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Functional Foods for the Management of Non-Alcoholic Fatty Liver Disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is increasingly evolving and a critical public health concern, raising the likelihood of liver cirrhosis, type 2 diabetes and cardiac problems. Existing epidemics of obesity and sedentary life style have lead to NAFLD's elevated prevalence. In recent years there is profound change in the diet pattern, particularly the hypercaloric fat and carbohydrates for preventing or treating chronic liver disorders such as NASH and NAFLD. Functional and nutritional foods have contributed significantly to NAFLD improvement and management. The justification for exploring functional foods as anti-NAFLD candidates for the chronic liver disease prevention is derived knowledge from *in vitro* and *in vivo* models. The findings from the *in vitro* and *in vivo* studies confirmed that these compounds are healthy, efficient, reversible inhibitors, when sufficiently consumed over a lifetime without severe toxicity, suitable for clinical trials and potentially becoming low-cost medication.

Keywords: non-alcoholic fatty liver disease, functional foods, phenolics, flavonoids, treatment, management

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the primary liver disease posing severe health and economic burden worldwide [1]. NAFLD is characterized with excessive fat storage in the liver, constituting up to 10% of the total liver weight [2]. NAFLD is typically caused due to reasons other than excessive alcohol intake such as obesity, insulin resistance, diabetes mellitus, high triglycerides, dyslipidemia, etc. [3]. Based on liver histology, NAFLD is further classified into the non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). NAFL is characterized by retention of fat in the liver without hepatocyte injury, whereas in NASH, significant inflammation, hepatocyte injury and liver steatosis are observed [4]. The progression of NASH is quite complicated and if unattended, will lead to liver fibrosis, cirrhosis, hepatocellular carcinoma and organ transplantation. At present, there is no FDA approved drug for the management of NAFLD. Functional foods are being prepared using scientific intelligence to provide the required amount of macro and micronutrients for once health. Compared to conventional foods, functional foods deliver potentially nutritious components that reduce the risk of several

chronic diseases [5]. Nutraceuticals are any food substance or part of a diet that afford significant health benefits [6]. This chapter will emphasize the importance of functional foods and nutraceuticals for the management of NAFLD.

2. Prevalence and pathogenies of NAFLD

NAFLD, the primary cause of chronic liver disease seen in developed countries, is presently highly prevalent among the Asian population. According to recent estimates, the global prevalence of NAFLD is reported at 25 percent and the combined prevalence rate is 27.4 percent in Asia [7]. Two hypotheses are framed for the pathogenesis of NAFLD. The first hit is a two-hit theory where fat accumulation in the liver is caused due to diet, obesity and insulin resistance and the first hit further exposes the liver to more insults called second hit which activate inflammatory pathways and fibrogenesis [8]. In continuation, a multi-hit hypothesis was suggested where numerous factors like environment, dietary habits and genetic led to the development of liver damage [9].

3. Key factors involved in the progression of NAFLD

3.1 Lipid accumulation and insulin resistance

Triglyceride accumulation in hepatocytes is a significant factor in the development of NAFLD. Glycerol and fatty acids undergo esterification to form triglycerides (TGs), which are usually stored or secreted. The fate of the fatty acids is to either undergo esterification or enter the β -oxidation pathway. Under normal conditions, TGs are not toxic; where they maintain free fatty acids [10]. Studies have shown increased de-novo lipogenesis and expression of transcription factors such as sterol regulatory element binding protein-1c (SREBP-1c), carbohydrate response element-binding protein (ChREBP) and peroxisome proliferator-activated receptor- γ (PPAR- γ) in NAFLD [11]. Insulin resistance is another critical factor in NAFLD that drive the activation of de-novo lipogenesis. Insulin receptor substrate-2 (IRS-2) is known to regulate SREBP-1c negatively. Insulin resistance also lead to decreased oxidation of free fatty acids; hence fat accumulate in the hepatocytes. Free fatty acids in hepatocytes also inhibit the insulin signaling through the serine-kinase pathway leading to insulin resistance [12]. Accumulation of fat in the liver also contribute to stress and dysfunction to mitochondria and endoplasmic reticulum (ER). Dysfunctional mitochondria lead to increased reactive oxygen species (ROS) generation and activation of inflammatory pathways leading to hepatic necro-inflammation and further damage of mitochondria (**Figure 1**) [13].

3.2 Cytokines

Studies have shown the involment of cytokines during liver inflammation, liver fibrosis, liver regeneration and hepatocyte apoptosis [14]. In obese individuals, adipose tissue is enlarged and release various adipokines, which further recruit macrophages resulting in the secretion of pro-inflammatory adipokines [15]. Increased leptin levels play a crucial role in NAFLD progression by inducing insulin resistance and steatosis development [16]. Adiponectin, an anti-inflammatory adipokine secreted exclusively by the adipocytes, plays a protective role in the liver by preventing lipid accumulation through enhanced β -oxidation of free fatty acids [17]. Lipid accumulation, insulin resistance, mitochondrial stress, ER stress

