Pathogenesis of NASH and Promising Natural Products

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[ABSTRACT] Nonalcoholic steatohepatitis (NASH) is a common clinical condition that can lead to advanced liver diseases. The mechanism of the disease progression, which is lacking effective therapy, remains obscure. Therefore, there is a need to understand the pathogenic mechanisms responsible for disease development and progression in order to develop innovative therapies. To accomplish this goal, experimental animal models that recapitulate the human disease are necessary. Currently, an increasing number of studies have focused on natural constituents from medicinal plants which have been emerged as a new hope for NASH. This review summarized the pathogenesis of NASH, animal models commonly used, and the promising targets for therapeutics. We also reviewed the natural constituents as potential NASH therapeutic agents.

[KEY WORDS] NASH; Natural medicine; Animal model; Target; Pathogenesis

[CLC Number] R965; R284

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most frequent liver disease worldwide, and is commonly associated with the metabolic syndrome. Within the general population, the overall global prevalence of NAFLD came up to 25% though substantial variability was noted across geographic regions [peak prevalence in the Middle East (31.8%) and South America (30.4%), while the lowest rates in Africa (13.5%)] [1]. In China, NAFLD, whose prevalence is approximately 29.2%, has become the most prevalent liver disorder [2]. NAFLD is defined by excess lipid accumulation in the liver of a patient in the absence of excessive alcohol intake [3]. The spectrum of NAFLD ranges from simple steatosis to NASH, fibrosis and cirrhosis, and NAFLD may even progress to hepatocellular carcinoma without apparent cirrhosis. According to clinical experiences, NASH is characterized by hepatocellular lipid accumulation (steatosis) along with lobular inflammation, hepatocellular ballooning, and often associated with fibrosis [4]. Currently, no highly sensitive and specific tests are available to differentiate NASH from simple steatosis, and liver biopsy remains the gold standard for the diagnosis of NAFLD and to distinguish steatosis from NASH. However, diagnostic accuracy can be improved by combining blood biomarkers and imaging tools [5]. Albeit the substantial progress of knowledge about pathophysiology and targets has been made, substantial challenges exist for the therapeutic methods for NASH. Due to the complicated pathogenesis and individual heterogeneity in NASH, there has been no approved agents to date for the treatment of NASH regardless of that many compounds are under clinical evaluation, which has been extensively reviewed [6]. In addition, animal models are vital for search of novel targets and better understanding of pathogenesis. Suitable models that resemble human NASH can help patients stratification and evaluation of therapeutic outcomes in the development.

Traditional Chinese medicines (TCMs) are abundant sources of biologically active substances that can be applied to prevent human diseases. Currently, an increasing number of studies have focused on natural products, which showed potential effects against NAFLD [7]. In China, the majority of clinical trials have focused on investigating the effects of herbal extracts and natural products on NAFLD [8]. These authors have no conflict of interest to declare.

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**Pathogenesis of NASH and Main Targets for the NASH Treatment**

The complex pathogenesis makes it hard to demonstrate the clear mechanism of NASH, whose initiation addresses multiple events. The pathogenesis of NASH is controversial for that there has been two widely recognized theories for the explanation of NASH, namely “two hit” along with “multiple hit”. “Two hit” theory suggests that in the context of simple hepatosteatosis alone, the second ‘hit’ which is responsible for liver injury was required for the development of NASH \[10\]. However, this view is outdated and “multiple hit” in which pathogenetic events occur simultaneously is more favored. All the events that lead to the development of NASH provide potential targets, including liver insulin resistance, lipotoxicity, oxidative stress (including reactive oxygen species), endoplasmic reticulum (ER) stress, mitochondrial dysfunction, adipose tissue dysfunction and dysregulated innate immunity, cytokine secretion and the gut-liver axis \[11-12\], which is affected by microbiota dysbiosis. In this review we primarily focus on several processes associated with cardinal signs including inflammation, fibrosis, carbohydrate metabolism and relevant targets under evaluation (Table 1).

Inflammatory cells and cytokines have been reported to participate in NASH pathogenesis. Apoptosis signaling kinase-1 (ASK-1)-JNK, MAP kinases, ERK, and NF-κB are potent mediators of inflammation and thus potential targets for therapy. ASK1 is a serine/threonine protein kinase in the mitogen-activated protein kinase kinase kinase (MAP3K) enzyme family and acts upstream of Jun N-terminal kinase (JNK) and p38 \[13\]. Multiple elements, such as tumor necrosis factor α (TNFα), lipopolysaccharide (LPS), ER stress, calcium influx, and oxidative stresses are capable to activate ASK1. ASK1 then phosphorylates and activates an intermediate kinase (MAPK kinase 4), which in turn activates the JNK and p38 pathways in hepatic cells. These pathways lead to apoptosis, inflammation, and fibrosis \[14\]. Studies with rodent models have supported the potential utility for ASK1 inhibitors as a treatment for NASH \[15\]. Of note, the antagonism of ASK-1 ameliorated NASH and alleviated fibrosis in some patients in a short-term clinical trial \[16\].

