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Revisiting Hepatoprotective Natural Products from a Biological Point of View

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Abstract – Naturally occurring small molecules from plants, microorganisms, and animals allow the design of drugs that can be beneficial in virtually all kinds of human diseases. Liver diseases with diverse etiologies such as viral infection, chemical intoxication, and metabolic fat accumulation are one of the leading causes of human mortality. Unfortunately, however, there are few effective drugs available capable of stopping or reversing the progress of liver disease. Here, we discuss the current advances in developing hepatoprotective natural products for several arrays of liver disease pathogenesis.
Keywords – The liver, Hepatoprotective, Natural Products, Apoptosis

Introduction

Secondary metabolites from natural resources with a variety of structural diversity are known (Firn and Jones, 2003; Newman *et al*., 2003). The occurrence of secondary metabolites in plants is considered to be a defense mechanism against environmental herbivores and microorganisms as well. It is now widely accepted that the metabolites are the result of a long-term evolutionary strategy of plants to inhibit or kill their enemies including competing plants.

From the dawn of human history, we have utilized the plants as food resources and medicinal purposes (Newman *et al*., 2003). Treatment of liver-related diseases with natural products has been one branch of Oriental medicine. Now the isolation and functional characterization of hepatoprotective components has become a fruitful area of therapy (Chien *et al*., 2011). Based on the historical literature and accumulated clinical experience, the search bioactive compounds or their mixtures to treat gastrointestinal diseases is a global pursuit. Regardless of the origins (synthetic or natural), however, few hepatoprotective agents are currently available. Ursodeoxycholic acid is an exception (Angulo, 2002).

The paucity of available compounds prompted us to review the status of research efforts in the context of the link between hepatoprotective natural products and the pathogenesis of hepatic diseases. A better understanding concerning how disease pathogenesis is mechanistically inter-connected would provide better directions to mine out hepatoprotective materials. Involvement of natural killer cells in apoptotic event of hepatocyte damage provides an example (Jeong and Gao, 2008) and widens the window of therapeutic targets of immune-modulation.

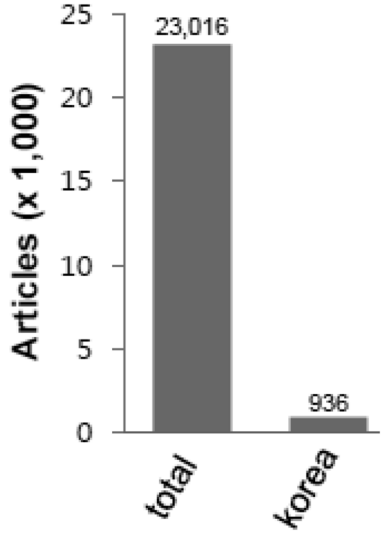

Fig. 1. The number of literatures on hepatoprotective phytochemicals contributed by Korean researchers.

As an approach, we conducted a literature search using the key words “hepatoprotective”, “liver protection and natural products”, and “phytochemicals”. The results were complied according to the origin of the articles (Korean and non-Korean). As is clearly evident in Fig. 1, the contribution of Korean scholars is paultry. The need for

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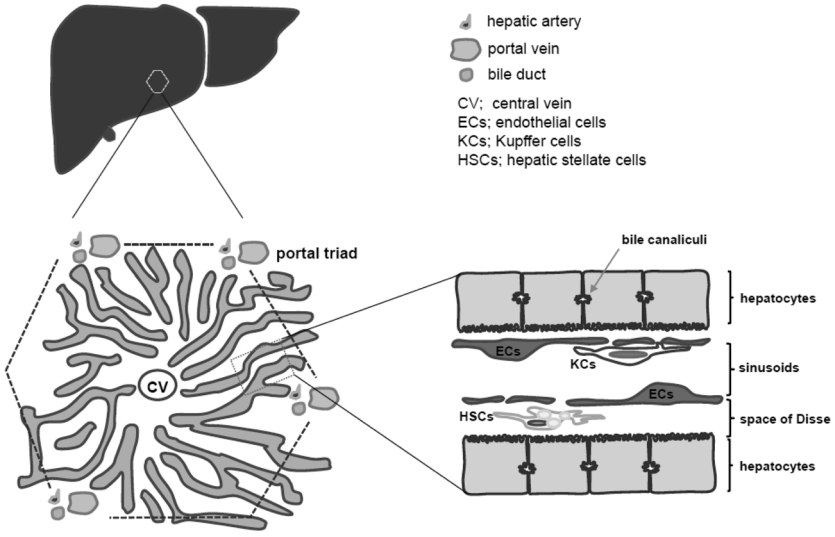


Fig. 2. Hepatic blood flow and resident cells in the liver.

Korean-based mechanistic approaches toward the development of successful hepatoprotective materials from natural resources is indicated.

Liver architecture and function – The liver is the largest internal organ in the human body, having a wide range of functions that include detoxification, production of biochemicals for digestion, and metabolism (Zakim and Boyer, 2003). It is also a complex organ in terms of its pleiotropic functions and blood vessel structure. The circulatory network in the liver is very complicated. Blood gains access to the liver by two routes. Two-thirds of the blood enters the organ via the portal vein, which is partially deoxygenated since it is collected from components of the gastrointestinal tracts, such as stomach, intestine, gallbladder, spleen, and pancreas. The hepatic artery, in contrast, accounts for the remaining one-third of the oxygenated blood issuing from the heart. In this way, the liver receives 25% of the blood of cardiac output even thought the liver mass accounts for only 2.5% of body weight (Fig. 2) (Rappaport, 1980). Both afferent systems merge at the sinusoidal bed, exiting the liver through the central vein. Therefore, the liver not only functions as a reservoir of blood volume but serves as a center for clearance and metabolism of endogenous or exogenous materials from various resources including food intake and toxins (Rappaport, 1980).

