disease (Cassard and Ciocan 2018). Thus, here we summarize the existing knowledge about the mechanism of action of probiotics and their potential therapeutic applications to deduce the effects of alcoholic liver disease.

8.2 Pathological Background of Alcohol-Induced Liver Disorder and Its Impact on Microbiota

Worldwide, alcohol consumption constitutes 4% of all deaths and ranks third among the several risk factors for disease and debility (Meroni et al. 2019). Alcohol toxicity and metabolism have most impact on the liver which affects the disease pathogenesis ranging from steatosis to hepatitis, cirrhosis, and hepatocellular carcinoma. The ALD pathophysiology varies according to the presence of genetic and nongenetic factors and the stage of the disease that affect its beginning and clinical development (Fig. 8.1) (Liu et al. 2020). Ethyl alcohol upon oral ingestion diffuses through cell membranes and is metabolized to a highly reactive molecule, i.e. acetaldehyde (Zakhari 2006). As a consequence, formation of acetaldehyde produces reactive oxygen species, depletion of cofactors like NADP, activation of pro-inflammatory and other signalling pathways causing adverse effects of alcohol consumption in all tissues (Waris et al. 2020).

Triggered by alcohol metabolism, the aldehydes produced in the intestine increase the levels of reactive oxygen species that activate pro-inflammatory cytokines and cause leaky gut pathophysiology, foremost to increased translocation of bacterial products, endotoxins, pathogen-associated molecular patterns (PAMPs), and bacterial DNA, from the gut lumen to the liver, instigating liver injury. Moreover, alcohol considerably brings about changes in gut microbial diversity, there occurs increase in the intestinal mucosal permeability. Intestinal epithelial tight junction protein expression is reduced leading to barrier dysfunction. Thus, endotoxin translocation into the blood occurs, inducing inflammatory responses and ROS production affecting the gut–liver axis causing hepatic steatosis and inflammation (Tuma et al. 1998; Teare et al. 1993).

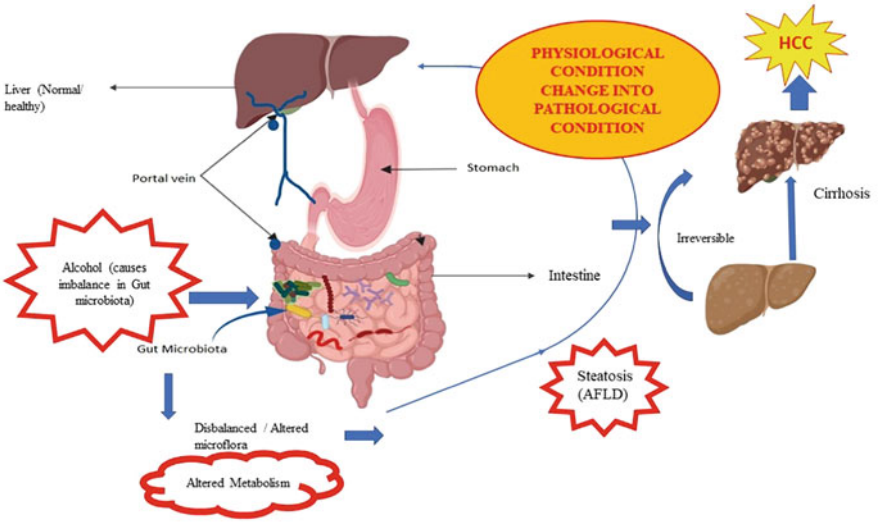

Fig. 8.1 Illustration of alcohol-induced abnormal metabolism causes ALD

ALD and gut microbiota correlation and association are not yet fully explored, however alterations in the microbiome both qualitative and quantitative have its sturdy impact on intestinal microbiota along with the increase in endotoxin levels, hepatic inflammation, and injury caused by ethanol and its vital role in ALD development. Due to intestinal bacterial dysbiosis which is a loss in balance of the different intestinal commensals, the gut homeostasis is perturbed. The consequences of dysbiosis both in the small and large intestine include loss of beneficial microbes, reduced bacterial diversity, and increase in the pathogenic species predominantly the overgrowth of Gram-negative bacteria, induced by both chronic and acute alcohol consumption (Bluemel et al. 2020). In human subjects with ALD, alcohol consumption led to leaky gut physiology with higher levels of systemic overload of detrimental bacteria as compared to healthy controls (Leclercq et al. 2014).

