Phytoestrogens in NAFLD: Potential Mechanisms of Action

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ABSTRACT

Many studies have shown that estrogen has a protective effect on premenopausal women with metabolic disorders and non-alcoholic fatty liver disease. Estrogen supplements may, at least in theory, prevent the development and progression of NAFLD, while the possibility of inducing cancer limits its application in practice. Phytoestrogen is extracted from plants, whose molecular structure and biological activity are similar to those of mammals' estrogen, therefore, could replace the role of estrogen and prevent the occurrence of adverse reactions to estrogen. This article reviews the published literature related to phytoestrogens and NAFLD as well as suggest the possible mechanisms that may underlie the association between phytoestrogens and NAFLD. It is hoped to provide basis for the treatment of NAFLD with phytoestrogen.

Phytoestrogen

Phytoestrogens are plant-derived xenoestrogens, also called "dietary estrogens", which are a group of naturally occurring nonsteroidal plant compounds. They have estrogenic or/and anti-estrogenic effects because of similar structure to estrogen by engaging and blocking receptor sites against estrogen. Coumestans, prenylflavonoids, and isoflavones are currently recognized the most active in estrogenic effects; isoflavones, which are commonly found in soy and red clover are the best-researched so far. Lignans have also been identified as phytoestrogens, although it cannot be classified as one of the above [1].

Phytoestrogens play their roles primarily by binding to estrogen receptors (ER). In addition, phytoestrogens may also adjust endogenous estrogens through binding or inactivating some enzymes. By inhibiting or stimulating the synthesis of hormone binding globulin (SHBG), the bioavailability of sex hormones can be affected. It is reported that phytoestrogens may have protective role against

cardiovascular disease and a variety of physical disorders [2], such as prostate, breast, bowel, and other cancers. Recently, the role of phytoestrogens in the treatment of fatty liver disease has begun to be closely watched.

Role of Gender and Menopausal Status in Sexual Dimorphic NAFLD

The mechanism of NAFLD is not clear, it is commonly believed that NAFLD is closely linked with insulin resistance (IR) and metabolic syndrome (MetS), and they can both cause and effect [3]. While NAFLD as a sexual dimorphic disease had got more attention in recent years because evidence based on animals and humans supports the cognition that women's gender can help prevent this kind of metabolic disorders, the occurrence of NAFLD was higher in men than in women, and longitudinal studies indicated that male gender was an independent predictor of NAFLD development [4]. Another important clue about the relation between estrogen and

non-alcoholic fatty liver disease is menopausal status related with the incidence of NAFLD. It was reported that NAFLD is more prevalent in postmenopausal and PCOS (polycystic ovarian syndrome)-affected women than premenopausal ones [5]. Same conclusion was found in a large cross-sectional population-based survey on 808 northeast Germany women aged 40–59 years; results showed that menopause status was independently associated with hepatic steatosis [4]. While It is worth noting that women with NAFLD exhibit a significantly lower concentration of serum estradiol (the principal active estrogen) than NAFLD-free (premenopausal, postmenopausal, and PCOS) controls [5]. A multicentric study from Italy examined 5408 healthy women after hysterectomy. Subjects were distributed randomly to receive tamoxifen (an estrogen inhibitor) or placebo for 5 years; results showed that tamoxifen was related to higher risk of NAFLD/NASH development, especially in overweight women [6]. There are more evidences that estrogen and menopause status play important roles in the development of non-alcoholic fatty liver disease. A lower prevalence of NAFLD has been reported in postmenopausal women receiving hormone replacement therapy (HRT) compared to those not receiving it [4]. A clinical trial (double-blind, randomized placebo-controlled) on about 50 women with T2D showed that HRT strategy including lowdose estradiol and norethisterone reduced serum levels of liver function enzymes significantly, probably due to decreased liver fat accumulation. These results suggest that HRT probably protects from NAFLD [7].

