Recent Advances in Plant Hepatoprotectives: A Chemical and Biological Profile of Some Important Leads

Arvind S. Negi, J.K. Kumar, Suaib Luqman, Karuna Shanker, M.M. Gupta, S.P.S. Khanuja

Central Institute of Medicinal and Aromatic Plants (CIMAP), P.O. CIMAP, Kukrail Picnic Spot Road, Lucknow 226 015, India

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Abstract: Medicinal plants have been traditionally used for treating liver diseases since centuries. Several leads from plant sources have been found as potential hepatoprotective agents with diverse chemical structures. Although, a big list of hepatoprotective phytomolecules was reported in the scientific literature, only a few were potent against various types of liver damages. Of which, silymarin, andrographolide, neoandrographolide, curcumin, picroside, kutkoside, phyllanthin, hypophyllanthin, and glycyrrhizin have largely attracted the scientific community. This review focuses discussion on the chemistry, biological activity, mode of action, toxicity, and future prospects of these leads. © 2007 Wiley Periodicals, Inc. Med Res Rev, 28, No. 5, 746–772, 2008

Keywords: hepatoprotective; andrographolide; neoandrographolide; curcumin; picroside; kutkoside; phyllanthin; hypophyllanthin; silymarin; glycyrrhizin

1. INTRODUCTION

Liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. According to WHO estimates, globally 170 million people are chronically infected with hepatitis C alone and every year 3–4 millions are newly added into the list. Also, there are more than 2 billion infected by hepatitis B virus (HBV) and over 5 million are getting infected with acute HBV annually.[1] Presently, there are nearly 5.5 million chronic liver disease patients in USA alone with

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more than 5,000 liver transplants performed in adults and more than 500 performed in children every year.[2] Thus, the impact of liver disorders on the overall population around the globe is considerable.

Liver is the largest organ in the human body that performs numerous interrelated vital functions. Some of the commonly known disorders include viral hepatitis, alcohol liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, metabolic liver disease, drug induced liver injury, gallstones, etc. Acute hepatitis may be asymptomatic and generally get resolved without sequelae, but unresolved inflammation that persists for more than 6 months leads to chronic condition. As a consequence of chronic liver disease, patient may develop portal hypertension and liver cirrhosis.[2]

Liver toxicity mainly occurs due to alcohol, viral and induced by drugs.

The first is the alcoholic liver damage which is differentiated by three main histological stages, that is, steatosis (fatty liver), acute alcoholic hepatitis and cirrhosis. Steatosis results from the redox imbalance generated by the metabolism of ethanol to acetate. On the other hand, acute alcoholic hepatitis is characterized by hepatocellular injury with associated inflammation and fibrosis. Both these histological stages can completely be reversed on discontinuation of alcohol. When the use of alcohol is quite intensified, inflammation leads to fibrogenesis and if it worsens cirrhosis may occur. In alcoholic liver disease, oxidative stress is caused by pro-oxidant formation, inadequate intake of antioxidants, antioxidant depletion, and alcohol-mediated inhibition of glutathione synthesis. Alcohol-induced liver diseases are mediated by cytokines, which are secreted by liver and other parts of the body. Cytokines regulate certain biochemical processes in the cells that produce them. In the liver, persistent cytokine secretion results in chronic inflammation leading to the conditions such as hepatitis, fibrosis, and cirrhosis. Cytokines, tumor necrosis factor (TNFα) and transforming growth factor (TGFβ) regulate apoptosis, which is in part responsible for alcohol-induced destruction of liver tissue. Fibrogenesis within the liver takes place due to the activation of collagen-producing stellate cells which is mediated through expression of interleukins (IL), such as TNF, IL1, IL6, IL8 ultimately causing precipitation of collagen deposition (Fig. 1).[3]
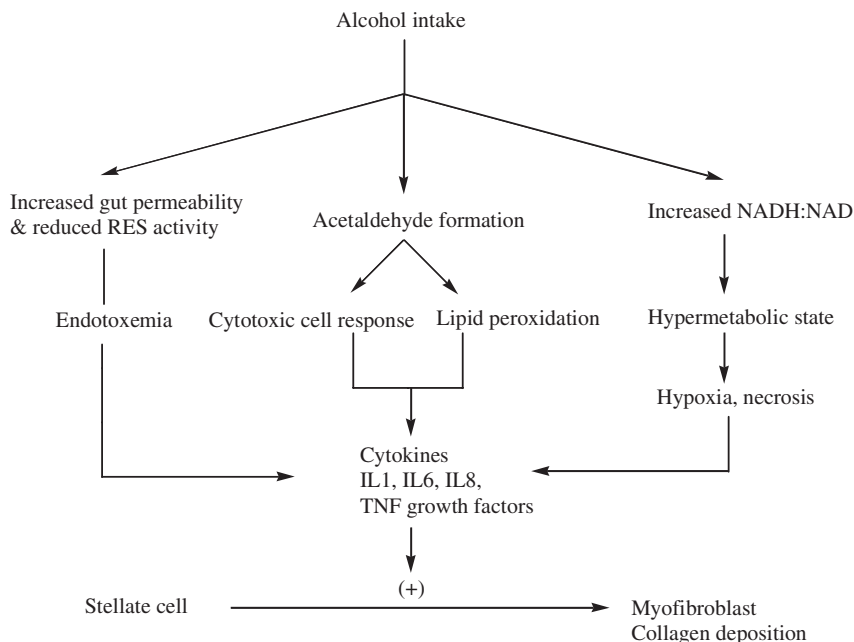