Parenchymal hepatocytes constitute about 70% of the total liver cell mass and 90% of the liver weight (Zakim and Boyer, 2003). As shown in Fig. 2, hepatocytes are only one to two cells thick and are separated from each other by capillary spaces called sinusoids. High permeability of the sinusoids allows each hepatocyte to be in close contact with blood. Hepatic stellate cells (HSCs) reside in the space of Disse, which is located between the hepatocyte plate and the capillary endothelium (Fig. 2). The major function of HSCs of the normal liver is the storage of vitamin A. Kupffer cells (KCs) are liver macrophages that reside in the sinusoid. Bile produced by hepatocytes is secreted into thin channels called bile canaliculi. The canaliculi are drained by bile ducts (Fig. 2), which in turn merged into hepatic ducts that carry bile juice away from the liver to the small intestine. As a result, blood flows in the sinusoids whereas bile travels in the opposite direction.

Pathophysiology of liver diseases

Hepatocytes are the most abundant cell type in the liver, and their apoptosis is prominent in virtually all forms of liver injury (Malhi and Gores, 2008). Liver injury encountered in clinical fields can be divided into acute and chronic types based upon the duration of the injury. In most cases, acute liver injury is reversible upon removal of the injurious insults, leading to a functional and structural restitution of the normal liver (Gressner *et al*., 2007; Pan *et al*., 2007). Hepatic fibrosis is a hallmark of chronic liver disease (Fig. 3), which eventually progresses to cirrhosis and/or hepatocellular carcinoma if it is not controlled properly. Chronic liver injuries reflect continuous cycles of acute liver injury and wound healing extended over time. Perpetuated cycles of the injury and healing result in fibrotic phenotypes of the liver, once

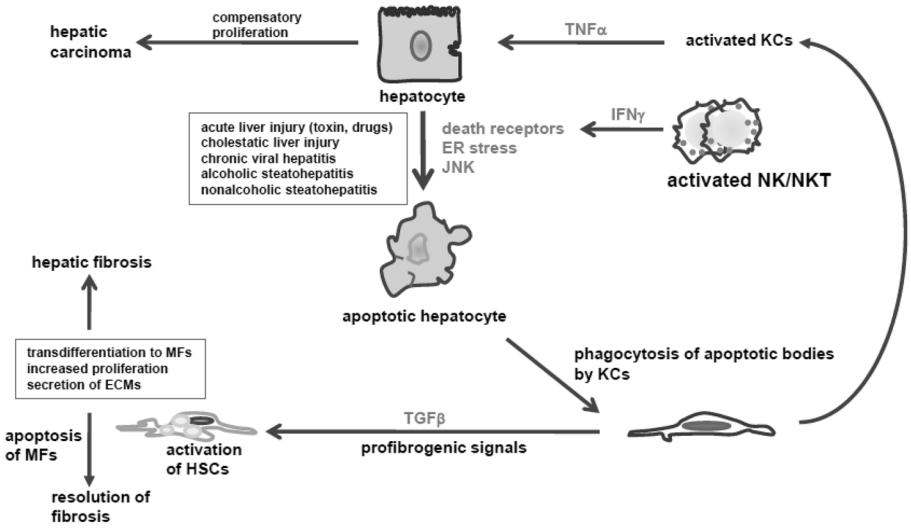


Fig. 3. Hepatocyte damage initiates the differential stage of liver diseases.

considered as one-way processes. With the paradigm shift that hepatic fibrosis may be reversible, inhibition and careful control of liver injury has become a therapeutic strategy aimed at preventing end-stage liver diseases (Gressner *et al*., 2007; Pan *et al*., 2007; Snowdon and Fallowfield, 2011).

Traditionally, the secretion of transaminase enzymes from damaged hepatocytes has been utilized as the readout of liver injury. The levels of blood serum glutamic oxaloacetic transaminase (GOT, also called aspartate aminotransferase) and serum levels of glutamic pyruvic transaminase (GPT, also called alanine amino-transferase) are elevated with liver damage (Zakim and Boyer, 2003). Recently, a novel biomarker of hepatic damage has emerged, which is well-correlated with disease severity and response to therapeutic potential of candidate drugs. The M30 neoantigen is an enzymatic cleavage product of cytokeratin 18 that is generated upon apoptotic insults. It has been detected in virtually all liver diseases including cholestasis or cholangitis, and graft-versus host disease during liver transplantation and steatohepatitis (Malhi and Gores, 2008). Since the biomarker reflects hepatic apoptosis, it should prove to be a useful diagnostic tool for monitoring disease progress and therapy.