Toll-like receptor 4 (TLR4) signaling has also been implicated in the pathogenesis of NASH by mediating innate and adaptive inflammatory responses \[17\]. TLR4 functions as a LPS sensor, and the activation of which will recruit adaptor protein myeloid differentiation primary response gene 88 (MyD88) and activate downstream NF-κB cascades \[18\]. Mice deficient in TLR4 exhibit low expression of proinflammatory cytokines even when these mice were fed a NASH-inducing diet \[19\]. JKB-121, a TLR4 antagonist, whose phase 2 trial for the treatment of NASH is ongoing (NCT02442687). In recent years, NOD-like receptor protein 3 (NLRP3) inflammasome, consisting of several sensor and signaling proteins, has been identified as another trigger for liver inflammation in NAFLD \[20\]. The signaling proteins combine to form active caspase 1 from procaspase 1. Caspase 1 is necessary for the secretion of interleukin (IL)-1β and IL-18 \[21\]. It has been proved that pharmaceutical blockade of NLRP3 activation effectively improve NAFLD pathology and fibrosis severity \[22\].

Chemokine receptors (CCR) are G-protein coupled receptors (GPCRs) that combine with chemokine ligands to trigger downstream cascade essential in facilitating basal and inflammatory leukocyte migration \[23\]. CCR2 and CCR5 are the most characterized chemokine receptors that involved in the progression of NASH and fibrosis. Inhibition of CCR2-CCR5 axis reversed short-term fibrosis in a clinical trial of NASH \[24\]. It was shown in mice that overexpression of CCR2 ligand, chemokine ligand 2 (CCL2), leads to adipose tissue inflammation and hepatic steatosis \[25\]. In rodent models of diet-induced NASH, treatment with a modified version of CCL5 that functions as an antagonist (Met-CCL5) or the small-molecule CCR5 antagonist maraviroc (a US Food and Drug administration (FDA)-approved inhibitor of CCR5-mediated entry of HIV into immune cells) ameliorated NASH and fibrosis \[26-28\]. Cenicriviroc, a dual CCR2 and CCR5 antagonist with nanomolar potency, had potent anti-inflammatory and anti-fibrotic activity in mouse models of NASH \[29\].

Metabolic syndrome (MetS), including increased waist circumference (i.e., obesity), hyperglycemia, dyslipidemia and systemic hypertension, is the strongest risk factor for NAFLD and NASH \[30\]. The metabolites induce hepaticcellular stress, injury and death, leading to fibrogenesis and genomic instability that predispose to end-stage liver diseases. The availability of metabolic targets for therapeutic strategies relies on reducing metabolic substrate delivery to the liver or

**Table 1  The review of main targets for the NASH treatment**

<table>
<thead>
<tr>
<th>Name</th>
<th>Function</th>
<th>Relevant agents</th>
</tr>
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<tbody>
<tr>
<td>ASK1</td>
<td>regulating inflammation and fibrogenesis</td>
<td>ASK1 antagonist: selonsertib (Ph 3)</td>
</tr>
<tr>
<td>TLR4</td>
<td>regulating inflammation, function as a LPS sensor</td>
<td>TLR4 antagonist: JKB-121 (Ph 2)</td>
</tr>
<tr>
<td>CCR2/5</td>
<td>regulating inflammation by controlling basal and inflammatory leukocyte trafficking</td>
<td>Dual CCR2 and CCR5 antagonist: cenicriviroc (Ph 3) CCR5 antagonist: Met-CCL5, maraviroc (FDA approved)</td>
</tr>
<tr>
<td>FGF21</td>
<td>regulating fatty acid and glucose metabolism, enhance insulin signaling</td>
<td>FGF21 analogues: BMS-986036 (Ph 2); AKR-001 (Ph 2) PEGylated FGF21 and Fc-FGF21(RG) (Ph 2a)</td>
</tr>
<tr>
<td>ACC</td>
<td>regulating fatty acid synthesis</td>
<td>ACC antagonist:PF-05221304 (Ph 2)</td>
</tr>
<tr>
<td>FXR</td>
<td>regulating bile acid synthesis and multiple metabolic pathways</td>
<td>FXR agonist: obeticholic acid (OCA) (Ph 2a)</td>
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</table>
Fibroblast growth factors (FGF) are peptide hormones that play important roles in NASH development involving fatty acid and glucose metabolism \[^{[19]}\]. FGF19 and FGF21, the most widely investigated members, together with their receptors are promising targets for NASH. FGF21 transcription is upregulated by ER stress, SIRT1 and transcription factors such as PPARα and PPARγ \[^{[14]}\]. FGF21 augments insulin sensitivity in adipose and hepatic tissues by increasing glucose transporter type 1 (GLUT1) expression, suppressing hepatic gluconeogenesis, inducing insulin signaling in adipocytes \[^{[30]}\], and reduces sterol-CoA response element binding protein-1c (SREBP1c) mediated lipogenesis in the liver \[^{[13]}\]. Several analogs of FGF21 have been advanced into clinical development \[^{[7]}\]. BMS-986036 (pegbelfermin, BMS) was tested in a 16-week phase 2 trial in NASH patients. Administration of the peptide by subcutaneous injection once daily resulted in dramatic reductions in liver fat at week 16 compared to placebo (ClinicalTrials.gov identifier NCT0241-3372). Acetyl-CoA carboxylase (ACC) is a biotin carboxylase that plays a key role in regulating fatty acid synthesis \[^{[52]}\]. There are two isoforms of ACC (ACC1 and ACC2) that have distinct tissue locations and metabolic functions \[^{[32-33]}\]. ACC1 is located in the liver, adipose tissue, and mammary gland, whereas ACC2 is primarily expressed in skeletal and heart muscle. ACC antagonism improved steatosis, inflammation, and fibrosis in both animal models and human derived cells in vitro \[^{[14]}\]. A phase 2a clinical trial results showed that ACC inhibitor PF-05221304 improved steatosis and liver injury in NAFLD patients (ClinicalTrials.gov identifier NCT03248882) \[^{[14]}\].

