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Alcohol Induced Gut Microbiota Modulation: The Role of Probiotics, Pufas, and Vitamin E in Management of Alcoholic Liver Disease

Faruqui AA*

Department of Pharmacology, Clinical Pharmacologist, Maharashtra, India

***Corresponding author:**

Arif A. Faruqui,
Department of Pharmacology,
Clinical Pharmacologist, Maharashtra, India
E-mail: drfaruqui@gmail.com

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1. Abstract

Chronic alcoholism is a global healthcare problem resulting in 3.3 million deaths or 6% of all deaths. In India, the alcohol consumption rates range from 23-73% in males and 24-48% in females. The liver suffers the greatest degree of injury, because it is the primary site of its metabolism, along with gut and brain. Alcohol metabolism in intestines may result in disruption of tissue homeostasis causing a chronic state of intestinal inflammation. Chronic alcohol consumption increases permeability of gut making it "leaky" or "permeable", allowing pathogens and other noxious materials to enter into the bloodstream. Once gut leakiness begins, endotoxins can enter the liver via the portal vein that drains from the gut. Approximately 20-30% of heavy drinkers develop clinically significant Alcoholic Liver Disease (ALD), including alcoholic steatohepatitis and cirrhosis. Treatment with probiotics prevent or significantly decrease alcohol-induced intestinal permeability, intestinal oxidative stress, inflammation of the intestine and liver, TNF-α production, expression of intestinal trefoil factor and attenuates endotoxemia and alcoholic steatohepatitis in humans with ALD. n-3 PUFAs may be useful in alleviating alcoholic steatosis and alcohol-induced liver injury through multiple pathways. Vitamin E deficiency has been well documented in ALD. Vitamin E supplementation has demonstrated hepatoprotective capabilities in the management of ALD. The current review provides an insight to alcohol induced gut microbiota modulation and its association with ALD. Moreover, the current review discusses the role of probiotics, n-3 PUFAs, and Vitamin E in restoring the gut function and management of ALD.

2. Introduction

Alcohol-associated worsening of systemic functions is the most common preventable disease worldwide. Globally elevated alcohol consumption results in 3.3 million deaths or 6% of all deaths are associated with alcohol [1]. In India; the use of alcohol is common in both, rural and urban regions. As per various clinical trials these rates range from 23-73% in males. The consumption of alcohol though being uncommon in females, the prevalence ranges from 24-48% in certain sections and communities [2].

Alcohol causes damage to internal end-organs, particularly liver, gut and brain. This damage to end-organ is individualized and unpredictable. About 20-30% of the people who misuse alcohol are likely to develop liver damage that may further worsen into liver cirrhosis or alcoholic hepatitis. Alcoholic Liver Disease (ALD) is a spectrum of disease that ranges from asymptomatic liver steatosis to fibrosis, cirrhosis and alcoholic hepatitis [1].

Gastrointestinal tract is the first-line of contact to ingested materials and is at risk when toxins are ingested. Therefore, materials causing GI damage may produce far harmful effects beyond intestine. Alcohol if consumed in larger amounts induces process in the gut that is responsible for inflammation throughout the body [3]. Current studies demonstrate the role of gut microbial milieu in the progression of ALD [1]. This review focuses on the effects of alcohol consumption on the gut microbiota and the consequences of microbiota dysbiosis on the liver resulting in ALD. The current

review also focuses on the role of probiotics, vitamin E and omega-fatty acids in prevention and treatment of ALD.

3. Alcohol Effects on Gut

Alcohol after consumption is absorbed in upper gastrointestinal tract by diffusion. Post-absorption alcohol enters the liver through the portal vein; therefore, the effect of alcohol on distal small intestine and colon is associated with its circulatory levels. Although the most part of alcohol metabolism occurs in hepatocytes, the enzymes resulting in oxidative metabolism of alcohol are seen in the intestinal mucosa. The intestinal mucosa is also responsible for formation of acetaldehyde in intestinal cells. Moreover, less common non-oxidative alcohol metabolism occurs in the intestines through reactions with membrane phospholipids and/or free fatty acids. This alternative pathway may be responsible for intestinal injury post chronic alcohol consumption. Therefore, alcohol metabolism through oxidative and non-oxidative pathways in intestines may result in disruption of tissue homeostasis resulting in chronic state of intestinal inflammation [3].

4. Alcohol Metabolism in Liver

Alcohol is majorly metabolized in parenchymal cells (hepatocytes) of the liver. Hepatocytes consist of highest levels of enzymes responsible for metabolism of ethanol (ethanol oxidizing enzymes, alcohol dehydrogenase (ADH)). These enzymes are located in the cytosol, and cytochrome P450 2E1 (CYP2E1), which is present in the smooth endoplasmic reticulum (Figure 1) [4].

