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Research Article

Gut-Liver Axis Modulation: A Potential Role of Probiotics and Omega-3 Fatty Acids in Management of NAFLD

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1. Abstract

Non-Alcoholic Fatty Liver Disease (NAFLD) has currently emerged as common liver disorder compared to alcoholic liver disease. The prevalence of NAFLD in India varies from 9% in rural areas to 32% in urban populations. This incidence is reported to be the lowest in western India (44.1%) compared to the highest prevalence in northern states (72.4%). Available treatments are associated with certain side-effects. Recently a relation between gut and liver, i.e. gut-liver axis has been studied extensively. Modulation of gut may provide a natural mechanism to improve NAFLD associated complications. Moreover, patients with NAFLD have lower levels of omega-3 poly unsaturated fatty acids (PUFAs). Thus, supplementation of omega-3 PUFA is important for both, prevention and treatment of NAFLD. Vitamin E provides a significant antioxidant action in prevention of NAFLD progression. Therefore, modulating gut microbiota with probiotics may be an effective alternative along with established therapies like vitamin E and PUFA for better outcomes in the management of NAFLD.

2. Keywords: NAFLD; Gut-Liver axis; Probiotics; Omega-3; Vitamin E

3. Introduction

Non-alcoholic fatty liver disease (NAFLD) has currently emerged as common liver disorder compared to alcoholic liver disease due to increased prevalence of obesity and diabetes [1]. Globally, NAFLD accounts for 25.24% of population with wide geographical variation. The highest prevalence of NAFLD is noted in Middle East and South American countries (about 30%) [2]. Limited studies conducted in Africa reports lower prevalence of 13%. In Europe, the prevalence of NAFLD is reported to be 24% [2]. The large-scale studies determining the prevalence of NAFLD in India are scarce. However, based on the available data, the prevalence of NAFLD varies from 9% in rural areas to 32% in urban populations. This incidence is reported to be the lowest in western India (44.1%) compared to the highest prevalence in northern states (72.4%) [3]. The first stage that signifies NAFLD is hepatic steatosis, predicted when the fat content in liver is elevated more than 5% of the liver volume [1]. NAFLD may progress into non-alcoholic steatohepatitis (NASH), cirrhosis, hepatocellular carcinoma (HCC), and liver failure (Figure 1); however, the exact natural course of the disease is still not completely defined [4].

4. Risk Factors

Metabolic syndrome (Mets) is strongly associated with NAFLD. The major components of Mets include hypertension, hyperglycemia, abdominal obesity, and dyslipidemia. NAFLD is both, consequence and predecessor of MetS [4]. Dietary factors such as, excessive caloric intake, fructose, physical inactivity, may also result in NAFLD [5, 6]. Alterations in hepatocellular lipid regulating genetic factors can contribute to NAFLD predisposition and progression towards NASH and fibrosis. A large number of extrahepatic conditions such as, atherosclerosis, Cardiovascular Disease (CVD), Chronic Kidney Disease (CKD), Polycystic Ovarian Syndrome (PCOS), Obstructive Sleep Apnea (OSA), extrahepatic malignancies, etc. are associated with NAFLD [4]. Gut microbiota alterations

and its metabolites are recently included as significant risk factors in development of NAFLD [5, 6].

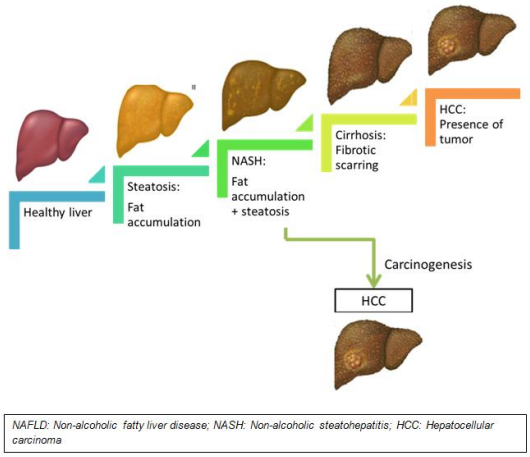


Figure 1: Progression of NAFLD [5].

5. Pathophysiology

The pathogenesis of NAFLD involves complex interaction of hormonal, genetic, nutrition and intestinal dysbiosis factors [7, 8].