The development and propagation of liver injury are intricately involved with the gut microbiota in patients with chronic alcohol abuse. The alcohol induces change in microbial functions which are a consequence of human gut microbiota alteration and have potential to accelerate this injury. Previous studies have demonstrated the role of gut initiated LPS as the dominant mediator of inflammation in alcoholic steatohepatitis and its modulation via LPS pathways have been proposed to treat patients with ALD (Liu et al. 2017). Furthermore, it has been shown that the altered gut microbiota and LPS signalling can be reversed by TLR4 antagonists and probiotics (Mandrekar and Szabo 2009). In patient study, increased levels of faecal Bifidobacteria and Lactobacilli were observed by treating with probiotics therapy containing *L. plantarum* and *B. bifidum* which led to decrease in liver injury markers in serum like AST in ALD patients compared to standard therapy. In the same study they concluded that bowel flora was restored when given short-term oral

supplementation with the probiotics and was also associated with improvement in alcohol-induced liver injury when compared to the standard therapy alone (Kirpich et al. 2008). In severe liver disease due to alcohol, the integrity of the intestinal barrier along with reduction in endotoxin levels (LPS) was confirmed on probiotic administration. Hence gut intestinal barrier is affected at numerous levels upon both acute and chronic alcohol consumption, therefore the change in gut microbiota should be involved as a therapeutic approach in strengthening intestinal related barrier functioning and in regression of ALD.

8.3 Does Alcohol Produce Deleterious Effects on Gut Microbiota?

The gut microbiota varies with development of alcohol liver disease. Pathogenesis of alcoholic liver disease relevant to intestinal dysbiosis has very diverse consequences (Woodhouse et al. 2018); therefore, it would be valuable to mention the influence of gut microbiota on alcohol liver disease progression and vice versa. Crosstalk between bacterial components such as LPS and various hepatic receptors like TLR's affects the gut–liver axis. Modulation of this interaction leads to worsening of hepatic disorders due to dysbiosis and altered intestinal permeability. In one such study a decline in the abundance and richness of both Firmicutes and Bacteroidetes at phylum level was observed upon chronic ethanol feeding along with a relative increase in Actinobacteria and Proteobacteria phyla. Moreover bacterial genera comprising Gram-positive Corynebacterium and Gram-negative alkaline tolerant Alcaligenes showed the greatest growth (Bull-Otterson et al. 2013). Metagenomic and metabolomics studies reveal how chronic alcohol administration lowers the gut microbial capacity to form new saturated long-chain fatty acids (LCFA). Lower bacterial synthesis of LCFA results in decrease in the abundance of good bacteria, such as Lactobacillus species, used saturated LCFA as a rich energy source. The decrease in these LCFA producing bacteria causes tight junction barrier disruption (Chen et al. 2015). The eubiosis is restored by supplementation of the saturated LCFA, which normalizes the intestinal gut barrier, and diminishes ethanol induced liver disease in mice. Thus, gut microbiota contributes to the progression of alcohol-related liver disease through different mechanisms. In alcoholic hepatitis the altered gut bacteria and produces immune depression, increase in inflammation of hepatocytes and changes in metabolites of microbial by various ways (1) *By Pathological bacterial translocation*, which is the most commonly known mechanism linking intestinal dysbiosis to alcoholic liver disease progression. The dysfunction of the gut barrier is brought about by ethanol with its metabolite acetaldehyde, these microbial products translocate to the portal venous blood from the intestine which activate Kupffer cells and hepatic stellate cells, thus further damage the hepatocytes (Mazagova et al. 2015), thus causing progression of alcoholic liver disease as a consequence of hepatotoxic outcome of alcohol and its metabolites. (2) *Changes in intestinal metabolites*, its challenging to know which metabolites from the dysbiotic microbiota provoke intestinal inflammation. Similar studies in rats following chronic

ethanol administration, the changes in the propionate and short-chain fatty acids (SCFAs) butyrate, known as faecal lipid metabolites have been reported (Parada Venegas et al. 2019). These SCFAs, such as acetate, propionate, and butyrate produced from the digestion of carbohydrates by the gut microbiota were found in high abundance in the healthy colon. (3) *Bile acid metabolism:* Bile acids are important correspondents between the intestine and the liver. Bile acids conjugates are modified in the intestine by bacteria and are secreted into the duodenum from the hepatic biliary system. A reduced bile flow is observed in patients with liver cirrhosis. Bile acids lead to produce antimicrobial molecules by activation of FXR signalling in intestinal epithelial cells therefore reduced bile flow leads to overgrowth of intestinal bacterial (Raedsch et al. 1983; Inagaki et al. 2006).