Interestingly, the role of HRT in improving metabolic changes associated with low estrogen levels is a controversial issue. Hamaguchi et al. [8] investigated the incidence of NAFLD in women. The results showed that compared to premenopausal women, NAFLD occurrence was higher in menopausal and postmenopausal women. However, post-menopausal conditions were associated with an increased risk of NAFLD at univariate, but multivariate analysis based on age, metabolic syndrome and weight gain does not lead to the same conclusion [8]. Therefore, whether the gender and menopause status are independent of the occurrence of non-alcoholic fatty liver is to be further studied. Hepatic steatosis and NASH can be further developed into hepatic fibrosis. Gender factors and menopausal status may also be involved in this process. One study found that men showed a higher risk of fibrosis than premenopausal women, while postmenopausal women displayed similar severity of liver fibrosis to men, suggesting that estrogen could prevent the development of liver fibrosis [9]. Further study focused on NAFLD non-obese women was confirmed by liver biopsy. The results showed that even in non-obese NAFLD subjects, postmenopausal women still had more severe fibrosis than premenopausal subjects [10].

In animal models, both estrogen and raloxifene (second-generation selective estrogen receptor modulating agents) can prevent the progression of liver fibrosis in diet-induced NASH in female ovariectomies mice by improving the inflammation and balloon changes of liver cell [11]. However, the value of HRT in liver disease has yet to be confirmed in humans. A randomized placebo-controlled study showed that HRT containing low doses of estradiol and norethisterone could reduce the concentration of liver enzymes in women with hysterectomy by reducing the accumulation of fat in the liver [7]. A North American study using data from the NHANES III survey also found that postmenopausal women who accepted HRT were much less at risk of NAFLD than those who did not (or 0.69; 95 % CI 0.48-0 .99) [12]. However, it remains uncertain whether HRT will reduce the risk of NASH and/or fibrosis in postmenopausal women. Two studies have shown that HRT has no or no significant effect on fibrosis in postmenopausal NAFLD women [9, 13]. A recent study has shown that the use of synthetic hormones can lead to more severe hepatocellular damage and lobular inflammation. Further analysis suggested that progesterone, rather than estrogen, was associated with more serious liver histological injury, suggesting that sex hormones have multiple effects on the development of NASH [14]. The sex dimorphism is also associated with prognosis in HCC. The survival rate of women is higher, but menopause seems to reduce these advantages [15, 16].

Phytoestrogens Currently Studied in NAFLD Research (**> Table 1**)

Based on the evidences about the decrease of estrogen production after menopause can increase the incidence of metabolic disorders, estrogen replacement therapy were once popular but were limited by side effects. A safer estrogen, namely extracted from plant estrogen was discovered.

The effects of black soybean on cholesterol metabolism and insulin resistance were investigated in high cholesterol/fat diets mice models. The results showed that black soybean could influences the balance between oxidative and antioxidative stress, these might

Phytoestrogens	Model of research	Possible roles of phytoestrogens	Researchers and Ref.
Genistein (GEN)	Rat	Improves liver function, attenuates non-alcoholic fatty liver disease	Mohamed Salih S et al. [27]
	ApoE(–/–) mice	Alleviates the development of nonalcoholic steatohepatitis	Jeon S et al. [18]
	Ovariectomized (OVX) mice	Improves metabolic dysfunction	Kim JH et al. [19]
	Neonatal male Sprague-Dawley rats	Prevents hepatic steatosis and NASH	Huang C et al. [20]
Isoflavones (SIFs)	C57BL/6J mice	Regulates hepatic lipogenesis/adipocytokines	Kim MH et al. [21]
	Ovariectomized Wistar rats	Relieves dyslipidemia and hepatic steatosis	Panneerselvam S et al. [22]
Biochanin A (BA)	C57BL/6 mice	Regulates hepatic lipid and glucose metabolism	Park HS et al. [23]

Table 1 Phytoestrogens currently studied in NAFLD research.

suggest that black soybean could be used to improve cholesterol metabolism, insulin resistance, and alleviates oxidative damage in NAFLD patients [17].

Genistein (GEN) has been proved to improve liver function and attenuates non-alcoholic fatty liver disease in a rat model of insulin resistance. Jeon et al. reported that genistein could alleviate the development of nonalcoholic steatohepatitis in ApoE(-/-) mice fed a high-fat diet [18]. Kim et al. [19] found that genistein has a dual role with estrogen receptor agonist and antagonist activity, which suggest that GEN may have potential for use as a SERM (natural selective estrogen receptor modulator). They also evaluated the effects of GEN (a conjugated estrogen) and their pairing effects as a tissue-selective estrogen complex (TSEC) treatment on estrogen-induced endometrial hyperplasia and metabolic dysfunction in high-fat diet ovariectomized (OVX) mice [19]. In addition, Huang et al. reported that genistein treatment of newborns has a longterm effect on the maintenance of liver fat metabolism after weaning, these results provided a potential way to prevent hepatic steatosis and NASH [20].