5.1. Hormonal Factors Associated with NAFLD

Over nutrition (increased food intake and reduced energy expenditure) results in activation of upload and dopamine receptors in the nucleus accumbens (craving generation area in brain) [7]. The fructose (macronutrient) in diet results in increased cerebral blood flow to brain areas associated with reward and motivation; thus, failing to reduce satiety [7]. The activation of reward centers contributes to reduced satiety promoting hormones i.e., glucagon-like peptide 1 (GLP-1) and increases the secretion of hormone that stimulates hunger i.e., ghrelin; which may contribute to elevated triglyceride levels in blood resulting in NAFLD pathogenesis [7]. Further adipose derived hormones, leptin and adiponectin may also play a significant role in NAFLD pathogenesis. Elevated levels of leptin are reported in patients with NAFLD signifying contribution of leptin resistance in NAFLD pathogenesis [7].

5.2. Genetic Factors Contributing To NAFLD Pathogenesis

Patatin-like phospholipase domain-containing 3 (PNPLA3) genes, a protein with both triacylglyerol lipase and acylglycerol transacylase activity is associated with NAFLD pathogenesis. PNPLA3 is associated with NAFLD risk in both, adults and children [7]. Recently reported other loci associated with NAFLD includes, neurocan (NCAN), glucokinase regulator (GCKR), lysophospholipase like 1 (LYPLAL1), transmembrane 6-superfamily member 2 (TM6SF2) and protein phosphatase 1 regulatory subunit 3B (PPP1R3B) [7].

5.3. Nutrition and Intestinal Microbiota Dysbiosis Associated with NAFLD

High saturated fat, low fibre and carbohydrate-rich diets contributing to obesity are associated with increased NAFLD risk. Gut Microbiota (GM) plays a crucial role in maintaining the liver function [7]. The liver is exposed to nutritional supply and GM derived metabolites from the gut via gut-liver axis. Fatty diet may modulate gut microbial composition in NAFLD patients [7].

Dysbiosis of gut microbiota, intestinal barrier impairment, and altered immunity status may cause transport of bacterial products from gut to liver through portal vein resulting in its recognition by specific receptors, activate immune systems, and induces activation of inflammatory pathways [8]. The activation of these pathways results in pro-inflammatory response, Insulin Resistance (IR), obesity, hepatic steatosis, and NASH progression and development [8].

5.3.1. Alterations in Intestinal Microbiota and Release of Inflammatory Mediators : Alterations in micro biome can be induced by variety of factors such as obesity, diet, alcohol intake, infection, and medication and causes impaired intestinal integrity, intestinal bacterial overgrowth, bacterial translocation, and releases lipopolysaccharide (LPS) which in turn enters the liver through portal circulation resulting in inflammatory response that causes liver injury and subsequently NAFLD (Figure 2) [8, 9].

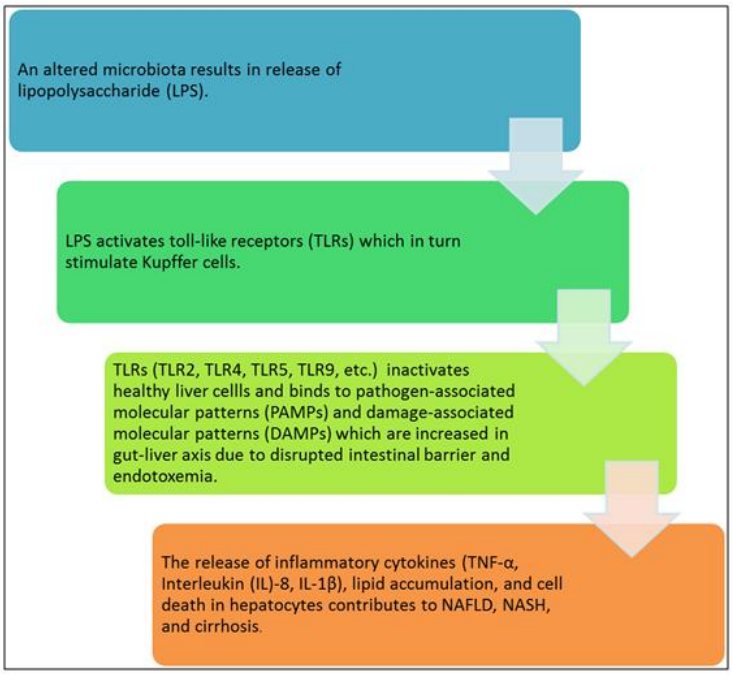
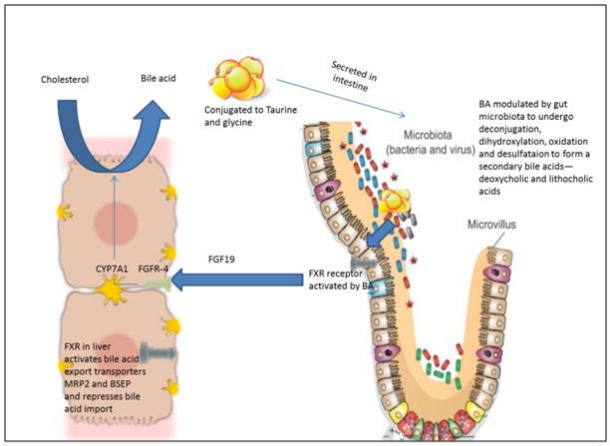