Figure 1. Diagramic presentation of mechanism of alcohol induced liver damage.[3]

The second is the viral hepatitis, mainly responsible for both acute and chronic liver diseases. So far hepatitis A, B, C, D, and E have been identified as causative in human. Hepatitis A is caused by picornovirus in which, inflammation of liver is due to food or drink contaminants. While hepatitis B is caused by hepadnavirus and may lead to acute and chronic liver hepatitis. Hepatitis C may lead to chronic form of hepatitis culminating to cirrhosis. Hepatitis A is rarely life threatening, while B and C are quite serious and may be fatal to life. The hepatocellular injuries caused by HBV infection are predominantly immune-mediated. Several cytokines, including interferon gamma (IFNγ) and TNFα, can purge viruses from infected cells non-cytopathically as long as the cell is able to activate antiviral mechanisms to which the virus is sensitive. The same cytokines also control viral infections indirectly by modulating the induction, amplification, recruitment, and effector functions of the immune response and by upregulation of antigen processing and display of viral epitopes at the surface of the infected cells.

The third factor for the cause of acute liver disease is the use of drugs like paracetamol, pain killers, and antibiotics. Drug induced liver injury is the main reason for a drug not reaching into the market or sometimes a cause for FDA withdrawal of an approved medicine.[2]

The use of herbal resources for the treatment of liver diseases is quite an old approach of various traditional systems of medicine. These medicinal systems conceptualize a general imbalance of the dichotomous energies leads to the disease and they focus on medicine that balance these energies and maintain good health. Mainly, herbs have been used for chronic hepatitis C and alcohol-induced liver diseases. Silymarin from *Silybum marianum*, andrographolide and neoandrographolide from *Andrographis paniculata*, curcumin from *Curcuma longa*, picroside and kutkoside from *Picrorrhiza kurroa*, phyllanthin and hypophyllanthin from *Phyllanthus niruri*, glycyrrhizin from *Glycyrrhiza glabra*, etc., are a few phytomedicines traditionally used in the treatment of liver disorders and are now included as complementary and alternative medicine for the liver patients. This review consists of a detailed chemical and biological profile of these leads with respect to isolation, characterization, quantification, biological activity, mode of action and toxicity.

The mechanism of hepatoprotection by these compounds is generally by exerting multiple effects. Although, they show hepatoprotection due to antioxidant effect but other effects like immunomodulatory, antiviral,[4] antiinflammatory,[5] antiprotozoal activities are also quite common. Other than these, they may affect by increasing protein synthesis in hepatocytes or decreasing formation of leukotrienes, prostaglandins and TNFα by kupffer cells.

