

## Potential Molecular Mechanism of Probiotics in Alcoholic Liver Disease

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### Abstract

Alcohol consumption and its abuse are significant prevalent cause for liver diseases and death worldwide. Increased bacterial endotoxin in the portal circulation, the plasma ratio of liver enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST) and triglyceride implies the symbiotic relation between the gut and liver plays a key function in alcoholic liver disease (ALD). Consumption of alcohol leads to dysbiosis and informalities of the intestinal barrier, hyper gut permeability, oxidative stress, inflammation and adversely affect adipose tissue metabolism, and those are mainly recognized as major factors for progression of alcoholic liver disease. Alteration of gut microbiota is referred to as bacterial overgrowth which leads to the release of bacterial products to change in commercial/pathogenic microbiota equilibrium. Lipopolysaccharide (LPS) derived inflammatory signal renders inflammation in alcoholic liver disease. Increase in concentration of lipopolysaccharide leads to activation of toll-like receptor 4 (TLR4) and alteration in micro RNA (miRNA) expression at the transcription level. Activation of myeloid differentiation factor 88 (MyD88) pathways eventually produces pro inflammatory cytokine activation that is an important mediator of alcoholic liver disease. However, there is no effectual Food and Drug Administration (FDA) approved treatment for any stage of alcoholic liver disease. Thus, the potential therapeutic approach for alcoholic liver disease is restoration and alteration of gut microbiota. With the increasing importance of gut microbiota in the onset and occurrence of a variety of diseases, the potential use of probiotics in ALD is receiving more exploration and clinical attention. Probiotic administration is nontoxic, inexpensive and non-invasive strategy with minimal side effects compared to antibiotic therapy and surgery. Yet, there is no substantial evidence on the efficient molecular mechanism regarding mode of action of probiotics on ALD as therapeutics. This review summarizes the research done on gut liver-axis and potential mechanism of probiotic in alcoholic liver disease.

**Keywords:** Alcoholic liver disease; Gut liver axis; Toll like receptors; Probiotics

### Abbreviations:

ALD: Alcohol Liver Disease; ALT: Alanine Aminotransferase; AP-1: Activating Protein-1; AST: Aspartate Aminotransferase; CD-14: Cluster of Differentiation 14; CFU: Colony Forming Unit; CYP2E1: Cytochrome P450 2E1; FoxO4: Forkhead Box O3; GGT: Gamma Glutamyl Transferase; HO-1: Heme Oxygenase-1; iNOS: Inducible Nitric Oxide Synthase; IL: Interleukin; IFN- $\beta$ : Inducing Interferon- $\beta$ ; LBP: LPS-Binding Protein; LDH: Lactate Dehydrogenase; LGG: Lactobacillus Rhamnosusgorbach-Goldin; LPS: Lipopolysaccharide, MAPKs: Mitogen-Activated Protein Kinases; MCP-1: Monocyte Chemoattractant Protein 1; MDA: Malondialdehyde; MyD88: Myeloid Differentiation Factor 88; NF- $\kappa$ B: Nuclear Factor-Kb; PAMPs: Pathogen Associate Molecular Patterns; Reg3b: Regenerating Islet-Derived Protein 3-Beta; TLR: Toll Like Receptor; TNF- $\alpha$ : Tumor Necrosis Factor- $\alpha$ ; s-TNF-R1/R2: Soluble Tumor Necrosis Factor Receptor 1/2; TG: Triglyceride; TGF- $\beta$ : Tumor-Growth Factor- $\beta$ ; WAT: White Adipose Tissue; 4-HNE: 4-hydroxynonenal

### Gut Liver Axis and Alcoholic Liver Disease

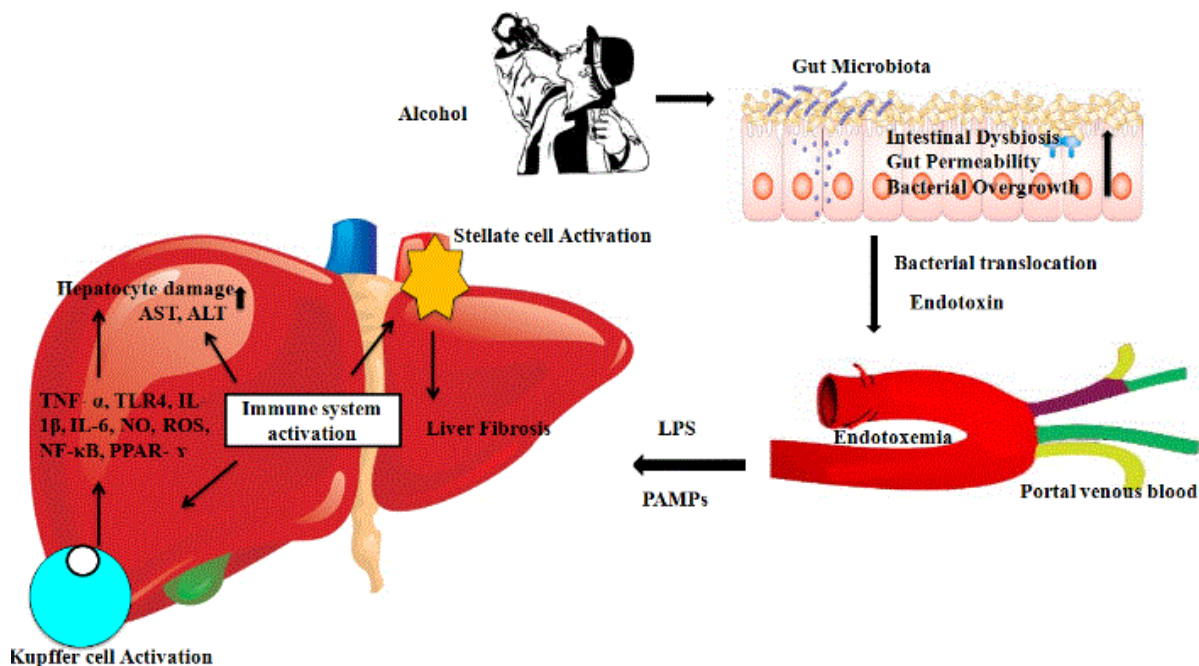
Alcoholic liver disease encompasses of fatty liver, hepatic steatosis, alcoholic steatohepatitis, alcoholic hepatitis, fibrosis, cirrhosis, and

hepatocellular carcinoma. On the persistent use of alcohol, fatty liver can result into cirrhosis, which leads to the development into hypertension in portal vein or liver malfunction [1] (Figure 1). Gut microbiota should be accepted an “externalized” organ placed within the body, as it provides fundamental physiological functions [2].