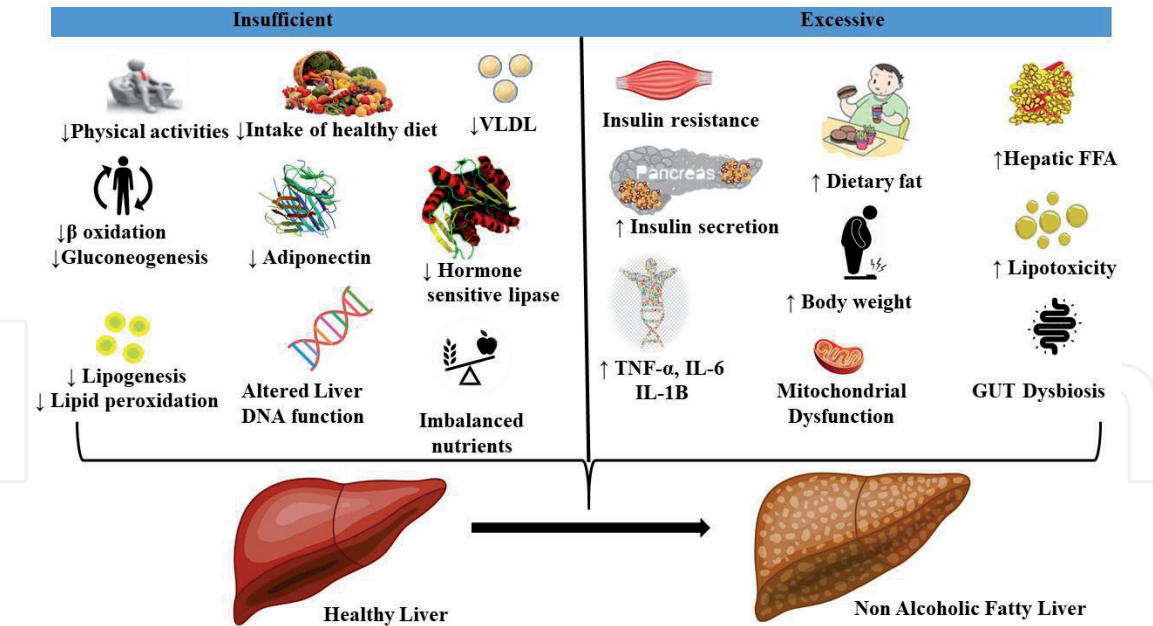


Figure 1.
Pathogenesis of NAFLD.

and fatty dysfunction have contributed to the generation of pro-inflammatory cytokines. c-Jun N-terminal kinase/activator protein 1 (JNK/AP-1), tumor necrosis factor- α (TNF- α) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) are some of the crucial players in the development of inflammation in NAFLD. JNK pathway lead to apoptosis and progression of NAFL to NASH. NF- κ B activation lead to chronic hepatocyte inflammation and insulin resistance [18].

3.3 Genetic, epigenetic and dietary factors

Gene mutations like single nucleotide polymorphisms (SNPs) influence the free fatty acids secreted from hepatocytes to release cytokines, further stimulating NAFLD progression [19]. An example of SNP that affect NAFLD progression is the patatin-like phospholipase 3 (PNPLA3) gene. Lipid accumulation in hepatocytes of a PNPLA3 gene mutant carrier is associated with lower lipoprotein secretion, which is rich in liver TGs [20]. Pirazzi et al. (2012) showed that humans with PNPLA3 mutation exhibited increased steatosis and fibrosis. Transmembrane 6 superfamily member 2 (TM6SF2) mutation is also associated with NAFLD [21]. Wild type TM6SF2 protein promote VLDL secretion, while its variant is associated with hepatic steatosis caused by lower VLDL secretion and high ALT levels [22].

Epigenetic modifications generally occur at the transcriptional levels, such as DNA methylation, histone modifications and microRNA (miRNA) expression. Studies have shown that aberration in epigenetics is known to increase susceptibility to NAFLD [23]. DNA methylation has a crucial epigenetic determinant in NAFLD progression, which is influenced by dietary methyl donor deficiency. Betaine, choline and folate are common methyl donors [24]. Sirtuins (SIRT) are a group of proteins with deacetylase activity mainly involved in epigenetic modification. SIRT have been implicated in regulating proteins involved in metabolic processes like lipid metabolism, oxidative stress, glucose metabolism and inflammatory pathways. Research findings in animal and human models showed that NAFLD is associated with lowered SIRT1 levels [25]. Recent studies demonstrated the association between miRNA levels and NAFLD pathogenesis. Krützfeldt et al. (2005) showed inhibition of miRNA-122 led to decreased plasma cholesterol levels and reduced expression of genes involved in hepatic cholesterol and fatty acid synthesis [26].

The pathogenesis of NAFLD is also dependent on dietary factors. The quantity of calorie intake and the diet's quality concerning nutrients decide a healthy diet. Diet rich in fructose is associated with NAFLD. Fructose is a lipogenic dietary factor with pro-inflammatory activity, causing oxidative stress and increased expression of TNF- α . Studies demonstrated that fructose intake in patients with NAFLD leads to increased fibrosis [27]. Diet rich in mono-unsaturated fatty acids showed protective effects by improving steatosis and insulin resistance in NAFLD patients [28]. Studies conducted in ob/ob mice showed less steatosis and lowered liver enzyme levels upon a moderate alcohol administration level [29].