4.1. Mechanism of Alcohol Metabolism [4]

- Alcohol Dehydrogenase (ADH) (in cytosol) and aldehyde dehydrogenase 2 (in mitochondria) catalyze sequential oxidations that convert ethanol to acetate by producing two mole equivalents of reduced nicotinamide adenine dinucleotide (NADH) (Figure 1).
- In the endoplasmic reticulum, CYP2E1 is a major inducible oxidoreductase that potentially oxidizes ethanol to acetaldehyde in the presence of molecular oxygen and converts reduced NAD phosphate (NADPH) to its oxidized form, by releasing water (Figure 1).
- A minor hepatic pathway of ethanol oxidation is peroxisomal catalase which uses hydrogen peroxide (H_2O_2) to oxidize ethanol to form acetaldehyde and water (Figure 1).

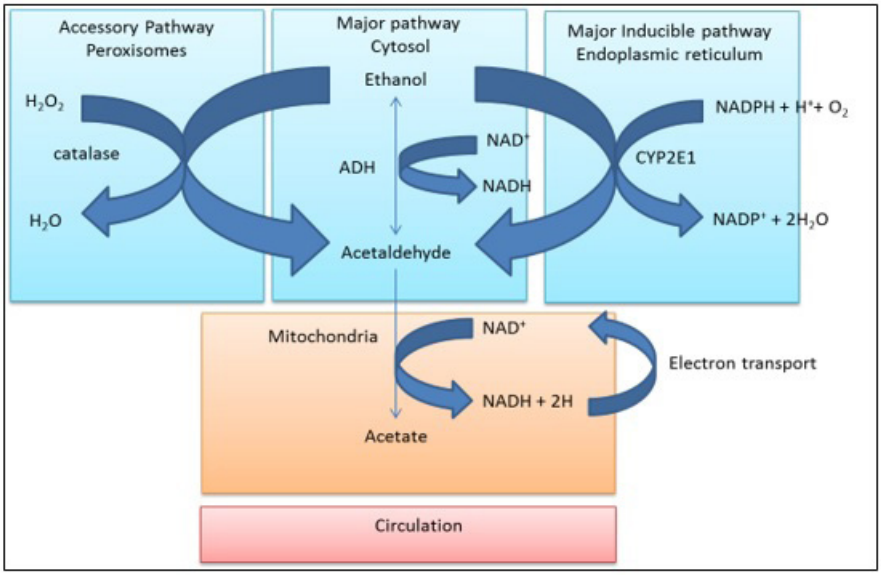

Figure 1: Major and minor pathways for alcohol metabolism [4] Abbreviations: ADH - Aldehyde dehydrogenase; NAD - Nicotinamide Adenine Dinucleotide; NADH - reduced Nicotinamide Adenine Dinucleotide; NADPH - reduced Nicotinamide Adenine Dinucleotide phosphate.

5. Alcohol - Induced Gut Inflammation

5.1. Alcohol Induced Intestinal Bacterial Overgrowth and Dysbiosis

Intestine consists of more than 500 species of bacteria and achieves bacterial homeostasis by creating a balance between good bacteria and pathogenic bacteria. This bacterial balance is disrupted by environmental or diseased condition resulting in bacterial 'dysbiosis'. Alcohol is reported to produce dysbiosis and overgrowth of gram-negative bacteria which release endotoxins. Endotoxins are responsible for activation of inflammatory mediators responsible for gut inflammation [3].

5.2. Bacterial Overgrowth

Bacterial overgrowth is stimulated directly by alcohol; however, some studies suggest that it could be an indirect by-product of poor digestive and intestinal function caused by alcohol consumption. Studies have also demonstrated a connection between alcohol, bile acid, and bacterial overgrowth. Alcohol may affect the bile acid metabolism which may in turn affect the intestinal bacteria [3].

5.3. Bacterial Dysbiosis

Clinical studies and molecular studies have demonstrated that alcohol consumption may alter the ratio between good bacteria and pathogenic bacteria. Connecting dysbiosis to alcohol-induced

health problems, several studies suggest that probiotic interventions attenuate liver injury in rats and liver dysfunction in cirrhotic patients.Alcohol-induced bacterial overgrowth elevates the risk of inflammation as intestinal bacteria can independently metabolize alcohol, producing excess acetaldehyde in the colon causing increased production of pro-inflammatory alcohol metabolites [3].

5.4. Alcohol Induces Intestinal Hyperpermeability

The intestinal barrier is responsible for regulating exchange of materials between GI tract and the bloodstream, allowing absorption of nutrient materials and preventing the absorption of noxious substances. It is made up of a layer of water, mucous gel, and epithelial and connective tissue. Chronic alcohol consumption may cause increased permeability of the epithelial layer making it "leaky" or "permeable," allowing pathogens and other noxious materials to enter into the blood stream. Studies demonstrate people with Alcohol Use Disorder (AUD) have increased intestinal permeability and are more likely to have liver disease (Figure 2) [3].