2. SILYMARIN

A. Introduction

Silymarin, derived from the seeds of *Silybum marianum* L. (Family: Asteraceae or Compositae), is a member of sunflower family and commonly called milk thistle. The plant has been used for centuries as a natural remedy for liver and biliary tract diseases.[6] Milk thistle protects and regenerates the liver in most liver diseases such as cirrhosis, jaundice, and hepatitis.[7] It acts as preventive medicine which protects liver cells from incoming toxicants such as alcohols, drugs, medications, mercury and other heavy metals, pesticides, etc., and cleanses the liver from these harmful chemicals. It was one of the ten best selling herbal dietary supplements in US in 2005.[8]

B. Chemistry

The active extract of *S. marianum*, known as silymarin, is a mixture of flavanolignans (Fig. 2) namely; silibinin (**1**), silydianin (**2**), and silychristine (**3**).[9] Although, the whole plant is used as medicinal, but seeds contain the highest content of silymarin (1.5–3.0%). Most of its hepatoprotective properties are attributed to silybin (silibinin), which is the main constituent (60–70%) of silymarin.[10] Silymarin
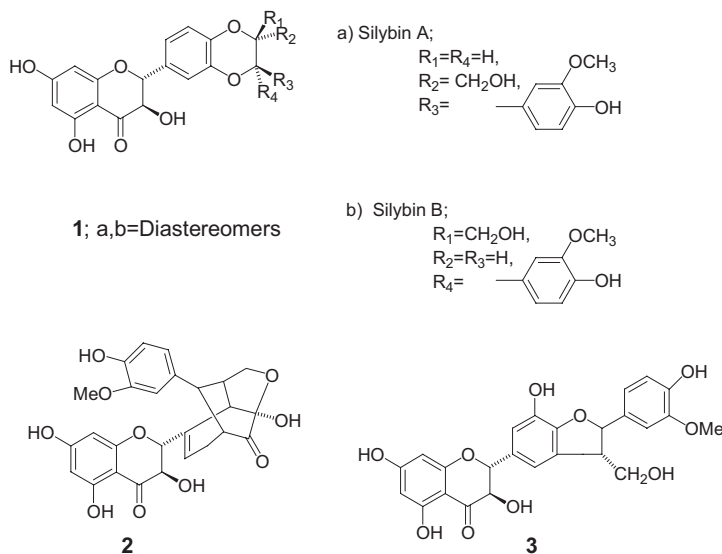
Figure 2. Structures of the components of silymarin: silybin (**1**), silydianin (**2**), and silychristin (**3**).

comprises at least 70% of standardized milk thistle. It can be extracted with aqueous alcohol (95%) as a bright yellow rich fraction. A hydro extraction technique was also developed to extract silymarin from milk thistle.[11] The silymarin content may vary in milk thistle extracts from 40 to 80%.[12] Various HPLC,[13] and LC-MS[14] methods have been developed to determine these constituents in the extracts.

C. Biological Activity

Silibinin is the most active constituent in silymarin mixture. It showed antihepatotoxic activity against *Amanita phalloides*,[15] ethanol, paracetamol (acetaminophen) and carbon tetrachloride induced liver injury.[16] It also produced hepatoprotective effects in acute viral hepatitis, alcohol related liver cirrhosis at doses ranging from 280 to 800 mg/day. Pharmacokinetic studies showed that silymarin is absorbed by the alimentary tract into blood.[17] The peak plasma concentrations in blood streams are reached after 2 hr and the elimination $t_{1/2}$ is 6 hr.[18,19] Approximately 3–8% of silymarin is excreted in urine whereas it can be recovered (20–40%) as glucuronides and sulfate conjugates from bile.[20,21] Double blind studies on human indicated that in acute viral hepatitis, silymarin therapy decreases complications[22–24] and recovers fast by developing immunity in a shorter interval. Similar studies conducted to assess the antihepatotoxic effects of silymarin in alcoholic liver disease revealed significant improvement in various parameters.[25]