Various molecular players and subcellular organelles are unequivocally involved in apoptotic program in liver diseases (Fig. 2). A distinct feature of hepatocyte damage depending on the etiology is discussed elsewhere in detail (Malhi and Gores, 2008). In acetaminophen-mediated acute liver injury, for example, upregulation of death receptors and activation of its downstream signaling pathway (Janus kinase, JNK) has been reported to be involved in liver cell death (Fig. 2) (Mitchell, 1998; Tujios and Fontana, 2011). Mice deficient in death-receptor are resistant to the development of hepatic fibrosis upon bile-duct ligation, which also implies the role of apoptosis in disease progression (Gujral *et al*., 2004). Consistent with the *in vivo* observation, caspase inhibition leads to attenuation of hepatocyte apoptosis and liver inflammation (Jeong and Gao, 2008). In a similar setting, however, triggering apoptosis in HSCs has been shown to decrease fibrosis (Siegmund *et al*., 2005).

In response to hepatocyte death, resident KCs phagocytose the corpse and remove the dead cells while become activated, as shown in Fig. 2. Active KCs synthesize and secrete proinflammatory cytokines including tumor necrosis factor (TNF)-α, resulting in sustained hepatic damage (Siegmund *et al*., 2005; Snowdon and Fallowfield, 2011). Alternatively, growth promoting profibrogenic mitogens secreted by activated KCs stimulate HSCs that are otherwise normally quiescent. Activated HSCs grow rapidly and vigorously synthesize extracellular matrix proteins, which in turn occupy the space where the damaged hepatocytes are eliminated (Fig. 2). Even though different types of cells are able to differentiate to fibroblastic cells and can participate in hepatic fibrogenesis, these cells, including HSCs, seem to undergo apoptosis during the resolution stage of liver injury (Malhi and Gores, 2008). In conditions of ongoing liver damage, selective apoptosis in HSC has been proven to uncouple hepatocyte apoptosis from hepatic fibrogenesis (Gressner *et al*., 2007). Conclusively, the above findings provide an important cue to handle liver damage; both

inhibition of hepatocyte apoptosis and promotion of HSC apoptosis may produce therapeutic benefits (Siegmund *et al*., 2005).

Natural product-derived components with hepatoprotective activity

From the brief overview of the pathogenesis in liver diseases, we may focus on various cell types and involved cellular processes such as apoptosis, immunomodulation, and differentiation programs to address the use of natural resources that contain bioactive constituents. Medicinal plants have been important resources for pharmaceutical drugs. As the demand for bioactive materials from natural resources increases, much effort has been invested to mine phytochemicals targeting human diseases including the liver. The versatility of natural remedies for the treatment of liver diseases has a long history (Firn and Jones, 2003). Herbal medicines and their ingredients are still used all around the world. Known liver protective plants constituents are represented in Table 1; the constituents are structurally diverse and include flavonoids, coumarins, terpenoids, and xanthenes. Silymarin, a flavonol lignan mixture extracted from *Silybum marianum*, has been a popular remedy for hepatic diseases. Baicalin (*Scutellaria baicalensis*), schizandrin B (*Schisandra chinensis*), tanshinones (*Salvia miltiorrhiza*), astragaloside (*Astragalus membranaceus*), glycyrrhizin (*Glychyrrhiza glabra*), and hypophyllanthin (*Phyllanthus niruri*) are potential candidates with hepatoprotective activity. Our lab has screened hepatoprotective and/or anti-fibrotic constituents from plant resources for the last two decades. The current review discusses latest accomplishment of isolated constituents with hepatoprotective activity, briefly summarized in Table 1.

Table 1. Classified hepatoprotective natural products from medicinal plants

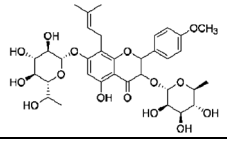
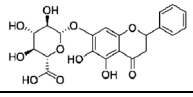
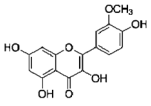
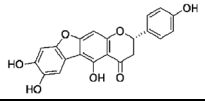
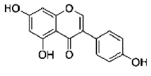
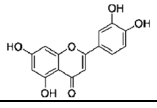
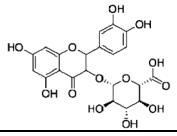
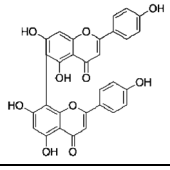
Class	Chemical Substances	Plant Source	Structure	References
Flavonoid	Icarrin and its glycoside	*Epimedium koreanum*		Lee (1995) Cho (1995)
	Baicalin	*Scutellaria baicalensis*		Wan (2008)
	Isorhamnetin	*Oenanthe javanica*		Lee (2008)
	Anastatin A, B	*Anantatica hierochuntica*		Yoshikawa (2003)
	Genistein	*Erycibe expansa*		Matsuda (2004)
	Luteolin	*Equisetum arvense*		Oh (2004)
	Quercetin-3O-β-D-glucuronopyranoside (methyl ester)	*Saururus chinensis*		Sung (1997)
	Agathisflavone	*Canarium manii*		Anand (1992)