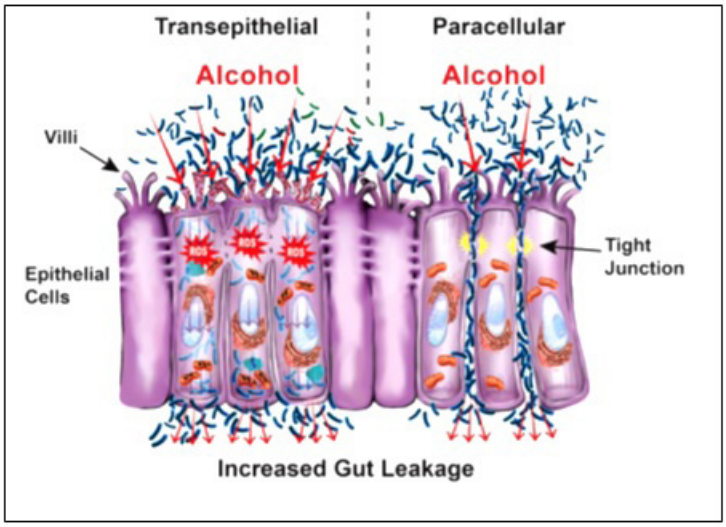

Figure 2: Alcohol induced intestinal hyper permeability [3].

6. Role of Gut-Liver Axis in Alcoholic Liver Disease

Approximately 20 - 30% of heavy drinkers develop clinically significant alcoholic liver disease, including alcoholic steatohepatitis and cirrhosis. Several factors, such as the amount and duration of alcohol consumption, obesity, and gender, may increase a person's risk for alcoholic liver disease. In addition, studies have reported that alcohol-induced gut inflammation can contribute to liver injury by increasing intestinal permeability and the likelihood that gut-derived endotoxins enter the liver [3].

A study found that people with AUD and liver disease are much more likely to have intestinal permeability: >40 times more likely than people without AUD and >20 times more likely than people with AUD who do not have liver disease [5].

Once gut leakiness begins, endotoxins can enter the liver via the portal vein that drains from the gut. In the liver, gut-derived substances interact with the liver's hepatocytes, parenchymal cells, and immune cells. Alcohol exposure increases LPS levels in portal and systemic circulation and that can have a host of deleterious effects as depicted in (Figure 3).

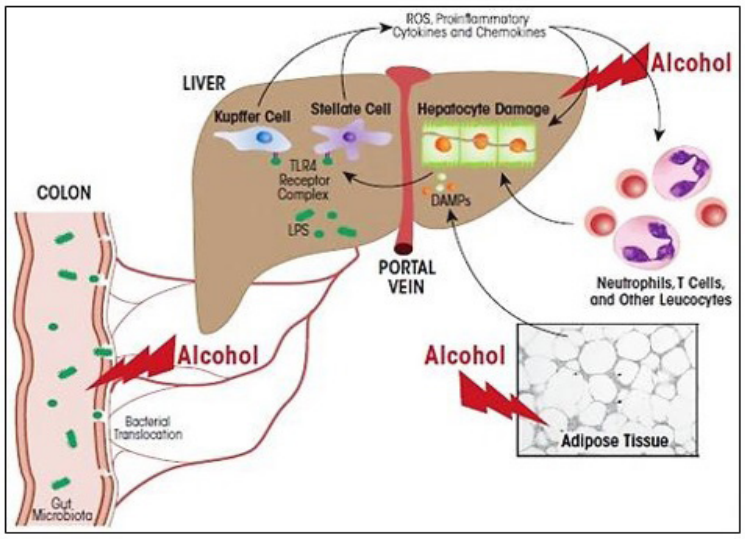

Figure 3: The gut–liver axis [4].

In the liver, LPS activates Kupffer cells and Hepatic Stellate Cells (HSCs) by interacting with toll-like receptor 4 (TLR4). These cells produce Reactive Oxygen Species (ROS) as well as pro-inflammatory cytokines and chemokines that together with alcohol contribute to hepatocyte damage. Other factors contributing to hepatocyte damage include alcohol-induced activation of various immune cells (i.e., neutrophils, T cells, and other leukocytes) as well as alcohol's effects on the adipose tissue, which results in production of damage-associated molecular pattern (DAMP) molecules [4].