For a well explanation of the liver–gut axis with respect to alcohol, identification of other pathways linking the alcoholic liver disease to gut microbiota is essential, which could be instrumental in designing interventional trials. To better define the bidirectional crosstalk of how the liver interconnects to the intestine, the interplay of gut microbiota and bile acids in ALD need to be explored for the development of novel pharmaceutical agents for treatment of patients with chronic alcohol abuse. Taken together, chronic alcohol administration leads to dysbiosis and is associated with a microbial shift, i.e. decline in healthy bacteria that are good for physiology such as Lactobacillus spp., whereas the pathogenic, i.e. bad bacteria such as Enterobacteriaceae increase (Yan et al. 2011). Thus, supplementing probiotics, prebiotics, or combination of both, i.e. symbiotic appear to be a usable option for treatment of alcoholic liver disease by inhibiting dysbiosis or restoring eubiosis.

8.4 Types and General Aspects of Probiotics

The term Probiotic was first introduced by Lily and Stillwell in 1965. The first probiotic species studied was lactic acid bacteria, the lactobacillus acidophilus identified by Hull in 1984 (Lilly and Stillwell 1965). Later in 1991, Holcombh identified *bifidobacterium bifidum*. Lactobacillae and Bifidobacterium are the main probiotics and other probiotics identified later were *Enterococcus, Escherichia, Saccharomyces, Bacillus, Propionibacteria,* and *Streptococcus* (Hamilton-Miller 2003). According to WHO, the probiotics are described as next most important elements in immune defence system following antibiotic resistance (Boden and Snapper 2008; Fonseca-Camarillo and Yamamoto-Furusho 2015).

8.4.1 Probiotics

Probiotics are the beneficial microorganisms with potential to maintain the healthy microbial balance, which are incorporated in the host to produce favourable effect. The administered probiotics have various health benefits as they lower the intestinal pH, decrease the abundance and colonization by pathogenic bacteria. Moreover the

probiotics make the microenvironment enriched with good microbes which have potential to modulate the host immune response (Williams 2010).

8.4.2 Composition of Probiotics

Most commonly used probiotics are comprised of yeast or bacteria which are marketed as dietary supplements and food in the form of dairy products like yoghurts, capsules, liquid drinks, and other fermented foods. These probiotic products constitute either a single strain or a mixture of several bacterial species. Probiotics are mostly available in the form of yeast, moulds, or bacteria; however, bacterial probiotics like lactic acid containing bacteria are more common (Hempel et al. 2011).

8.4.3 Criteria for Probiotics

In order to define probiotics, Fuller back in 1989 defined the important criteria for a probiotic to be classified as a good one. (1) A probiotic strain should have a beneficial effect on the host phenotype, such as probiotics should increase resistance to disease and proliferation capabilities. (2) A probiotic should be non-pathogenic in nature and no toxic effect on the host. (3) The probiotic organisms should be viable with a capacity to be given in large numbers. (4) The probiotics administered strains should be sustainable and have good storage properties (Markowiak and Slizewska 2017).

8.4.4 Commonly Used Bacterial Probiotics

(i) Lactobacillus*: rhamnosus, reuteri, acidophilus, and fermentum*; (ii) Bifidobacterium: *bifidum, longum, infantis, thermophilum*; (iii) Streptococcus: *lactis, intermedius, cremoris, salivarius*; (iv) Propionibacterium; (v) Pediococcus; (vi) Leuconostoc; (vii) Bacillus; (viii) Enterococcus; (ix) E. faecium (Doron and Gorbach 2006).

8.4.5 Important Functions of Probiotics

There are various health benefits probiotics provided to the host as being an essential component of the host gut microbiota. (a) Reduces disease progression particularly in chronic liver disease and others. (b) Increases calcium absorption from gut to prevent osteoporosis. (c) Probiotics protect from pathogenic microorganisms like Candida by competing for their colonization. Probiotics are also useful as they inhibit growth of harmful bacteria by producing various inhibitory substances

which include antibiotics and make the microenvironment acidic and unfavourable for pathogenic bacteria. (d) Liver toxicity reduction. (e) Promotion of healthy digestive tract colonization by maintaining peristalsis, better digestion of proteins, fats, carbohydrates, and nutrients re-absorption. (f) Maintain balance of oestrogen levels. (g) Boost levels of vitamin B and K. (h) Increase in host immunity and resistance to various infectious diseases. (i) Improves lactose intolerance (Rastogi et al. 2011; Madsen 2001; Ewaschuk et al. 2007; Singh et al. 2013).

