Beneficial and detrimental effects of natural dietary products on the risk of hepatocellular carcinoma, and their roles in its management

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ABSTRACT
Hepatocellular carcinoma (HCC) is a common solid malignancy and a leading cause of cancer-related death worldwide. The mechanisms underlying the pathogenesis and development of HCC are complex and heterogeneous. Although mainly related to hepatitis B and C chronic infection; HCC may also arise from diet-associated conditions such as non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Furthermore, toxins and nutrients such as mycotoxins and alcohol have an established role in the pathogenesis of chronic liver diseases, whereas specific diet patterns or foods have been associated with a reduction in HCC risk. The aim of this review is to provide a thorough overview of the clinically relevant effects - either beneficial or detrimental - of natural products consumed by humans on HCC risk and management.

Key words: Hepatocellular carcinoma; natural products; diet; dietary supplements

INTRODUCTION
The risk of hepatocellular carcinoma (HCC), associated with nutritional and metabolic factors, has been underestimated until recently. HCC may represent a late complication of non-alcoholic steatohepatitis-related cirrhosis,[¹] which in turn is strongly related to diet-associated conditions such as obesity, type 2 diabetes mellitus and dyslipidemia.[²] Furthermore, several foods, beverages and food contaminants are known to affect the risk of developing HCC. Nutritional compounds that display anti-inflammatory and antioxidant effects may have specific applications in preventing oxidative stress-induced injury, which characterizes the pathogenesis of cirrhosis and steatosis.[³] The pivotal role of diet is highlighted by the results of two large case-control
studies conducted in Italy and Greece, indicating that strong adherence to the Mediterranean diet may be protective against HCC (approximately a 50% reduction in risk), with potential benefits also in patients with chronic viral hepatitis. As for patients with established chronic liver disease, nutritional interventions to support sufficient energy intake significantly improve patient survival. A better knowledge of the detrimental or beneficial effects of foods is therefore important in the prevention and management of HCC, and the evaluation of dietary supplements potentially able to reduce the risk and/or the progression of cirrhosis and steatosis is of the highest interest.

The potential protective and therapeutic mechanisms of natural compounds in the prevention and treatment of hepatotoxicity and HCC have been recently reviewed. The aim of the present review is to provide an insight on the clinically relevant effects—either beneficial or detrimental—of natural products consumed by humans on HCC risk and management.

**DETRIMENTAL NATURAL PRODUCTS**

**Foods and beverages**

**Alcohol**
The detrimental effects of alcohol on the liver are well known; ethanol exerts toxic effects that can cause cell injury and a reactive response culminating in alcohol-induced hepatic cirrhosis. More in detail, reactions catalyzed by the main enzymes involved in alcohol metabolism, namely alcohol dehydrogenase and aldehyde dehydrogenase, lead to the production of reactive oxygen species (ROS) that can exert toxic effects such as lipid peroxidation, enzymes inactivation, DNA mutations, and destruction of cell membranes; in addition, in conditions of chronic alcohol abuse there is an increased production of acetaldehyde from ethanol, due to induction of the microsomal system and in particular of the Cytochrome P450 enzyme Cytochrome P450 2E1. Acetaldehyde is one of the main mediators of alcohol-induced fibrogenesis in the liver, as it can stimulate synthesis of fibrillar-forming collagens and structural glycoproteins of extracellular matrix in hepatic stellate cells, and increase the secretion of transforming growth factor-β. Eventually, these events may lead to hepatic cirrhosis, which is associated with a 5-year cumulative risk for HCC of 8%. The immunosuppressive effects of alcohol and alcohol-induced epigenetic modifications may also contribute to the development of HCC in patients with alcoholic liver disease.

**Red meat**
Red meat consumption has been reported to be associated with an increased risk of HCC. Meat processing, e.g. curing and smoking, can in fact result in the formation of carcinogenic chemicals, including N-nitroso-compounds and polycyclic aromatic hydrocarbons. cooking, especially if high-temperature, can also produce known or suspected carcinogens, including heterocyclic aromatic amines and polycyclic aromatic hydrocarbons. The International Agency for Research on Cancer has recently classified red meat and processed meat as “probably carcinogenic to humans” (Group 2A) and “carcinogenic to humans” (Group 1), respectively. However, the strongest association appears to be with colorectal cancer, pancreatic cancer and prostate cancer, and currently available evidence supporting a causative role for red meat in HCC is inconsistent.