D. Mode of Action

The mechanism of action of silymarin is not well understood. However, reports indicate that it acts in multiple ways.[26–28] Silymarin increases superoxide dismutase activity in erythrocytes and lympocytes thus showing antioxidant activity.[29,30] It stabilizes the membrane structure of hepatocytes and thus prevents toxins from entering the cell through enterohepatic recirculation. It promotes liver regeneration by stimulating nucleolar polymerase A and by increasing ribosomal protein synthesis. It also prevents depletion of glutathione in human hepatocyte cultures thus protecting cells from methotrexate and ethanol induced damage *in vitro*.[31] In a recent report, silymarin has been found to modify specifically the functions related to various transporters and receptors located in the cell membranes.[32] Overall, its mechanism of action for hepatoprotection appears from its antioxidant effect to scavenge free radicals and inhibit lipid peroxidation.[33] Its

hepatoprotective property against wider range of liver damage inducing agents makes it a unique drug amongst others in the race.[34]

E. Toxicity and Side Effects

Silymarin showed low level of toxicity with no mortality or any sign of adverse effects at oral doses of 20 g/kg in mice and 1 g/kg in dogs. After intravenous infusion its LD_{50} was 400 mg/kg in mice, 385 mg/kg in rats and 140 mg/kg in rabbits and dogs.[35] Although, silymarin showed a good safety record, but there are few reports of associated occurrence of gastrointestinal disturbances and allergic skin rashes.[36,37] These data demonstrate that the acute, subacute, and chronic toxicity of silymarin is very low.

F. Future Prospects

Silymarin is one of the most successful examples of developing a modern drug from traditional information. However, standardization of silymarin is still lacking in its various formulations and effective dosages. In spite of its popularity as herbal hepatoprotective drug, medical practitioners are not fully confident on its efficacy and safety parameters. Well-designed double blind placebo-controlled studies for specific liver disorders are necessarily required.

3. ANDROGRAPHOLIDE AND NEOANDROGRAPHOLIDE

A. Introduction

Andrographolide (**4**) and neoandrographolide (**5**) are obtained from *Andrographis paniculata* Nees (Family: Acanthaceae), a well known plant for liver diseases.[38] The plant is basically originated from south-east Asia and commonly called; Chuan xin lian in China, Kalmegh and Bhunimba in India, Hempedubumi in Malaysia, etc. The plant is also known as ''king of bitters'' due to its bitterness. The hepatoprotective activity of andrographolide is well established[39−41] and other constituents (Fig. 3) of the plant like neoandrographolide (**5**), andrographoside (**6**), and andrograpanin (**7**) also showed significant activity against various types of liver damages.

B. Chemistry

Bioguided phytochemical investigation of *A. paniculata* yielded andrographolide as the main hepatoprotective principle.[42] Chemically, andrographolide (**4**) is a labdane diterpene lactone named as, 3-[2-{decahydro-6-hydroxy-5-(hydroxymethyl)-5,8α-dimethyl-2-methylene-1-naphthalenyl} ethylidene]dihydro-4-hydroxy, 2(3H)-furanone. It is present in all parts of the plant and maximum in leaves (over 2%). Its structure and stereochemistry were fully established.[43,44] Various techniques have been developed to separate and determine andrographolide in the plant extract.[45−49]

C. Biological Activity

Methanolic extract of *A. paniculata* showed 32% recovery in CCl_4 induced liver damage in rats.[50] Andrographolide exhibited protective effects comparable to that of silymarin against liver damage in rats induced by carbon tetrachloride, paracetamol, galactosamine and *t*-butylhydroperoxide.[39−41] The protective effect of the leaf extract against carbon tetrachloride induced hepatotoxicity was found more significant than that of andrographolide.[51] Whereas andrographolide was found more potent than silymarin against paracetamol induced damage of hepatocytes. It normalizes the elevated levels of certain enzymes (GOT, GPT, and alkaline phosphatase) induced by paracetamol in serum as well as in isolated hepatic cells.[52] Andrographolide and neoandrographolide had significant antihepatotoxic effect against *Plasmodium berghei* K173-induced hepatic damage of *Mastomys*
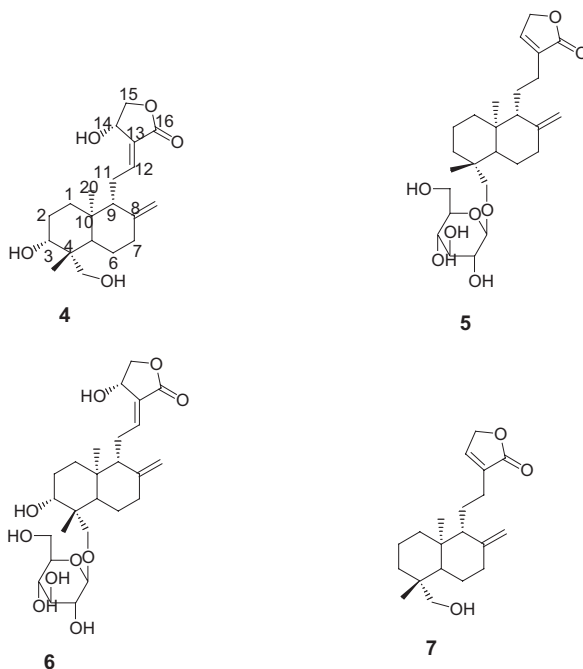
Figure 3. Structures of andrographolide (**4**), neoandrographolide (**5**), andrographoside (**6**), and andrograpanin (**7**).