Isoflavones (SIFs) are the most studied phytoestrogens so far. Kim et al. reported that isoflavones play an antisteatotic role by regulating hepatic lipogenesis/insulin resistance or adiposity – a variety of adipocytokines related to hepatic steatosis. Results showed that daidzein might relieve NAFLD by directly regulating hepatic de novo lipogenesis and insulin signaling, and indirectly controlling adiposity and adipocytokines through changes in adipocyte metabolism [21]. Panneerselvam et al. investigated the molecular mechanism of soybean isoflavones on hypertriglyceridemia and hepatic steatosis in postmenopausal obese animal models. Results suggested that with the coexistence of postmenopausal state and fat-rich diet intake, the development of deranged lipid metabolism has a synergistic effect. SIF extract could obviously relieve the lipid metabolism disorder, suggesting that this natural phytoestrogen could be used as a strategy to relieve dyslipidemia and hepatic steatosis in postmenopausal women [22].

Biochanin A (BA), an agonist of PPAR- α , was demonstrated to improve hepatic steatosis and insulin resistance by regulating hepatic lipid and glucose metabolism by Park et al. [23]. In conclusion, a large number of studies have confirmed the preventive and therapeutic effects of phytoestrogens on NAFLD. Since phytoestrogens contains a variety of species, the active ingredients and mechanism need to be further explored.

Possible Mechanism of Phytoestrogens in NAFLD (► Table 2)

Phytoestrogens have been reported presenting protective effect on a number of physiological and pathological processes related to NAFLD, most of these roles are related to its act like estrogen, specific mechanisms involved lipid and glucose metabolism, adipose tissue mobilization, antioxidant, immunity, and inflammation signaling pathway as well as microbiota.

Phytoestrogens and Lipid Metabolism

In addition to estrogenic activities by binding directly to the estrogen receptors, phytoestrogens have also emerged as potential regulators of hepatic lipogenic signaling, although research is much more limited in this area. Kim et al. [21] reported that dietary genistein, a phytoestrogen derived from soy, could reduce hepatic steatosis in male mice fed a high fat diet for 12 weeks. These findings are further supported by observed downregulation of lipogenic genes in both human lung cancer cells and within the HepG2 cell line after treatment with genistein [24]. Daidzein, another kind of phytoestrogen, has also been linked with reducing hepatic lipogenesis and steatosis in high fat diet-fed mice as observed by reduced lipogenic gene expression and lipid concentrations [21]. The mechanisms associated with these effects may be related to the effects of the signaling pathways involved in fat metabolism.

Steroid regulatory element binding protein (SREBP) and liver X receptor (liver X receptor, LXR) are members of nuclear transcription factors family, they are important transcriptional regulatory factors for fat synthesis genes and cholesterol metabolism. Both of them were suggested to be influenced by phytoestrogens and therefore related to NAFLD [25, 26]. Panneerselvam et al. [22] studied the effects of SIF on hypertriglyceridemia and hepatic steatosis in postmenopausal obese animal models. Ovariectomized (OVX) and sham-operated Wistar rats were fed with high-fat diet (HFD) and normal diet for 8 weeks with and without SIF extract. The combination of OVX and HFD can cause hyperglyceridemia, hypercholesterol, and atherosclerotic lipid profile. Hepatic lipogenic proteins, such as LXR, SREBP1, PPARy, ACC, and FAS were involved in these mechanism. Results suggested that the combination of postmenopausal conditions and fat-rich dietary intake has a synergistic effect on the development of abnormal lipid metabolism. SIF extract might relieve lipid metabolism disorders by interrupting this effect thus could be a strategy to relieve NAFLD in postmenopausal women.

