Effects of natural compounds in treatment and prevention of hepatotoxicity and hepatocellular carcinoma

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ABSTRACT
Liver diseases are most common disorders in the world and characterized by rapid changes from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). Natural products that attained great attention is to be used in the prevention and treatment of multiple diseases in humans. Several researches have been reported numerous natural and phytochemical compounds that may counteract or prevent the hepatic injury and primary liver cancer. The conservative treatment of liver toxicity and HCC may face awkward challenges in chemotherapy such as therapeutic failure or drug resistance. Accordingly, there is an actual need for safe and effective therapeutic and preventive modalities for liver disorders. The present review aims to focus on the potential protective and therapeutic effects of natural compounds in the prevention and treatment of hepatotoxicity and HCC. It also demonstrates the mechanism of the natural products in enzymatic regulation of antioxidants and its role in apoptosis and proliferation of cancerous lesions of hepatocytes. Accordingly, it highlights the promising role of natural bioactive compounds and provides the rational for further transitional researches, and emphasize on the scientific validation of natural compounds for therapeutic portfolio for clinical use in liver diseases.

Key words: Antioxidant; hepatocellular carcinoma; hepatotoxicity; liver; natural compounds

INTRODUCTION
Chronic liver diseases are common worldwide disorders characterized by bad sequels started with steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC).⁴ Indeed, HCC is the fifth most commonly leading cancer, the major cause of death in patients with liver cirrhosis, and the second common cause of cancer-related death in the world.⁵ The major target strategy in the treatment of liver diseases is to terminate the serial consequences at the pre-fibrotic stage of the liver.⁶ To date, modern medicines have little to offer for alleviation of hepatic diseases. However, natural-based preparations are successfully employed for the treatment of liver disorders.⁷ Accordingly, there is an increased attention in natural products that may counteract...
the detrimental effects of environmental or chemical toxic compounds and prevent multiple hepatic disorders in humans.[9]

POLYPHENOLS

Polyphenols, commonly presented in vegetables, herbs, seeds, fruits, and other natural sources, represent more than 8,000 different compounds, classified in different classes based on their chemical structure, they are composed of at least one aromatic ring with one or more hydroxyl groups attached.[7] Polyphenols may be a promising candidate for preventing ethanol-induced liver injury through regulating alcohol metabolic enzymes in a cyclic AMP-dependent manner, polyphenols play a crucial role in the protection of liver against hepatitis due to its potential activity in the reduction of early proinflammatory cytokines [tumor necrosis factor-α and interleukin (IL)-1β], activation of anti-inflammatory IL-10, and inhibition of lipopolysaccharide-induced activation of nuclear factor kappa B (NF-κB) in hepatocytes.[8] Polyphenols are composed of two formulas; phenolic acids and flavonoids and account for 60% and 30%, respectively, of dietary polyphenols.[9]

Phenolic compounds (PhCs), which are ubiquitously found in plants, have a potent antioxidant activity mainly due to their ability in redox reactions, so they act as reducing agents, singlet oxygen quenchers, hydrogen donators, and chelating agents of metal ions.[10] Moreover, previous studies revealed that PhCs played an important role in the prevention of hepatotoxicity through increase in the level of reduced glutathione (GSH).[11]

Flavonoids are a group of polyphenolic compounds, different in chemical structure and characteristics, naturally founded in plants. More than 9,000 different flavonoid compounds were described in plants till now and they play major biological roles through affecting several developmental and important processes.

Flavonoids showed versatile health benefits such as anti-inflammatory, antioxidant, anti-proliferative and anticancer activity, free radical scavenging activity, and antihypertensive effects.[12] It has been reported that one of the flavonoid compounds luteolin showed a hepatoprotective effect and antioxidant properties against methanol hepatotoxicity.[13]

HERBAL AGENTS

Milk thistle (Silybum marianum) is one of the most famous herbal agents used to treat liver diseases since the 16th century. Major constituents of milk thistle are the flavonoids, such as silybinin, silidianin, silichristin, and isosilibinin.[14] Silymarin showed antioxidant properties and hepatoprotective activity, through inhibition of lipid peroxidation, depletion of liver GSH, inhibition of genotoxicity, and enhancement of hepatogenesis.[15]