natalensis.[53] Andrographolide has shown choleretic activity in the rats and guinea pigs, stimulating bile production.[54]

Andrographolides are distributed throughout the viscera when consumed orally and 90% is excreted within 48 hr of consumption. Four main metabolites (Fig. 4) of andrographolide as sulphonates (M-1, M-2, M-3, and M-4) were isolated from rat urine and feces after 48 hr of oral administration at room temperature.[55]

D. Mode of Action

Andrographolide, andrographoside, and neoandrographolide protect liver against the hepatotoxins by reducing the levels of the lipid oxidation product, malondialdehyde (MDA), and by maintaining high levels of the reduced form of glutathione (GSH).[56] The lowering of MDA formation revealed the free radical scavenging properties of diterpene lactones. On the other hand, neoandrographolide has
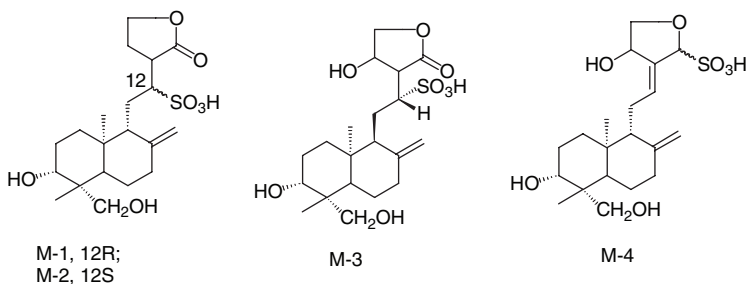

Figure 4. Metabolites of andrographolide.

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been reported to possess antiradical mechanism, scavenging free radicals by donating the allylic hydrogen atoms of the α/β unsaturated lactone either by homolytic cleavage or by deprotonation-oxidation mechanism (Fig. 5).[57]

E. Toxicity and Side Effects

Andrographolide and neoandrographolide were found to be safe. But, their oral administration may cause poor appetite and sometimes vomiting, due to its extreme bitter taste.[58] Extended oral administration of these compounds on rats and rabbits at 1 g/kg dosage for a week did not produce any significant changes in the body weight, blood chemistry, hepatic and kidney functions. The LD_{50} for andrographolide and neoandrographolide was found to be more than 40 and 20 g/kg in oral doses in mice.[59]