Phytoestrogens and Antioxidant Oxidative Stress and Lipid

Peroxidation can lead to fatty hepatitis and liver fibrosis, which is an important reason for the progress of NAFLD. A study discussed the antioxidant role of phytoestrogens in vivo. Male Wistar rats were fed with starch (control group) and 60% fructose (insulin resistance model). After 15 days, each group was divided into two groups and fed separately with either genistein (1 mg/kg per day) in dimethyl sulfoxide (DMSO) or 30% DMSO alone. After 60 days, the oxidative stress in rat plasma and liver, interleukin (IL 6), tumor necrosis factor (TNF α) spectrum, lipid, and lipoprotein profiles were detected, and liver 3-nitrotyrosine (3 NT) expression and pathological damage were studied. Results showed that more serious hyperlipidemia, significant changes in plasma lipoprotein profile, and increases in IL-6 and TNF α levels could be found in fructose-fed rats if compared with control. The administration of genistein can significantly reduce these biochemical and histological abnormalities [27]. This study provides the basis for the treatment of NAFLD by the anti-oxidative stress mechanism of genistein. There are other studies reported that GEN could reduce NASH and hypercholesterolemia in obese ApoE(-/-) mice fed with HFD, the restoration in cholesterol metabolism and the recovery of oxidative stress might be related to the protective effect of GEN on NASH [18].

Table 2 Possible mechanisms of phytoestrogens in NAFLD.

Targets	Phytoestrogens	Research model	Roles of phytoestrogens	Researchers and Ref.
Lipid metabolism	Genistein	HepG2 cell human lung cancer cells	Downregulation of lipogenic genes, such as SCD1	Hess D et al. [24]
	Daidzein	High fat diet-fed C57BL/6J mice	Reduced lipogenic gene expression and lipid concentrations	Kim MH et al. [21]
	Resveratrol	Mice	Sterol regulatory element binding protein	Kay HY et al. [25]
	Sauchinone		(SREBP) and Liver X receptor (liver X receptor, LXR)	Kim YW et al. [26]
	Soy isoflavones	Ovariectomized (OVX) and sham-operated Wistar rats	Hepatic lipogenic proteins, such as LXR, SREBP1, PPARy, ACC and FAS	Panneerselvam S et al. [22]
Antioxidant oxidative stress and lipid	Genistein	Male Wistar rats	Oxidative stress in rat plasma and liver, interleukin (II) 6, tumor necrosis factor (TNF)- α , liver 3-nitrotyrosine (3 NT) expression	Mohamed Salih S et al. [27]
	Genistein	Obese ApoE(-/-) mice fed with HFD	Downregulates hepatic expressions of scavenger receptors involved in oxidized LDL uptake, CD36 and scavenger receptor A	Jeon S et al. [18]
Adipose tissue mobilization	Genistein	C57BL/6J mice	Upregulating genes involved in fatty acid β-oxidation and by downregulating genes associated with adipogenesis or lipogenesis	Kim MH et al. [28]
	Phytoestrogens including resveratrol and genistein	PEMT, pemt(-/-) mice (mice lacking phosphatidylethan- olamine N-methyltrans- ferase)	Promote lipolysis	Gao X et al. [29]
	Synergistic combination of phytoestrogens	Menopausal women	Anti-adipogenic	Miller CN et al. [30]
Glucose metabolic pathways	ВА	C57BL/6 mice	Inhibited gluconeogenesis related metabo- lites and related enzymes, glucose 6-phos- phatase and pyruvate kinase expression	Park HS et al. [23]
Immunity and inflammation pathway	Genistein	ApoE(–/–) mice	Reducing mRNA levels of monoacylglycerol O-acyltransferase 1 downregulated The expression of the scavenger receptors in liver (including oxidized LDL uptake, CD36 and the scavenger receptor A)	Jeon S et al. [18]
	Genistein	NASH rats induced by HFD	Zhiobarbituric acid reaction substance (TBARS), tumor necrosis factor- α , interleu- kin-6, and transforming growth factor β 1, mitogen-activated protein kinase. and nuclear factor kappa B pathway c-Jun N-terminal kinase	Ji G etal. [31]
	Isoflavones	SH-SY5Y human neuroblas- toma cells	Decrease the expressions of the immunoglob- ulin binding protein (BiP) mRNA, spliced X-box binding protein-1 (Xbp-1) mRNAs, and C/EBP homologous protein (CHOP).	Park YJ et al. [32]
Microbiota	Daidzein	Ovariectomized mice, osteoclast cell	Metabolized by gut bacteria to O-ODMA and S- equol. Its structure is similar to that of estrogen and can activate ER/or ERb	Ohtomo T et al. [34]
	Soy isoflavones, such as genistein and glycolic	Postmenopausal women	Alters the structure and composition of fecal bacterial communities	Nakatsu CH et al. [35] Hong SW et al. [36]
	Chalconoid isoliquiriti- genin, a low affinity ER ligand, found in licorice root	Mice	Improves metabolic syndrome	Madak-Erdogan Z et al. [38] Chen KL et al. [39]