7. Mechanisms Involved in Liver Fibrosis/Cirrhosis

Hepatic Stellate Cells (HSCs) are key players in the development of fibrosis. Chronic ethanol consumption initiates a complex activation process that transforms these quiescent HSCs into an activated state. Activated HSCs secrete copious amounts of the scar-forming extracellular matrix proteins (e.g., collagen type 1). This, in turn, contributes to structural changes in the liver, such as the loss of hepatocyte microvilli and sinusoidal endothelial fenestrae, ultimately forming scar tissue (fibrosis) that can progress to cirrhosis. The scar tissue forms bands throughout the liver, destroying the liver's internal structure and impairing the liver's ability to regenerate itself and to function (Figure 4) [4].

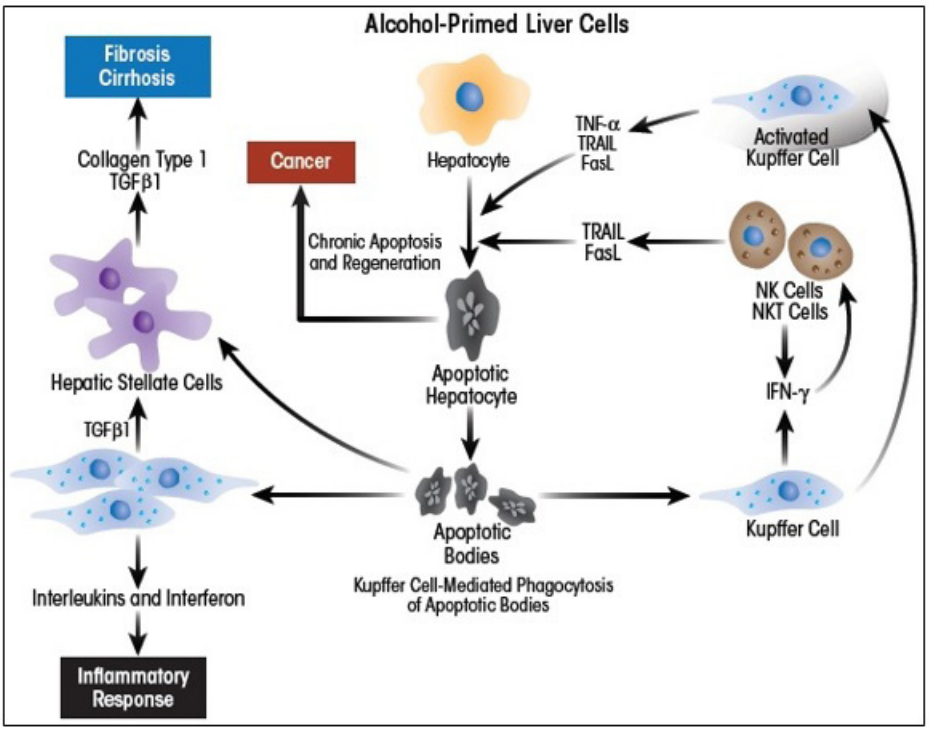

Figure 4: Role of Kupffer cells and hepatic stellate cells in promoting alcohol-induced inflammatory changes and progression to fibrosis and cirrhosis [4].

TNF α-tumor necrosis factor alpha, IFN-γ - interferon gamma, TRAIL - tumor necrosis factor-related apoptosis-inducing ligand, FasL - Fas ligand, NK cells - Natural killer cells, NKT cells - Natural Killer T cells.

8. Probiotics

WHO defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [6]. The physiological functions of probiotics are achieved directly or indirectly by adjusting the composition of the host intestinal microbiota, activating the endogenous microbial community and regulating the immune system. Oral probiotics can cure or alleviate a variety of gastrointestinal diseases. Some studies have confirmed that oral probiotics effectively alleviate lactose intolerance; prevent gastroenteritis, constipation, and diarrhea; and modulate the gut microbiota [7].

Therapeutic intervention studies indicate that in ALD rodent models and Minimal Hepatic Encephalopathy (MHE) alcohol-cirrhosis humans, probiotic and synbiotic intervention increases *Lactobacillus* and *Bifidobacterium*. These findings suggest that the intestinal microbiota play a role in attenuating alcohol-induced dysbiosis and liver injury. In addition, the modulation of intestinal microbiota could be a viable therapeutic strategy to prevent or normalize alcohol-induced dysbiosis and which would be expected to have beneficial effects on alcohol-induced liver injury as well as other inflammatory-mediated diseases resulting from chronic alcohol consumption [8].

Treatment with probiotics prevent or significantly decrease alcohol-induced intestinal permeability, intestinal oxidative stress, inflammation of the intestine and liver, TNF-α production, expression of intestinal trefoil factor and its transcriptional regulator

hypoxia-inducible factor-2α (HIF-2α) and attenuates endotoxemia and alcoholic steatohepatitis in rodent models and in humans with ALD. Probiotics also restore stool microbiota community structure and liver enzymes in ALD human patients. Thus, these studies suggest that probiotics transform the intestinal microbiota community composition, which may prevent alcohol-induced dysbiosis, intestinal permeability, bacterial translocation, endotoxemia, and the development of ALD. Transformation of the intestinal microbiota may be a therapeutic target for the treatment of intestinal barrier dysfunction and the development of ALD [8].