Glycyrrhizin, the active constituent obtained from aqueous extraction of the liquorice root (Glycyrrhiza glabra), has been used in traditional medicine to alleviate bronchitis, gastritis, and jaundice. The major constituents of licorice are glycyrrhetic acid, flavonoids, hydroxycoumarins, and beta-sitosterol. The latter is likely possessing glucocorticoid and mineralocorticoid properties.[16] Licorice and their products have been reported to be useful in the treatment of human hepatitis, animal inducible hepatocarcinogenesis, and attenuating titanium dioxide nanoparticles-induced hepatotoxicity.[17,18]

Ginseng (Panax ginseng), a valued Chinese and Korean traditional medicinal herb, has been clinically used in China for thousands of years. Red ginseng elicits a protection against aflatoxin B1 and fumonisins-induced hepatic pre-cancerous lesions in rats and synergistic action with honey against CCl4-induced hepatonephrotoxicity.[19,20]

Ginkgo biloba extract has been shown antioxidant property due to its ability to scavenge free radicals and inhibition of lipid peroxidation.[21] The most recent discovered G. biloba components are polyphenols from which flavonoids and terpene lactones were derived and widely used for treating cardiovascular, non-alcoholic fatty liver, and cerebrovascular diseases.[22-24]

Dandelion (Taraxacum officinale), dandelion water extract (DWE), a herbal medication, may have an effect on the activity of messenger RNA expression of hepatic antioxidant enzymes due to its components that includes sesquiterpene lactones, phenylpropanoids, triterpenoid saponins, and modify lipid profile in streptozotocin-induced diabetes in rats.[25-27] It has been reported that DWE has anti-fibrotic effect through inactivation of hepatic stellate cells (HSCs) and the enhancement of hepatic regenerative capabilities.[28]

Garlic (Allium sativum) has been widely used as a foodstuff and a traditional medicine for many centuries throughout the world. Garlic is available in different forms such as powder or garlic oil. Garlic has a beneficial value such as anti-atherosclerotic, antihypertensive, antimicrobial, anticancer, immunomodulatory, antioxidant, and radioprotector effects.[29] On the other hand, allicin (diallyl thiosulfonate), which is the main biologically active
component of freshly crushed garlic cloves, has been produced by the interaction of the non-protein amino acid alliin with the enzyme allilinase (alliin lyase). It has anti-hepatocarcinogenic effect through the p53 gene modulating apoptosis and autophagy.\textsuperscript{30}

Turmeric (\textit{Curcuma longa}) has been found in the Far East and tropical regions. It had been used to treat menstrual disorders, colic, inflammation, bruising, dyspepsia, hematuria, and flatulence. It also has anticancer and antioxidant actions due to the presence of three chemical components, for example, curcumin I, II, and III.\textsuperscript{31} It suppresses the activation of NF-\kappaB, so it may be useful in preventing liver disease such as hepatonephrotoxicity.\textsuperscript{32}

Colchicine (\textit{Colchicum autumnale}) is the major alkaloid obtained from \textit{C. autumnale}. Pharmacological properties of colchicine included antimitotic effects and can be used for the treatment of gout.\textsuperscript{33} Moreover, colchicine has been acting as an anti-tumor agent.\textsuperscript{34} Colchicine has been reported to be a safe anti-fibrotic agent when used for long-term treatment of liver disease.\textsuperscript{35}

Thyme (\textit{Thymus vulgaris}) is cultivated in Central and Southern Europe, Africa, and Asia. It is rich with essential oils and anti-oxidative phenolic substances.\textsuperscript{36} It is widely used in folk medicine for the treatment of a variety of diseases including gastroenteric and bronchopulmonary disorders. It is also effective as anthelmintic, antispasmodic, carminative, sedative, diaphoretic, antimicrobial, antioxidant, and antifungal agents.\textsuperscript{37} \textit{T. vulgaris} also showed hepatoprotective effects against aflatoxicosis in rats.\textsuperscript{38}

Marigold (\textit{Calendula officinalis}) is an annual herb native to the Mediterranean region. In Europe and America, it is cultivated for ornamental and medicinal purposes. \textit{C. officinalis} as the marigold or maravilla has been widely used in folk therapy. \textit{Calendula} flower decoction or tincture showed more than 35 properties and its preparations have been used as valuable remedies for burns. \textit{C. officinalis} is mainly used for cutaneous and internal inflammatory diseases of several origins.\textsuperscript{39} Its extract has a protective effect against ultraviolet-induced oxidative stress.\textsuperscript{40} It has been well documented that \textit{Calendula} extract showed anti-genotoxicity and ameliorative effect against hepatotoxicity induced by aflatoxin due to high percentage of total PhCs.\textsuperscript{41,42} The effect of herbal agents has been summarized in Table 1.