Phytoestrogens and Adipose Tissue Mobilization

Non-alcoholic fatty liver disease (NAFLD) is closely related to visceral adiposity, adipose tissue inflammation and multiple adipocytokines. Previously, it had been reported that genistein inhibited NAFLD by increasing the catabolism of fatty acids. However, this molecular approach focuses on hepatic metabolism. In order to determine whether the anti-steatotic effect of genistein is related to the metabolism of visceral adipocyte, Kim et al. [28] discussed the mechanism of genistein supplementation on adipocyte metabolism pathway.

C57BL/6J mice were fed with normal fat (NF) diet, high fat (HF) diet and HF diet supplemented with genistein (1, 2, and 4 g/kg diet) for 12 weeks, respectively. As a result, unlike the NF diet mice, mice fed the HF diet had increased body weight, increased visceral fat mass, increased serum and hepatic lipids level, and developed NAFLD. However, genistein supplements (2 and 4 g/kg diet) normalized these changes. In the linear regression analysis, among other NAFLD-related parameters, visceral fat (R0.77) and TNF α (R0.62) were closely related to NAFLD.

By upregulating genes involved in fatty acid β -oxidation and by downregulating genes associated with adipogenesis or lipogenesis, genistein supplementation suppresses adipocyte hypertrophy. In addition, genistein supplementation enhances anti-steatohepatitic adiponectin TNF and reduces steatohepatitic TNF α . In conclusion, these findings suggest that genistein may prevent NAFLD by regulating visceral adipocyte metabolism and adipocytokines.

Moreover, phytoestrogens including resveratrol and genistein were reported to reduce hepatic lipogenesis, inflammation, and apoptosis. Such compounds also appeared to promote lipolysis and protection from high fat diet-induced weight gain in vivo [29]. However, the human equivalent dose is too high and it is difficult to achieve efficacy in human population. Miller et al. [30] identified a lower dose, synergistic combination of phytoestrogens that has proven to be anti-adipogenic. The application of these compounds may not only benefits the weight management of menopausal women and reduces the risk of disease, but also prevents the lipotoxicity of NAFLD.

Phytoestrogens and Glucose Metabolic Pathways

Various pathways have been suggested to involve in the biological activity of phytoestrogens, including glucose metabolic pathways and insulin signaling pathway.

BA stimulated the transcriptional activation of PPAR- α in vitro and increased the expression of PPAR- α and its regulatory proteins in the liver [23]. CE-ToF/MS analysis showed that BA administration promoted the recovery of phosphatidylcholine synthesis, lipogenesis and beta oxidation metabolites in the liver of obese mice. BA also inhibited gluconeogenesis related metabolites and related enzymes, glucose 6-phosphatase, and pyruvate kinase expression. These results suggest that BA improves metabolic disorders, such as hepatic steatosis and insulin resistance, by modulating lipid and glucose metabolism in diet-induced obesity. Therefore, BA may be a potential therapeutic agent to prevent obesity mediated liver steatosis and insulin resistance.

Phytoestrogens and Immunity and Inflammation Pathway

Inflammation and immune signaling pathways are closely related to NASH and the development of NAFLD. Jeon et al. [18] reported that GEN also alleviated hepatic steatosis by reducing mRNA levels of monoacylglycerol O-acyltransferase 1, a target gene of peroxisome proliferator-activated receptor y. The expression of the scavenger receptors in liver (including oxidized LDL uptake, CD36 and the scavenger receptor A) were downregulated by GEN. Anti-inflammatory activity of genistein has been revealed in animal studies. [i et al. [31] investigated the anti-inflammatory effect of genistein on NASH rats induced by HFD and its potential mechanisms. Rats were fed with normal diet or HFD for 12 weeks with or without low (4 mg/kg/day) or high (8 mg/kg/day) dose of genistein. Serum aminotransferase, thiobarbituric acid reaction substance (TBARS), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) and transforming growth factor beta (TGF- β 1) were measured, and liver inflammation, liver TBAR, IL-6, TNF- α , and TGF- β 1 levels were detected, proteins involved in mitogen-activated protein kinase (MAPKs) and nuclear factor kappa B (NFkB) pathway were determined. The results showed that Nash model rats replicated the typical pathological and histopathological features of Nash in human. Genistein improved the liver function, delayed the progress of Nash, reduced the levels of TBARS, TNF- α and IL-6 in the serum and liver, and inhibited I kappa B-alpha phosphorylation, NFkB p65 subunit nuclear translocation and activation of c-Jun N-terminal kinase (JNK). Studies above suggested genistein may be a promising drug to inhibit the inflammatory process and prevent liver damage in patients with NASH.