The most common probiotic representatives are *Lactobacillus* and *Bifidobacterium* that promote anti-inflammatory environment by enhancing intestinal epithelium growth and survival as well as counteract the pathogenic bacteria by producing anti-microbial agents and modulating immune system and host defence [6].

8.1 Mechanisms of Probiotics in Improvement of Alcoholic Liver Injury

8.1.1. Enhance Intestinal Barrier Function and Modulate Mucosal Immune System: Probiotics directly or indirectly affect intestinal epithelial cells, strengthening intestinal mucosal barrier function and regulating the immune system. Probiotics significantly increase the content of short-chain fatty acids (SCFA) that provide the energy metabolic substrate in the intestine and promote absorption of sodium ions, proliferation of colon cells, and intestinal mucous growth. Probiotics also promote intestinal barrier integrity via enhancing the expression of tight junction (TJ) proteins claudin-3 and occludin, thereby ameliorating alcohol liver injury [7].

Alcohol consumption decreases the expression of the hypoxia-inducible factor (HIF). HIF is a master transcription factor involved in maintaining barrier function. It increases the expression of the intestinal trefoil factor (ITF), xenobiotic clearance by P-glycoprotein (P-gp), and various other nucleotide signaling pathways. Probiotics preserve barrier function by increasing HIF expression in chronic and acute alcohol liver diseases [7].

8.1.2 Regulate Intestinal Flora: One of the major mechanisms of probiotic function is alteration of gut microbiota. Gut microbiota plays a key role in the immune system. Imbalance of the intestinal microbiota stimulates the immune system, promoting chronic liver inflammation. *Bifidobacteria* and *Lactobacillus* are the predominant genera when there is a significant difference between the host and individual bacterial species, which prevents the growth of anaerobic Gram-positive bacteria, inhibits the growth of Gram-negative bacteria, enhances phagocytic activity, and promotes the secretion of IgA, thereby enhancing cellular immune function. Supplementation with probiotics naturally produce aryl hydrocarbon receptor (AhR) ligands, promote IL-22 production and intestinal regenerating islet-derived 3 gamma (Reg3g) expression mediated by IL-22 which improves alcohol-induced liver injury and prevents microbiota disorder [7].

8.1.3. Reduce Inflammatory Cytokine Expression: Alcohol-induced barrier dysfunction results from local and systemic production of proinflammatory cytokines such as TNF-α and IL-1β. Once the gut barrier function is compromised, there is translocation of bacteria and bacterial products released into the blood; subsequently, a large number of Kupffer cells accumulate and activates toll-like receptors (TLRs) on the surface of liver. Kupffer cells combine with endotoxin to activate mitogen-activated protein kinase (MAPK) and nuclear factor $\kappa\beta$ (NF-$\kappa\beta$), producing inflammatory cytokines, including TNF-α and interleukin (IL-6, IL-1β).

Probiotics decrease alcohol-induced inflammatory cytokine (TNF-α) production via inhibition of TLR-mediated endotoxin activation [7].

8.1.4. Antioxidant Activity: Reactive oxygen species (ROS) are highly reactive oxygen-containing molecules, ions, or groups that interact with one another and damage cellular molecule complexes, especially in the liver. Alcohol consumption induces overproduction of ROS and inhibits fatty acid oxidation in the liver, leading to ROS-mediated liver injury [7].

Alcohol disrupts intestinal barrier and increases its permeability in two ways: via transepithelial mechanisms, which allow material to pass directly through the epithelial cells, and paracellular mechanisms, which allow material to pass through the junctions between the epithelial cells. Alcohol and its metabolites trigger transepithelial mechanisms by damaging the cells directly and weakening cell membranes via several mechanisms including oxidative stress caused by Reactive Oxygen Species (ROS). Alcohol's metabolites trigger paracellular mechanisms by disrupting the proteins that create the tight junctions linking cells and proteins that stabilize cells' cytoskeletons. Increased permeability of the intestinal barrier allows bacteria and the toxins they create to leave the gut and infiltrate other organs through the bloodstream [3].

Probiotics reduce oxidative stress and promote the production of antioxidants to alleviate alcohol-induced liver injury [7] Probiotics could also contribute to intestinal barrier function by modulating certain gut bacteria leading to reduced metabolism of alcohol and ROS production in the intestine [9].