Table 1. continued

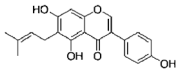
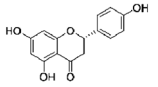
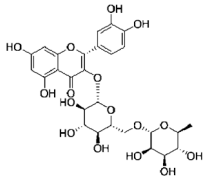
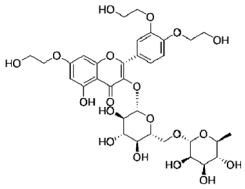
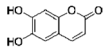
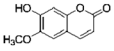
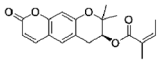
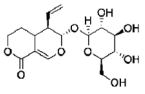
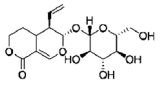
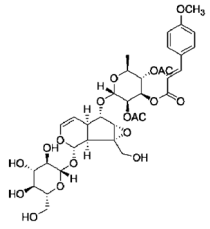
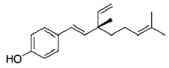
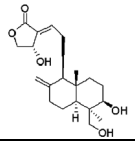
Class	Chemical Substances	Plant Source	Structure	References
Flavonoid	Wighteone	*Cudrania cochinchinensis*		Lin (1996)
	Naringenin	*Cudrania cochinchinensis*		Lin (1996)
	Rutin			Janbaz (2002)
	Troxerutin			Zhang (2009)
	Kolaviron	*Garcinia kola*	Fraction containing Garcinia biflavonoids	Farombi (2009)
Coumarin	Esculetin	*Cnidium monnieri*		Shin (2011)
	Scopoletin	*Solanum lyratum*		Kang (1998)
	decursinol angelate	*Angelica gigas*		Yoo (2007)
Iridoid	Genitopicroside	*Swertia japonica*		Hase (1997)
	Sweroside	*Swertia japonica*		Hase (1997)
	Scropolioside A	*Scrophularia koelzii*		Garg (1994)
	Picroliv	*Picrorhiza kurrooa*		Visen (1991)
Monoterpenoid	(S)-bakuchiol	*Psoralea corylifolia*		Hyun (2001)
Diterpenoid	Andrographolide	*Andrographis paniculata*		Chander (1995)

Table 1. continued

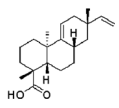
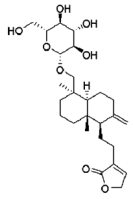
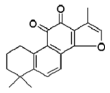
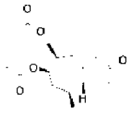
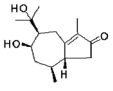
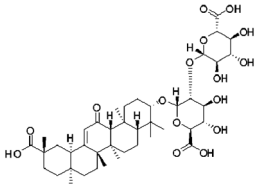
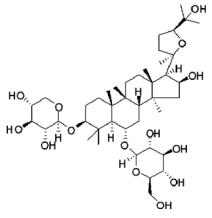
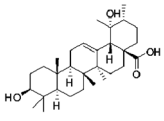
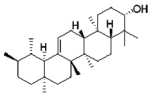
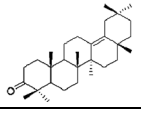
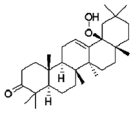
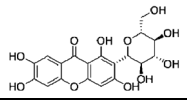
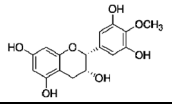
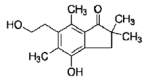
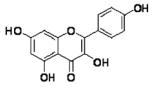
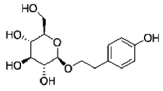
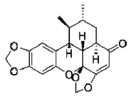
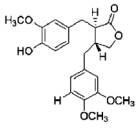
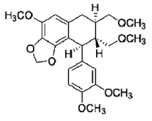
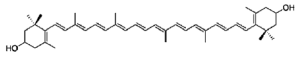
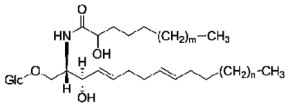
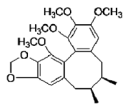
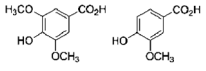
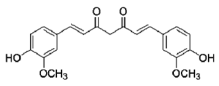
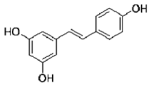
Class	Chemical Substances	Plant Source	Structure	References
Diterpenoid	Acanthoic acid	*Acanthopanax koreanum*		Park (2004)
	Neoandrographolide	*Andrographis paniculata*		Chander (1995)
	tanshinone	*Salvia miltiorrhiza*		Park (2009)
Sesquiterpenoid	Torilin	*Cnidium monnieri*		Oh (2002)
	Torilolone	*Cnidium monnieri*		Oh (2002)
Triterpenoid	Glycyrrhizin	*Glychyrrhiza glabra*		Numazaki (1994)
	Astragaloside IV	*Astragalus membranaceus*		Liu (2009)
	pomolic acid	*Astragalus membranaceus*		Liu (2009)
	Amyrin (α-, β-, γ)	*Sedum sarmentosum* *Protium heptaphyllum*		Amin (1998) Oliveira (2005)
	γ-amyrone	*Sedum sarmentosum*		Amin (1998)
	18β-hydroperoxy-olean, 12-en-3-one	*Sedum sarmentosum*		Amin (1998)

Table 1. continued

Class	Chemical Substances	Plant Source	Structure	References
Phenolic	Mangiferin	*Salacia reticulate*		Yoshikawa (2002)
	4-O-methylepigallocatechin	*Salacia reticulate*		Yoshikawa (2002)
	Onitin	*Equisetum arvense*		Oh (2004)
	Kaempferol	*Rhodiola sachalinensis*		Song (2003)
	Salidroside	*Rhodiola sachalinensis*		Song (2003)
Lignan	Sauchinone	*Saururus chinensis*		Sung (2000a) Sung (2000b)
	Arctigenin Traxillagenin 4'-Dimethyltraxillagenin	*Torreya nucifera*		Kim (2003)
	Hypophyllanthin	*Phyllanthus niruri*		Negi (2008)
Msc	Cerebrosides	*Lycium chinensis*		Kim (1997b) Kim (1999) Kim (2000)
	Zeaxanthin Zeaxanthin dipalmatate	*Lycium chinensis*		Kim (1997c) Kim (1997a) Kim (2002)
	Schisandrin B	*Schisandra chinensis*		Tang (2003)
	Syringic acid Vanillic acid	*Lentinula edodes*		Itoh (2010)
	Curcumin			Zhao (1996)
	Resveratrol	*Acer mono*		Yang (2005)
	Betaine	*Lycium chinensis*		Kim (1998)