**Pickled foods**
A possible carcinogenic effect of pickled vegetables was first reported in 1992. Traditionally, pickled vegetables are prepared by packing moist vegetables in a jar for weeks to months, allowing fermentation and growth of fungi and yeasts. This process can potentially yield carcinogenic compounds such as the N-nitroso compound Roussin’s red (dimethylthiotetranitrosodiiron). Consistently, a large systematic review and meta-analysis revealed that those who consume pickled vegetables/foods have an about 50% increase in risk of gastric cancer vs. those who consume little or no pickled vegetables/foods. An association between pickled food and HCC has also been reported.

**Sugar**
Non-alcoholic fatty liver disease (NAFLD) is considered as the hepatic manifestation of the metabolic syndrome. It is characterized by an increase in intrahepatic triglyceride content (i.e. steatosis), with or without inflammation and fibrosis [i.e. non-alcoholic steatohepatitis (NASH)]. Hepatic de novo lipogenesis (DNL) has been suggested to be abnormally increased in NAFLD, and to contribute to its development. As glycolysis and the metabolism of carbohydrates are the main providers of substrates for DNL, a high-carbohydrate diet can prime the DNL pathway with a large substrate load and increase rates of DNL. Dietary fructose may contribute to NAFLD by promoting DNL, insulin resistance, oxidative
stress, bacterial overgrowth, and inflammation.[24] Both NAFLD and NASH can further progress to hepatic fibrosis and eventually to cirrhosis,[25] older age and deterioration of metabolic status being major risk factors for fibrosis progression.[26] NAFLD/NASH that progresses to cirrhosis carries the highest risk for HCC, due to the erratic liver remodeling with repeated cycles of hepatocellular destruction and compensatory regeneration that characterizes cirrhosis. However, there is increasing concern that NAFLD-associated HCC may also occur in non-cirrhotic liver, due factors specifically associated with NAFLD (e.g. lipotoxicity associated with DNL and increased levels of proinflammatory adipokines/cytokines).[27] Recent findings indicate that the incidence rate of HCC in NAFLD and NASH is 0.44 and 5.29 cases per 1,000 person-years, respectively.[28] Although these rates are lower than those reported for patients with hepatitis B virus (HBV) or hepatitis C virus (HCV), the number of patients with NAFLD and NASH-related HCC is projected to increase, given the increasing prevalence of these conditions. Epidemiological evidence linking dietary sugar, and specifically, fructose consumption, with cancer derives from case-control studies that found an association between high dietary glycemic load and increased risk for HCC, especially in patients with chronic viral hepatitis.[16,17] However, a recent analysis of the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort revealed an association between higher total sugar intake and risk of HCC, but not between glycemic index/glycemic load and HCC.[18]

Overnutrition
Hepatic cirrhosis and an associated increased risk of developing HCC independent of viral hepatitis frequently occurs secondary to NASH and NAFLD,[29] which often has a increasing prevalence of these conditions. Exposure to AFB1 has been associated with HCC in several cohort studies, supporting a role of AFB1 in liver cancerogenesis-particularly among subjects who are carriers of hepatitis B surface antigen.[37] It has been estimated that aflatoxin exposure may account for 5-28% of total HCC cases worldwide.[38]

Fumonisins are ubiquitous mycotoxins that contaminate cereal grains, primarily maize. More than 10 compounds have been isolated and characterized; fumonisin B1 is believed to be the most toxic among them, as it has been shown to be hepatocarcinogenic in rodents.[39-41] Fumonisins are thought to impair the de novo synthesis of ceramide and sphingolipid metabolism due to a structural resemblance with ceramide; this may lead to disruption of signal transduction pathways in the target cells.[42] A pathogenic role of exposure to fumonisins through consumption of moldy corn in human HCC has been suggested by studies carried out in China.[43-45]

Ochratoxin A is another mycotoxin that may have a role in the development of HCC.[46] It may be found in cacao and derived products, dried fruits, wine, cereals, green coffee, and spices (mainly nutmeg, paprika, coriander, and pepper powder).[47] The carcinogenic effect of ochratoxin A is the result of both direct genotoxic (covalent DNA adduct formation and mutagenicity)[48] and epigenetic mechanisms leading to protein synthesis inhibition, oxidative stress and the activation of specific cell signaling pathways.[49] In a recent case-control study, high performance liquid chromatography analysis of serum samples from HCC patients and controls indicated that the incidence of elevated ochratoxin A was highest in the HCC group, being 5-fold higher than in the control group.[50] These findings support a strong association between the presence of ochratoxin A and HCC. Ochratoxin A is a stable compound that is not destroyed by common food preparation procedures.