3.4 Gut microbiota

Recent studies showed the involvement of the gut-liver axis in the pathogenesis of NAFLD [30]. Bacterial toxins like lipopolysaccharides (LPS) are the potent toxins released by gut bacteria. LPS activate inflammatory response by activating stress-activated protein kinase, JNK, p38 affecting insulin resistance, obesity, hepatic fat accumulation and NASH development [31]. Patients with NAFLD have increased gut permeability and higher bacterial growth than normal subjects [32].

4. Functional foods for NAFLD

Human history's survival has always been highly reliant on food to avoid or battle the diseases. Hippocrates, the renowned physician, quoted, "Let food be thy medicine and medicine be thy food." Functional foods should not contradict the scientific advancements made to treat degenerative diseases over the last two centuries. Due to modern nomadic lifestyle and the shift in natural resources, traditional agricultural practices and dietary habits are not standard in this decade. Anti-NAFLD compounds, in general, must be discerning and innocuous. They should look at molecular and metabolic levels to reduce fat deposition in the liver. Flavones such as quercetin, hesperetin from onions and citrus fruits were shown to reduce fatty acid deposition in the liver. Anti-NAFLD compounds' mechanism must be reversible in the event NAFLD needs, for example, to promote the tissue darning subsequent injury. Most food-derived compounds show reversible activity [33]. The flavonoid, naringenin dose-dependently and reversibly inhibited transforming growth factor- β (TGF- β) and vascular endothelial growth factor (VEGF), thereby modulated the fatty acid oxidation and trafficking dependent liver inflammation [34].

Pre-clinical and clinical models have proven their worth to test natural compounds for anti-NAFLD activities. Many anti-NAFLD compounds such as flavones, anthraquinones, stilbenes, naphthols and polysaccharides showed potency at cellular levels, phase I and II clinical trials [35]. Epidemiological studies illustrated that populations gobble such bioactive substances have low disability rates as their main diets. Since these compounds are food endogenous, the fortifying meal is a relatively cheap way of delivering them throughout a lifetime, as many people tend to forget pills over long periods. It was suggested that the use of non-alcoholic steatohepatitis inhibitors might not prove relevant, as these inhibitors may be more efficient against the progression of disease at an early stage as comparing when a metastatic illness has progressed to advanced stages [36]. Meanwhile, the onset or evolution of the process of non-alcoholic steatohepatitis and cirrhosis generally take months or years; continual intake of anti-NAFLD compounds might be the ultimate approach to inhibit NAFLD-related molecular and metabolic stimulators chronically.

Most NAFLD inhibitors of diet can inversely inactivate more than one stimulator of fatty liver disease, making them favorable over irreversible inhibitors with one specificity and substantial lethal side effects. Inhibitors of nutrition sources, such as silymarin and morin, are reversible and appear to target multiple activators and inhibitors of NAFLD [37]. NAFLD inhibitors seem immune or could delay drug resistance in standard therapy to develop drug resistance by prolonging cells [38]. These assets converse to these bioactive composites to bypass drug resistance and be effective against several types of non-alcoholic fatty liver dependent diseases. NAFLD is a widely down-regulated co-fetal mechanism in healthy populations. NAFLD targeting does not lead to side effects even after chronic exposure to naturally occurring and physiological anti-NAFLD compounds.

Low molecular weight anti-NAFLD functional foods such as antioxidants, pre- or probiotics, tangeritin and lycopene may offer novel strategies in NAFLD dependant insulin resistance, obesity and abnormal fatty acid metabolism-related hepatocellular carcinoma [39]. Orally administered lycopene was detected in the liver and plasma and brain cortex of the rat model of various diseases with numerous concentrations [40]. Reviews on the work of several types of research on a diversity of anti-NAFLD functional foods, the non-alcoholic fatty liver-associated enzymes they inhibit, the right sources of NAFLD compounds, the molecular mechanisms of inhibition and references for additional information. The list is not meticulous. Minerals have some negative role on NAFLD and the existed clinical data showed that Zinc (Zn), selenium (Se) and Copper (Cu) have some negative impact on anti-NAFLD [41].

4.1 Phenolic compounds

4.1.1 Catechins

Oolong tea polyphenols markedly inhibited the formation of oxidized lipids, reduced body fat and the risk of developing arteriosclerosis [42]. A double-blind placebo-controlled study by Sakata et al. (2013) demonstrated the health benefits of green tea extracts for NAFLD [43]. The green tea polyphenols showed higher bio-availability in the serum following its consumption. Antioxidant, anti-inflammation, lipid metabolism-related biomarkers, as well as SREBPs and related genes, are critical mediators of NAFLD. Epigallocatechin-3-gallate (EGCG), a critical bioactive from green tea, prevented NAFLD in several experimental models (**Table 1**) [57].

4.1.2 Curcumin

Curcumin, a yellow-pigmented bioactive of *Curcuma longa*, is most commonly used as a dietary spice. Numerous investigations described its pharmacological activities, including antioxidant, lipid-modifying, anti-inflammatory, anti-cancer effects [58]. Oral administration of curcumin (50 mg/kg body weight) synergistically regulated both endogenous and exogenous Nrf2/LXR α pathways in high sucrose diet-induced NAFLD rats [44]. Jazayeri et al. (2019) showed curcumin regulated the PPAR- γ activity, inhibited cyclooxygenase controlled inflammation and improved NAFLD [44, 59]; Curcumin (100 mg/kg body weight) administration for three weeks to methionine and choline-deficient (MCD) diet-fed mice significantly upregulated superoxide dismutase 1 (SOD1), SIRT1 levels and inhibited O-GlcNAcylation pathway [60]. Saadati et al. (2019) conducted a randomized placebo-controlled clinical trial with 52 NAFLD subjects where they showed curcumin (1500 mg) administration for 12 weeks significantly reduced serum cholesterol, glucose and liver fibrosis [61].