Conclusion: toward molecular targeting

Secondary metabolites originating from natural resources are synthesized by organisms under pressure to protect against environmental competitors or to ensure their reproductive success. Thus, they represent a population of molecules that can interact with multiple protein targets in nature (Evans *et al*., 1988). Natural products, therefore, are developed and selected by molecular evolution to specifically bind these protein targets.

A representative example is rapamycin, which is a bacterial metabolite from soil Actinomycetes that show broad cellular effects via the binding to its protein target, a small (12 kDa) FK506-binding protein (FKBP12) and, in turn, the rapamycin-FKBP12 complex inhibits mammalian target of rapamycin (mTOR). In this manner, rapamycin has been utilized as a chemical tool to study the mTOR-related signaling pathways and serves to lead the design of potently-active anti-cancer and anti-aging drugs.

Advances in molecular biology, cellular biology, proteomics and genomics dramatically increase in the number of molecular protein targets. High-throughput screening systems utilizing chemical libraries may be the first option to test and choose active materials to the molecular targets. Natural products also provide benefits in such efforts since they have unique chemical diversity found in their structures. In addition, new technologies are available to overcome the traditional bottlenecks in the drug discovery process from natural origins. However, the natural product library is composed of crude or semi-pure compound mixtures. This heterogeneity surely adds another layer of complexity to screening system with natural products.

As mentioned above, natural products represent a valuable source with diverse chemical structures for drug development. Current technical advances that we witness in assay methods, chemical separation and structure elucidations lower the inherent difficulties in handling mixtures of these complex molecules.

In the context of the candidate liver molecules to target, the ample attempts that modify a cellular process, autophagy, by small molecules provides a cogent example. Autophagy is a newly-introduced cellular process in a variety of clinical and basic research fields. Some gastroenterologists have already noted that autophagy appears in the liver under basic and pathophysiological processes. Autophagy is a cellular process in which cells degrade their own organelles and unfolded protein aggregates through lysosomal engagement. Accumulating evidence indicates that the autophagic process is involved in a wide range of cell physiological activities ranging from homeostatic stress response to inflammation to aging. Moreover, it actively participates in disease progress including neurodegenerative diseases, tumorigenesis, and inflammatory bowel disease. Although its importance in biological aspects is recognized, the link to the etiology of liver disease is relatively unexplored. Hidvegi *et al*. (2010) recently investigated how the well-known compound, cabamazepine, stimulates the autophagic process to successfully clear the toxic effects of misfolded α1-antitryptin protein in cells and in a mouse model of associated hepatic fibrosis. Similarly, silibin has been explored with respect to its capacity to activate and induce autophagic cell death, by which it can kill fibrosarcoma cells. These observations imply that autophagy may have a role in hepatic pathophysiology. Research focusing on defining the underlying mechanisms of disease process often lead to new treatment strategies. Rather than correcting the genetic mutations, targeting the autophagy responsible for the elimination of unfolded protein aggregates or damaged organelles would provide an excellent example of the new concept of therapy and drug development.

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References

Adewusi, E.A. and Afolayan, A.J., A review of natural products with hepatoprotective activity. *J. Med. Plants Res.*, **4**, 1318-1334 (2010).

Amin, H., Mingshi, W., Hong, Y.H., Decheng, Z., and Lee, K.H., Hepatoprotective triterpenes from *Sedum sarmentosum*. *Phytochemistry*, **49**, 2607-2610 (1998).

Anand, K.K., Gupta, V.N., Rangari, V., Singh, B., and Chandan, B.K., Structure and hepatoprotective activity of a biflavonoid from *Canarium manii*. *Planta Med.*, **58**, 493-495 (1992).

Angulo, P., Use of ursodeoxycholic acid in patients with liver disease. *Curr. Gastroenterol. Rep.*, **4**, 37-44 (2002).

Chander, R., Srivastava, V., Tandon, J.S., and Kapoor, N.K., Antihepatotoxic activity of diterpenes of *Andrographis paniculata* (Kalmegh) against Plasmodium berghei induced hepatic damage in Mastomys natalensis. *Int. J. Pharmacogn.*, **33**, 135-138 (1995).

Chien, C.F., Wu, Y.T., and Tsai, T.H., Biological analysis of herbal medicines used for the treatment of liver diseases. *Biomed. Chromatogr.*, **25**, 21-38 (2011).

Cho, N.J., Sung, S.H., Lee, H.S., Jeon, M.H., and Kim, Y.C., Anti-hepatotoxic Activity of Icariside II, a Constituent of *Epimedium koreanum*. *Arch. Pharm. Res.*, **18**, 289-292 (1995).