Figure 2: Gut microbiota and NAFLD: The link [9]

5.3.2. Gut Microbiota and Bile Acid (BA) Secretion: Gut microbiota (GM plays a crucial role in BA homeostasis. GM regulates the expression of bile acid enzymes for synthesis of BA. GM influences BA metabolism processes (conjugation in the liver, reabsorption in the terminal ileum, deconjugation in the small intestine, conversion to secondary bile) through associated enzymes, transporter expression, or activity [9].

BA regulates metabolism and inflammation via farnesoid X receptor (FXR) and transmembrane G protein-coupled receptor 5 (TGR5) (Figure 4). Primary BA (cholic acid and chenodeoxycholic acid) and secondary BA (lithocholic acid and deoxycholic acid) activate FXR and TGR5, respectively (Figure 3) [9].



In Intestine, Bile acid (BA) modulated by gut microbiota activates FXR. FXR induces fibroblast growth factor-19 (FGF-19) expression causing modulation of CYP7A1 via activation of FGF receptor-4. This results in reduced cholesterol to bile acid formation. In hepatocytes, FXR activates the expression of BA export transporters, multidrug resistance-associated protein 2 (MRP2) and bile salt export pump (BSEP)

Figure 3: Bile regulation through FXR [10, 11, 12, 13].

The metabolic effects of FXR includes inhibition of de novo lipogenesis, increased fatty acid oxidation, regulates glucose and triglyceride metabolism through inhibition of gluconeogenesis, TG synthesis and very low-density lipoprotein (VLDL) export and promotes TG clearance [9, 10]. TGR5 by inducing Glucagon-like peptide 1 (GLP-1) secretion which increases energy expenditure and attenuates diet-induced obesity affects glucose homeostasis [9].

BA, through FXR and TGR5 signalling pathways, reduces hepatic inflammation and fibrosis [9]. BA maintains the intestinal barrier integrity to protect liver against the GM-related inflammatory cascades. The disruption of GM alters the BA metabolism and altered BA metabolism is associated with metabolic and immune reaction that contributes to NAFLD [9].

Treatments considered for NAFLD management

The primary goal of NAFLD treatment is to improve steatohepatitis and fibrosis, along with prevention of cardiovascular (CV) and liver-associated mortality [14].

The strategies for NAFLD treatment involve [15]:

- Treatment of any existing associated metabolic conditions such as diabetes and hyperlipidemia;
- Lifestyle changes, weight loss, exercise, or pharmacotherapy for improving insulin resistance (IR);
- Use of antioxidants as hepato-protective agents for protection of liver from secondary insults.

5.4. Diet and Life-Style Modifications

Weight reduction and increased physical activity are associated with reduced risk of NAFLD and its benefit in CV disease is well established. The liver histology, IR, and quality of life is improved with modest (7–10%) reduction in weight and exercise. Caloric intake reduction may have positive outcomes; however, evidences suggest role of fructose consumption for NAFLD pathogenesis [14].

5.5. harmacological Treatments Used for NAFLD Management

5.5.1. Insulin Sensitizers: IR is known to be associated with NAFLD patients and it plays a crucial role in lipid accumulation in liver ultimately resulting in NASH. The association between IR and NAFLD provided the hint of using insulin sensitizing drugs (metformin and thiazolidinediones) for NAFLD treatment [15].

Metformin: Metformin is suggested to improve transaminase levels and hepatic steatosis; however, its effect on inflammation was insignificant, and only one study reported improvement in fibrosis [14]. In largest metformin trial, treatment of non-alcoholic fatty liver disease in children (TONIC), metformin failed to show superiority against placebo for primary outcome of sustained reduction in transaminase levels [15]. Moreover, metformin failed to demonstrate any significant improvement in fibrosis, steatosis, inflammation or NAFLD activity score (NAS) [14].