Farnesoid X receptor (FXR) is a nuclear hormone receptor as well as a bile-acid sensor, regulating bile acid levels through modulating multiple pathways including bile acid synthesis, de novo lipogenesis, and glucose metabolism \[^{[35]}\]. In the liver, FXR agonists enhance insulin sensitivity \[^{[36]}\], increase triglyceride clearance and mitochondrial fatty acid ß-oxidation, and suppress lipogenic gene transcription \[^{[37]}\]. In rodent models of diet-induced NASH, FXR agonists prevent the development of NASH and promoted the resolution of steatohepatitis and fibrosis \[^{[38]}\]. Obeticholic acid (OCA) is a semi-synthetic derivative of chenodeoxycholic acid with picomolar agonistic activity on FXR \[^{[39]}\]. A small randomized trial in type 2 diabetic patients with NAFLD showed an improvement in insulin sensitivity as measured by euglycemic clamp, a modest but dose-related weight loss, and a reduction in ALT levels \[^{[40]}\]. Nevertheless, appeal of small molecule agonists targeting FXR is the most advanced modality and is a highly promising strategy for treating NASH, either as a standalone therapy or in combination with other drugs \[^{[14]}\]. Controversially, several studies have suggested that depletion or antagonism of FXR, specifically in the intestine, may have therapeutic potential in NASH \[^{[41-43]}\]. Interestingly, treatment of mice with an intestine-selective FXR antagonist alleviated metabolic function in diet-induced and genetic obesity \[^{[44]}\].

**Animal Models Used for NASH**

The progress of drug development for NASH relies primarily on available animal models, which can mimic the real situation of human NASH. It is a pity that no single model perfectly displaying the full spectrum of the NASH pathophysiology. Herein we focus on the animal models highly applicable in researches. Animal models of NASH can be widely categorized as dietary, genetic, toxins-based, and combination models (Table 2).

**Dietary models**

Large portion of animal models are aimed at triggering liver damage. Therefore, diets deficient in nutrition are theoretically available for establishing models of NASH. The methionine and choline deficient (MCD) diet lack two essential factors, namely methionine and choline, whereas it’s rich in sucrose and fat (40% sucrose, 10% fat). Normal mice fed MCD and choline-deficient l-amino acid (CDAA)-defined diet develop a histological appearance similar to NASH with fat, inflammation, and fibrosis, but they fail to recapitulate the accompanying symptoms of obesity and insulin resistance, rather they will cause body weight loss. Hence, mice on CDAA or MCD diet bears little resemblance to the etiology and metabolic features of human NASH. In addition, the CDAA diet often takes longer to elicit the histological liver changes than the MCD diet does \[^{[45-47]}\].

The strong association between obesity and NAFLD has spurred the development of various diet-induced obesity (DIO) models. The high-fat diet (HFD) induces liver injury by providing saturated fats, which supply 60% caloric value of the diet, results in slight pathogenic outcomes but practically no inflammation or severe liver injury. The mice fed on HFD progress to obesity and insulin resistance after about 9 weeks with perisinusoidal hepatic fibrosis. HFD does not develop hepatic inflammation until approximately 19 weeks on the diet \[^{[46,48-49]}\].