Duan, W.J., Li, Q.S., Xia, M.Y., Tashiro, S.I., Onodera, S., and Ikejima, T., Silibinin activated p53 and induced autophagic death in human fibrosarcoma HT1080 cells via reactive oxygen species-p38 and c-Jun

N-terminal kinase pathways. *Biol. Pharm. Bull.*, **34**, 47-53 (2011).
Farombi, E.O., Shrotriya, S., and Surh, Y.J., Kolaviron inhibits dimethyl nitrosamine-induced liver injury by suppressing COX-2 and iNOS expression via NF-kappaB and AP-1. *Life Sci*. **84**, 149-155 (2009).
Firn, R.D. and Jones, C.D., Natural products: a simple model to explain chemical diversity. *Nat. Prod. Rep.*, **20**, 382-391 (2003).
Gao, B., Radaeva, S., and Park, O., Liver natural killer and natural killer T cells: immunobiology and emerging roles in liver diseases. *J. Leukoc. Biol.*, **86**, 513-528 (2009).
Garg, H.S., Bhandari, S.P.S., Tripathi, S.C., Patnaik, G.K., Puri, A., Saxena, R., and Saxena, R.P., Antihepatotoxic and immunostimulant properties of iridoid glycosides of *Scrophularia koelzii*. *Phytother. Res.*, **8**, 224-228 (1994).
Gressner, O.A., Weiskirchen, R., and Gressner, A.M., Evolving concepts of liver fibrogenesis provide new diagnostic and therapeutic options. *Comp. Hepatol.*, **6**, 7 (2007).
Gujral, J.S., Liu, J., Farhood, A., and Jaeschke, H., Reduced oncotic necrosis in Fas receptor-deficient C57BL/6J-lpr mice after bile duct ligation. *Hepatology,* **40**, 998-1007 (2004).
Hase, K., Li, J., Basnet, P., Xiong, Q., Takamura, S., Namba, T., and Kadota, S., Hepatoprotective principles of *Swertia japonica* on D-galactosamine/lipopolysaccharide-induced liver injury in mice. *Chem. Pharm. Bull.,* **45**, 1823-1827 (1997).
Hidvegi, T., Ewing, M., Hale, P., Dippold, C., Beckett, C., Kemp, C., Maurice, N., Mukherjee, A., Goldbach, C., Watkins, S., Michalopoulos, G., and Perlmutter, D.H., An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis. *Science,* **329**, 229-232 (2010).
Hyun, C., Jun, J.Y., Song, E.K., Kang, K.H., Baek, H.Y., Ko, Y.S., and Kim, Y.C., Bakuchiol: A hepatoprotective compound of *Psoralea corylifolia* on tacrine-induced cytotoxicity in Hep G2 cells. *Planta Med.,* **67**, 750-751 (2001).
Itoh, A., Isoda, K., Kondoh, M., Kawase, M., Watari, A., Kobayashi, M., Tamesada, M., and Yagi, K., Hepatoprotective effect of syringic acid and vanillic acid on CCl4-induced liver injury. *Biol. Pharm. Bull.*, **33**, 983-987 (2010).
Janbaz, K.H., Saeed, S.A., and Gilani, A.H., Protective effect of rutin on paracetamol and CCl_4-Induced hepatotoxicity in rodents. *Fitoterapia*, **73**, 557-563 (2002).
Jang, Y.P., Lee, Y.J., Kim, Y.C., and Huh, H., Production of a hepatoprotective cerebroside from suspension cultures of *Lycium chinense*. *Plant Cell Rep.,* **18**, 252-254 (1998).
Jeong, W.I. and Gao, B., Innate immunity and alcoholic liver fibrosis. *J. Gastroenterol. Hepatology*, **23**, S112-S118 (2008).
Kang, S.Y., Sung, S.H., Park, J.H., and Kim, Y.C., Hepatoprotective scopoletin, a constituent of *Solanum lyratum*. *Arch. Pharm. Res.,* **21**, 718-722 (1998).
Kim, H.P., Kim, S.Y., Lee, E.J., Kim, Y.C., and Kim, Y.C., Zeaxanthins dipalmitate from *Lycium chinense* has hepatoprotective activity, *Res. Comm. Mol. Pathol. Pharm.,* **97**, 301-314 (1997a).
Kim, H.P., Lee, E.J., Kim, Y.C., Kim, J., Park, J.H., and Kim, Y.C., Zeaxanthin dipalmitate from *Lycium chinense* fruits reduces experimentally induced hepatic fibrosis in rats. *Biol. Pharm. Bull.*, **25**, 390-392 (2002).
Kim, S.H., Jang, Y.P., Sung, S.H., Kim, C.J., Kim, J.W., and Kim, Y.C., Hepatoprotective dibenzylbutyrolactone lignans of *Torreya nucifera* against CCl_4-induced toxicity in primary cultured rat hepatocytes. *Biol. Pharm. Bull.*, **26**, 1202-1205 (2003).
Kim, S.K., Kim, Y.C., and Kim, Y.C., Effects of singly administered betaine on hepatotoxicity of chloroform in mice. *Food Chem. Toxicol.,* **36**, 655-661 (1998).