Thiazolidinediones (TZD): TZD has demonstrated beneficial impact on IR, hepatocyte fatty acid metabolism and adiponectin levels thus, suggesting promising improvement in treatment of NAFLD. The combination of TZDs with metformin may result in reduced TZD effect. TZD have demonstrated reduction in transaminase levels and steatosis. Most clinical trials have showed improvement in metabolic end points and steatohepatitis. The improvement in regression of fibrosis is not convincing with TZD. TZD is associated with two major drawbacks that limits its beneficial effects and results in treatment discontinuation (Box 1) [15].

Box 1: Drawbacks of TZD [15].

- After discontinuation of drug there is reversion of improvement resulting in its long-term administration.
- Most patients complains about side-effects such as lower extremity edema and weight gain (average 2 kg to 5 kg)

5.5.2. Incretin-Based Therapies: Currently the discovery of neuroendocrine hormones known as incretins [GLP-1 and glucose-dependent insulinotropic polypeptide (GIP)] has established a direct relation between the gastrointestinal (GI) and endocrine system. The intestinal tract produces incretins in response to food intake. Incretins stimulates glucose dependent insulin release, reduce glucagon release and prolong gastric emptying. These effects may be associated with improved glycemic control, increased weight loss, increased insulin sensitivity, and may benefit NAFLD patients. Levels of GLP-1 and GIP are reduced after secretion of enzyme dipeptidyl peptidase-4 (DPP-4) and GLP-1 receptors are reduced in patients with diabetes. DPP-4 inhibitors are newly developed agents approved for

diabetes but its use in NAFLD is not yet been studied. GLP-1 receptor agonists are also approved for its use only in diabetes.

5.5.3. Anti-Tumour Necrosis Factor-Alpha Agents: NAFLD pathogenesis is associated with inflammatory activation. Inflammatory mediators such as, tumour necrosis factor-alpha (TNF-α) has a significant role in obesity and IR. TNF-α antagonist like pentoxifylline have demonstrated improvement in steatosis, inflammation and ballooning in small NAFLD clinical trials assessing the histological response [15].

5.5.4. Lipid-lowering agent: Use of statins, fibrates, and omega-3 polyunsaturated fatty acids (PUFAs) for dyslipidemia has demonstrated to possess potential antioxidant properties and favourable effect on adiponectin levels, suggesting a significant role in NAFLD management. Multiple retrospective studies have demonstrated significant effect of statins in improving steatosis and decreasing fibrosis progression. Moreover, statins are reported to be safe for its use in patients with dyslipidemia and NAFLD. However, statins and fibrates have not demonstrated significant improvement in liver fibrosis in prospective studies [15].

The balance between omega-3 and omega-6 PUFA is crucial for human health. The patients with NAFLD have demonstrated increased concentration of omega-6 and a lower level of omega-3 PUFA. Thus, supplementation of omega-3 PUFA is important for both, prevention and treatment of NAFLD. A meta-analysis including seven randomized controlled trials involving 442 patients reported that ω-3 PUFA supplementation significantly reduced alanine aminotransferase (ALT), Total Cholesterol (TC), triglyceride (TG) and increased high-density lipoprotein cholesterol (HDL-C) in patients with NAFLD. Omega-3 PUFAs demonstrates beneficial effects in NAFLD patients [16].

5.5.5. Antioxidant agents: Oxidative stress is known to play a major role in NAFLD pathogenesis. Antioxidants such as ursodeoxycholic acid (UDCA), vitamin E, silymarin (milk thistle) and betaine may prove to be beneficial therapeutic options for NAFLD management [15].

- UDSA: UDSA, a hydrophilic bile acid, is known to possess cytoprotective and antioxidant properties. However, Clinical studies of UDSA (moderate dose or at highest dose) failed to demonstrate any significant benefit in NAFLD management [15].
- Silymarin: Silymarin also known as milk thistle is used in liver disease for its antioxidant properties. Due to being natural product from seeds of the Silybum marianum plant, it is considered to have lesser side-effects. The studies demonstrating its beneficial effects in management of NAFLD are lacking. Moreover, standardized formulations of silymarin and effective dosages are lacking till date [15].
- Betaine: Betaine is a naturally occurring choline metabolite. It has demonstrated to increase S-adenosylmethionine levels and reduce oxidative stress. However, clinical studies failed to report any improvement in steatosis or other histological outcomes. Therefore betaine is not recommended in patients with NAFLD [15].
- Vitamin E: Vitamin E is a fat soluble vitamin with excellent anti-oxidant properties. It has been assessed by many small clinical trials. However, recent two large clinical trials, Pioglitazone or Vitamin E for NASH Study (PIVENS) and TONIC reported vitamin E effect in adults and children with NAFLD. Vitamin E in both the studies demonstrated improvement in hepatocellular ballooning and NAS; suggesting reduced risk of disease progression and cytoskeletal injury [15].