Bioactive compound	Disease effect reported	NAFLD biomarkers and enzymes activities investigated	Sources	Mechanisms of action	References
PHENOLICS i. Catechins (EGCG, ECG) ii. Curcumin iii. Genistein (Isoflavones) iv. Daidzein (Isoflavones) v. Resveratrol	Antioxidant, anti-inflammatory, anti-fibrotic, antitumor and anti-arteriosclerotic	upregulate Insulin-Degrading Enzyme; downregulate AMP, SREBPs; promote glycogen synthase kinase	Fresh tea leaves, black grapes, strawberries, apples, blackberries, broad beans, pears and raspberries	Reduced liver fat deposition in liver. Prevention of NAFLD related liver tumorigenesis	[43]
	Anti-oxidant, antimicrobial, anti-inflammatory, anti-cancer, lipid-modifying,	activate Nrf2; upregulate FXR, LXR- α ; reduce serum levels of total cholesterol and TG	Turmeric	Suppressed fat accumulation and hepatic injury	[44]
	Antioxidant, anti-inflammatory, anti-fibrotic, anti-tumorigenesis	activate AMPK; upregulate PPAR- α ; downregulate COX-1, hepatic thromboxane A ₂ receptor; reduce serum ALT	Soybeans, Soy milk and soy-based beverages	Normalize hepatomegaly and liver steatosis Control of aminotransferase abnormalities and glucose tolerance	[45, 46]
	Anti-cancer, anti- osteoporosis, anti- ischemic heart disease and hepatoprotective	Downregulate ChREBP, Akt activation, Improve phosphatidylinositol 3-kinase; glucose transporter-2, SOD-2 and glutathione S-transferase α 3	Leguminous Plants, Textured soy proteins, Herbal teas	Hepatic de novo lipogenesis, inhibition of hepatic fat accumulation	[47]
	Anti- obese, anti-diabetic, anti- cancer	downregulate TNF α , COX-2, IL-6; reduce ALT and AST, inhibit (PI3K)/Akt pathway, NF- κ B pathway	Skin of grapes, Peanuts and Berries	Autophagic mediated fat depletion	[48]
PROANTHOCYANIDINS i. GSP ii. Tannins iii. Silybin	Lipid-lowering, antioxidant.	Downregulate SREBP1c, HMG CoA reductase	Grape seeds	Prevented oxidative liver injury	[49]
	Antioxidant, Anti-lipogenic, antimicrobial	Inhibit histone acetyltransferase, FAS, ACC; upregulate Carnitine palmitoyltransferase-1	Coffee, Tea, Cocoa, Sorghum grain	Reduced liver steatosis	[50]
	Antioxidant, anti-inflammatory, antipyretic, anti-fibrotic	Inhibit NF κ B activation, SCD1, AOX, Insulin resistance; reduce AST & ALT	Milk thistle	Reduced liver steatosis	[51]

Bioactive compound	Disease effect reported	NAFLD biomarkers and enzymes activities investigated	Sources	Mechanisms of action	References
SAPONINS & TERPENES i. Ginsenoside ii. Andrographolide iii. Glycyrrhetic acid	Antioxidant, antimicrobial, antifungal, antidiabetic, hepatoprotective	Upregulate PPAR- α induced fatty acid oxidation; activate SIRT6; Downregulate inflammatory cytokines, SREBP-1C	Panax species	Alleviated hepatic lipid accumulation	[52]
	Anti-inflammatory, anti-platelet aggregation, antineoplastic.	Inhibit CYP3A and CYP2C9; downregulate HNF4 α ,	<i>Andrographis paniculata</i>	Improved glucose metabolism and liver function	[53]
	Antiviral, anti-inflammation, analgesia, anti-tumor, Immunomodulation, hepatoprotective.	Inhibit AKR1B10, Upregulate Glycogen synthesis, PDase and GSK3 β	Herbliquorice	Decreased hepatic lipogenesis	[54]
PHYTOSTEROLS	Antioxidant, Lipid-modifying	Downregulate TGF- β , IL-6, IL-10, C-reactive protein, lipoprotein cholesterol (LDL-C) and hepatic TG	Unrefined plant oils, Nuts	Increased liver lipid metabolism	[55]
CAROTENOIDS	Antioxidant, anti-inflammatory	Downregulate Malondialdehyde, TNF- α	Carrots, guava, mangoes, collard greens	Decreased fat accumulation and bleedings	[56]

Table 1.
Mechanism of action of functional foods against NAFLD.