F. Future Prospects

These diterpenoid lactones have shown potent hepatoprotective activity against various kinds of liver disorders mainly as antidiarrhoeal agents. However, their hepatoprotective activity against viral hepatitis is questionable. Extensive research attempts possibly through double blind clinical trials are required to establish its potency. Extremely high bitterness is another problem associated with this drug and efforts to suppress or hide this property might recommend it for oral administration.
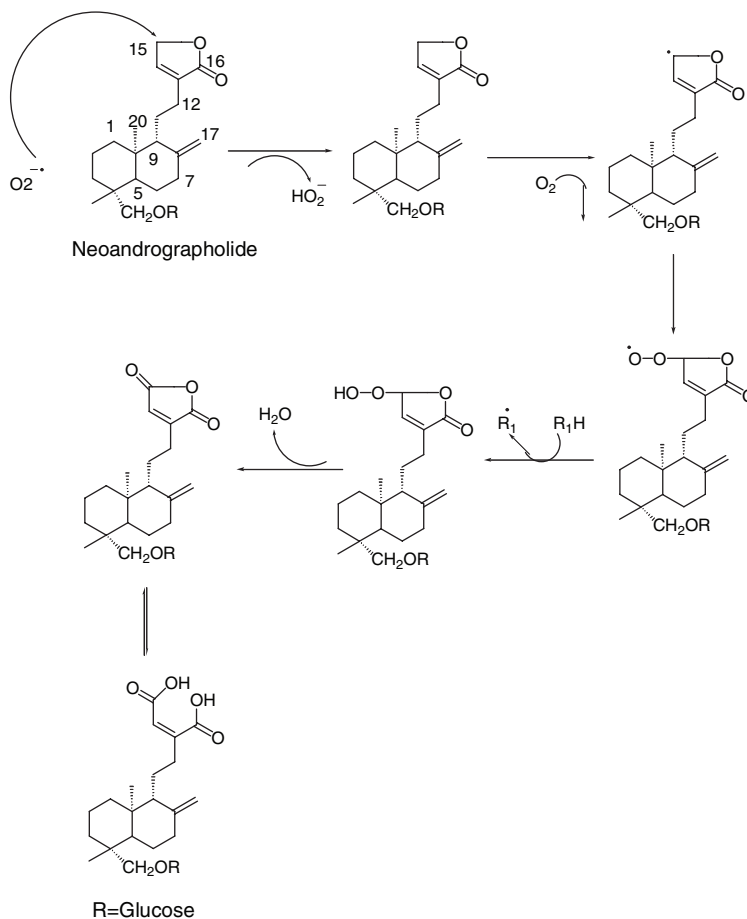

Figure 5. Proposed reaction mechanism between neoandrographolide and superoxide.

4. CURCUMIN

A. Introduction

Curcumin (**8**) is a main component of rhizomes of ancient spice, turmeric (*Curcuma* spp. Family: Zingiberaceae). The genus *Curcuma* consists of hundreds of species that possess rhizomes and underground root like stems. Turmeric is grown in warm and rainy regions of the world such as China, India, Indonesia, Jamaica, and Peru. Apart from culinary use, turmeric has been used in traditional medicine for the treatment of jaundice and other disorders of liver, parasitic infections, ulcers, inflammation of joints, various skin diseases, etc.

B. Chemistry

Curcuminoids are a mixture of several structurally close phenolic compounds present in the rhizomes of turmeric (approximately 3–5% w/w). Three curcuminoids of major occurrence are curcumin (60–80%), demethoxycurcumin (10–20%), and bisdemethoxycurcumin (5–10%).[60] Chemically, curcumin is a diferuloylmethane having a diferulic acid moiety fused with another carbon atom or methylene moiety. Thus, it has a methylene-1,3-diketo group showing keto-enol tautomerism due to stabilization by hydrogen bonding. Curcumin exists mainly in keto-enol form rather than in a diketo form (Fig. 6).[61]

Turmeric got much attention due to its varied medicinal properties, and thus, various techniques like SFC, Microwave assisted, hydrotropy based, high speed countercurrent chromatography, etc., have been developed to extract and isolate curcumin.[62–66] Although, numerous methods are available to isolate curcumin, its purification is still time consuming and hence, mostly it is available in the market as a mixture of three main curcuminoids (**8**, **9**, and **10**, Fig. 7).

Curcuminoids are highly conjugated phenolic compounds which show a strong UV absorbance between 420 and 430 nm.[67] Several HPLC methods have been developed to determine the curcumin content in the curcuminoids rich fraction.[68–70] Hepatoprotective activity of curcuminoids were also determined by bioactivity guided fractionation of ethyl acetate soluble fraction of rhizomes of *C. longa*.[60]

C. Biological Activity

The extracts of *C. longa* rhizomes exhibited protective activity against CCl$_4$-induced liver injury *in vivo* and *in vitro*.[71] Curcumin has a very good antioxidant activity. Most of its biological activities are considered due to this only. It inhibits lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates. A few are described below:

(i) Curcumin has been found to have protective effects on CCl$_4$ induced hepatic cytochrome P450 (CYP) damage in rats.[72] The CYP isozyme inactivation in rat liver caused by CCl$_4$ was inhibited by curcumin. Treatment with curcumin on fibrotic rats, after hepatic damage, showed significant improvement as well as restoration of lipid profile, marker enzymes, and thiobarbituric acid reactive substances to normal.[73]
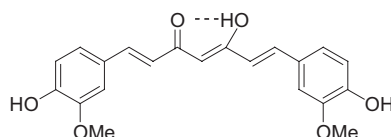

Figure 6. Keto-enol tautomerism in Curcumin (**8**).