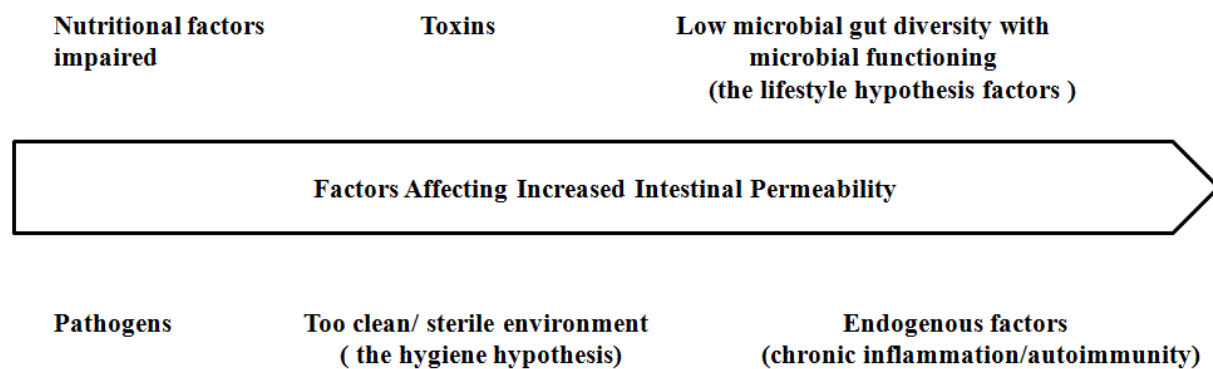
### Alcoholic Liver Disease and Enteric Dysbiosis

Presently recognized pathogenic factor connecting enteric dysbiosis and ALD appears to be pathological bacterial translocation [3]. The factors behind the pathogenesis of the increased intestinal permeability are shown in (Figure 2) [4]. The intestinal permeability of Caco2 cell monolayers seems to be increased due to exposure of ethanol and acetaldehyde, which causes tight junction disruption [5]. Intestinal CYP2E1 plays a key role in alcohol-induced intestinal permeability and oxidative stress [6].

Recent evidences provide information about the control of host inheritance and metabolism on the composition of the intestinal microbiota [7]. The correlation between alcoholic liver injury and imbalance of certain bacteria phylum are largely unidentified. Increased gut permeability along with consumption of alcohol also altered activity and composition of the gut microbiota such as Clostridiales Family XIV *Incertae Sedis*, *Ruminococcaceae* and *Bifidobacterium* spp.



**Figure 1:** Mechanism of alcoholic liver disease. Ethanol exposure sensitizes Kupffer cells to the activation by lipopolysaccharide (LPS) via toll-like receptor 4 (TLR4). This sensitization enhances the production of various pro-inflammatory mediators, such as tumour necrosis factor (TNF- $\alpha$ ) and ROS that contribute to hepatocyte dysfunction (necrosis or apoptosis) leading to fibrosis/cirrhosis.



**Figure2:** Regulation of gut permeability: what to blame for an increased intestinal permeability?

**Figure 2:** Regulation of gut permeability: What to blame for an increased intestinal permeability? Increased intestinal permeability may precede and promote translocation of bacteria, endotoxins (LPS), and pathogens associated molecules into the portal venous system. There is a close link between the liver and the gastrointestinal tract; the liver is constantly exposed to nutrients, toxins, food-derived antigens, microbial products and gastrointestinal tract microorganisms.

Dysbiosis might lead to differences in intestinal fermentation products like SCFAs. After ethanol administration, levels of short-chain fatty acids (SCFAs) get lower except for acetic acid levels: whose level increases as it is a metabolite of ethanol [8]. In mice,

administration of the butyrate recovers the intestinal barrier function in all stages of alcohol exposure, but the liver injury was reduced only in case of acute or short-term alcohol exposure [9]. In alcohol-fed mice, induction of long chain fatty acids (LCFAs) reduces ALD by

escalating the levels of *Lactobacillus* spp., improving intestinal barrier function and reducing intestinal inflammation [10]. After chronic alcohol feeding, microbial metabolites blend together with small amounts of *Lactobacillus* also reduces intestinal barrier dysfunction and ALD. This is a superior example of a correlation between how the host and microbial genome has been recognized.

Another mediator of intestinal dysbiosis is intestinal inflammation. Recent studies in mice demonstrated that monocytes and macrophages elevate TNF- $\alpha$  production after chronic alcohol consumption; which eventually increases cytokine production [11]. Researcher showed that by using non-absorbable antibiotics resulted in the reduction of intestinal bacterial overgrowth, inflammation and permeability [12]. The oral dose of an antibiotic drug metronidazole, resulted into an elevated level of intra colonic acetaldehyde due to increase in aerobic bacteria and decrease in anaerobic bacteria [8]. On the other hand, antibiotic ciprofloxacin, prevented accumulation of intra colonic acetaldehyde, which lead to decreased fecal alcohol dehydrogenase activity and colonic microbiota [8]. *In vivo* studies, showed gut permeability to macromolecules also increased during co-relation with alcohol induced liver damage and endotoxemia [13]. This evidence links intestinal dysbiosis with gut barrier dysbalanced, yet the triggering of microbial products and the metabolites currently not known.