Kim, S.Y., Choi, Y.H., Kim J., Huh, H., and Kim, Y.C., A new antihepatotoxic cerebroside from *Lycium chinense* fruits. *J. Nat. Prod.,* **60**, 274-276 (1997b).
Kim, S.Y., Kim, H.P., Huh, H., and Kim, Y.C., Antihepatotoxic zeaxanthins from the fruits of *Lycium chinense*. *Arch. Pharm. Res.,* **20**, 529-532 (1997c).
Kim, S.Y., Lee, E.J., Kim, H.P., Kim, Y.C., Moon, A., and Kim, Y.C., A novel cerebroside from *Lycii Fructus* preserves the hepatic glutathione redox system in primary cultures of rat hepatocytes. *Biol. Pharm. Bull.,* **22**, 873-875 (1999).
Kim, S.Y., Lee, E.J., Kim, H.P., Lee, H.S., and Kim, Y.C., LCC, a cerebroside from *Lycium chinense*, protects primary cultured rat hepatocytes exposed to galactosamine. *Phytother. Res.,* **14**, 448-451 (2000).
Farombi, E.O., Shrotriya, S., and Surh, Y.J., Kolaviron inhibits dimethyl nitrosamine-induced liver injury by suppressing COX-2 and iNOS expression via NF-kappaB and AP-1. *Life Sci.*, **84**, 149-155 (2009).
Lee, M.K., Choi, Y.J., Sung, S.H., Shin, D.I., Kim, J.W., and Kim, Y.C., Anti-hepatotoxic activity of icariin, a major constituent of *Epimedium koreanum*. *Planta Med.*, **61,** 523-526 (1995).
Lee, M.K., Yang, H., Ha, N.R., Sung, S.H., and Kim, Y.C., Isorhamnetin from *Oenanthe javanica* attenuates fibrosis in rat hepatic stellate cells via inhibition of ERK signaling pathway. *Nat. Prod. Sci.*, **14**, 81-85 (2008).
Lee, M.K., Lee, K.Y., Jeon, H.Y., Sung, S.H., and Kim, Y.C., Antifibrotic activity of triterpenoids from the aerial parts of *Euscaphis japonica* on hepatic stellate cells. *J. Enzyme Inhib. Med. Chem.*, **24**, 1276-1279 (2009).
Lin, C.C., Lee, H.Y., Chang, C.H., Namba, T., and Hattori, M., Evaluation of the liver protective principles from the root of *Cudrania cochinchinensis* var. gerontogea. *Phytother. Res.*, **10**, 13-17 (1996).
Malhi, H. and Gores, G.J., Cellular and molecular mechanisms of liver injury. Gastroenterology, **134**, 1641-1654 (2008).
Liu, H., Wei, W., Sun, W.Y., and Li, X., Protective effects of astragaloside IV on porcine-serum-induced hepatic fibrosis in rats and in vitro effects on hepatic stellate cells. *J. Ethnopharmacol.*, **122**, 502-508 (2009).
Matsuda, H., Morikawa, T., Fengming, X., Ninomiya, K., and Yoshikawa, M., New isoflavones and pterocarpane with hepatoprotective activity from the stems of *Erycibe expansa*. *Planta Med.*, **70**, 1201-1209 (2004).
Mitchell, J.R., Acetaminophen toxicity. *N. Engl. J. Med.*, **319**, 1601-1602 (1998).
Negi, A.S., Kumar, J.K., Luqman, S., Shanker, K., Gupta, M.M., and Khanuja, S.P., Recent advances in plant hepatoprotectives: a chemical and biological profile of some important leads. *Med. Res. Rev.*, **28**, 746-772 (2008).
Newman, D.J., Cragg, G.M., and Snader, K.M., Natural products as a source of new drugs over the period 1981-2002. *J. Nat. Prod.,* **66**, 1002-1037 (2003).
Numazaki, K., Umetsu, M., and Chiba, S., Effect of glycyrrhizin in children with liver dysfunction associated with cytomegalovirus infection. *Tohoku J. Exp. Med.*, **172**, 147-153 (1994).
Oh, H., Kim, J.S., Song, E.K., Cho, H., Kim, D.H., Park, S.E., Lee, H.S., and Kim, Y.C., Sesquiterpenes with hepatoprotective activity from *Cnidium monnieri* on tacrine-induced cytotoxicity in Hep G2 cells. *Planta Med.*, **68**, 748-749 (2002).
Oh, H., Kim, D.H., Cho, J.H., and Kim, Y.C., (2004). Hepatoprotective and free radical scavenging activities of phenolic petrosins and flavonoids isolated from *Equisetum arvense*. *J. Ethnopharmacol.*, **95**, 421-424 (2004).
Oliveira, F.A., Chaves, M.H., Almeida, F.R.C., Lima, R.C.P., Silva, R.M., Maia, J.L., Brito, G.A.C., Santos, F.A., and Rao, V.S., Protective effect of α- and β-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. *J. Ethnopharmacol.*, **98**, 103-108 (2005).