Food contaminants

**Mycotoxins**

Aflatoxin B1 (AFB1) is a mycotoxin (i.e. toxic compounds produced by fungal secondary metabolism) produced by *Aspergillus flavus* and *Aspergillus parasiticus*, widely represented in nature. The mycotoxin is found in many foods such as corn, rice, oil seeds, dried fruits, and peanuts that have been improperly stored in hot, humid, and unsanitary conditions.[44] Metabolism of aflatoxins by hepatic enzymes may generate reactive epoxide species with the potential of forming a covalent bond with guanine,[35] generating adducts that can promote cellular and macromolecule damage, including mutations in the p53 tumor-suppressor gene.[36] Exposure to AFB1 has been associated with HCC in several cohort studies, supporting a role of AFB1 in liver cancerogenesis-particularly among subjects who are carriers of hepatitis B surface antigen.[37] It has been estimated that aflatoxin exposure may account for 5-28% of total HCC cases worldwide.[38]
Available data on the presence of mycotoxins in grains and foods indicate that there may be a continuous low-level exposure to these toxic metabolites. Foods mainly contributing to the intake of mycotoxins with diet are cereals, maize being the most risky commodity due to the potential co-occurrence of more than one mycotoxin. It has been postulated that individuals with increased maize-based products consumption such as celiac patients could be particularly at risk of mycotoxin exposure. However, studies have shown that the intake of mycotoxins in these potentially vulnerable populations is generally below the tolerable daily intake.

Pyrrolizidine alkaloids
Pyrrolizidine alkaloids such as riddelliine, which is found in Senecio riddellii (Riddell groundsel) and Senecio longilobus (also known as woolly groundsel and thread-leaf groundsel), can be found as a contaminant in foods such as meat, grains, seeds, milk, herbal tea and honey. In hepatocytes, Cytochrome P450s convert dehydropyrrolizidine alkaloids to 6,7-dehydropyrrolizine esters, i.e. the toxic metabolites. Dehydroretronecine and dehydroheliotridine that are produced from the initial toxic metabolites via ROS react rapidly with the SH, OH, NH groups on nucleotides, as well as with proteins to form adducts, eventually leading to DNA damage and carcinogenesis. There is a large body of evidence from studies in animals supporting the carcinogenicity of pyrrolizidine alkaloids. Of note, there are published reports of primary liver tumors in natives of Central and South Africa associated with the consumption of traditional medicinal plants containing of pyrrolizidine alkaloids. Honey and tea have been reported to be a significant source of pyrrolizidine alkaloids in Western countries. Although health impairment due to chronic intake of pyrrolizidine alkaloids is improbable for adult consumers with average amounts of consumption of honey and tea, longer-term regular consumption of products with containing high amounts of pyrrolizidine alkaloids could be associated with a risk of health impairment.

BENEFICIAL NATURAL PRODUCTS

Foods and beverages
Coffee
A protective effect of coffee against HCC was first suggested by Gallus et al. in 2002. Since then, several other studies have confirmed this hypothesis. Meta-analyses of epidemiological studies found that an increased consumption of coffee may reduce the risk of liver cancer. In a recent analysis of EPIC, a large epidemiological study designed to investigate the association between diet, lifestyle and environmental factors and the incidence of various types of cancer and other chronic diseases, coffee consumers in the highest compared to the lowest quintile had 72% lower risk of developing HCC. Consistently, high levels of coffee consumption were associated with reduced risk of incident HCC and chronic liver disease mortality in a population-based prospective cohort study of more than 215,000 men and women from Hawaii and California. Coffee has been shown to exert beneficial effects on body weight, development of diabetes, the prevention of hepatic fibrosis in NAFLD, and other chronic liver diseases, including chronic hepatitis C. There are approximately 1,000 substances in coffee, including caffeine, diterphenoic alcohols and chlorogenic acid (CGA), a polyphenol. It is uncertain which are the exact substances and mechanisms responsible for the beneficial effects of coffee on the liver. Several substances as well as the method of preparation are thought to be of importance. As an example, filtered coffee may provide the most benefit due to a reduction in cafestol and kahweol, which can raise serum cholesterol, while maintaining CGA and caffeine content.