4.1.3 Isoflavones

Isoflavones exhibited an excellent therapeutic effect for NAFLD through de novo lipogenesis via ChREBP and anti-adipogenic Wnt signaling [62]. Genistein derived from soybean is the most investigated isoflavone with higher potency against NAFLD. Genistein supplementation (2 and 4 g/kg diet) for 12 weeks markedly reduced serum and liver lipids and downregulated SREBP-1c, PPAR- γ in NAFLD mice [63]. Genistein significantly suppressed the expression of cyclooxygenase-1 and hepatic thromboxane A2 receptor expression through the thromboxane A2 (TXA2) pathway [46]. Daidzein, a naturally occurring phytoestrogen occurring in soybean and legumes, reduced NAFLD risk and inhibited hepatic fatty acid β -oxidation in high fat supplemented mice [47]. Liu et al. (2017) showed administration of soy isoflavone (10 or 20 mg/kg) to NAFLD animals inhibited fatty acid synthesis. It promoted fat oxidation in the liver by regulating the expression of SREBP-1c and PPAR α [64].

4.1.4 Resveratrol

Resveratrol (3, 5, 4'-trihydroxystilbene) is widely present in the skin of grapes, berries and peanuts. Oral administration of resveratrol (50 mg/kg body weight) to high-fat diet (HFD)-induced C57BL/6 J mice reduced inflammation and fibrosis risk by modulating the I κ B α -NF- κ B and autophagic pathway [48]. Resveratrol by upregulating SIRT1 decreased liver lipogenesis markers and improved lipid metabolism in HFD fed mice [65]. Intragastric administration of resveratrol improved hepatic steatosis mediated by a SIRT1/ATF6-dependent mechanism in HFD fed mice [66]. Theodotou et al. (2019) showed supplementation of trans-resveratrol as a micronized formulation to NAFLD subjects reduced TG accumulation and improved insulin resistance via activation of 5' adenosine monophosphate-activated protein kinase (AMPK) and SIRT1 [67].

4.2 Proanthocyanidins and flavonoids

The oligomeric and polymeric components of the flavonoid biosynthetic pathway are proanthocyanidins, also known as condensed tannins. Proanthocyanidins are widely distributed in seeds, fruits, flowers, nuts, barks of several plants and are typically made up of catechin and epicatechin [68]. The number of anti-NAFLD proanthocyanidins published studies is less, but findings are similar to those of other flavonoids (**Figure 2**). The grape seed proanthocyanidins (GSP) exhibited anti-NAFLD effect mainly by lipid-lowering and high antioxidant activities [49]. In another study, GSP suppressed high calorie diet-induced hepatic injury in animals [69]. A significant number of studies demonstrated the hepatoprotective and anti-fibrotic effect of morin in NAFLD models, mainly by modulating the key signaling pathways associated with fibrosis [70]. Administration of polymethoxylated flavones enriched Daoxianyeju extracts (0.2% and 0.5%) to HFD fed mice prevented liver inflammation and steatosis by activating nuclear factor erythroid-2 related factor 2 (Nrf2) signaling [71]. Tannic acid supplementation to the western diet-fed mice for 12 weeks suppressed histone acetyltransferase activity and prevented lipid accumulation [72]. Silybin, also known as silibinin, derived from *Silybum* (milk thistle) plant extracts in combination with tangeretin (75–150 mg/kg) showed potent antioxidant, anti-inflammatory and anti-fibrotic activities [73]. Citrus peel extract composed of hesperidin, narirutin, synephrine and tangeretin prevented *in vivo* lipid accumulation and fatty liver development by regulating AMPK activation [74].

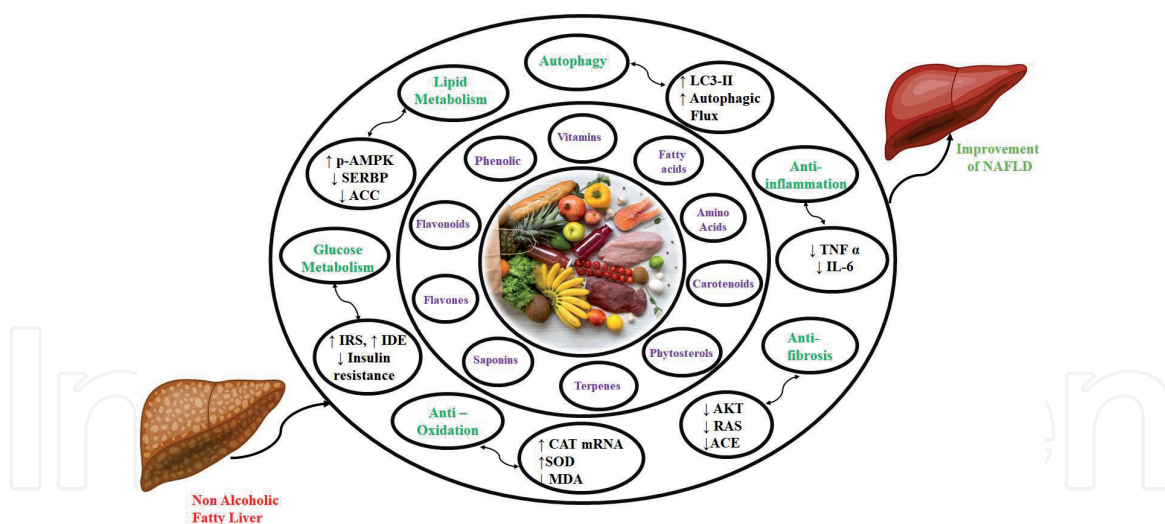


Figure 2.
 Significance of functional foods for NAFLD.