The DIO models with macronutrient composition resembling that of obese humans are popular mouse model of NASH; however, the distinction between strains and species must also be taken into consideration. For instance, the choline-deficient l-amino-defined high fat diet (CDA-HFD) is able to induce an enlarged fatty liver with fibrosis in 6 weeks in C57BL/6J mice while gaining or maintaining body weight, yet in a different strain, A/J mice, a steady decline in body weight is observed \[^{[50]}\].

**Genetic models**

Genetic alterations can induce energy consumption, and fatty acids are delivered substantially to the liver, where ultimately excessive lipids accumulate. The commonly used models are the leptin (ob/ob)-deficient, leptin-receptor (db/db)-deficient mice, foz/foz mice (deficient in the Alstrom syndrome 1 gene), the Zucker (fa/fa) rat, and several
transgenic or conditional knockout mice. Ob/ob mice have high phenotypic concordance to db/db mice, the phenotype is characterized by obesity, hyperphagia, hyperglycaemia, insulin resistance, hyperlipidaemia and the spontaneous development of fatty liver [81-82]. Both ob/ob and db/db mice have the advantage of phenotypes similar to human metabolic syndromes; however, both models fail to spontaneously develop hepatic fibrosis or NASH without a secondary insult, such as the addition of a NAFLD-inducing or NASH-inducing diet [83]. Mice which are mutated or deficient in Alms1 are called foz/foz mice. Like ob/ob and db/db mice, these mice are also obese, insulin resistant and display steatosis [84-85].

KK-Ay/a mice possess a heterozygous mutation in the agouti gene. These mice are hyperphagic as a result of

<table>
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<th>Table 2  Commonly used animal models of NASH</th>
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<tbody>
<tr>
<td><strong>Model</strong></td>
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<td>-----------------</td>
</tr>
<tr>
<td>Dietary</td>
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<tr>
<td>Methionine and choline deficiency (MCD)</td>
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<tr>
<td>Choline-deficient L-amino-defined diet (CDAA)</td>
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<tr>
<td>High-fat diet (HFD)</td>
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<tr>
<td>High-fat, fructose and cholesterol (AMLN diet)</td>
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<tr>
<td>Toxins/Diet-based</td>
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<tr>
<td>Carbon tetrachloride (CCl₄)</td>
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<td>Thiouacemide (TAA)</td>
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<tr>
<td>streptozotocin (STZ)</td>
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<tr>
<td>Genetic</td>
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<td>Both leptin-deficient (ob/ob)</td>
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<td>Leptin receptor-deficient (db/db)</td>
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<tr>
<td>The Zucker (fa/fa) rat</td>
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<tr>
<td>MUP-uPA transgenic</td>
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<td>PTEN KO</td>
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<tr>
<td>KK-Ay/a</td>
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<td>PTEN−/−</td>
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<td>PPARα−/−</td>
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<tr>
<td>Combined</td>
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<tr>
<td>STAM (STZ + HFD)</td>
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<tr>
<td>DIAMOND mouse</td>
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paired hypothalamic food intake suppression \[56\]. They develop obesity and associated hyperglycemia, hyperinsulinemia, insulin resistance, and liver steatosis. Liver steatosis in these mice does not spontaneously advance to steatohepatitis, which, however, can be induced by additional stimulus, such as the MCD diet \[57\]. In addition, Nakagawa \textit{et al.} created a model of NASH utilizing major urinary protein (MUP)-urokinase-type plasminogen activator (uPA) mice, which can induce hepatocyte ER stress and transient liver damage \[58-59\]. It has been reported that HFD-fed MUP-uPA mice show substantial upregulation of collagen gene expression, hepatic stellate cells (HSCs) activation, and upregulation of fibrogenic markers. In conclusion, the HFD-fed MUP-uPA model is almost identical to human NASH in its pathological features \[60\].

Other genetic models such as SREBP-1c transgenic mice, and mice with global deficiencies in phosphatase and tensin homolog (PTEN), PPAR-\(\alpha\), acyl-coenzyme A oxidase (AOX), and methionine adenosyltransferase 1A (MAT1A) have also been studied. As previously reviewed \[53\], these models have limitations since they fail to display obesity. Moreover, RNA-sequencing (RNA-seq) analysis had shown that the liver gene expression profile of Pten \(-/\) mice is quite discordant from other mouse models of NASH \[61\].