8.4.6 Mechanism of Action of Probiotics

Clinical benefits produced by the probiotics are a consequence of the combined effect of several mechanisms. The probiotics most likely modulate immune system at both cell-mediated immune response and humoral immune functions. Probiotics produce organic acids and are important for both gut microbiota and probiotic–host interactions. They improve the barrier function and produce various small metabolites with local and non-local effects (Sanders et al. 2019).

8.4.7 Role of Probiotics

In many previous studies, the role of probiotics in the modulation of various physiological processes like immunological, respiratory, and gastrointestinal functions has been defined (Floch et al. 2011). Probiotics have been shown to release antibacterial substances such as bacteriocins to decrease the harmful bacterial growth playing a protective role by competing with intestinal pathogens (Cotter et al. 2005). Probiotics produce various metabolites such as acetic acid and lactic acid having favourable effect on host health (Servin 2004). Earlier Metchnikoff discovered that healthy bacteria in the form of lactic acid bacteria (LAB) were reported to have a marked influence on both digestive and the immune system (Perdigon et al. 1995). In current times gram-positive probiotic strains such as Lactobacillus and Bifidobacterium are being used as treatments of intestinal dysfunctions (Marco et al. 2006). However, Escherichia coli Nissle 1917 (EcN), a gram-negative bacteria are also used as probiotics in the treatment of chronic bowel disease and colitis (Mollenbrink and Bruckschen 1994). Besides, engineering these natural probiotics may aid in escalating the benefit to the host by producing various immunomodulatory molecules. The gut microbiota thus has an essential role in many disorders and its modulation in the form of probiotics could be an effective therapy to treat various diseases. Therefore, probiotic intervention increases the community of beneficial microorganisms and its product could serve better therapeutic option for various disorders including chronic liver disease.

8.4.8 Role of Probiotics in Liver Disease

Probiotic intervention modulates gut microbiota and is known to alleviate progressive liver disease. Previous preclinical studies in rats with ALD probiotic and prebiotic treatment prevented dysbiosis in colon lumen (Mutlu et al. 2009). In the same line several other studies have also supported the role of probiotics by its supplementation which alleviated alcohol-induced liver injury by restoring gut microbiota homeostasis (Loguercio et al. 2002; Dhiman et al. 2014; Lata et al. 2007). It was shown that 2 week treatment with LGG positively changed the alcohol-induced dysbiosis in mice with continued alcohol intake (Wang et al. 2011). Importantly, the LGG supplementation prevented ethanol-persuaded pathogenic variations in the microbiome and decreased the liver disease (Wang et al. 2012) thereby establishing the beneficial effects of probiotics by restoration of gut microbiota in ALD highlighting the important function of microbiota in gut-liver pathophysiology.

8.5 Mechanism of Action of Probiotics for the Management of Alcohol-Induced Liver Disorders

Probiotics are preparations containing living microorganisms that play a vital role in altering the balance of gut microflora (Lambert et al. 2003). Favourable physiological conditions can be attained by adjusting the composition of the host microflora (Lata et al. 2007). It is already established that excessive intake of alcohol leads to dysbiosis, that damages gut mucosa and results into increased intestinal permeability, which in turn increases bacterial translocation across epithelium. As a result of increased bacterial translocation, bacterial products such as lipopolysaccharides (LPS) increase in circulation and enhance the production of free radicals or reactive oxygen species (ROS) and various pro-inflammatory cytokines, leading to ROS mediated liver injury (Thurman et al. 1998). Endotoxins derived from the superficial membrane of Gram-negative bacteria such as LPS act via recognition of CD4T mediated receptors like toll-like receptors (TLRs), articulated in Kupffer cells and induce the release of various cytokines and chemokines which are responsible for activation of TNF-α and NFκB and these are the major involved in liver injury. A study showed that reduction in TLR4 complex or CD14 proved to protect mice from alcohol-induced liver injury (Mutlu et al. 2009). Here, probiotics come in role, they restrain the altered gut microflora and improve alcohol-induced gut dysfunction thereby decreases gut permeability and prevents intestinal bacterial translocation.