4.3 Saponins and terpenes

Saponins consist of a broad family of structurally similar substances with steroid or triterpenoid glycone (Sapogenin) containing more than a fraction of oligosaccharides. The saponins and their derivatives are reported in several edible legumes. Several *in vivo* studies documented legume saponins' health benefits, including antioxidant, antidiabetic, hepatoprotective, hypocholesterolemic, anti-cancer, antitumor, antiviral [75]. Treatment of Akebia saponin D (100 μ M) to oleic acid-induced BRL cells reduced lipid accumulation, increased BNip3 levels and mitophagy [76]. Hou et al. (2020) demonstrated oral administration of ginsenoside-Rg1 (30 mg/kg/day) reduced SREBP-1c expression, lipid accumulation and alleviated liver inflammation in NAFLD rats [52]. Sea cucumber-derived saponins echinoside A (EA) ameliorated orotic acid induced-NAFLD mainly by inhibiting lipogenesis genes. Andrographolide, a diterpene lactone present in *Andrographis paniculata* treatment to choline-deficient amino acid-defined mice, prevented liver inflammation, reduced macrophage infiltration and inflammation activation [53, 77].

Wang et al. observed glycyrrhizic acid (a natural triterpene glycoside) administration to MCD diet-fed mice significantly inhibited hepatic stellate cell activation and collagen deposition [54]. In a similar study, glycyrrhizic acid suppressed lipid accumulation and reduced the levels of SREBP-1c, FAS, SCD-1 in HFD fed mice [78]. Glycyrrhetinic acid, a bioactive triterpenoid from licorice, reduced the inflammation and fat content in the mouse liver and inhibited AKR1B10 activity [79].

4.4 Phytosterols

Phytosterols or plant sterols are cholesterol-like molecules which perform vital structural functions in plants. Phytosterols are best known for their cholesterol-lowering effects and recent investigations highlighted their anti-fibrotic developments in key NAFLD models [80]. Plant sterol and stanol ester supplementation significantly reduced plasma lipids and prevented HFD induced inflammation in experimental animals [81]. In a high-fat Western-style diet-induced mice study, stigmasterol and β -sitosterol markedly reduced the liver TGs, cholesterol, intestinal bile acid levels and alleviated NAFLD [55]. Intragastric administration of phytosterol esters for 12 weeks to HFD fed rats reduced liver size, lipid content and improved intestinal flora [82]. In combination with EPA and DHA, phytosterol

esters significantly reduced the levels of TGs, cholesterol, LDL cholesterol and decreased the pro-inflammatory cytokines in NAFLD subjects [83]. β -sitosterol supplementation for 12 weeks mitigated high-fructose diet-induced macrovesicular steatosis and progression of steatohepatitis [84].

4.5 Carotenoids

Carotenoids are a family of poly-isoprenoid structured and fat-soluble pigments that occur naturally in plants and microbes. The primary sources of carotenoids in the human diet are yellow, orange and red-colored fruits and vegetables. In the last few decades, carotenoids have been the main focus of research mainly due to their potent antioxidant, anti-inflammatory and anticancer properties [85]. Besides, carotenoids were also reported for their anti-fibrotic effect in several experimental NAFLD models [86]. Several clinical studies revealed circulatory carotenoid levels to NAFLD risk [56, 87]. β -carotene is reported for strong antioxidant potential and a vast number of *in vitro* and *in vivo* studies revealed the hepatoprotective and anti-fibrotic effect of β -carotene [88–90]. Lycopene, a non-provitamin A carotenoid, mainly exhibited hepatoprotective effect through scavenging ROS. Supplementation of lycopene significantly lowered steatosis and obesity-induced inflammation in NAFLD animals [91–94]. The xanthophyll carotenoid, astaxanthin is reported for various biological effects such as free radical scavenging, ocular protective, hepatoprotective, anti-aging, anti-diabetic, anti-inflammatory, anticancer, etc. [95–97]. In experimental liver fibrosis models, astaxanthin offered hepatoprotection by reducing liver pro-inflammatory cytokines, attenuating insulin resistance, downregulating key signaling pathways [98–102]. Other carotenoids like α -carotene, lutein and zeaxanthin also exerted hepatoprotection in experimental NAFLD models [103–105].

4.6 Functional foods from plant/animal origin/carbohydrates

Oats (*Avena sativa*) rich in β -glucan, a polysaccharide responsible for its functional properties and other active compounds such as antioxidants, vitamins, minerals and phenolic compounds and dietary fibers. A study conducted in Sprague–Dawley rats found that a diet rich in oats increased liver LDLR, reduced liver TGs and cholesterol, thereby preventing NAFLD development to liver cirrhosis [106]. Flaxseed (*Linum usitatissimum*) is a highly nutritional functional food due to active components such as polyunsaturated fatty acid (PUFA), α -linolenic acid, proteins, lignans, soluble and insoluble dietary fibers, antioxidants and phytoestrogens. In a clinical trial conducted with 50 subjects, supplementation of flaxseed diet significantly reduced the body weight, liver enzymes, insulin resistance, hepatic fibrosis and steatosis. A significant difference was observed between control and flaxseed groups in ALT, AST, GGT, fibrosis score and steatosis score, etc. [107].