Oxidative stress is a liable factor for organ malfunction and tissue injury occurring due to alcohol induction [14]. iNOS can be one of the cause for intestinal inflammation after chronic alcohol consumption. As expression of intestinal iNOS is primarily dependent on TNF-receptor 1 enterocytes [15]. Besides, an inhibitor of iNOS also participated into gut permeability, liver injury and endotoxemia in the induction of alcohol [16]. Whether iNOS affects the gut microbiota or not, requires further investigation.

miR-221 is also concerned with the down regulation of the tight junction proteins in the mouse model of alcohol-induced gut

permeability [17]. The alcohol-induced intestinal inflammation correlated with reduced levels of mRNA and protein in Reg3b and increased expression of miR-155 in the small intestine. Further studies demonstrated that miR-155-deficient mice were secured from intestinal inflammation occurred due to chronic alcohol administration. In addition, there was no elevation in serum endotoxin levels in the miR-155-deficient mice after chronic alcohol induction suggesting that miR-155 may have a function in alcohol-induced disruption of the gut integrity. Another recently identified regulator of gut permeability found to be FoxO4 which increases on induction of alcohol [18].

An outcome of increased intestinal permeability, leads to increase in plasma levels of gut microbial products along with pathological bacterial translocation. Administration of acute alcohol, showed increase plasma levels of peptidoglycan in a rat model [19]. Gut-liver axis is affected by chronic alcohol consumption leads to the activation of inflammatory cascade and TLR signalling which is topical for researchers as well as physicians, as it will be useful for translating novel findings into clinical practice and for understanding ALD pathophysiology. Discrepancies in these studies may be due to different factors such as species, treatment, the model used to induce alcohol, duration and alcohol dose (drinking water, intra gastric feeding, tube diet, gastric gavage).

### The role of lipopolysaccharide and toll like receptors in alcoholic liver disease

The activation/inhibition of several molecular pathways is required as the pathogenesis of ALD. Toll-like receptors (TLRs) play a major role in managing the inflammation process, promoting the fabrication of several chemokine, cytokines that may contribute to tissue repair or escalate tissue damage in diseases like ALD [20]. Specific TLRs and their location (expression) are shown in Table 1 [21-25].

| TLRs | Expressed   | References  |
|------|---|---|
| TLR1 | Hepatocytes, Kupffer cells, Hepatic stellate cells, biliary epithelial cells                              | Yang L, Seki E (2012)                                 |
| TLR3 | Kupffer cells, biliary epithelial cells   | Seki et al. (2001) [23]                               |
| TLR4 | Hepatocytes, Kupffer cells, Hepatic stellate cells, biliary epithelial cells, sinusoidal epithelial cells | Seki and Brenner (2008) [24]                          |
| TLR5 | Bacterial flagellin   | Takeda K et al. (2003) [27]                           |
| TLR7 | Macrophages, dendritic cells, recognizes single stranded RNA in endosomes                                 | Wu J et al. (2010) [25], Seki and Brenner (2008) [24] |
| TLR9 | Hepatic stellate cells, sinusoidal epithelial cells   | Wu J et al. (2010) [25]                               |

**Table 1:** Mainly Kupffer cells, hepatocytes and hepatic stellate cells show the expression of TLRs in liver. Kupffer cells also express TLR2, TLR3, and TLR9 and respond to their corresponding ligands. Cultured hepatocytes respond to TLR2 and TLR4 ligands, their responses in vivo are quite weak. Biliary epithelial cells express a variety of TLRs, and at least TLR2 and TLR4 signalling activates NF- $\kappa$ B through MyD88. Liver sinusoidal endothelial cells (LSECs) express and respond to all TLR ligands except for TLR5 ligand. Hepatic DCs express all TLRs; however, TLR5 expression is low.

A Gram-negative bacteria wall component, Lipopolysaccharide (LPS) consists of Lipid A (ligand of TLR4) and O-antigen (oligosaccharide region). LPS recognition occurs through cluster of differentiation 14 (CD14) co-receptor that helps in the shifting of LPS to TLR4 and MD-2. LBP is another cofactor that commutes LPS to the

CD14 co-receptor. The composite of these supplementary molecules initiates the signal, which eventually results in the dimerization of TLR4 molecules [26].

The alternative adapter molecule as TIR-domain containing adapter-inducing interferon- $\beta$  (TRIF) helps in distinguishing TLR downstream signalling pathways (MyD88-dependent or MyD88-independent). Interaction of TLRs to their specific ligands leads to MyD88-dependent cascade resulting in the activation of activating protein-1 (AP-1) and NF- $\kappa$ B with the help of mitogen-activated protein kinases (MAPKs) and I $\kappa$ B kinase  $\beta$  (IKK $\beta$ ) complex, respectively [27]. Activation of TLR4 by LPS and signalling via MyD88-dependent and independent pathways contribute to chronic ethanol-induced liver injury. Studies reported that LPS elevates the pro-inflammatory cytokine levels by reactive oxygen species (ROS) production in macrophages and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase by means of the NF- $\kappa$ B pathway [28]. KC-dependent production and release of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-8 will further lead to the activation of immunocytes (neutrophils, monocytes and lymphocytes). Such defensive cells will react to TLR activation in Kupffer cells (KCs), secrete anti-microbial peptides, generate ROS and escalate phagocytosis [29].

Increasing in serum LPS involving TLR4 activates Kupffer cells along with the consequent inflammation in ethanol-fed rats [30]. Also, it leads to decreased steatosis, inflammation and focal necrosis in TLR4 non-functional mice fed ethanol model [31]. Studies reported that, with chronic alcohol consumption, NF- $\kappa$ B, TGF- $\beta$  and TNF- $\alpha$  level decreases in CD14 knockout mice [32]. The study demonstrated the involvement of TLR2, TLR4 and TLR5 resulted in increased steatosis, aminotransferase levels and liver microRNA (mRNA) expressions in alcohol fed mice [33]. In TLR4-knock-out alcohol-fed mice showed protection from liver damage, inflammation and ROS production [34]. ROS play a critical role in the modulation/regulation of a number of signal transduction cascades, including LPS-stimulated signaling pathways both in cells of the innate immune system (monocytes/macrophages, neutrophils, etc.) and non-immune cells. Few data suggested that the chronic ethanol exposure resulted in an increase in ROS is an important contributor to the dysregulation of LPS-mediated signal transduction and inflammatory cytokine production in Kupffer cells [35]. *In vitro* studies, direct interaction between NADPH oxidase isozyme-4 (Nox-4) and TLR4 leads to the activation of NF- $\kappa$ B and ROS generation [36]. This supports the function of MyD88 adapter in TLR4-mediated liver damage.