Recent guidelines by American Association for the Study of Liver Disease (AASLD) and the European Association for the Study of the Liver (EASL) recommend the use of vitamin E 800IU in patients with biopsy-proven NASH and without diabetes [17].

6. Obeticholic Acid

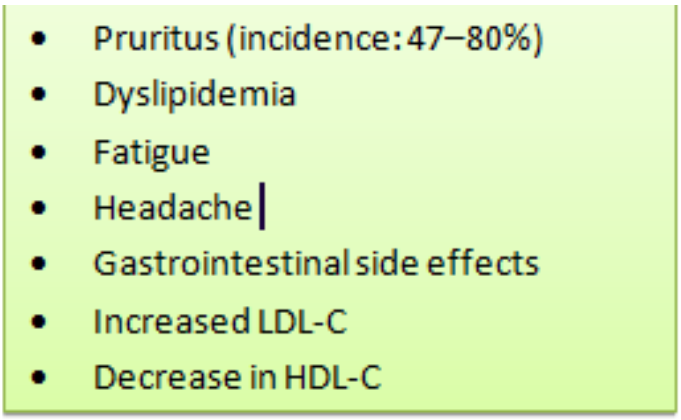
A Proposed treatment for NASH/NAFLD and its limitations.

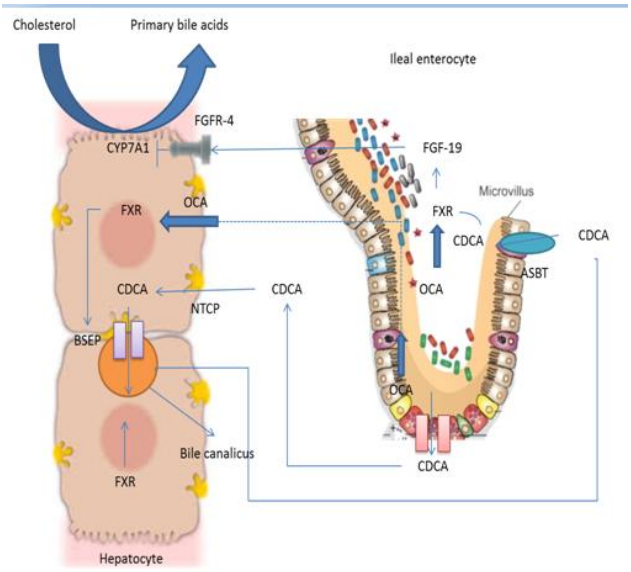
A new drug specifically targeting FXR has been developed and may prove to be effective in pharmacological management of NASH/NAFLD [18].

Obeticholic acid (OCA), a first-in-class selective FXR agonist, reported to possess anticholestatic and hepato-protective properties, is approved for primary biliary cholangitis [19]. It has currently grabbed the attention for its off-label use in NAFLD as it is a liver-specific treatment.

OCA has ability to reduce the BA synthesis by acting on ileal enterocyte FXRs to release FGF-19 which enters the portal circulation and binds FGFR-4 which inhibits CYP7A1 gene resulting in reduced cholesterol to BA synthesis. In hepatocytes, OCA also stimulates the bile salt export pump (BSEP) resulting in downregulation of BA uptake in the portal circulation. This reduces the exposure of liver to toxic BA levels (Figure 4) [13].

Box 2: Common adverse events associated with obeticholic acid [19]





ASBT: Apical sodium-dependent bile acid transporter; BSEP: Bile salt export pump; CYP7A1: Cholesterol 7α-hydroxylase; FGF-19: Fibroblast growth factor-19; FGFR-4: Fibroblast growth factor receptor-4; FXR: Farnesoid X receptor; NTCP: Na+-taurocholate cotransporting polypeptide; OCA: obeticholic acid.

Figure 4: Mechanism of obeticholic acid [13]

In various animal studies, OCA is reported to improve insulin sensitivity, control glucose homeostasis, and modulate lipid metabolism. It has also showed various anti-inflammatory and anti-fibrotic effects in hepatic, renal, and intestinal tissues (where FXR is expressed) [19].