**MICRONUTRIENTS (VITAMINS AND MINERALS)**

Vitamin B\textsubscript{12} (cyanocobalamin) molecule contains a cobalt complex, it is known as cobalamin. Molecular weight of vitamin B\textsubscript{12} is the highest among all vitamins; therefore, it is known to accumulate at high levels in the liver. Vitamin B\textsubscript{12} is a complex organometallic cofactor associated with three subfamilies of enzymes: The adenosylcobalamin-dependent isomerases, the methyl cobalamin-dependent methyltransferases, and the dehalogenases.\textsuperscript{43} In chronic feeding regimen without a methyl-donor, vitamin B\textsubscript{12} may lead to the development of HCC.\textsuperscript{44} Previous studies reported that vitamin B\textsubscript{12} showed

**Table 1: Effect of herbal agents against hepatic disorders**

<table>
<thead>
<tr>
<th>Name</th>
<th>Family</th>
<th>Constituents</th>
<th>Mechanism of action</th>
<th>Major effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Silybum marianum}</td>
<td>\textit{Asteraceae}</td>
<td>Silibinin</td>
<td>Inhibit GSH depletion and genotoxicity</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>\textit{Glycyrhiza glabra}</td>
<td>\textit{Fabaceae}</td>
<td>Glycyrrhetic acid</td>
<td>Enhance GSH formation</td>
<td>Anti-hepatocarcinogenesis</td>
</tr>
<tr>
<td>\textit{Panax ginseng}</td>
<td>\textit{Araliaceae}</td>
<td>Ginsenosides</td>
<td>Improve GSH synthesis</td>
<td>Hepatorenoprotective effect</td>
</tr>
<tr>
<td>\textit{Ginkgo biloba}</td>
<td>\textit{Ginkgoaceae}</td>
<td>Polyphenols</td>
<td>Free radical scavenger Prevention of tumor initiation</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>\textit{Taraxacum officinale}</td>
<td>\textit{Asteraceae}</td>
<td>Taraxacin</td>
<td>Enhance mRNA expression of hepatic antioxidant enzymes Prevention of tumor initiation</td>
<td>Anti-fibrotic effect</td>
</tr>
<tr>
<td>\textit{Allium sativum}</td>
<td>\textit{Amaryllidaceae}</td>
<td>Allicin</td>
<td>Modulation of p53 gene Delay or arrest of the tumor development</td>
<td>Anticancer</td>
</tr>
<tr>
<td>\textit{Curcuma longa}</td>
<td>\textit{Zingiberaceae}</td>
<td>Curcumin I, II, and III</td>
<td>Suppresses the activation of nuclear factor kappa B Prevention of tumor initiation</td>
<td>Anticancer and antioxidant</td>
</tr>
<tr>
<td>\textit{Colchicum autumnale}</td>
<td>\textit{Colchicaceae}</td>
<td>Colchicine</td>
<td>Inhibition of cellular mitosis Delay or arrest of the tumor development</td>
<td>Anti-tumor anti-fibrotic agent</td>
</tr>
<tr>
<td>\textit{Thymus vulgaris}</td>
<td>\textit{Lamiaceae}</td>
<td>Thymol</td>
<td>Increase GSH synthesis Prevention of tumor initiation</td>
<td>Antioxidant, antimicrobial</td>
</tr>
<tr>
<td>\textit{Calendula officinalis}</td>
<td>\textit{Asteraceae}</td>
<td>Triterpenoids</td>
<td>Enhance antioxidant enzymes Prevention of tumor initiation</td>
<td>Antioxidant, anti-inflammatory</td>
</tr>
</tbody>
</table>

GSH: glutathione; HCC: hepatocellular carcinoma
Vitamin C (ascorbic acid) is one of the most required nutrients for a variety of biological functions. The health-promoting effects of vitamin C can be attributed to its biological functions as a cofactor for a number of enzymes, most notably hydroxylases involved in collagen synthesis and as a water-soluble antioxidant. However, it can exert its antioxidant properties in both aqueous and non-aqueous environments. Vitamin C is able to decrease hepatic apoptosis and necrosis against cholestatic liver injury in experimental animals.