Fish
By virtue of its high content in omega-3 fatty acids, which may have anti-carcinogenic and anti-inflammatory effects, fish might be protective against HCC. Evidence supporting a protective role of fish comes from the EPIC study. In EPIC, total fish intake was inversely associated with HCC risk (20% reduction in risk per 20 g/day of fish, after calibration). Lean/white fish (cod, haddock, and plaice), fatty fish (salmon, tuna, trout, herring, kippers, and mackerel, and crustaceans and mollusks were independently associated with lower HCC risk, even after adjusting for HBV/HCV status and liver function biomarkers.

Olive (Oleaeuropaea)
Epidemiological studies have shown that intake of virgin olive oil is associated with low incidences of several types of cancer, likely due to its high content inphenolicantioxidants. These include hydroxytyrosol and oleuropein. Hydroxytyrosol (HT) is a natural polyphenolic compound with significant antioxidant properties. It has been recently demonstrated.
that HT inhibited the proliferation and induced apoptosis in HCC in vitro and in a tumor model of HCC,[74] and exerted antiproliferative, antioxidant and anti-inflammatory effects on human hepatoma HepG2 and Hep3B cell lines.[75,76] Oleuropein, a major constituent of O. europaea, was shown to effectively inhibit cell viability and to induce apoptosis in HepG2 human hepatoma cells in a dose-dependent manner, through activation of the caspase pathway.[77]

Oleanolic acid (3β-hydroxyolean-12-en-28-oic acid) is a pentacyclic triterpenoid found in olive leaves. Antitumor effects of oleanolic acid have been investigated recently both in vitro and in vivo. It exhibited inhibitory effect on HCC through induction of apoptosis and cell cycle arrest both in transplanted tumors in mice and in HepG2 cells, indicating that oleanolic acid has significant antitumor activities on HCC both in vitro and in vivo models.[78] Olive fruit pulp is a rich source of antioxidants and possesses very good hepatoprotective activity against CCl₄-induced hepatic damage in mice.[79]

Other foods and beverages
Several other foods and beverages have been reported to have a protective role against HCC, including tea polyphenols (i.e. green and black tea),[80] tomatoes and tomato-based products[81] (a rich source of lycopene, an antioxidant carotenoid that has even been shown to prevent HCC metastases in animal studies[82]), dietary fiber,[18] green-yellow vegetables and fruit.[83]

Nutraceuticals and dietary supplements
A large proportion of HCC patients use dietary supplements.[3] However, only in few cases their use is supported by clinical evidence.

Branched chain amino acids
Branched chain amino acids (BCAA) may suppress hepatocarcinogenesis by several mechanisms, including improvement of immune function, reduction of oxidative stress and improvement of insulin resistance.[84] Supplementation of BCAA for 2 years in patients with cirrhosis (Child-Pugh class A) has been associates with increases albumin synthesis in a multicenter, randomized controlled trial.[85] However, another randomized controlled trial did not find an improvement in serum albumin levels with BCAA supplementation, possibly due to different patient characteristics (patients were Child-Pugh class B or C).[86] Clinical trials have reported that long-term oral supplementation with BCAAs is associated with decreased frequency of development of HCC in obese patients with cirrhosis and hepatitis C virus infection,[87] significant reduction in HCC incidence rate and improvement of event-free survival rate in patients with cirrhosis,[88] and reduced HCC recurrence after treatment with radiofrequency ablation in patients with cirrhosis.[84,89] Finally, BCAAs have been also shown to improve health-related quality of life[85,86] and sleep disturbances in patients with cirrhosis.[90]

Milk thistle (Silybum marianum)
Milk thistle is an herbal agent that has been used to treat liver diseases for centuries. Silymarin, the main active constituent of milk thistle, is a mixture of polyphenols, including flavonolignans and flavonoids. Despite a strong anticancer activity against human HCC cells are demonstrated in vitro,[91] clinical studies supporting the use of silymarin as a hepatoprotective agent have yielded conflicting results.