Choline is an essential nutrient present in eggs, liver, soy wheat and vegetables. Choline is either produced in the body or is absorbed from a diet rich in phospholipids such as phosphatidylcholine. Phosphatidylcholine is a significant component of cell membranes and present in egg yolk and soy. Choline and betaine supplementation effectively alleviated NAFL in dairy cattle, PEMT- deficient mice by increasing AMPK, reducing mRNA levels of DGAT2 and lipid accumulation, decreased expression of genes such as acyl-CoA synthase-1 and -4, mitochondrial glycerol phosphate acyltransferase, etc. [108]. Studies conducted in humans showed that betaine supplementation reduced serum concentrations of ALT and AST and lowered hepatic steatosis [109, 110]. In Balb/c mice, administration of betaine increased serum ALT, decreased hepatic and visceral mass accumulation by reducing glucose

production through inhibiting gluconeogenesis and promoting the use of glucose in glycogen production leading to improved serum glucose levels. Thereby, betaine reversed insulin resistance by promoting IRS1 phosphorylation and enhanced downstream pathways of gluconeogenesis and glycogen synthesis and effectively alleviated NAFLD [111].

4.7 Functional foods from microbial origin

Monascus is a fungi class that includes *M. purpureus*, *M. pilosus* and *M. ruber* relevant in the field of functional food due to the presence of bioactive metabolites such as monascin and ankaflavin. These compounds possess pharmacological properties such as antioxidant, anti-inflammatory, antidiabetic, immunomodulatory and anticancer [112]. A study conducted in FL83B hepatocytes and male C57BL/6 J mice observed that both monascin and ankaflavin inhibited fat accumulation in hepatocytes by preventing fatty acid uptake, lipogenesis and accelerating fatty acid β -oxidation. Monascin and ankaflavin also improved AMPK phosphorylation and downregulated expression of steatosis related genes. Treatment with monascin and ankaflavin suppressed expression of SREBP-1c, FAS, ACC and upregulated FXR, PGC-1 α and PPAR- α . This result suggested that monascin and ankaflavin are potential bioactives for NAFLD [113, 114].

Sargassum serratifolium is a brown macroalgae that possess several bioactive compounds such as sargahydroquinic acid, sargachromenol, sargaquinic acid, etc. This seaweed is widely used in culinary preparations of Korea and China. *S. serratifolium* have many pharmacological properties such as anti-inflammatory, anti-obesity, lipid-lowering, etc. A review of C57BL/6 J mice treated with a rich ethanol fraction of *S. serratifolium* demonstrated lipid-lowering effects by activating AMPK-mediated fatty acid oxidation signaling and prevented SREBP-1c signaling related lipogenesis in the liver and fatty tissues. The extract was also able to downregulate FAS and SCD-1 along with SREBP-1c and inhibited TG synthesis and cholesterol and activated fatty acid oxidation by promoting AMPK. This showed that *S. serratifolium* is a practical, functional ingredient for alleviating NAFLD by controlling lipid accumulation in liver [115].

Freshwater clams (*Corbicula fluminea*) is well-known hepatoprotective used in Chinese traditional medicine. The major active components are brassicasterol, campesterol, stigmasterol, α -linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid and carotenoids. In HepG2 cells, ethanolic extract of residual clam meat significantly decreased lipid accumulation by suppressing FAS. In tilapia and mice models, the clam extract reduced AST levels, ALT, total cholesterol, accumulation of triglycerols, etc. The extract was also able to downregulate SCD-1 index, promoted PUFA n3/n6 ratio and reduced ballooning, PGE2, total fatty acids, triacylglycerol level, hepatocyte size and inflammation, etc. This result showed that freshwater clam extract is a useful functional component for developing hepatoprotective supplements against NAFL [116].

5. Role of functional foods for NAFLD

There may be limitations to their use in disease prevention in Anti-NAFLD functional foods, while there are opportunities to assess functional foods' ability as anti-NAFLD compounds.

1. It will be essential to address the suitable intake of anti-NAFLD compounds that confer prevention without toxicity.

2. Knowledge about the kinetics of functional foods or their blood metabolite levels, which may differ from individuals and diseases, would be required over the individual's lifespan for a sustained level of NAFLD inhibitor in the blood.
3. If clinical evidence shows that a dietary component is successful in disease prevention and broad intervention trials prove effective in reducing those disease-related biomarkers, then the medical community can embrace the element.
4. How early in life is anti-NAFLD food being consumed?
5. When does one stop using anti-NAFLD functionally in the event of liver regeneration, such as fat dissipation?
6. How does one assess whether the prevention of disease progression with anti-NAFLD functional foods has been affected?
7. Nevertheless, the use of anti-NAFLD compounds, some from natural sources and others from synthesis, such as LPSF/GQ-02, have shown successful clinical efficacy. However, Thiazolidinediones (TZD) were addressed by suggesting that acting as an insulin sensitizer effective against insulin resistance and fat accumulation could be good anti-NAFLD agents at an early stage NAFLD. Anti-NAFLD functional foods can, therefore, be beneficial at the early stages of the non-alcoholic fatty liver cycle [117].
8. Data on the toxicity of the anti-NAFLD compound will be needed for diseases such as diabetes, where both excessive and insufficient NAFLDs exist, compounds that may inhibit excessive NAFLDs and worsen insufficient NAFLDs may need to be established.
9. Most human NAFLD or liver steatosis cells are deficient in PNPLA3 (Patatin like phospholipase domain-containing gene. This deficiency in PNPLA3 indicates that high-fat deposition activity after eating and decreases during periods of fasting food. It plays a role in toxic clearance hence their responsiveness to Anti- NAFLD compounds [118].