8.1.5. Improve Alcohol-Induced Lipid Metabolism: The adenosine monophosphate-activated protein kinase (AMPK) signaling pathway is an important energy metabolic pathway characterized by a series of cascade reactions that activate catabolism and deactivate anabolism. AMPK regulates lipid metabolism by manipulation of several transcription factors. Chronic or acute alcohol consumption decreases AMPK and acetyl-CoA carboxylase phosphorylation and increases malonyl-Co-A production, leading to abnormal lipid metabolism in the liver [7].

Probiotics may function as a direct mediator in regulating hepatic lipid metabolism and apoptotic cell death. Probiotic administra-

tion prevents alcohol-increased expression of genes involved in lipogenesis and alcohol-decreased genes involved in fatty acid β-oxidation. Importantly, these lipid regulatory effects were mediated through probiotic action on AMPK phosphorylation. Probiotics also decreases Bax expression and increases Bcl-2 expression, which attenuates alcohol-induced hepatic apoptosis. Thus, probiotics likely exert their beneficial effects, at least in part, through modulation of hepatic AMPK activation and Bax/Bcl-2-mediated apoptosis in the liver [9].

9. Eicosapentaenoic Acid (Epa) and Docosahexaenoic Acid (Dha)

EPA ($C_{20}H_{30}O_2$; 20:5n-3) and DHA ($C_{22}H_{34}O_2$; 22:6n-3) are long-chain ω-3/n-3 polyunsaturated fatty acids (PUFAs). EPA is a non-essential n-3 fatty acid as the human body can convert essential n-3 alpha-linolenic acid (ALA) into EPA and DHA. However, this conversion is not efficient enough in humans to meet the EPA and DHA demand to impart beneficial health effects; thus, it is expected to obtain these fatty acids from dietary sources. EPA and DHA are among the most studied bioactive compounds of marine origin; fish oil being the major and well-known source. Further, due to its recognized health benefits and recommendation by several health agencies, primarily DHA and EPA have become very popular as dietary supplements [10].

9.1. Beneficial effects of EPA and DHA

EPA and DHA have been healthy nutritional supplements for long time due to numerous health benefits summarized in figure 5 [10].

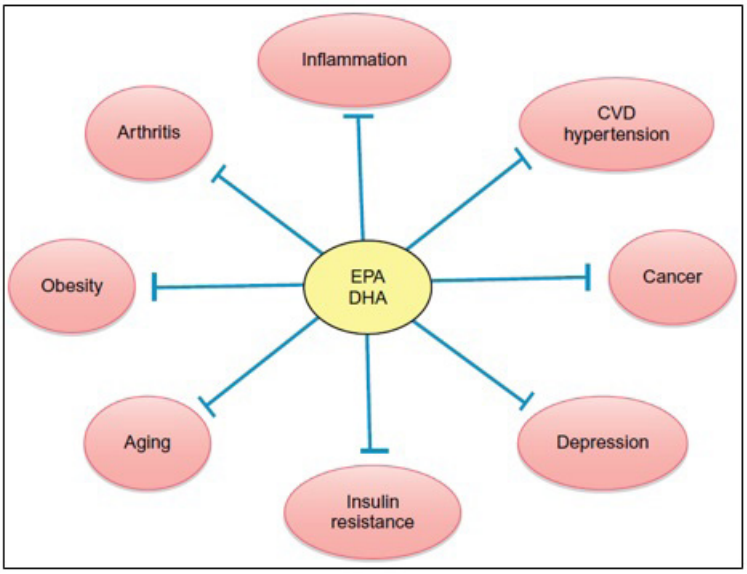

Figure 5: Beneficial effects of EPA and DHA [4].

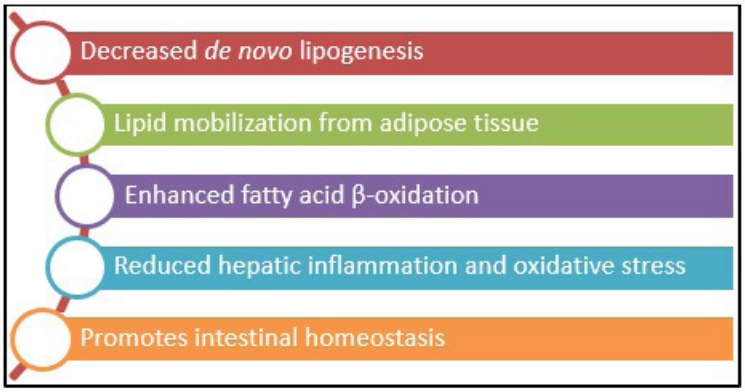

Figure 6: Mechanisms of EPA and DHA in ALD

9.2. EPA and DHA in ALD

n-3 PUFAs may be useful in alleviating alcoholic steatosis and alcohol-induced liver injury through multiple mechanisms mentioned in figure 6 [11].