A Cochrane systematic review revealed that the evidence supporting a role of milk thistle for the treatment of patients with alcoholic and/or hepatitis B or C virus liver diseases is scanty, and that milk thistle vs. placebo or no intervention had no significant effect on mortality, complications of liver disease or liver histology.[92] Milk thistle was not associated with increased risk of adverse events.[92] Silymarin use in 1,049 patients with advanced fibrosis or cirrhosis unsuccessfully treated with peginterferon plus ribavirin has been associated with reduced progression from fibrosis to cirrhosis.[93] In a 24-week multicenter, double-blind, placebo-controlled trial that included 154 patients with chronic HCV infection and elevated serum alanine aminotransferase (ALT) unsuccessfully treated with interferon-based therapy, silymarin did not significantly reduce serum ALT levels.[94] Silymarin has also been used as an adjuvant therapy in conjunction with chemotherapy and other supplements (α-tocopheryl acetate and a product containing stem cell differentiation stage factors) in a case report of a patient with locally advanced HCC, with encouraging results.[95]

Omega-3 fatty acids
Preliminary data indicate that omega-3 polyunsaturated fatty acids (PUFAs) inhibit HCC cell growth and might therefore be useful for the chemoprevention and treatment of human HCC.[96] This hypothesis is supported by the results of a population-based prospective cohort study of 90,296 Japanese subjects, in which consumption of omega-3 PUFAs, particularly...
eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, protected against the development of HCC, irrespective of HCV or HBV status. The effect of treatment with high dose purified long chain omega-3 fatty acids on liver fat percentage and scores for liver fibrosis in patients with NAFLD is currently being investigated in a randomized double blind placebo controlled trial.

**Spirulina platensis**

Spirulina is a blue-green alga (cyanobacterium) available as a dietary supplement. In vitro studies have demonstrated that spirulina may exert hepatoprotective effects. In patients with chronic hepatitis C infection, viral load and ALT levels tended to improve after 6 months of treatment with spirulina in a small, active-controlled trial. Another small, uncontrolled trial reported significant improvements in aspartate aminotransferase, alanin aminotransferase, gamma-glutamyltransferase, triglycerides, low-density lipoprotein-cholesterol, total cholesterol, and the ratio of total cholesterol to high-density lipoprotein cholesterol after 6 months of treatment in patients with NAFLD. According to the authors, spirulina supplementation resulted also in a significant reduction in weight and insulin resistance, and a significant improvement in health-related quality of life was observed. However, no changes in sonographic findings were observed.

**Antioxidants**

Reduced glutathione (GSH) is a potent antioxidant naturally occurring in the body, and is available for parenteral administration. Very few studies have assessed the therapeutic role of GSH in liver diseases. In an Italian study that compared the effects of reduced GSH and vitamin K in patients with alcoholic liver disease, those treated with reduced GSH showed a greater improvement of hepatic function than patients treated with vitamin K. A study published several years ago assessed the effect of GSH treatment on HCC in 8 patients with biopsy-proven HCC not amenable to surgery, but results were inconclusive. Besides direct GSH supplementation, hepatic GSH deposits can be restored by administering compounds such as S-adenosyl-L-methionine and N-acetylcysteine. Both compounds are generally very well tolerated, although of limited clinical value in improving liver function in chronic liver diseases.

Finally, it has been observed that patients with HCC have low levels of serum vitamin B and vitamin D which suggests that these patients might benefit from supplementation with these vitamins.

**Traditional Chinese medicines**

It has been suggested that use of Chinese herbal medicines might result in the protection of liver function during chemotherapy. The herbal formulation PHY906 consists of four commonly used herbs, i.e. Scutellaria baicalensis Georgi, Paeonia lactiflora Pall, Glycyrrhiza uralensis Fisch, and Ziziphus jujube Mill, at a ratio of 3:2:2:2. Studies have shown that PHY906 not only reduces gastrointestinal toxicity and enhances the antitumor efficacy of some anticancer drugs but also alleviates chemotherapy-induced side effects, such as diarrhea. Preliminary clinical data indicate that PHY906 can serve as an adjuvant to chemotherapy in the treatment of advanced HCC. Other traditional Chinese medicines that may have a role in the treatment of HCC are bufotoxin (toad skin secretion), astragalus and products containing ginseng (Panax ginseng), astragalus and mylabris (dried body of the Chinese blister beetle).

**CONCLUSION**

Identifying modifiable risk factors such as diet is important to counteract HCC. Dietary patterns are complex to assess, and are entangled with other aspects of lifestyle. To date, conclusive evidence supporting a detrimental or beneficial role in the prevention of chronic liver diseases is available only for few products. Available information on coffee, fish and BCAAs supplementation is of acceptable quality and supports a beneficial role for these products in the prevention and management of HCC. On the other hand, the detrimental effects of alcohol and aflatoxins are widely recognized. Excessive sugar and calorie consumption should also be avoided.

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**REFERENCES**