Zinc (Zn) is an essential trace element with various biological effects, depending on its catalytic and structural role in an enormous number of enzymes and “Zn-finger” proteins. Zn ions (Zn2+) control cell proliferation, differentiation, and have a role in both apoptotic and necrotic cell death. Zn also has anti-oxidative and anti-inflammatory properties and it postulates hepatoprotective effect against cisplatin-induced oxidative stress, which may be attributed to down-regulations of NADPH oxidase gene expression.

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Vitamin E (α-tocopherol) is a potent lipid-soluble and chain-breaking antioxidant required nutrient for humans because it is necessary for the prevention of several symptoms, including peripheral neuropathy and hemolytic anemia. It plays a significant role in preventing or minimizing peroxidation damage in biological systems. Supplementation with vitamin E inhibits DNA damage due to free radical scavenging activity and its exerting anti-cytotoxicity and anti-genotoxicity. Moreover, α-tocopherol showed hepatoprotective activity against cisplatin-induced oxidative stress, which may be attributed to down-regulations of NADPH oxidase gene expression.

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**DIETARY SUPPLEMENTS**

N-acetyl cysteine (NAC) is a derivative of the sulfur-containing amino acid cysteine and an intermediary (along with glutamic acid and glycine) in the conversion of cysteine to GSH. Oral NAC administration leads to an increase in intracellular cysteine and GSH levels. NAC is the primary antidote for acetaminophen-induced hepatotoxicity. NAC is able to inhibit genotoxicity due to reactive oxygen species (ROS), protect DNA and nuclear enzymes, and prevent the formation of carcinogen-DNA adducts. NAC succeeded in the treatment of severe hepatic injury induced by a dietary fitness supplement.

Alpha lipoic acid (ALA) influences oxidative status by scavenging ROS, regenerating endogenous antioxidants, repairing oxidative damage, and chelating metal ions. ALA has been proven to be a natural, yet very powerful free radical scavenger and antioxidant. ALA has a protective effect against CCl_4-induced hepatotoxicity and prevents against liver fibrosis due to inhibition of transforming growth factor (TGF)/ platelet-derived growth factor-stimulated HSCs activation and ROS generation.

L-carnitine (CAR) is a conditionally essential nutrient, synthesized endogenously from lysine and methionine in the liver, kidney, and brain and it induces its effects on both fat and glucose metabolism. CAR binds to fatty acyl-coenzyme A and regulates their transport into mitochondrial matrix for β-oxidation. L-CAR is a superoxide scavenger, antioxidant, and DNA cleavage protector. L-CAR has shown a protective effect against radiation-induced detoxification metabolism. Se administration increases the antioxidant capacity of several intracellular systems. In addition, Se showed hepatoprotective effect against malathion-induced liver injury and diabetic rats. Table 2 demonstrated the effect of micronutrients on hepatic lesions.

<table>
<thead>
<tr>
<th>Name</th>
<th>Mechanism of action</th>
<th>Major effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>Suppresses genetic expression of α-smooth muscle actin and heat-shock protein 47, Inhibit hepatic fibrosis</td>
<td>Hepatoprotective effect</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Free radical scavenger, Prevention of tumor initiation</td>
<td>Antioxidant, anti-apoptosis</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Genomic expression NADPH, DNA damage, Prevention of tumor initiation</td>
<td>Anti-cytotoxicity and anti-genotoxicity</td>
</tr>
<tr>
<td>Zinc</td>
<td>Free radical scavenger, control cell proliferation, Prevention of tumor initiation</td>
<td>Anti-inflammatory, anti-apoptosis</td>
</tr>
<tr>
<td>Selenium</td>
<td>Catalysis of redox reaction, Prevention of tumor initiation</td>
<td>Antioxidant</td>
</tr>
</tbody>
</table>
organotoxicity via induction of endogenous antioxidants.\cite{71} Reduction of concentration of CAR in blood and tissues is accompanied with hyperlipidemic condition.\cite{72} It has been well reported that hepatoprotective effect of L-CAR against CCl₄-induced hepatotoxicity is due to significant increase of GSH level.\cite{73}