Additionally, phytoestrogens, much like E2, have been demonstrated to affect XBP1 activity. X-box binding protein 1, also known as XBP1, is a protein which in humans is encoded by the XBP1 gene, which is a transcription factor that regulates the expression of genes important to the proper functioning of the immune system and in the cellular stress response. In neuroblastoma cells, both genistein and diadzein reduced XBP1 expression and activity in an estrogen receptor-dependent fashion [32]. This finding suggests that phytoestrogens might work in a similar fashion within the liver, but further research is necessary.

Phytoestrogens and Microbiota

Microbiota consists of a large population of microorganisms including fungi, viruses, and bacteria, present in and on the body. Emerging evidence suggests microbial communities contribute to the development of insulin resistance and NAFLD. HFDs (high-fat diets) change gut microbiome composition and increase the number of Gram-negative bacteria, which produce lipopolysaccharide (LPS); an endotoxin associated with inflammation in T2D [33]. LPS production and increased inflammation could drive NAFLD that is strongly influenced by obesity and insulin resistance. Evidences showed that the combination of estrogen and probiotics can improve intestinal leakage and inflammation caused by obesity and

prevent NAFLD effectively. Daidzein is metabolized by gut bacteria to O-ODMA and S-equol. Its structure is similar to that of estrogen and can activate ER/or ERb [34]. In addition, soy isoflavones, such as genistein and glycolic, can significantly alter the structure and composition of fecal bacterial communities in postmenopausal women by increasing the concentration of beneficial Gram-positive Bifidobacteria and inhibit the Clostridium, which could be the cause of several human diseases [35, 36]. A diet containing more phytoestrogens has been shown to improve weight gain, and the high level of gut microbial metabolites of the estrogen plant lignans in the blood are associated with a lower rate of overweight and obesity. For example, individuals who do not carry ODMA producing bacteria are more likely to be obese than producers. In addition, 50% of obese individuals are equol non-producers [37]. Equol may contribute to the reduction of blood glucose and low density lipoprotein levels, which indicates that bacteria play a vital role in the activation of soybean phytoestrogens, which are associated with obesity. Recent studies have shown that supplementation of the chalconoid isoliquiritigenin, a low affinity ER ligand, found in licorice root, improves the weight gain associated with ovariectomy and metabolic syndrome in mice, and does not stimulate reproductive tissue [38, 39]. As mentioned above, phytoestrogen may affect the development of NAFLD by adjusting the gut microbiota, possibly through its estrogen-like role, and there may be other mechanisms. Further clarification is needed.

Possible mechanisms of phytoestrogens in NAFLD described above are summarized in **> Fig. 1**

Phytoestrogens and NAFLD Related Hepatocellular Carcinoma (HCC)

It has been reported that about 3–15% of the obese patients with NASH progress to cirrhosis and about 4–27% of NASH with cirrhosis patients transform to HCC. It is also known that HCC can develop de novo in patients with NASH without the presence of cirrhosis [40]. The mechanism is also complicated, which may involve obesity, IR, genetic mutations related to oxidative stress and reactive oxygen species (ROS), inflammatory response with increased release of cytokines such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) and nuclear factor NF κ B, reduce the adiponect in synthesis [41].

It has been founded that women have a lower risk of HCC than men, and this risk is more pronounced in individuals who are under 50 years of age. The reason is thought to be related to sex hormone levels. Estrogen has been discovered to play a major role in HCC retardation, E2 has been indicated to inhibit cell growth and induced apoptosis in hepatocellular carcinoma (HCC) PLC/PRF/5 cell line [42]. Nowadays researchers have focused on the effects and mechanism of action of estrogen as an HCC therapy.