OCA is associated with high rates of pruritus in all clinical trials. The incidence of pruritus ranges from 47–80% based on the increase in dose i.e. 10 to 50 mg. Pruritus associated with OCA has been the reason for dose adjustment or discontinuation of the drug. In Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT) trial 23% patients developed pruritus after treatment with OCA compared to 6% in placebo group. This symptom is managed by use of concomitant medications such as, bile acid sequestrants, antihistamines, dose reduction, or symptomatic treatment. Other side-effects include dyslipidemia, fatigue, headache, and gastrointestinal side effects. OCA is associated with increased low-density lipoprotein-cholesterol (LDL-C) and a decrease in high-density lipoprotein-cholesterol (HDL-C) and triglycerides [13, 19].

OCA is a promising therapeutic agent for management of NAFLD; however, it is associated with several drawbacks (Box 2) such as elevated LDL levels and pruritus and requires concomitant treatment for the management of other adverse effects [19].

7. Saroglitazar: An approved treatment for NASH

Saroglitazar, a novel dual peroxisome proliferator activated receptor (PPAR) α/γ agonist is currently approved for its use in India for its use in diabetic dyslipidemia [20]. Reports of post marketing analysis have demonstrated promising efficacy and safety of saroglitazar in Indian diabetic dyslipidemic patients [21]. Saroglitazar has predominant PPAR- α agonist action and moderate PPAR- γ effect. Some clinical trials have evaluated the role of saroglitazar in NAFLD patients with diabetic dyslipidemia. These studies have demonstrated significant effect of saroglitazar on TG, ALT, and fatty liver index (FLI) in NAFLD patients with diabetic dyslipidemia. The commonly reported adverse events with Saroglitazar include asthenia, gastritis, dizziness, and tremors [22].

8. Probiotics: Gut Microbiota Modulation and its Effectiveness in NAFLD

Dysbiosis of gut microbiota is associated with liver damage and restoring gut microbiota may prove to be potential therapeutic strategy to prevent liver damage. Probiotics are non-harmful, live microorganisms that provide health benefits by modulating GM when administered in sufficient amounts. The commonly used bacteria in probiotics include Lactobacilli, Streptococci, and Bifidobacteria [9]. Probiotics promote anti-inflammatory environment and counteract on pathogenic bacteria through immune system modulation and activation of host defence [6, 9].

8.1. Probiotics: Anti-Inflammatory Action of Gut-Liver Axis

Probiotics restores intestinal barrier integrity by its positive effects on ZO-1 expression, mucus thickness and restoring commensal bacteria proportion. Probiotics causes bowel inflammation shutdown, including T regulatory cells, dendritic cells (DCs), and macrophages to secrete anti-inflammatory cytokines [transforming growth factor-beta (TGF-β) and interleukin-10 (IL-10)] to induce anti-inflammatory effects. In liver, probiotics decreases endotoxemia that results in halting of hepatic damage (observed through reduction of ALT and AST). Probiotics contributes to the recovery of the hepatic function, affects the lipid composition of fatty-laden hepatocytes, favoring endotoxins clearance, and negatively impacts inflammatory and fibrogenic processes (i.e., lower nitric oxide synthase (iNOS), matrix metallopeptidases (MMP) and NF-kB) [6].

8.2. Probiotics: Effects on Bile Acid Modulation

A recent study conducted in mice demonstrated that probiotics induced microbiota modulation results in changed BA absorption, down regulation of FGR-FGF15 (FGF-19 in humans) axis, and increased BA neosynthesis in hepatocytes thus, enhancing the BA deconjugation and fecal excretion. These effects are opposite to the effects induced by FXR agonist, OCA [23, 24] (Figure 5).

8.3. Clinical Findings with Probiotics in NAFLD

8.3.1. Monotherapy with Probiotics: Experimental models of probiotics in NAFLD have demonstrated promising results thus, providing better perspective for conduction of clinical trials for evaluation of probiotics in patients with NAFLD (Table 1) [6]. Sepideh et al. conducted randomized clinical trial including 42 NAFLD patients treated with 2 capsules/day probiotic or placebo for 8 weeks. Primary endpoints assessed were Fasting Blood Sugar (FBS), insulin, insulin resistance, tumor necrosis factor alpha (TNF-α), and interleu-