6. Bioavailability and synergy of anti-NAFLD functional foods

Low molecular weight anti-NAFLD compounds such as phenolics, terpenes, phytosterols, small carbohydrates and amino acids might be more bioavailable than high and moderately soluble proteins, polypeptides and large carbohydrate molecules. Nonetheless, large molecules such as proteins and carbohydrates have shown a protective effect against NAFLD. In contrast, substances with low molecular weight might help liver regeneration. Combining one or more anti-NAFLD compounds has been shown to result in improved behavior in several studies. Silybin treatment, in combination with vitamin E and phosphatidylcholine, significantly improved liver enzymes and liver steatosis in NAFLD patients [119]. Another study by Han et al. showed metformin (500 mg orally three times daily) in combination with vitamin E (100 mg) and bicyclol (25 mg) synergistically prevented NAFLD in human subjects by improving liver enzymes and liver histology parameters. The combination of blueberry juice and probiotics 1.5 mL per 100 g (0.07 mg/mL concentration) weight showed protection to hepatocyte mitochondrial function in the HFD induced animals [120].

7. Conclusion and future prospects

Even though there is no FDA approved anti-NAFLD compound, industrial and academic researchers are still investigating for naturally occurring bioactive(s) for potential and safe anti-NAFLD compounds. Prior consideration is necessary for designing anti-NAFLD functional foods, especially for complex diseases such as insulin resistance, chronic liver diseases, etc. Food bioactive such as curcumin, proanthocyanidins that have shown hepatoprotective and anti-NAFLD properties, are the right candidates for incorporating into functional foods. Also, research support is highly essential for accessing the anti-NAFLD properties of other food components. Anti-NAFLD functional foods could be a low-cost strategy to prevent obesity-related complications. NAFLD inhibitors are recognized as one of the targets for obesity therapy [121]. The development of post-genomic functional foods may need to focus on molecular targets such as NAFLD factors that drive the early stages of chronic disease onset/progression. A comprehensive and successful work in functional foods will involve knowledge of ethnobotany, chemotaxonomy, transgenic plants or animal animals (as bioactive compound factories) and interdisciplinary approaches involving foods, nutrition scientists, and biomedical scientists for design. In this context, researchers must have in-depth knowledge in the field of ethnobotany and chemotaxonomy. To determine the efficacy of functional food, the post-genomic wave of functional foods would need to span the entire spectrum, from primary to clinical trials.

Acknowledgements

All authors greatly acknowledge CSIR-CFTRI (MLP277) for the support.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

ACC	Acetyl-CoA carboxylase
AKR1B10	Aldo-keto reductase family 1 B10
ALT	Alanine transaminase
AMPK	5' adenosine monophosphate-activated protein kinase
AP-1	Activator Protein 1
AST	Aspartate transaminase
ATF6	Activating Transcription Factor 6
ChREBP	Carbohydrate-responsive element-binding protein
DGAT2	Diacylglycerol O-acyltransferase 2
DPA	Docosapentaenoic acid
EGCG	Epigallocatechin-3-gallate
EPA	Eicosapentaenoic acid
FABPs	Fatty-acid-binding proteins
FXR	Farnesoid X receptor
GGT	Gamma-glutamyl transferase
GSP	Grape seed proanthocyanidins
HFD	High Fat Diet

IRS-1	Insulin receptor substrate 1
IRS-2	Insulin receptor substrate 2
JNK	c-Jun N-terminal kinase/
LDL	Low-density lipoprotein
LPS	Lipopolysaccharides
LPSF/GQ-02	Benzylidene thiazolidinedione
MCD	Methionine and choline-deficient
NAFLD	Non-Alcoholic fatty liver disease
NAFL	Non-Alcoholic fatty liver
NASH	Non-Alcoholic steatohepatitis
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
Nrf2	Nuclear factor erythroid 2-related factor 2
PEMT	Phosphatidylethanolamine N-methyltransferase
PGC-1 α	Pparg coactivator 1 alpha
PGE2	Prostaglandin E2
PNPLA3	Patatin-like Phospholipase 3
PPAR- γ	Peroxisome proliferator-activated receptor gamma
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species
SCD1	Stearoyl-Coenzyme A desaturase-1
SIRT	Sirtuins
SNPs	Single Nucleotide Polymorphisms
SOD1	Superoxide dismutase 1
SREBP-1c	Sterol regulatory element-binding protein-1c
TG	Triglyceride
TGF- β	Transforming growth factor- β
TM6SF2	Transmembrane 6 superfamily member 2
TNF- α	Tumor Necrosis Factor- α
TXA2	Thromboxane A2
TZD	Thiazolidinediones
VEGF	Vascular endothelial growth factor
VLDL	Very low-density lipoprotein

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