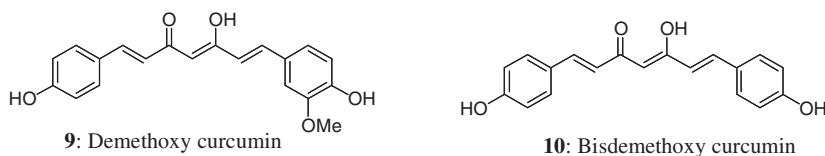**9**: Demethoxy curcumin   **10**: Bisdemethoxy curcumin

Figure 7. Structures of other two curcuminoids found in *C. longa*.

(ii) The hepatotoxic effects of ethanol are attributed to generation of hydroxyethyl radicals, which induce lipid peroxidation.[74] The polyunsaturated fatty acids of cellular membranes are particularly susceptible to this oxidative attack leading to membrane lesions and loss of cellular homeostasis. Lipid peroxidation in liver slices was found to be doubled in presence of ethanol and curcumin reduced the condition significantly. Naik et al.[75] clearly pointed out that curcumin mitigates the ethanol induced liver cell damage by decreasing lipid peroxidation. Presumably, curcumin functions as an antioxidant to scavenge free radicals. Since curcumin also reduces H_2O_2 induced renal cell injury[76] it seems to serve as an antioxidant in cells in general.

Also, the hepatoprotective activity against alcohol induced toxicity was assessed by monitoring the changes in the serum enzyme levels of aspartate transminase (AST) and alkaline phosphatase.[77,78] The levels of serum lipids and thiobarbituric acid reactive substances (TBARS) in alcoholic rats were also assessed (Table I). It was found that administration of curcumin reduced the levels of serum lipids and TBARS due to either scavenging of peroxides and other activated oxygen species or neutralization of the free radicals.[79] Curcumin also showed antihepatotoxic activity against paracetamol induced liver toxicity in rats.

Curcumin is poorly absorbed from intestine after oral administration. It was shown that oral consumption of curcumin in rats resulted in approximately 75% being excreted in the feces and only traces appeared in the urine.[80] Curcumin is biotransformed into dihydrocurcumin and

Table I. Activities of Serum AST, ALP and Levels of Serum Cholesterol, Phospholipids, Free Fatty Acids, and TBARS in Control, Alcohol and Alcohol + Curcumin Treated Rats

Parameter	Group I Control (Saline 9.875g/kg)	GroupIIa 25% Aqueous alcohol (9.875g/kg)	Group IIb 25% Aqueous alcohol +curcumin (80mg/kg)
AST (U/L)	8.2±1.6	57.8±4.11	35.4±1.85
ALP (U/L)	123.6±5.31	185±5.21	141.4±2.8
Cholesterol (mg/100 mL of serum)	101±10.04	183.33±8.29	145.33±7.45
Phospholipids (mg/100 mL of serum)	98.12±10.87	163.52±8.656	134.15±7.45
Free fatty acids (mg/100 mL of serum)	82.38±5.4	169.44±8.48	123.23±7.61
TBARS (nmol/ mL of serum)	1.336±0.206	3.428±0.371	2.06±0.06
Groups IIa and IIb compared with group I $p<0.001$. Group IIa compared with group IIb $p<0.001$.			

tetrahydrocurcumin, which are further converted to monoglucuronide conjugates[81] to tetrahydrocurcumin and hexahydrocurcumin in human and rodents (Fig. 8).[82]