Some studies identified an IL-10 and heme oxygenase-1 (HO-1) dependent pathway in Kupffer cells mediates the anti-inflammatory effects of globular adiponectin (gAcrp) on LPS stimulated TNF- $\alpha$  expression [37-38]. Research showed that when mice were treated with cobalt protoporphyrin (CoPP) to induce HO-1 expression, ethanol-induced sensitivity to LPS was ameliorated [39]. Mice were treated with CoPP along with carbon monoxide releasing molecule-A1 (CORM-A1), a CORM to induce HO-1 expression during ethanol feeding or once injury had been established. This experimental result proved that induction of HO-1 or treatment with a CORM during chronic ethanol exposure protects and/or reverses ethanol-induced liver injury [39]. Study showed that chronic ethanol feeding increased LPS-stimulated TNF- $\alpha$  expression by Kupffer cells, linked with a shift to an M1 macrophage polarization. Both gAcrp and fAcrp suppressed TNF- $\alpha$  expression in Kupffer cells; however, only the effect of gAcrp was dependent on IL-10. Their data demonstrated that gAcrp and fAcrp utilize differential signaling strategies to reduce the sensitivity of macrophages to activation by TLR4 ligands, with fAcrp utilizing an IL-4/STAT6-dependent mechanism to shift macrophage polarization to the M2/anti-inflammatory phenotype [40]. Some recent literature suggested an increase in LPS (TLR4) resulting in elevation of miR-155

and TNF- $\alpha$  stability in isolated Kupffer cells in ALD mouse model [41]. Administration along with CpG and LPS involving TLR9 showed an increase plasma level of miR-155, miR-122 and miR-146 in the same mouse model [42]. In TLR4-knockout mouse model, studies also showed increased LPS results in the elevated levels of both miR-155 and miR-122 along with protection from ALD [42].

There are minimal clinical reports for the TLR contribution on hepatic inflammation in response to ALD. Studies reported that overexpression of TLR 2, 4 and 9 may play a critical role in the neutrophils dysfunction in LPS induced alcoholic hepatitis patients. It also reported that TLR antagonists were incapable of preventing neutrophils dysfunction along with the use of an endotoxin scavenger which may decrease the inflammatory response in the clinical result [43]. Studies revealed that in a stable activation of NF- $\kappa$ B transcription factor, there is an upregulation of TLR3 and TLR7 expression and elevated levels of pro-inflammatory cytokine which were linked with end stage of ALD in humans [43].

Therefore, advance discussion on lineage with the gut microbiota based treatments; as a prospective strategy for ALD patients is justified.

## Adipose Tissue and Alcohol

Another aspect of the review focuses on the current knowledge related to regulation of adipose tissue function by alcohol. Recently, it has been established that the role of adipose tissue as a key controller for all metabolic control with actions that spread beyond than just being the energy storage endocrine organ. Adipose tissue is the primary site for glucose uptake as it is necessary for glucose homeostasis. The finding of interaction with adipose tissue has been interest; as fatty acid released from adipose tissue is transported to the liver, which contributes to hepatic steatosis [44-47]. Chronic alcohol exposure amends metabolism of adipose tissue which includes inappropriate activation of lipolysis, disrupted insulin-glucose mechanism, adipokine secretion leading to the expression of the inflammatory cytokines. These changes not only affect adipose tissue but throughout the body.

Among 600 different protein or adipokines: leptin, adiponectin, and resistin are mainly measured and these play a vital role in the development of ALD. An anti-inflammatory adipokine is an adiponectin. Adiponectin has an important role in insulin sensitizing effect by altering the signaling pathway of AMP-activated protein kinase (AMPK) pathway, metabolism of glucose and fatty acid oxidation in tissues. In animal models, many data generated from chronic alcohol liver disease showed the decreased levels of circulating adiponectin [48-51]. While in humans converse results were obtained, when low or moderate level alcohol was added to the diet for short period, the result suggested serum adiponectin increase in men and women, while in chronic heavy drinker (>50 g/day) adiponectin was decreased. These results suggested that higher dose of alcohol in animals will lead to decrease in adiponectin, while in humans adiponectin level may improve; an explanation to these species specific response has not been completely studied [52-55].

Leptin receptor is found in several tissues, which regulates utilization of energy, food intake, lipid breakdown: lipolysis, fatty acid oxidation, lipid formation as well as insulin sensitivity indicating dual activity of a hormone as paracrine and autocrine function [56]. An increase in leptin receptor, leptin protein, mRNA is detected within adipose tissue in chronic alcoholic rat model [57]. While in the human decrease of serum leptin was observed following acute alcohol, but