\textit{Toxins-based models}

Chemically induced parenchymal liver damage and fibrosis is specifically used for studying mechanisms of hepatic fibrosis progression and regression. The frequently applied chemotoxins contain carbon tetrachloride (CCl\(_4\)), thioacetamide (TAA), and streptozotocin (STZ). CCl\(_4\) is the most widely used toxin for inducing reproducible and predictable hepatic fibrosis, contributing to severe cirrhotic necrosis in hepatocytes through its metabolite trichloromethyl radical \[62\]. STZ is particularly toxic to pancreatic \(\beta\) cells, leading to progressive loss of insulin generation, but STZ can also have hyperglycemia-independent direct hepatotoxic effects \[63\]. Of note, CCl\(_4\) and TAA (with or without high-fat dieting) are used in adult mice, STZ is administered to neonatal mice (STAM model) \[64\]. Generally, hepatic chemotoxins are contributable to weight reduction in mouse models. The corresponding alterations which are similar to CDAA and MCD diet lead to the limitations of their utilities for demonstrating etiology and nature history of NASH, thus the primary applications are on initial pathogenic factors.

\textit{Combined models}

The limitations of existing models led us to develop mouse models of NASH that meets many criteria for a relevant NASH model outlined above. The combination of multiple interventions could make up for respective disadvantages and more faithfully mimic the human NASH. For instance, a model administered a Western diet (WD) with weekly CCl\(_4\) also appears to closely resemble human NASH \[65\]. As mentioned above, ob/ob and db/db mice fail to develop fibrosis without a second hit, so their combination with MCD diet will supply the deficiency.

In the streptozotocin high-fat diet model (STAM), C57BL/6J mice are given low-dose streptozotocin immediately after birth, surviving mice are then started on HFD. These mice develop steatohepatitis, fibrosis and HCCs in approximately 20 weeks \[66\]. This model recapitulates several important histological aspects of human NAFLD and is also associated with oxidative stress \[67\], however, it differs from the human state as \(\beta\) cell function loss is induced by streptozotocin rather than a systemic, inflammatory, insulin resistant milieu.

Another recently developed model is the Diet Induced Animal Model of Non-alcoholic Fatty Liver Disease (DIAMOND) mouse, which also incorporates a high fat and fructose diet on a specific genetic strain (129S1/SvImJ and C57BL/6J cross) \[67\]. The DIAMOND mouse is able to recapitulate all histological features of NASH including ballooning. Although this model better represents clinical human NASH pathological process, it requires a long course of dietary intervention (months to years) to present many features of NASH.

\textbf{Potential Natural Constituents for NASH Therapy}

Based on the reality that there have been no approved agents for the treatment of NASH, we summarize the natural constituents which have anti-steatohepatitis activity. The deeper exploration of natural compounds may be promising direction for curing NASH. Here, we categorize them into four subsets according to their functions in the review (Table 3). The potential natural compounds and the regulated aspects are showed in Fig.1.

\textit{Regulating metabolism}

Numerous natural compounds have been reported to regulate metabolisms (Fig. 2). Emodin (1), isolated form \textit{Rheum palmatum L.}, has been used in the treatment of ischemic disease and inflammatory disease previously \[68\]. Dong \textit{et al.} established a rodent mouse model fed with high caloric laboratory chow, and they found emodin (1) treatment improved liver injury and weight normalization. In addition, blood lipid and hepatic triglyceride in emodin group markedly reduced, which may contribute to the increased expression of PPAR\(\gamma\) \[69\]. It has also been reported that myricetin (2) and plumbagin (3) lowered hepatic lipid levels possibly by altering PPAR signaling \[70-71\].

AMPK is a ubiquitous heterotrimetric serine/threonine kinase that acts as a vital cellular energy sensor and a pivotal regulator of cellular metabolism. Therefore, some natural compounds have been found to regulate AMPK activity and functions as lipid modulator in NASH models. Guo and his colleagues reported that baicalin (4) impeded NASH progression in HFD-fed rats through promoting AMPK and ACC phosphorylation \[72\]. Berbamine (5), a natural bisbenzylisoquinoline alkaloid derived from \textit{Berberisamurensis}, was reported to allay intracellular lipid accumulation. Sharma \textit{et al.} found that berbamine (5) could attenuate lipid accumulation via AMPK/mTOR/SREBP-1c axis \[73\]. In addition, Neet...
andrin B (6) and daphnetin (7) have been proved to participate in the activation of AMPK \[16-20\]. Theacrine (8) and nuciferine (9) were reported to effectively decreased fatty acid, further molecular investigations indicated their improvement.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Summary of natural compounds displaying anti-nonalcoholic steatohepatitis activity</th>
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<tbody>
<tr>
<td>Biological function towards NASH</td>
<td>N°</td>
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<tr>
<td>Regulating metabolism</td>
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<tr>
<td>Regulating inflammation</td>
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<td>Biological function towards NASH</td>
<td>Nº</td>
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<td></td>
<td>(36)</td>
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<tr>
<td>Regulating fibrosis</td>
<td>(37)</td>
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<tr>
<td>(38) Geraniol</td>
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<tr>
<td>(39) Genistein</td>
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<tr>
<td>(40) Naringenin</td>
<td></td>
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<tr>
<td>(41) Scoparone</td>
<td></td>
</tr>
<tr>
<td>(42) Epigallocatechin-3-gallate (EGCG)</td>
<td></td>
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<tr>
<td>(43) Isorhamnetin</td>
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<tr>
<td>(44) Astragaloside IV</td>
<td></td>
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<tr>
<td>(45) 1,8-Cineole</td>
<td></td>
</tr>
<tr>
<td>(46) Glycyrrhizic acid</td>
<td></td>
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<tr>
<td>(47) Xanthohumol</td>
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<tr>
<td>(48) Curcumin</td>
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<tr>
<td>(49) Thymoquinone</td>
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<tr>
<td>(50) Isochlorogenic acid B</td>
<td></td>
</tr>
<tr>
<td>(51) Conophylline</td>
<td></td>
</tr>
<tr>
<td>Regulating intestinal microbiota</td>
<td>(52)</td>
</tr>
<tr>
<td>(53) Salidroside</td>
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![Fig. 1](image-url)  
*The natural compounds and the relevant events in NASH*
in energy expenditure \(^{76-77}\).