10. Vitamin E

As demonstrated earlier, oxidative stress plays a key role in the pathogenesis of ALD. Alcohol metabolism includes increased synthesis of reduced form of nicotinamide adenine dinucleotide

hydrogen and suppression of mitochondrial β oxidation and increased lipid peroxidation in liver. This liberates oxygen-free radicals and decrease in mitochondrial glutathione and S-adenosyl-L-methionine levels, thus depleting the endogenous antioxidant capabilities [12].

Vitamin E deficiency has been well documented in ALD. Alcoholics with cirrhosis often have low Vitamin E levels in the liver. Some observational studies also suggest that Vitamin E deficiency increases the liver's vulnerability to alcohol. Vitamin E has experimentally proven hepatoprotective capabilities including membrane stabilization, reduced nuclear factor-kappa B (NF-κB) activation, reduced tumor necrosis factor (TNF) production, and inhibition of hepatic stellate cell activation which are the primary pathological factors involved in the development of ALD. An antioxidant like Vitamin E could likely be beneficial in patients with ALD having least adverse effects and is economical [12].

Table 1: Role of n-3 PUFAs in ethanol-induced liver injury

n-3 PUFAs	ALD Model	Animal	Hepatotoxicity	Mechanisms
Endogenous n-3 PUFAs	Chronic-plus-single binge ethanol feeding	fat-1 mice	↓AST; ↓ALT; ↓TB; ↓ALP; ↓TG; ↓TC	Adipose lipolysis: ↑GPR120, ↑CaMKKb, ↑PDE3B, ↓p-AMPK, ↓cAMP, ↓HSL, ↓ATGL; Adipose inflammation: ↓IL-1b, ↓TNF-α; Hepatic FFA uptake: ↓CD36, ↓FATP5, ↓FATP2; Hepatic inflammation: ↓IL-1b, ↓TNF-a, ↓MCP-1 [17]
Endogenous n-3 PUFAs	3 acute doses of ethanol every 12 h	fat-1 mice	↓ALT; ↓TG; ↑HDL-C	Lipogenesis: ↓SREBP-1c, ↓FAS, ↓SCD-1; β-oxidation: ↑PPARα; Inflammation: ↓IL-6, ↓TNF-α, ↓MCP-1; Oxidative stress: ↓CYP2E1, ↑HO-1 [18]
DHA	3 acute doses of ethanol every 12 h	C57BL/6 mice	↓TG; ↓ALT	Lipogenesis: ↓SCD1; Inflammation: ↓IL-6, ↓TNF-a; Oxidative stress: - ROS, ↑HO-1 [19]
DHA	50mM ethanol for 1, 1.5 or 5 h	Adult rat hepatocytes	N.A.	Oxidative stress: ↓ROS, ↓4-hydroxy-nonenal, -gluthatione peroxidase, -glutathione [20]
EPA	50mM ethanol for 1, 1.5, or 5 h	Adult rat hepatocytes	↑TC	Oxidative stress: ↑ROS, ↑MDA, ↑C11 Bodipy oxidation [21]

IL-1β, interleukin-1β; TNF-α - tumor necrosis factor-alpha; CD36 - fatty acid translocase; FATP2 - fatty acid transporter protein 2; MCP-1 - monocyte chemo-attractant protein-1; SREBP-1c - sterol regulatory element binding protein-1c; FAS - fatty acid synthase; SCD-1 - stearoyl-CoA desaturase; PPARα -peroxisome proliferator-activated receptor α; IL-6 - interleukin-6; HO-1 - heme oxygenase-1; MDA – malondialdehyde; C11-BODIPY, a polyunsaturated fatty acid-like probe.

11. Evidences of Probiotics, Epa and Dha, and Vitamin E in Ald

11.1. Probiotics in ALD

Dhiman et al. reported that daily intake of probiotic VSL#3 for 6 months significantly reduced the risk of hospitalization for hepatic encephalopathy (HE), as well as Child–Turcotte–Pugh and model for end-stage liver disease scores, in patients with cirrhosis. Patients with cirrhosis who had recovered from an episode of HE during the previous month were randomly assigned to a probiotic preparation (VSL#3, 9 X 10^{11} bacteria) (n = 66) or placebo (n = 64) daily for 6 months. The study showed a reduction in the development of breakthrough HE in patients receiving probiotic (34.8% in the probiotic group vs. 51.6% in the placebo group; hazard ratio [HR], 0.65; 95% confidence interval [CI], 0.38–1.11; p = 0.12). Fewer patients in the probiotic group were hospitalized for HE (19.7% vs. 42.2%, respectively; HR, 0.45; 95% CI, 0.23–0.87; P = 0.02) or for complications of cirrhosis (24.2%) than in the placebo group (45.3%) (HR - 0.52; 95% CI - 0.28–0.95; p = 0.034). Child–Turcotte–Pugh and model for end-stage liver disease scores improved significantly from baseline to 6 months in the probiotic group, but not in the placebo group. There were no adverse events related to VSL#3 [13].