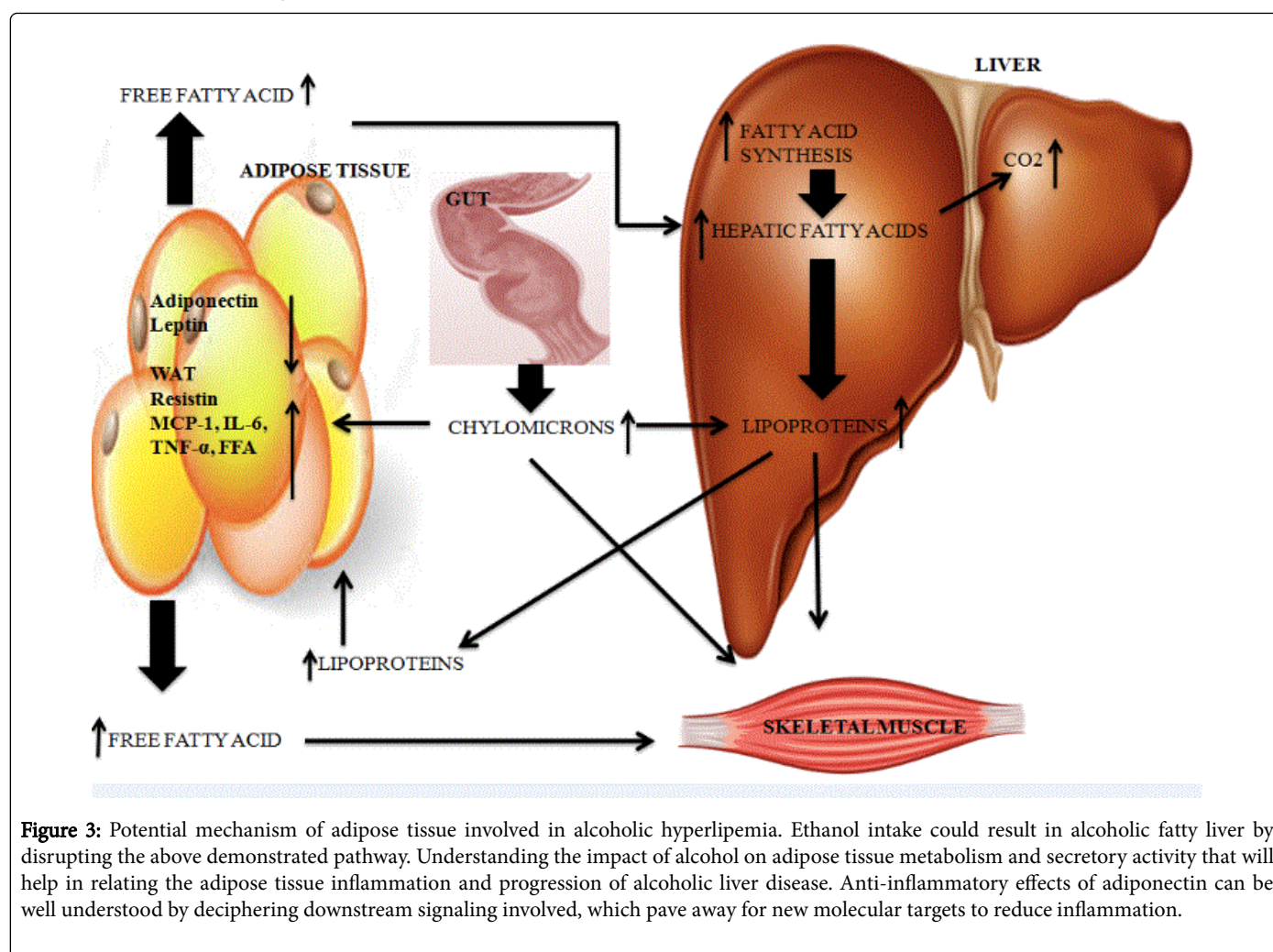


nutrient status and time of the day when a test was conducted influenced the result. Therefore, leptin levels both in serum and adipose tissue vary depending on the intake of chronic alcohol intake [58].

Resistin can develop insulin resistance and regulates food intake, thus inversely acting to adiponectin [59]. The effect of alcohol intake can be clearly demonstrated by elevation of resistin. Moreover, impaired glucose hemostasis can also be seen [60].

Adipokines like resistin, adiponectin, leptin, inflammatory mediators TNF- $\alpha$ , IL-6, MCP-1 may also play a significant role in modulating lipid metabolism. Alcohol consumption potentially disrupts lipid homeostasis at liver-adipose tissue axis metabolism results in elevated levels of adipose tissue lipolysis. As lipolysis proliferation occurs; ectopic fat deposition in liver and other organs also take place; further leading to the development of alcoholic liver

disease (Figure 3). The onset of long-term alcohol intake tries to balance the lipid metabolism, which leads to the loss of adipose tissue and fatty acid efflux. The degree of lipid accumulation depends on the dietary components like fat. Progressive changes of the mitochondria lead to down regulation of tri-carboxylic acid (TCA) activity (due to interference of free fatty acid oxidation). Alcohol assists esterification of fatty acid to triglyceride, phospholipid and cholesterol esters. Further triglycerides from adipose tissue and diet as well as those synthesized by de novo pathway are taken up by the liver which contributes to hepatic lipid accumulation [47]. It has also been known that catecholamine effects positively, while insulin affects negatively in adipose lipolysis [61]. There are reports which suggested that catecholamine is not responsible for stimulated triglyceride turnover (reversely transported and deposited in the liver) in adipose tissue but insulin mediated negative regulation is the main reason [62].



In the gut, chronic alcohol can also reduce the GLUT 4 (glucose transporter-4) availability on the membrane due to which glucose tolerance on adipose tissue is also affected. *In vivo* experiment of mice for the reflective effect of the metabolism on chronic alcohol consumption showed decreased weight of epididymal white adipose tissue (WAT), perirenal adipose tissue, mesenteric adipose tissue [46], and adipocyte size [63-64]. The former study has shown variation in the result depending on the pair-feeding technique [65-66], a

difference in the macronutrient composition of the diet, dose of alcohol, animals receiving complete nutritional adequate liquid diet [67]. *In vivo* study, performed with a high dose of alcohol (5 g/kg/day) and low fat (10%) chow diet showed elevated WAT and perirenal lipid depots [68]. Non-significant increase of WAT was seen upon administration of lower dose (2.5 and 0.5 g/kg/day) along with the same feeding pattern [69]. High alcohol dose (5 g/kg/day) along with high-fat diet (59%) delayed WAT mass to increase, indicating that

dietary factor plays an important role in modifying alcoholic effect [70]. Lower fat mass is linked with higher liver fat in alcoholics. In rodents, alcohol exposure reduces adipose mass while increase fatty acid uptake by hepatocytes [71]. Reduction in adipose mass could be linked with a reduction in uptake of triglyceride synthesis or by an increase in adipose lipolysis. A study demonstrated that chronic alcohol exposure enhances WAT lipolysis in association with activation of major adipose triglyceride hydrolases [72]. CYP2E1 is also expressed in adipose tissue, thus up regulation of CYP2E1 on alcohol consumption leads to oxidative stress and affect the metabolism [67]. *In vitro* experiment on 3T3-L1 with alcohol revealed an overexpression for CYP2E1, decreased adiponectin secretion similar to in vivo experiment in rat [61]. In another experiment, 3T3 L1 adipocytes with acetaldehyde resulted in decrease lipogenic regulators like PPAR  $\gamma$ , lipid [67].