Constituents regulating metabolism are often characterized by the interference in de novo lipogenesis or fatty acid oxidation. Luteolin (10), tomatidine (11), oxymatrine (12) and oleanolic acid (13) were reported to regulate glucose homeostasis and lipid synthesis via reducing SREBP-1c, ACC and FAS expression \(^{78-81}\). Nordihydroguaiaretic acid (14) or schizandrin A (15) supplement alleviated obesity partially contributing to the elevated fatty acid oxidation \(^{82-85}\). In common leptin-deficient murine models, dioscin (16) or acetylschikonin (17) treatment suppressed total triglyceride synthesis and attenuated liver injuries in ob/ob and db/db mice respectively \(^{86-89}\). Betulinic acid (18), tiliamosine (19) and cycloastragenol (20) can activate FXR to improve ER stress, cholesterol metabolism, and simultaneously repress metabolic disorders \(^{90-93}\). Ginsenoside Rb1 (21), the major bioactive component of ginseng, exerts anti-diabetic and anti-insulin resistance effects. Ginsenoside Rb1 (21) treatment could increase insulin sensitivity, and reduce hepatic lipid content and liver weight. The beneficial effect of ginsenoside Rb1 (21) was primarily attributed to the reduction of ectopic lipid accumulation and lipolysis in adipocytes, which may result from the increasing level of adiponectin \(^{94}\). The elevated leptin and adiponectin were also observed in OA-induced primary hepatocytes from rats after sophocarpine (22) treatment \(^{95}\).

**Regulating inflammation**

Silymarin, an extract from a milk thistle plant, has been extensively documented for prevention from liver injuries \(^{96-99}\). Silybinin (23), the major bioactive component of silymarin, was capable of attenuating hepatic steatosis, hepatocyte ballooning and lobular inflammation in db/db mice fed the MCD diet \(^{99}\). Cyanidin 3-O-β-D-glucoside (24) was also reported to abate hepatic steatosis, neutrophil infiltration in db/db mice \(^{99}\).

Resveratrol (25) and quercetin (26) have been elucidated to have anti-inflammation activity for NASH based on similar mechanisms. Resveratrol (25) is established for the antiox-

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**Fig. 2** The natural compounds regulating metabolism against NASH
Excessive accumulation of ECM as aspects of pathology [98]. Resveratrol (25) treatment suppressed hepatic steatosis, insulin resistance, and inflammation in HFD-induced mouse models of NASH [66, 97]. Mechanically, resveratrol (25) upregulates autophagy and Nrf2-mediated antioxidant defence, downregulates NF-κB-mediated inflammatory response in hepatocytes and adipocytes [98]. In HFD-induced mice and rats, carnosic acid (27), scutellaran (28) and matrine (29) treatment morphologically alleviated inflammatory histology involving in modulation of NF-κB pathways [99-101]. Quercetin (26), a flavonoid typically exists in fruits including broccoli, onions and leafy green vegetables [102]. In HFD-induced rodent NASH models, quercetin (26) treatment improved hepatic steatosis, reduced inflammatory cell infiltration and portal fibrosis. Except for the mechanisms overlapped with resveratrol (25), quercetin (26) also curbs the generation of CYP2E1-mediated reactive oxygen species (ROS), which is crucial in the pathogenesis of NASH [98]. Oxidative stress determined by ROS production together with the SOD level was markedly reduced after treatment with geniposide (30) and salvianolic acid A (31) in palmitic acid (PA)-induced HepG2 cells [103-104]. NACHT, LRR, and PYD domains-containing protein 3 (NLPR3) inflammasome activation tightly relevant to ROS production was inhibited by digoxin (32) in HFD-fed mice [105].