Lata et al. demonstrated in a double-blind, randomized study that treatment with a probiotic *Escherichia coli* Nissle for 42 days in 34 cirrhosis patients (19 on probiotics; 15 on placebo) who had an alcoholic etiology of their cirrhosis improved colonic colonization and liver function. The study found a trend of significant lowering of the endotoxemia (p = 0.07) and improvement of liver functions evaluated by Child-Pugh score (p = 0.06) [14].

Data from a small open-labelled study by Stadlbauer V et al. provide a proof-of-concept that probiotics restore neutrophil phagocytic capacity in cirrhosis, possibly by changing IL10 secretion and TLR4 expression. The study evaluated the effectiveness of the probiotic *Lactobacillus casei* Shirota on alcoholic cirrhosis (AC) patients (n = 12) and healthy controls (n = 13). Compared to control group, cirrhotic patients who received the probiotics for 4 weeks had a significantly lower TLR4 expression and Il-10, sTNFR1 (soluble TNF receptor), and sTNFR2 levels, along with a restored neutrophil phagocytic activity which suggested that the probiotic is safe and may be effective in the treatment of patients with defective immunity [15].

Kirpich et al. demonstrated that, short-term oral supplementation with *Bifidobacterium bifidum* and *Lactobacillus plantarum* 8PA3 was associated with restoration of the bowel flora and greater improvement in alcohol-induced liver injury than standard therapy alone. In this open-labelled study 66 patients who were diagnosed with alcoholic psychosis and liver disease randomly received 5 days of *Bifidobacterium bifidum* and *Lactobacillus plantarum* 8PA3 or standard therapy alone (abstinence plus vitamins). Results were compared between groups and with 24 healthy, matched controls who did not consume alcohol. Mild alcoholic hepatitis patients had a significant end-of-treatment reduction of ALT and AST, lactate dehydrogenase, and total bilirubin. Compared to standard therapy, probiotic treatment significantly reduced serum ALT. This liver function improvement was associated with changes in the fecal commensal bacteria *Bifidobacteria* and *Lactobacilli* [16].

11.2. EPA and DHA in ALD

Studies on n-3 PUFAs, EPA and DHA have revealed potential role in ALD. The liver with large lipid accumulation (hepatic steatosis) is more susceptible to further injury that leads to advanced stages of ALD. Thus, reducing lipid accumulation in liver is an important strategy to halt or postpone the progression of ALD [11]. (Table 1) lists studies of n-3 PUFAs in ethanol-induced liver injury and their outcomes.

11.3. Vitamin E in ALD

Oxidative stress plays a key role in the pathogenesis of ALD and alcoholics with cirrhosis often have low vitamin E levels in the liver. A prospective, open labeled, randomized comparative study of eight weeks by Kolasani BP et al. showed that vitamin E will be a useful addition for treating alcoholic liver disease. The study enrolled 30 adult patients with ALD who received standard treatment (group A) and vitamin E (group B) along with standard treatment. Biochemical parameters like Liver Function Tests, De Ritis Ratio, Hb and TLC; prognostic parameters like Child Pugh Score and Model for End-Stage Liver Disease score were recorded before and after the treatment period in each group and compared. In group A, the change observed in total protein and child pugh score were significant ($p < 0.05$) whereas that seen in prothrombin time (PT) was highly significant ($p < 0.001$). In group B, the changes observed in total protein, A: G ratio, bilirubin, PT, model for end-stage liver disease (MELD) score, hemoglobin (Hb), total leukocyte count (TLC) were significant ($p < 0.05$) whereas those seen in albumin, PT-INR, Child Pugh Score were highly significant ($P < 0.001$). The changes in albumin, globulin and A: G ratio observed in Group B were statistically significant compared to their respective changes observed in Group A [12].

12. Rationale for The Combination of Probiotics, Pufas and Vitamin E

- Probiotics restore gut microbiota along with intestinal barrier function.
- PUFAs promotes intestinal homeostasis along with reduced hepatic inflammation and
- Vitamin E is an excellent antioxidant that provides hepatoprotective action and relieves oxidative stress
- Probiotics, PUFAs, and Vitamin E all work synergistically resulting in gut microbiota modulation, restoration of altered intestinal function and reduced oxidative stress and hepatic inflammation.

13. Conclusion

Alcohol consumption is associated with altered gut microbiota that may result in increased inflammation, oxidative stress and liver cirrhosis. Probiotics, PUFAs and Vitamin E all work synergistically to reduce the liver injury in alcoholics.

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