As adipokines and lipid metabolism is getting affected simultaneously metabolic disturbances leads to the release of pro-inflammatory cytokines like TNF- $\alpha$ , IL-6, MCP-1 from adipose tissue. TNF- $\alpha$  can cause hepatic insulin resistance [73], which disrupts the WAT insulin uptake [74]. Upregulation of WAT leads to elevation IL-6 and MCP-1 expression in chronic alcohol fed rodents [68]. As a result, more macrophages are attracted to the site of inflammation leading to tissue necrosis. Finally, elevation of pro inflammatory cytokines contributes to the alcohol induced lipolysis and ectopic lipid storage up regulation. Thus lipid released by lipolysis of adipose tissue contributes to hepatic steatosis. The mechanism through which alcohol induces its action on adipose tissue is yet to be answered.

## Probiotics

Live bacteria that are good for health, particularly gastrointestinal system is termed as "Probiotics". According to WHO/FAO, 2001 define probiotics as live microorganisms that, when consumed in adequate amounts, confer a health benefit to the host. By this definition, a native bacterial species is not a probiotic until the bacteria are isolated, purified, and proven to have a health benefit when administered in proper amount [75]. The precise mechanisms for the benefits of probiotics on the gut-liver axis are not yet recognized. Many of the beneficial therapeutic effects of probiotics may result from (1) transition of the intestinal microbiota composition as well as production of antibacterial factor; (2) improvement of intestinal epithelial permeability and function; and/or (3) modulation of both immune system: local and systemic levels (4) Probiotic microorganisms may affect he micropod and cannabinoid receptors expression, as these receptors is having analgesic properties with respect to intestinal pain [76].

Current research has contributed to a potential therapeutic role of probiotics on liver health. Though the evidence suggested the effectiveness of probiotic on the alcohol liver disease in patients and on experimental models, but potential mechanism by which probiotic functions are still poorly understood. However, we found in some studies that focused on the known mechanism of probiotics in different diseases (Figure 4).

## Barrier function

A gut lumen barrier function plays a significant role in endotoxemia of gut-liver axis in multiple disease conditions. The intestinal epithelial cells form a lining sealed by tight junction and adhere junctions which is covered by mucin layer which block the direct contact of particles

[77]. Induction of alcohol will disrupt the structure of gut epithelial cells. Probiotics have the capacity to affect components of epithelial barrier function, by decreasing the phenomenon of apoptosis of intestinal cells or producing mucin. LGG and supernatant of LGG was able to prevent cytokine induced apoptosis in colonic epithelial cells by blocking TNF and ROS [78]. In another study LGG has also demonstrated that it plays a positive role in preventing inflammation and exerts mitogenic effect, while simultaneously enhancing mucosal regeneration [79]. Probiotic LGG-s administrated has also shown to decrease the levels of miR122a in the intestine, which are correlated with TNF- $\alpha$  levels that further increase occluding expression [80].

## Competition for adherence

Probiotic strains provide competitive inhibition for pathogenic bacteria for binding sites. Probiotic *L. helveticus* R0052 inhibited *E. coli* O157:H7 adherence and increases its permeability while inhibiting the growth of pathogen [81]. *S. boulardii* secretes a heat labile factor while has shown to be responsible for decreased bacterial adherence [82].

## Quorum signalling

Auto inducers help the bacteria to communicate with each other and this phenomenon is known as quorum sensing. It plays an important role in cell-to-cell signaling mechanism, which helps in the regulating traits of enteric microbes, and allows them to colonize as well as infect the host successfully. A study reported that *L. acidophilus* secretes molecule that inhibits the quorum sensing or interaction with *E. coli* O 157:H7, as a result bacterial toxicity is restricted [83].