kin 6 (IL-6). Mean insulin, insulin resistance, and IL-6 decreased significantly in probiotic group compared to placebo group ($p < 0.05$). No statistically significant difference was reported in TNF- α levels between both the groups [25]. Another double-blind single-centre randomized controlled trial assessed live multi-strain probiotic vs. placebo in 58 diabetic patients with NAFLD. The primary outcomes included fatty liver index (FLI) and liver stiffness (LS) and changes in aminotransferase activity, serum lipids and cytokines (TNF-α, IL-1β, IL-6, IL-8, and IFN-γ) levels were assessed as secondary outcomes. FLI was significantly reduced from the baseline in probiotic group ($p < 0.001$) and no difference was noted in placebo group. LS were slightly improved but the findings were not statistically significant. Among secondary outcomes, significant reduction in serum levels of AST and GGT followed by TNF-α and IL-6 levels were reported with probiotics [26]. A systematic review and meta-analysis conducted involving 22 clinical studies reported that probiotics supplementation in NAFLD patients results in reduction of weight, body mass index, improved liver function (reduced ALT and AST), improved lipid profile (total cholesterol, low-density lipoprotein cholesterol, and triglycerides), reduced plasma glucose levels, and decreased inflammatory cytokines [27]. In conclusion, all the studies favours the use of probiotic in NAFLD, and it may prove to be a promising therapeutic method for NAFLD treatment [23].

Table 1: Clinical studies that evaluated monotherapy and combined therapy of probiotics and omega 3 polyunsaturated fatty acids in patients with NAFLD.

Authors	Study design and patients	Duration	Treatment	Primary and secondary end points	Outcomes
Sepideh et al. [25]	Randomized clinical trial (RCT)	8 weeks (2 months)	Multistrain Probiotic	Fasting blood sugar (FBS), insulin, IR, tumor necrosis factor alpha (TNF-α), and interleukin 6 (IL-6) measured at baseline and end of the study	Mean FBS, insulin, insulin resistance, and IL-6 reduced significantly ($p < 0.05$)compared to values at baseline
Kobyliak et al. [26]	Double-blind single center RCT	8-weeks	Multi-strain probiotic	Primary endpoints fatty liver index (FLI) and liver stiffness (LS)	FLI reduced significantly in probiotic group compared to placebo group ($p < 0.005$).
	Patients with NAFLD (n = 58) were included		or placebo	Secondary endpoints included AST, serum lipids and cytokines TNF-α, IL-1β, IL-6, IL-8, and IFN-γ levels	Slight reduction in LS
					AST was reduced in probiotic group
					TNF-α and IL-6 levels changed significantly in probiotic group.
Tang et al.[27]	A systematic review and meta-analysis	NA	probiotics	Changes in anthropometric parameters (weight, BMI), liver function (ALT, AST, alkaline phosphatase levels, the glutamyl transpeptidase	Significant reduction in weight (2.31 kg), and BMI 1.08 kg/m^2 was observed post-probiotic treatment.
	22 studies assessing effectiveness of probiotics in NAFLD patients were included			levels),	Reduction in liver enzymes such as ALT (7.22 U/l), AST (7.22 U/l), alkaline phosphatase levels (25.87 U/l), the glutamyl transpeptidase
				lipid profiles(total cholesterol, low-density lipoprotein cholesterol, and	Levels (−5.76 U/l) were noted.
				triglycerides), and cytokines (TNF-α and leptin) and Degree of liver fat infiltration (DFI) was reported	Lipid profile improved significantly by reduction in Total cholesterol low-density lipoprotein cholesterol and
					triglycerides.
					Probiotic also decreased inflammatory mediators such as tumor necrosis factor alpha by 0.62 and leptin by 1.14
					The risk of probiotics for restoring DFI was 2.47
Rajkumar et al. [28]	A Randomized, placebo-controlled trial	6-weeks	Probiotic monotherapy	Insulin sensitivity, blood lipids, and inflammation	Monotherapy with probiotics reduced total cholesterol, triglyceride, LDL, and VLDL increased HDL. It also improved insulin sensitivity and reduced hsCRP.
	Overweight patinets (n = 60; age: 40–60 years) were included		(VSL#3) omega-3 fatty acid monotherapy		Omega-3 PUFA monotherapy had significant effect on insulin sensitivity and hsCRP
			Placebo and combination of probiotic and omega-3 PUFAs		Combination of probiotics and omega-3 PUFAs demonstrated more pronounced effects on HDL, insulin sensitivity and hs CRP

Kobyliak et al. [29]	Randomized placebo-controlled trial (RCT) including type-2 diabetic patients (n = 42) with	8-weeks	Probiotics + omega-3 vs. placebo	Primary outcomes were change in fatty liver index (FLI) and liver stiffness (LS)	FLI reduced significantly from baseline in Probiotics + omega-3 whereas no changes were reported in placebo group pre and post-treatment
	NAFLD			Secondary outcomes included changes in transaminases level, serum lipids and	Changes in LS were insignificant in both groups.
				cytokines levels	Significant reduction in serum gamma-glutamyl transpeptidase, triglycerides, and total cholesterol was reported in combination group
					Inflammatory markers were reduced significantly in combination group.