As a result, level of alcohol-induced pro-inflammatory cytokines, chemokines, and reactive oxygen species (ROS) decreases in the intestine and liver. Probiotics are known to reverse the inhibited fatty acid β-oxidation due to alcohol, i.e. reduced lipogenesis, thus beneficial in the management if alcohol-induced hepatic steatosis (Gu et al. 2019). Another pathway involved in alcohol-induced liver injury is

AMP-activated protein kinase (AMPK) signalling pathway, responsible for regulation energy balance by lipid metabolism via alteration of various transcription factors such as sterol regulatory element-binding protein 1 (SREBP-1) and peroxisome proliferator-activated receptor-α (PPARα), which are involved in lipogenesis and fatty acid oxidation (Li et al. 2011). Excessive alcohol intake decreases acetyl-CoA carboxylase (ACC) and AMPK phosphorylation and increases malonyl-Co-A (MCA) production, which is a primary cause of abnormal lipid uptake in the liver. Most often used probiotic strain, Lactobacillus rhamnosus GG (LGG) has been proved to be beneficial in treating ALD, it increases fatty acid oxidation and decreases lipogenesis in liver (Zhang et al. 2015).

8.6 Preclinical Evidences of Probiotics for the Management of Alcohol-Induced Liver Disorders

The favourable properties of probiotics have been studied in various animal models in the management of non-alcoholic steatohepatitis (NASH) and ALD (Kirpich and McClain 2012). Nowadays the most commonly used probiotic strains are Bifidobacteria, Lactobacillus rhamnosus GG (LGG), lactic acid bacteria (LAB), and Saccharomyces boulardii. Various strains of Lactobacilli such as Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactobacillus casei, and Lactobacillus helveticus are widely being used. Numerous probiotics are reported to be used for the treatment and management of a various disorders. Among them, the most frequently used strain is Lactobacillus rhamnosus GG (LGG) (Lee and Salminen 2009). In some of the ALD models of rats and mice, administration of LGG revealed significant improved liver enzymes such as alanine transaminase (ALT) and aspartate transaminase (AST), reduction in plasma endotoxin level. Wang et al., in 2011 showed that LGG supplementation in mice causes significant reduction in alcohol-induced hepatic steatosis and endotoxemia (Wang et al. 2011). Nanji et al., in 1994 were one of the groups experimentally demonstrated the efficacy of LGG in treating ALD (Nanji et al. 1994). They administrated LGG concentrate (10^{10} CFU per mL) to Wistar rats and there was a significant reduction in alcohol-induced liver endotoxemia. In another study, rats with alcohol pancreatitis-related liver damage were fed with a combination of Lactobacillus helveticus, Lactobacillus acidophilus, and Bifidobacterium which effectively protected the rats against bacterial translocation and liver damage during acute pancreatitis (Marotta et al. 2005). Additional studies on LGG in rats showed significant reduction in alcohol-induced intestinal permeability, oxidative stress, and inflammation (Forsyth et al. 2009) and improve intestinal dysbiosis by restoring gut microflora (Mutlu et al. 2009). We have represented several preclinical experimental studies on probiotics for the treatment of ALD in Table 8.1.

Table 8.1 Preclinical evidences associated with the use of probiotics in the management of alcohol-induced liver disorders