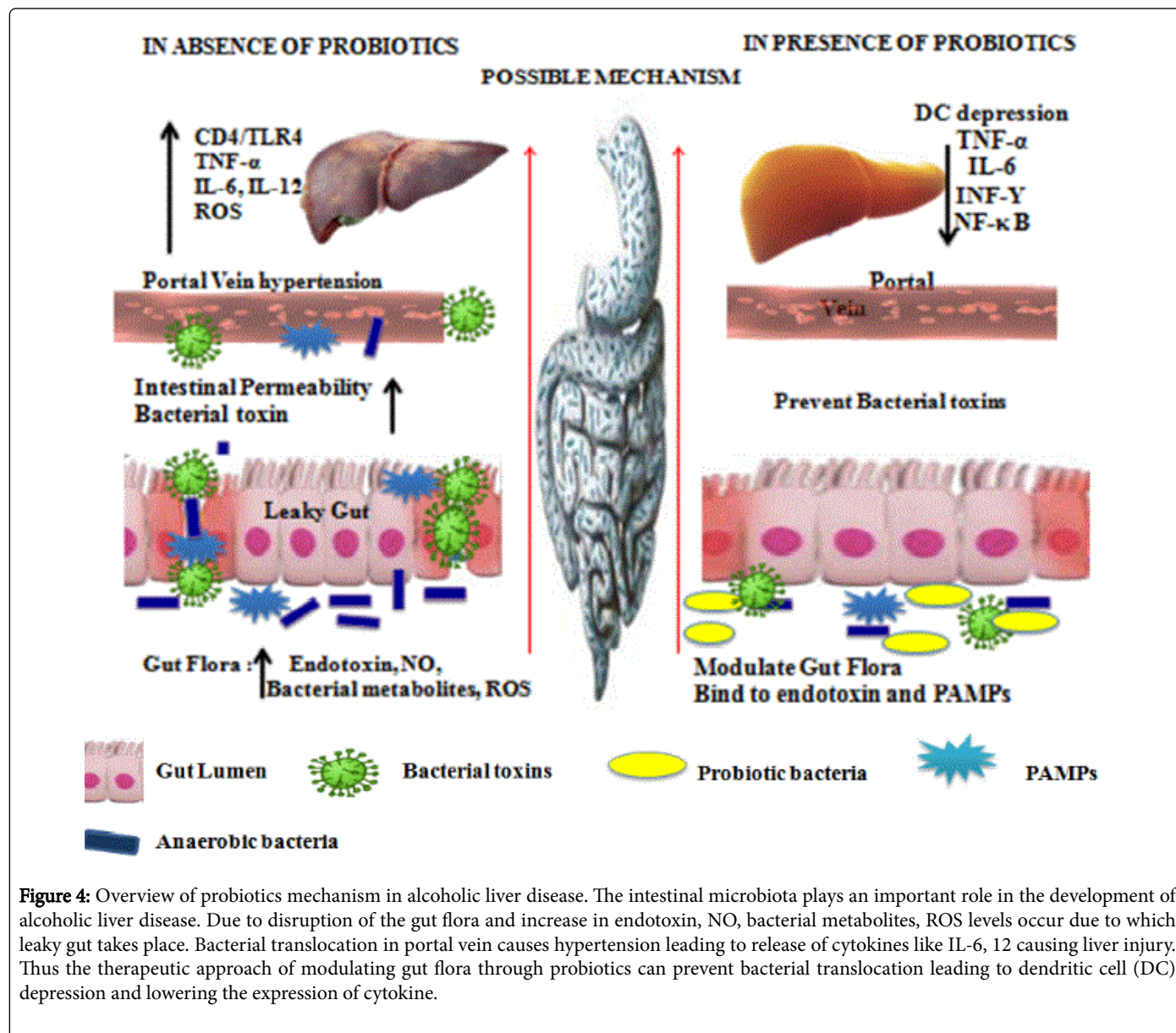
## Metabolism

Microbes mainly help to metabolize food to produce metabolites, which can be either harmful or beneficial to the host. A study demonstrated that the injection of the LCFA as energy source e.g. heptadecanoic acid (C17:0) reduced alcohol ingestion and attenuated ALD in experimental models. Upon the administration of probiotic, SCFA levels reported to be increased in the ALD model [84]. The study also claimed that LGG-s attenuates ALD by a mechanism involving increasing intestinal fatty acid and amino acid metabolism.

## Probiotic as Treatment in Alcoholic Liver Disease

Probiotics have shown to modulate gut flora and demonstrated positive effects on the alcohol induced rat experimental models depending upon the bacterial species used. Studies have shown that treatment with antibiotics to sterilize the gut and eliminate the source of endotoxin can prevent alcohol induced liver injury [85]. Probiotics also validated that it helps in decreasing ammonia production, which prevent hepatic encephalopathy in patients with cirrhosis [86]. Rat model of alcoholic steatohepatitis has also suggested that injection of probiotic strain like LGG significantly decreases the severity of liver injury [87]. The above studies strongly support the concept of the gut microbiota playing the key role in liver injury and gut leakiness that allows pro-inflammatory bacterial products to initiate alcoholic liver disease. Another study reported that the combination of LGG with oats helps in preventing alcohol induced dysbiosis [88]. Rat with acute liver injury when administered with *Bifidobacterium animalis* NM2/*Lactobacillus acidophilus* NMI/*Lactobacillus rhamnosus*/*Lactobacillus*

*rhamnosus* DSM 6594/*Lactobacillus plantarum* DSM 9843 resulted in prevention of alcohol induced dysbiosis [88].



**Figure 4:** Overview of probiotics mechanism in alcoholic liver disease. The intestinal microbiota plays an important role in the development of alcoholic liver disease. Due to disruption of the gut flora and increase in endotoxin, NO, bacterial metabolites, ROS levels occur due to which leaky gut takes place. Bacterial translocation in portal vein causes hypertension leading to release of cytokines like IL-6, 12 causing liver injury. Thus the therapeutic approach of modulating gut flora through probiotics can prevent bacterial translocation leading to dendritic cell (DC) depression and lowering the expression of cytokine.

Other probiotic strains like *Escherichia coli* Nissle, *Lactobacilli* and *Bifidobacilli* have also been used for the treatment purpose of ALD, which helps to restore the intestinal microflora, reduces endotoxins and improve liver function [89]. The results were reconfirmed with the larger study with the probiotic combination of *Lactobacillus* and *Bifidobacilli* [90]. While for the patients with mild alcoholic hepatitis showed decrease in levels of ALT, AST, lactate dehydrogenase and total bilirubin levels when treated with *Bifidobacterium bifidum* and *Lactobacillus plantarum* 8PA3 strains for 5 days [91]. Developing data also reported a beneficial effect of *Lactobacillus casei* Shirota in improving the neutrophils phagocytic capacity, which is used as an indicator for risk of infection and mortality in ALD [92]. A trial carried out on alcoholic cirrhosis patients evaluated that patients who received probiotic *L. Casei* Shirota for 4 weeks had a noteworthy decrease in TLR4, IL-10 and TNF receptors complied by restoration of neutrophils phagocytic activity, indicating that a probiotic is safe and