Natural compounds have also shown to exert immunomodulatory properties by inhibition of proinflammatory cytokines release through altering percentages of immune cells in the liver. Koumine (33) was documented to enhance the proportion of hepatic CD4⁺/CD8⁺ T cells, Treg cells in HFD-fed rats, indicating its regulating activity in immune system [106]. In NASH murine models, schisandrin B (34) or kukoamine B (35) treatment downregulated TNF-α, IL-1β and IL-6 levels [107-108]. Geraniol (36) or betaine (37) supplement showed anti-inflammatory function through ameliorating mitochondrial dysfunction in rats and mice fed with MCD [106-109]. Genistein (38) is a phytoestrogen mainly found in soy and functions as antioxidant [111]. Genistein (38) treatment alleviated liver inflammation and fibrosis via decline in TNF-α and IL-1β in NASH mice induced by MCD diet [112]. Similarly, naringenin (39) and scoparone (40) were discovered to markedly inhibit hepatic inflammation in MCD mice [113-114].

In preobese mice induced by HFD diet, evident metabolic improvements in hepatic insulin sensitivity, glucose tolerance were observed after intervention with withaferin A (41) (WA), which has been reported to alleviate many metabolic disorders [115-116]. Abu Bakar and his colleagues found that WA (41) improved hepatic oxidative functions via augmented antioxidant enzyme activities [117]. Naural constituents exhibiting anti-inflammatory potential are shown in Fig. 3.

Regulating fibrosis

Liver fibrosis is characterized by activation of HSCs and excessive accumulation of ECM as aspects of pathology [118]. Liver fibrosis is the primary determinant of clinical disease progression and outcomes of patients with NASH [119]. Hepatic iron deposition is inevitably disrupted when excessive fibrogenesis occurs, and fibrosis ordinarily indicates a higher likelihood of progression to cirrhosis and other end-stage liver diseases in NASH [120].

Concerns were raised on the protective role of epigallocatechin-3-gallate (EGCG) (42) against NASH, which is one of the major green tea catechins [121]. Apart from that EGCG has been previously reported for counteracting the activity of TGF/SMAD, PI3K/Akt/FoxO1 and NF-κB cascades [122], it was found by Kochi et al. that EGCG (42) administration impeded the development of liver fibrosis in a SHRSP-ZF rat model induced by HFD and CCl₄ [123]. And isorhamnetin (43) treatment showed protective effect on fibrosis through reducing fibrogenic markers expression in mice induced by combinations of HFD diet and CCl₄. Astragaloside, the major active components of Radix Astragalii, significantly ameliorated liver fibrosis via inhibition of TGF/1/Smad pathway in CCl₄ induced mouse model [124]. And astragaloside IV (44) has been proved by much evidence for its various pharmacological activities including antioxidant stress, anti-inflammatory, anticancer and antifibrosis [126-127]. Administration with 1,8-Cineole (45) in PTEN−/− mice improved fibrosis stage and reduced Collagen 1 expression [128].

The activation of HSCs, which is the central mediator of liver fibrogenesis, was suppressed by interventions with glycyrrhizic acid (46) and xanthohumol (47). In MCD-diet fed mice, glycyrrhizic acid (46) also exerted multiple hepatoprotective effects including inflammation amelioration and lipid metabolism regulation apart from limiting pericellular fibrosis and collagen deposition [129]. Xanthohumol (47) is a promising compound for treating fibrosis in NASH for inducing activated HSC apoptosis in vitro in a dose dependent manner without impairing viability of human primary hepatocytes at high doses [130].

Curcumin (48) is a polyphenol derived from the herbal remedy and spice turmeric [131], emerging as a hepatoprotective compound. Previous studies have proved the protective effects of curcumin (48) toward several types of chemically induced hepatotoxicity, including CCl₄ and alcoholic induced liver disease [132-133]. In a mouse model established by feeding MCD diet, curcumin (48) treatment significantly reduced α-smooth muscle actin and restricted the fibrosis stage [134]. With regard to the integrated prevention against NASH, its insulin-sensitizing function depends on decreasing hepatic PTP1B expression and activity [135]. Thymoquinone (49) has been reported by Awad et al. for the potential of improving hepatic steatosis, inflammatory, apoptotic status and fibrosis in high-fat high-cholesterol (HFHC) diet-feeding rats [136]. Besides, in a mouse model induced by MCD-diet, isochlorogenic acid B (50) exhibited comprehensive anti-fibrosis actions, which encompass minimal fibrosis state, downregulating genes involved in fibrosis, suppressing multiple profibrogenic factors [137]. Nakade et al. and his colleagues investigated the effect of conophylline (51) (CnP), a vinca alkaloid isol-
Regulating intestinal microbiota

Despite the detailed mechanisms of microbiota dysbiosis in the intestine on the pathogenesis of NASH are obscure to date, the intestinal microecology might hinge on regulating energy balance and metabolism to promote NASH. It has been reported that HFD- or fructose-induced dysbiosis may enhance energy harvest from ingested food and, together with inflammation, promote energy storage and insulin resistance [140]. Dysbiosis can contribute to deterioration of the intestinal epithelial barrier, resulting in translocation of inflammation-provoking bacterial products and metabolites (and even intact bacteria) that reach the liver via the portal circulation to further enhance NAFLD and NASH progression [140].