S. No	Animal model	Intervention	Observation	Pathological outcome	Reference
1.	57BJ/6N mice (Lieber DeCarli diet containing 5% alcohol for 8 weeks). [protein (17%), corn oil (40%), carbohydrate (7%) and alcohol (35%)]	Lactobacillus rhamnosus GG (LGG) supplementation (2 weeks). (10^9 CFU/mouse per day)	– Histology of liver and intestine – Barrier function analysis and Caco-2 monolayer cell culture	Significant reduction in alcohol-induced hepatic steatosis and endotoxemia	(Wang et al. 2011)
2..	Male Wistar rats (diet containing corn oil and ethanol)	Ethanol with lactobacilli GG concentrate (10^{10} CFU per mL)	Measurement of plasma endotoxin and severity of pathological variations in the liver.	Mean ± SE of the pathology score was significantly higher in the CO + E group compared to the CO + E + L group.	(Nanji et al. 1994)
2.	Male Sprague–Dawley rats were gavaged with alcohol twice daily (8 gm/kg) for 10 weeks.	Rhamnosus Gorbach–Goldin (LGG) or vehicle (V) [Once a day]	Analysis of hepatic tissues for oxidative stress and inflammatory biomarkers.	Significant reduction in alcohol-induced intestinal permeability and oxidative stress.	(Mutlu et al. 2009)
3.	Sprague–Dawley rats (alcohol-rich diet for 2 weeks)	Pre-treatment for 1 week with a mixture of synbiotics (Lactobacillus acidophilus, Lactobacillus helveticus, and Bifidobacterium).	Measured transaminase and endotoxemia levels before treatment, after 6 h, after 24 h and 2 weeks later, at the time when rats were sacrificed.	Synbiotics improved the acute pancreatitis-induced increase in endotoxemia and transaminase levels.	(Marotta et al. 2005)
4.	Mice (ethanol containing diet for 4–5 weeks)	Oral administration- Heat-killed L. brevis (dose −100 or 500 mg/kg once a day for 35 days)	Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum, triglyceride content (TG) and total cholesterol	Inhibited an increase in the level of serum ALT and AST, TG, and total cholesterol. Suppression of overexpression of TNF-α, SREBP-1, and SREBP-2 mRNA in the liver	(Segawa et al. 2008)

8.7 Clinical Evidences Associated with the Use of Probiotics for the Management of Alcohol Induced Liver Disorders

Till date, several studies have reported the beneficial effects of probiotics in experimental ALD, still there is lack of clinical data and limited clinical trials. Some of the clinical evidences and clinical trials associated with the use of probiotics for the treatment of ALD have been shown in Tables 8.2 and 8.3. In a clinical study,

Table 8.2 Clinical evidences associated with the use of probiotics for the management of alcohol-induced liver disorders

Disease/ condition	No. of patients/ subjects (each group)	Intervention	Pathological outcomes	Reference
Alcoholic cirrhosis	10	De Simone Formulation (Lactobacillus rhamnosus, Plantarum, Bifidus, Salivarius, Lactis, acidophilus, Casei, Bulgaricus, Breve, Fructooligosaccharides, folic acid, Fe gluconate, and Zn oxide) for 3 months.	Reduced plasma ALT, AST, and GGT levels; Normalized plasma TNF-α, IL-10, and IL-6 levels and decreased 4-HNE, MDA, and S-NO levels	(Loguercio et al. 2002)
Alcoholic cirrhosis	20	*Lactobacillus casei* Shirota treatment for 4 weeks.	Reduction in sTNFR1, sTNFR2, TLR4, and IL10 levels	(Loguercio et al. 2005)
Alcoholic psychosis and liver disease	66	*Lactobacillus plantarum* 8PA3 and *Bifidobacterium bifidum* treatment for 5 days.	Increased lactobacilli and Bifidobacteria; reduction in AST, ALT, LDH, GGT, and total bilirubin	(Kirpich et al. 2008)
Alcoholic cirrhosis	12	A combination of different strains of lactic acid bacteria for 2 months	Improvement in gut microflora, reduced ALT, γ-GT, and TNF-α levels	(Stadlbauer et al. 2008)
Alcoholic and non-alcoholic cirrhosis And hepatic encephalopathy patients	89	De Simone Formulation treatment for 6 months	Lesser risk of hospitalization for hepatic encephalopathy, improvement in CTP (Child-Turcotte-Pugh) and MELD (model for end-stage liver disease) scores	(Dhiman et al. 2014)

Table 8.3 Clinical trials on probiotics for the management of alcohol-induced liver disorders

S. N	Study title	Study type	No. of subjects	Intervention/ treatment	Recruitment status	Phase	Primary outcome measures	Secondary outcome measures	Clinical trials.gov identifier:
1.	Effect of Probiotics on gut–liver axis of alcoholic liver disease (EPALD)	Interventional (Clinical Trial)	130	Drug: hepatitis, alcohol, probiotics Drug: alcohol, hepatitis, Placebo	Completed	4	Liver Enzymes (ALT) [Time Frame: 7 days after probiotics]	Lipopolysaccharide (LPS) and Pro-inflammatory Cytokines [Time Frame: 7 days after probiotics]	NCT01501162
2.	Profermin®: Prevention of progression in alcoholic liver disease by modulating dysbiotic microbiota (SYN-ALD)	Interventional (Clinical Trial)	40	Dietary Supplement: Profermin Plus, FSMP, probiotics Dietary supplement: Fresubin, dietary supplement	Recruiting	NA	Hepatic stellate cell activity [Time Frame: 24 weeks]	1. Hepatic a-SMA acti vity 2. Alfa-smooth muscle actin concentration [Time Frame: 24 weeks]	NCT03863730
3.	Effect of Probiotics on gut–liver axis of alcoholic hepatitis	Interventional (Clinical Trial)	140	Drug: Probiotics (Lacidofil®) Drug: Placebo	Unknown	4	Liver enzymes [Time Frame: 7 days after probiotics]	1. LPS and pro-inflammatory cytokines. 2. Stool culture and stool Polymerase chain reaction denaturing gradient gel electrophoresis [Time Frame: 7 days after probiotics]	NCT02335632