effective in the treatment of ALD [92]. A recent study on colonic microbiota with or without alcohol and in normal healthy individuals showed dysbiosis as *Bacteroidetes* decreases and *Proteobacteria* increases in correlation to endotoxemia [93]. *In vivo* studies, alcohol fed models was given corn oil for 1 month along with a daily dose of *Lactobacillus rhamnosus* GG at 1010 CFU/ml resulted in an improved liver pathology and lowered plasma levels of endotoxin which showed an increase intestinal barrier function [94]. In the same line study with dose of 15 g/kg/day ethanol consumption for two weeks normalized AST/ALT levels, liver function and endotoxin [95]. Mouse with Lieber DeCarli diet (5%EtOH, w/v) and heat killed *Lactobacillus brevis* SBC8803 administered orally at 500 mg/5 ml/kg/day for five weeks showed reduced serum levels of ALT, AST, TG and liver total cholesterol [96]. While administration of *Lactobacillus rhamnosus* GG at 109 CFU/mouse/day for 2 weeks along with Lieber DeCarli diet resulted an increase mucosal protecting factors, tight junction proteins,



positive modification of gut flora and desensitization of macrophage [97-99]. Similar *in vivo* study with Lieber DeCarli diet and *Lactobacillus rhamnosus* GG supernatant dose (109 CFU/mouse/day) for four weeks results in decreased hepatic steatosis and inflammation; normalization of fatty acid levels by restoration of occludin in ileum, increased hepatic AMPK activation, inhibition of hepatic fatty acid and increased concentration of amino acid [100-102].

Further, many pharmaceutical preparations using multiple strains of bacteria are currently available and indeed are new therapeutic approach. For example VSL#3, a mixture containing 450 billion bacteria of different strains has shown positive effects on liver injury by indicating the lowest level of plasma cytokines and oxidative stress markers in ALD patients, as it helps to modify intestinal microflora *in vivo* [92]. In germ free mice, study revealed that alcoholic hepatitis severity can be transferred via fecal microbiota transplantation. For the study, the characterization of dysbiosis was carried out in the patients with alcoholic hepatitis which showed that the number of *Bifidobacteria*, *Streptococci*, and *Enterobacteria* strains seems to be increased while the cluster of *Clostridium leptu* or *F. prausnitzii* strains were decreased which are known as anti-inflammatory strains of bacteria [103]. Three months treatment with VSL#3 in ten alcoholic cirrhosis patients resulted in reduced plasma ALT, AST, GGT levels; while normal plasma TNF- $\alpha$ , IL-6, IL-10 levels; and decreased MDA, 4-HNE and S-NO levels [104]. Similar study conducted in alcoholic, non-alcoholic cirrhosis and hepatic encephalopathy patients treated for 6 months resulted in reduced risk of hepatic encephalopathy and improved liver disease [105]. A study was carried out in 20 alcoholic liver patients given a dose of *Lactobacillus casei* Shirota for four weeks showed normalized phagocytic capacity, decreased TLR 4, sTNF R1, sTNF R2 and IL-10 levels [106]. Treatment with *Escherichia coli* Nissle in 34 patients resulted in improvement of intestinal colonization, reduced endotoxin levels in blood and restored microflora in 42 days [107]. A mixture of different lactic acid bacterial strains given to alcoholic patient resulted in positive effect on balance of commensal bacteria, reduced ALT, TNF- $\alpha$  level [108]. Combination of *Bifidobacterium bifidum* and *Lactobacillus plantarum* 8PA3 for 5 days of treatment demonstrated increased colonization of *Bifidobacteria* and *Lactobacilli*, reduction in ALT, AST, LDH, and total bilirubin levels [109].

Diet has the potential to either improve or enhance the disease condition. An interesting study performed *in vivo* with an unsaturated diet (corn oil) aggravated ethanol induced endotoxemia and negatively affects liver disease in comparison to saturated diet (triglyceride). These results were also correlated with reduction in *Bacteroidetes* strains and increased *Proteobacteria* and *Actinobacteria* strains [110].

However, these treatments can only be effective when the live microbes must colonize the gut and confer their beneficial effects, but the complication is that the pathogenic bacteria level varies from patient to patient. As well as the standard treatment which uses antibiotics are harmful for the live microbes, therefore the variable effect may occur on the administration of live probiotics.

A symbiotic study carried out for two months with different bacteria strains and a prebiotic in ALD patients whose daily intake was 150 g, significantly improved liver damage and function when compared to basal values [111]. Further, the same group also reported the effect of VSL#3 treatment, which drastically improved the plasma levels of MDA, 4-HNE, TNF- $\alpha$ , IL-6 and IL-10 in alcoholic cirrhosis patient [111]. Pharmacotherapy approach is also being used in ALD patients where the specific drug treatment including glucocorticoids,

pentoxifyline were given. But, long term use of this drug in chronic alcoholic patient has not been approved yet due to complication of fibrosis. Though, there is a future perspective in which those drugs can be combined with probiotics, prebiotics, caspase inhibitors, osetopontin, and endocannabinoids to treat alcoholic patients [112]. Along with the line of symbiotic approach, *in vivo* study was performed with Microstructured Synbox system (a phenolic compound: epigallocatechin gallate (EGCG which is prebiotic for *L. plantarum*) resulted in effective therapy for ALD [113]. Microbiome composition, growth, function are associated with metabolite profile affecting the host immune metabolic systems which eventually disrupts metabolic homeostasis [114].

## Conclusion

Alcoholic consumption remains to be one of the predominant causes of liver disease and liver-related death worldwide. The gut microbiota, metabolites and adipose tissue response prove to be one of the key factors that added to the pathogenesis of ALD. Identification of alternative pathways connecting the gut microbiota to ALD is challenging, but could be an important key for an improved perceptible towards gut-liver axis. TLR4 mediates the progression of alcoholic liver injury, most likely by responding to higher levels of circulating endotoxin. Therefore, according to pharmacological strategy, i.e. targeting the endotoxin-CD14/TLR4 signalling pathways may prove to be beneficial in ALD. Although the researchers showed positive effects of probiotics in animal experimental studies as well as in patients, there are few limitations to the probiotic treatment approach. The functional mechanism of probiotic is specific to strains, thus recognizing the exceptional strains with the characteristic of highest prophylactic properties and few preventing properties on liver disease is still required. Similarly, engineered probiotic strains whose viability and stability in the gut should be taken into consideration for the treatment of ALD.

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