Pan, Q., Zhang, Z.B., Zhang, X., Shi, J., Chen, Y.X., Han, Z.G., and Xie, W.F., Gene expression profile analysis of the spontaneous reversal of rat hepatic fibrosis by cDNA microarray. *Dig. Dis. Sci.*, **52**, 2591-2600 (2007).

Park, E.J., Zhao, Y.Z., Young, H.K., Jung, J.L., and Sohn, D.H., Acanthoic acid from *Acanthopanax koreanum* protects against liver injury induced by *tert*-butyl hydroperoxide or carbon tetrachloride *in vitro* and *in vivo*. *Planta Med.*, **70**, 321-327 (2004).

Park, E.J., Zhao, Y.Z., Kim, Y.C., and Sohn, D.H., Preventive effects of a purified extract isolated from Salvia miltiorrhiza enriched with tanshinone I, tanshinone IIA and cryptotanshinone on hepatocyte injury in vitro and in vivo. *Food Chem. Toxicol.*, **47**, 2742-2748 (2009).

Rappaport, A.M., Hepatic blood flow: morphologic aspects and physiologic regulation. *Int. Rev. Physiol.*, **21**, 1-63 (1980).

Shin, E., Lee, C., Sung, S.H., Kim, Y.C., Hwang, B.Y., and Lee, M.K., Antifibrotic activity of coumarins from *Cnidium monnieri* fruits in HSC-T6 hepatic stellate cells. *J. Nat. Prod.*, **65**, 370-374 (2011).

Siegmund, S.V., Uchinami, H., Osawa, Y., Brenner, D.A., and Schwabe, R.F., Anandamide induces necrosis in primary hepatic stellate cells. *Hepatology*, **41**, 1085-1095 (2005).

Snowdon, V.K. and Fallowfield, J.A., Models and mechanisms of fibrosis resolution. *Alcohol Clin. Exp. Res.*, **35**, 1-6 (2011).

Song, E.K., Kim, J.H., Kim, J.S., Cho, H., Nan, J.X., Soku, D.H., Ko, G.J., Oh, H., and Kim, Y.C., Hepatoprotective phenolic constituents of *Rhodiola sachalinensis* on tacrine-induced cytotoxicity in Hep G2 cells. *Phytother. Res.*, **17**, 563-565 (2003).

Sung, S.H., Won, S.Y., Cho, N.J. and Kim, C.Y., Hepatoprotective flavonol glycosides of *Saururus chinensis* herbs. *Phytother. Res.*, **11**, 500-503 (1997).

Sung, S.H. and Kim, Y.C., Hepatoprotective diastereomeric lignans from *Saururus chinensis* herbs. *J. Nat. Prod.*, **63**, 1019-1021 (2000a).

Sung, S.H., Lee, E.J., Cho, J.H., Kim, H.S., and Kim, Y.C., Sauchinone, a lignan from *Saururus chinensis*, attenuates CCl_4-induced toxicity in primary cultures of rat hepatocytes. *Biol. Pharm. Bull.*, **23**, 666-668 (2000b).

Tang, M.H., Chiu, P.Y., and Ko, K.M., Hepatoprotective action of schisandrin B against carbon tetrachloride toxicity was mediated by both enhancement of mitochondrial glutathione status and induction of heat shock proteins in mice. *Biofactors*, **19**, 33-42 (2003).

Tujios, S. and Fontana, R.J., Mechanisms of drug-induced liver injury: from bedside to bench. *Nat. Rev. Gastroenterol. Hepatology*, **8**, 202-11 (2011).

Visen, P.K.S., Shukla, B., Patnaik, G.K., Kaul, S., Kapoor, N.K., and Dhawan, B.N. Hepatoprotective activity of picroliv, the active principle of *Picrorhiza kurrooa*, on rat hepatocytes against paracetamol toxicity. *Drug Dev. Res.*, **22**, 209-219 (1991).

Vogel, G., Natural substances with effects on the liver. In: Wagner H, Wolff P, eds. New Natural Products and Plant Drugs with Pharmacological, Biological or Therapeutic Activity. Heidelberg: Springer-Verlag; 1977.

Wan, J.Y., Gong, X., Zhang, L., Li, H.Z., Zhou, Y.F., and Zhou, Q.X., Protective effect of baicalin against lipopolysaccharide/D-galactosamine-induced liver injury in mice by up-regulation of heme oxygenase-1. *Eur. J. Pharmacol.*, **587**, 302-308 (2008).

Yang, H., Sung, S.H., and Kim, Y.C., Two new hepatoprotective stilbene glycosides from *Acer mono* leaves. *J. Nat. Prod.*, **68**, 101-103 (2005).

Yoo, H.H., Lee, M.W., Kim, Y.C., Yun, C.H., and Kim, D.H., Mechanism-based inactivation of cytochrome P450 2A6 by decursinol angelate isolated from Angelica gigas. *Drug Metab. Dispos.*, **35**, 1759-65 (2007).

Yoshikawa, M., Xu, F., Morikawa, T., Ninomiya, K., and Matsuda, H., Anastatins A and B, new skeletal flavonoids with hepatoprotective activities from the desert plant *Anastatica hierochuntica*. *Bioorg. Med. Chem. Lett.*, **13**, 1045-1049 (2003).

Yoshikawa, M., Ninomiya, K., Shimoda, H., Nishida, N., and Matsuda, H., Hepatoprotective and antioxidative properties of *Salacia reticulata*: preventive effects of phenolic constituents on carbontetrachloride-induced liver injury in mice. *Biol. Pharm. Bull.*, **25**, 72-76 (2002).

Zakim, D. and Boyer, T.D., Hepatology: A Textbook of Liver Disease. (4^{th} Ed), Philadelphia: Saunders (2003).

Zhang, Z.F., Fan, S.H., Zheng, Y.L., Lu, J., Wu, D.M., Shan, Q., and Hu, B., (2009). Troxerutin protects the mouse liver against oxidative stress-mediated injury induced by D-galactose. *J. Agric. Food Chem.*, **57**, 7731-7736 (2009).

Zhao, Z. S., O'Brien, P. J., The prevention of CCl4-induced liver necrosis in mice by naturally occurring methylenedioxybenzenes. *Toxicol. Appl. Pharmacol.*, **140**, 411-21 (1996).

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