Berberine (52), distributed majorly in the Chinese medicine Coptis chinensis, is originally known for its clinical use for bacterial diarrhea [142]. In HFD-fed mice, berberine (52) has been reported to regulate the relative abundance of both Firmicutes and Bacteroidetes in the gut [143]. Subsequently, Zhang et al. found that berberine (52) treatment could effectively curb the progression of NASH in HFD-fed rats via altering the gut microbiota construction [144]. Salidroside (53) is the main active component of Roseroot, which has been established for its glycolipid metabolism-regulating and anti-inflammation properties [145-147]. Salidroside (53) can attenuate liver lipid accumulation, inflammatory responses, and insulin resistance in rats with NAFLD [148]. In murine NASH models induced by HFD, treatment with salidroside (53) significantly ameliorated HFD-induced intestinal bacteria, and bile acid disorder, thus proving the potential role of salidroside (53) in NASH treatment via the gut microbiota-bile acid-FXR axis [149]. In addition, resveratrol (25) was reported to maintain gut barrier integrity and restrict inflammation in intestines. Resveratrol (25) treatment led to metabolic endotoxemia reduction and gut microbial distribution alterations in SD rats fed with HFD [140]. Natural compounds which can regulate fibrosis and intestinal microbiota are shown in Fig. 4.
Discussion

NASH is a worldwide chronic liver disease which brings huge economic and health burden. The pathological initiation of NASH is complicated and no drugs have been approved. There is a large body of events such as glycolipid metabolism dysregulation, ER stress, mitochondrial dysfunction, impaired immune systems, cytokine secretion, and the microbiota dysbiosis, correlating positively with NASH development. The progression to other end-stage liver diseases render it more urgent to develop available agents for clinical application.

The applications of existing agents and compounds under evaluation are limited for the lack of effectiveness or various side-effects. Accumulating evidences from experimental studies indicates that a number of natural products from TCMs can attenuate NASH through underlying mechanisms. Over the past centuries, TCMs have exerted preventative and curative applications on NAFLD. Quantities of TCMs are under clinical evaluation despite the obscure of primary active components. It’s essential to search potential natural products in the treatment for NASH, but their appeal for clinical use warrants further investigation.

Abbreviations

NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease; TCMs, traditional Chinese medicines; ER, endoplasmic reticulum; ASK-1, apoptosis signaling kinase-1; NF-κB, nuclear factor-kappa B; MAP3K, mitogen-activated protein kinase kinase kinase; JNK, Jun N-terminal kinase; TLR4, Toll-like receptor 4; MyD88, myeloid differentiation primary response gene 88; NLRP3, NOD-like receptor protein 3; IL-1β, interleukin-1β; TNFα, tumor necrosis factor α; LPS, lipopolysaccharide; CCR, Chemokine receptors; GPCRs, G-protein coupled receptors; CCL2, chemokine ligand 2; FDA, Food and Drug administration; MetS, metabolic syndrome; HFD, high-fat diet; MUP, major urinary protein; uPA, urokinase type plasminogen activator; PTEN, phosphatase and tensin homolog; AOX, acyl-coenzyme A oxidase; MAT1A, methionine adenosyltransferase 1A; RNA-seq, RNA-sequencing; CCl4, carbon tetrachloride; TAA, thioacetamide; STZ, streptozotocin; WD, western diet; DIAMOND, Diet Induced Animal Model of Non-alcoholic Fatty

Fig. 4 Regulating fibrosis (42–51) and intestinal microbiota (52, 53) anti-NASH

strong potential for natural products in the treatment for NASH, but their appeal for clinical use warrants further investigation.
Liver Disease; ROS, reactive oxygen species; NLRP3, NACHT, LRR, and PYD domains-containing protein 3; WA, withaferin A; OA, oleic acid; HFDHFr, high-fat and high-fructose diet; HFHC, high-fat-high-cholesterol diet; ALIOS, American Lifestyle-Induced Obesity Syndrome; DEN, diethylnitrosamine; BDL, bile duct ligation; PA, palmitic acid; HFDfr, high-fat diet/high-fructose; EGCG, epigallocatechin-3-gallate.

References


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