As a group of natural compounds with estrogen-like activity and similar structure to estradiol, phytoestrogens structurally mimic the mammalian estrogen 17β -estradiol (E2) and plays important roles in NAFLD related HCC. In 2002, to investigate the role of phytoestrogens in HCC, Lei et al. performed an investigation of dietary intake of phytoestrogens. Results showed the diet in regard to estrogen-like substances may be relevant in modulating the risk of developing HCC in cirrhotic patients [43]. Afterwords, several researches proved that genistein alter cell cycle, inhibit cell proliferation, induced apoptosis and thereby inhibits invasive potential of human hepatocellular carcinoma [44–46]. Thereafter, mechanisms of phytoestrogens in HCC have been widely studied, however, most studies were focused on genistein.

Lee et al. reported that genistein-mediated p-AMPK activation increases hepatocyte apoptosis, suppresses the inflammatory response in resident liver macrophages by increased cellular respiration, and consequently inhibits the initiation and progression of HCC [47]. Recent research showed that epigenetic changes such as DNA methylation and histone acetylation play important roles in determining gene expression. Sanaei et al. found that DNA demethylating agents such as genistein and histone deacetylase inhibitors (HDACIs) such as trichostatin A (TSA) may strongly reactivate silenced genes, enhance estrogen receptor alpha (ER α) reactivation and induction of apoptosis. These results may provide new avenues and therapeutic strategies for cancer therapy [48].

The role of genistein in the glycometabolism of hepatocellular carcinoma has also been studied. Glucose metabolism in HCC cells



Fig. 1 Possible mechanism of phytoestrogens in NAFLD.

is characterized by two major biochemical events: increased glucose uptake and aerobic glycolysis. Li et al. reported that genistein may inhibit glycolysis and induced apoptosis in HCC cells. Further investigation showed that genistein inhibited the expression and activity of HIF-1 α to suppress GLUT1/HK2. In addition, Gen improved the sensitivity to sorafenib (Sora) in Sora-resistant HCC cells with activated glycolysis in vitro and in vivo, providing evidence for its potential clinical application in the treatment of HCC [49]. It has been also reported that genistein inhibits tumor invasion by suppressing multiple signal transduction pathways in human hepatocellular carcinoma cells [50]. More interestingly, some studies have found that phytoestrogens combined with some anti-HCC drugs may improve the effica-

gens combined with some anti-HCC drugs may improve the efficacy or reduce the side effects of treatment. Youssef et al. reported that combination of sorafenib and biochanin-A synergistically may enhance the anti-proliferative and pro-apoptotic effects on hepatocellular carcinoma cells, which could serve as a potential effective regimen for treatment [51]. Rigalli et al. evaluated the effect of genistein on the expression and activity of P-gp, MRP2, MRP3 and BCRP in HCC-derived HepG2 cells. The results found that genistein (at 1.0 and 10 µM) increased P-qp and MRP2 protein expression and activity, correlating well with an increased resistance to sorafenib cytotoxicity as detected by the methylthiazole tetrazolium (MTT) assay. These suggest the possibility of nutrient-drug interactions leading to enhanced chemoresistance in HCC when genistein is ingested with soy rich diets or dietary supplements [52]. Genistein also has been found to reinforce the inhibitory effect of cisplatin on liver cancer recurrence and metastasis after curative hepatectomy. The two drugs exhibited synergistic effects even at relatively low concentrations [53]. In addition, Ma et al. demonstrated that genistein not only potentiated the proliferation-inhibiting and apoptosis-inducing effect of arsenic trioxide(ATO) on human HCC cell lines in vitro, but also dramatically augmented its suppressive effect on both tumor growth and angiogenesis in nude mice. These data suggest that the combination of anti-tumor agents with genistein presents a promising therapeutic approach for the treatment of HCC [54].

In conclusion, due to alarming rise in metabolic diseases, the incidence of NAFLD is increasing in recent years, it is expected that the incidence of NAFLD/NASH-HCC would rise manifold in future. So far, there is no specific effective treatment and no definite guidelines have been drawn for surveillance and management of NAFLD/ NASH-associated HCC. As a natural, minor side effects compound, phytoestrogens have potential as a preventive and therapeutic agent for NAFLD and NAFLD related HCC in the future. Present research mainly focuses on animal experiments, and the relevant mechanisms and specific effects are still to be studied in the human body.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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