Lycopene is the red pigment of tomatoes. Lycopene concentration in human serum tends to be higher than those of all other carotenoid pigments.\cite{74} Lycopene showed potent anti-inflammatory effects through its action as an antioxidant and free radical scavenger, which may reduce cellular damage.\cite{75} It plays a crucial role in the protection of cell membranes from lipid peroxidation by neutralizing hydroxyl radicals and may bind to DNA, promoting further protection beyond antioxidant activity.\cite{76} Lycopene demonstrated potential beneficial effects against oxidative stress. These beneficial functions are due to enhancement of cellular gap junction communication, induction of phase II enzymes through activation of the antioxidant response element of transcription system, and suppression of insulin-like growth factor-1-stimulated cell proliferation. Its effects also include anti-angiogenesis, inhibition of cell proliferation, and induction of apoptosis.\cite{77} Lycopene showed potent protective effect against hepatic steatosis in knockout mice.\cite{78}

S-adenosyl-l-methionine (SAMe) is an endogenous agent that is a critical precursor for transmethylation and transsulfuration reactions. SAMe plays an important role such as a cofactor for many transmethylation reactions of amino acids, proteins, nucleotides, and neurotransmitters and a vital precursor for the transsulfuration pathway that ultimately generates GSH.\cite{79} SAMe has potent activity against acetalaminophen-induced hepatotoxicity as compared to NAC.\cite{80} SAMe reduced the cytotoxicity of other hepatotoxins such as carbon tetrachloride, which may lead to liver fibrosis and alcohol-mediated damage.\cite{81} SAMe was reported to protect liver against hepatic injury and fibrosis through the inhibition of oxidative stress and HSCs formation due to activation of Smad7 (an inhibitor of TGF-beta signaling; regulator of hepatic fibrosis) messenger RNA expression.\cite{82}

Whey protein concentrates (WPCs) are heterogeneous compounds obtained from milk after casein precipitation at pH 4.6.\cite{83} WPCs play an important biological role since they act as antioxidants, antihypertensive and anti-tumor, hypolipidemic and antiviral, antibacterial, and chelating agents. WPCs counteract oxidative stress and DNA damage in rats that fed an aflatoxin-contaminated diet.\cite{84,85} The effect of various supplements has been depicted in Table 3.

### Table 3: Effect of dietary supplement against hepatotoxicity and hepatic cancer

<table>
<thead>
<tr>
<th>Name</th>
<th>Mechanism of action</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-acetyl cysteine</td>
<td>Increase intracellular cysteine and GSH levels; Prevention of tumor initiation</td>
<td>Antidote for acetaminophen-induced hepatotoxicity</td>
</tr>
<tr>
<td>Alpha lipoic acid</td>
<td>Inhibition of TGF/PDGF-(HSC); Prevention of tumor initiation and hepatic fibrosis</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>L-carnitine</td>
<td>Superoxide scavenger, and DNA cleavage protector; Prevention of tumor initiation</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Lycopene</td>
<td>Suppression of insulin-like growth factor-1-stimulated cell proliferation; Prevention of tumor initiation and hepatic fibrosis</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>S-adenosyl-l-methionine</td>
<td>Cofactor for amino acids, inhibition of HSC</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Whey protein concentrates</td>
<td>Free radical scavenger; Prevention of tumor initiation</td>
<td>Antioxidants, hypolipidemic agent</td>
</tr>
</tbody>
</table>

GSH: glutathione; TGF: transforming growth factor; PDGF: platelet-derived growth factor; HSC: hepatic stellate cell

It can be concluded that natural bioactive compounds are promising candidate in the treatment and prevention of hepatic injury as well as HCC. The effects may be due to their anti-oxidative properties, modulatory effects in several cytokines, and anti-genotoxic efficacy. The current article highlights on the potential mechanism of the action of natural compounds against hepatotoxicity and suggests further studies for developing novel therapeutic tools in the treatment of hepatic lesions.

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**Conflict of interest**

There is no conflict of interest.

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