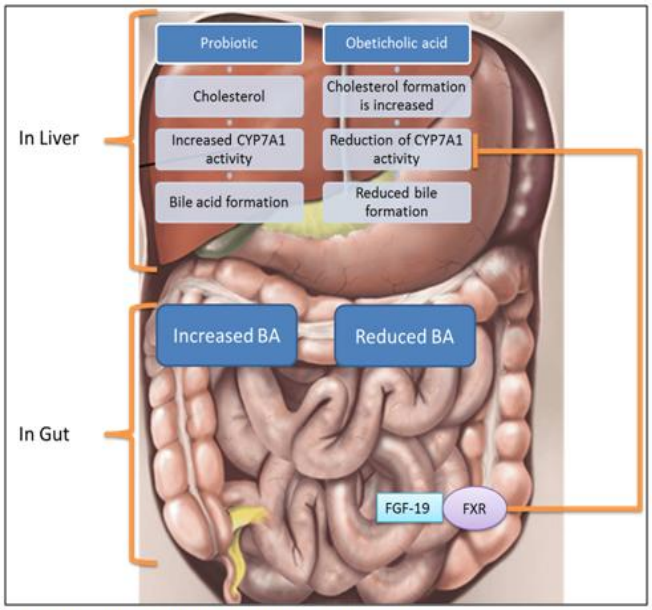


Figure 5: Bile regulation with probiotics and obeticholic acid [21]

832. Probiotics and Omega-3 Pufas: Combined Effect in NAFLD: Two clinical trials have studied the combined effect of probiotics and omega-3 PUFAs (Table 1). The combination of probiotics with PUFAs has demonstrated significant reduction in lipid parameters and chronic inflammatory markers; and improved insulin sensitivity and FLI. A randomized, placebo-controlled trial conducted by Rajkumar et al. evaluated the effects of probiotic (VSL#3) and omega-3 fatty acid on insulin sensitivity, blood lipids, and inflammation in 60 overweight (BMI > 25), healthy adults, aged 40–60 years. The probiotic supplementation resulted in significant reductions of total cholesterol, triglyceride, LDL, and VLDL and increased HDL-C levels ($p < 0.05$). Moreover, probiotics also improved insulin sensitivity ($P < 0.01$), decreased high sensitivity C-reactive protein (hsCRP), and favourably affected gut microbiota composition. Omega-3 demonstrated a significant effect on insulin sensitivity and hsCRP [28]. Another double-blind single centre randomized placebo-controlled trial assessed the efficacy of administration of probiotics with omega-3 vs. placebo in type-2 diabetic patients (n = 48) with NAFLD demonstrated significant reduction in FLI from baseline (83.53 ± 2.60 to 76.26 ± 2.96; $p < 0.001$) while no significant changes were observed in the placebo group. Serum gamma-glutamyl transpeptidase, triglycerides, and total cholesterol levels were reduced with probiotics-omega-3 combination. Chronic systemic inflammatory markers decreased significantly in probiotics-omega-3 combination group [29].

9. Conclusion

NAFLD still remains a major health concern with various pathways (hormonal, genetic, and dietary factors) playing a crucial role in its pathogenesis. Alteration in gut microbiota is strongly associated with pathogenesis of NAFLD. Available treatments only focus on issues associated with liver without looking into the point of origin i.e the gastrointestinal tract. OCA, an approved treatment for biliary cholangitis & used off label for NASH, is associated with significant pruritus resulting in patient discomfort. In such conditions, modulation of gut microbiota may reduce the liver worsening and provide cure with lesser side-effects. Many clinical trials have evaluated efficacy of probiotics in NAFLD and has reported promising results. The addition of PUFAs to probiotics has demonstrated a pronounced effect in reducing insulin resistance, inflammatory cytokines, improving lipid profile, FLI, and liver stiffness of NAFLD patients.

Therefore, modulating gut microbiota with probiotics may be an effective alternative along with established therapies like vitamin E and PUFA for better outcomes in the management of NAFLD.

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