(continued)

Table 8.3 (continued)

S. N	Study title	Study type	No. of subjects	Intervention/ treatment	Recruitment status	Phase	Primary outcome measures	Secondary outcome measures	Clinical trials.gov identifier:
4.	Novel therapies in moderately severe acute alcoholic hepatitis (NTAH-Mod)	Interventional (Clinical Trial)	130	Dietary Supplement: Lactobacillus rhamnosus GG Drug: Placebo for probiotic	Recruiting	NA	MELD score [Time Frame: 180 days] Improvement in MELD score over 180 day study duration.	Gut mucosal permeability [Time Frame: 180 days] Gut mucosal permeability will be measured by changes from baseline in the gut mucosal integrity as assessed by the lactulose/ mannitol test.	NCT01922895

Loguercio et al., in 2002 stated that a probiotics mixture, known as De Simone Formulation (Lactobacillus rhamnosus, Plantarum, Bifidus, Salivarius, Lactis, acidophilus, Casei, Bulgaricus, Breve, Fructooligosaccarydes, folic acid, Fe gluconate, and Zn oxide) has protective effects in liver disease. De Simone Formulation treatment showed significant improvement in plasma levels of 4-hydroxynonenal (4-HNEand) malondialdehyde (MDA) in NAFLD and alcoholic cirrhotic (AC) patients (Loguercio et al. 2002). Kirpich et al., in 2008 demonstrated the effectiveness of probiotics in the management of ALD patients and reported that probiotics (L. plantarum 8PA3B and bifidum) have shown significant increase in Bifidobacterium and Lactobacillus in human faecal matter and improved the levels of low-density lipoprotein (LDL), ALT, and total bilirubin (STB) (Kirpich et al. 2008). In an open-labelled study, Loguercio et al. assessed the efficacy of the probiotic containing Lactobacillus casei Shirota on healthy controls and patients suffering from alcoholic cirrhosis (AC). As compared to control group, patients receiving probiotic treatment of 4 weeks had a lesser TLR4 expression with a decrease in level of sTNFR1 (soluble TNF receptor 1) and sTNFR2, which clearly suggests that probiotic therapy is effective as well as safe for the patients with weak immune system (Loguercio et al. 2005). Another study revealed that treatment with a symbiotic mixture of different strains of bacteria with a prebiotic significantly improved liver function in AC patients. In a study Stadlbauer and co-workers used a combination of different strains of lactic acid bacteria for the treatment of alcoholic cirrhosis and observed a significant decrease in γGT (Gamma Glutamyl Transferase) and ALT levels (Stadlbauer et al. 2008).

8.8 Future Prospective and Conclusion

In preclinical studies, excessive consumption of ethanol causes dysbiosis leading to bacterial overgrowth in gut, which subsequently causes impairment of gut mucosa layer along with the damage of. The damaged tight junctions result into endotoxemia. Elevated endotoxins cause activation of Kupffer cells and stimulate inflammation and hepatic steatosis. Probiotics and prebiotic supplements prevent from alcohol-induced intestinal and liver injury via multiple mechanisms: (a) Alteration of gut microbiota; (b) reduction of free radicals or ROS production in liver and intestine; (c) improvement in mucosal layer and CRAMP, antimicrobial peptide, and expression of claudin-1 protein through increased HIF signalling; (d) inhibition of miR122a expression leading to upregulation of occludin; and (e) activation of hepatic AMPK. There is still requirement of exhausted research in the field of probiotics for the management of alcohol-induced liver disorders. From the above discussion, it was proposed that probiotics exert anti-inflammatory, antioxidant properties and help in the normal functioning of various vital organs involved in metabolism.

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