D. Mode of Action

It is believed that the hepatoprotective activity of curcumin is due to its antioxidant activity which is comparable to vitamins C and E.[83,84] Curcumin was demonstrated as a potent scavenger of a variety of reactive oxygen species including superoxide anion radicals, hydroxy radicals,[85] nitrogen dioxide radicals[86], singlet oxygen, etc.[87] Curcumin exhibited potent inhibitory activity against P450 in rat liver.[88] One of its metabolites, tetrahydrocurcumin was found to have better protective effect when compared with silymarin.[89] The hydroxyl and the methoxyl groups of phenyl ring and the 1,3-diketo systems are important structural features to contribute to these effects. Further, the antioxidant activity increases when the phenolic hydroxyl is at ortho to the methoxyl group. Based on bond dissociation enthalpies using density function theory (DFT), it has been understood that the antioxidant mechanism of curcumin is due to hydrogen atom abstraction from the phenolic group and not from the central methylene group in the heptadienone link.[90]
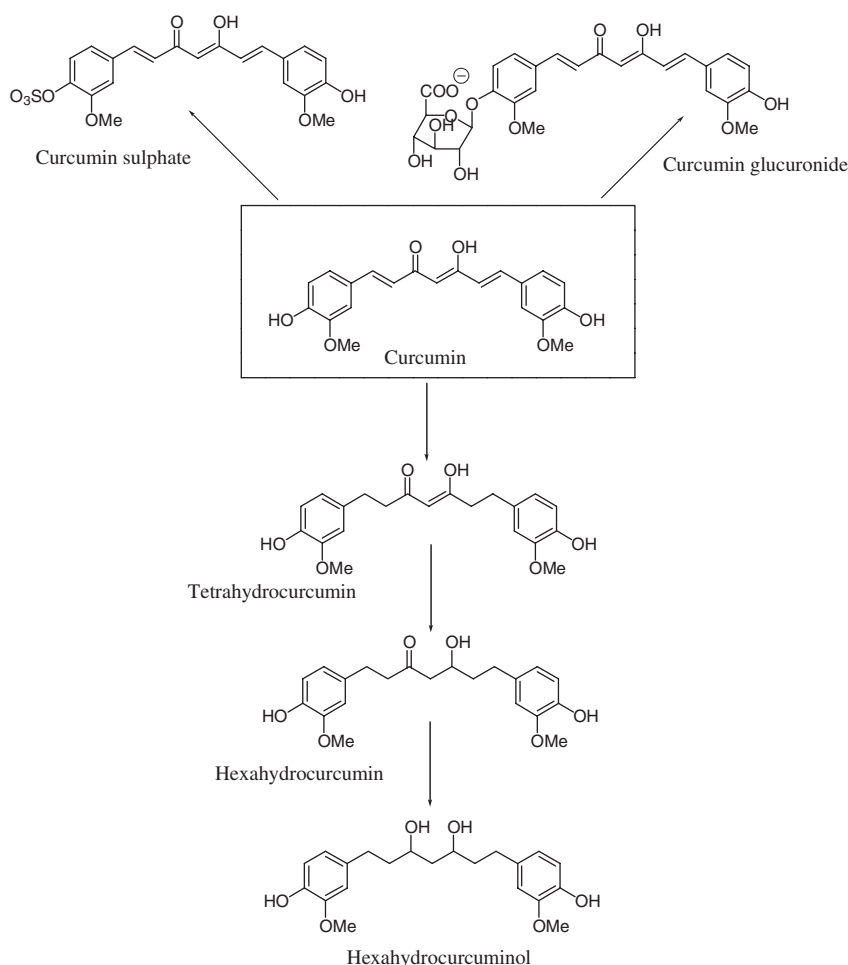

Figure 8. Major metabolites of curcumin in rodents and humans.

E. Toxicity and Side Effects

Curcumin is being consumed all over the world for centuries and no toxicity is reported so far.[91] Curcumin was found harmless up to a dose of 2 g/kg with no mortality. The clastogenic potential of *C. longa* in experimental rats in *in vivo* condition has been evaluated. A single acute dose of 500 mg/kg body weight could not induce micronucleated polychromic erythrocytes but caused higher chromosomal aberrations.

F. Future Prospects

Curcumin is one of the most commonly used indigenous molecules. Wide ranges of medicinal properties have proved its use not only in kitchens, but also in various health protective activities. Curcumin has very poor bioavailability, which reduces its efficacy. Curcumin is sensitive even at mild temperatures and light hence, unstable. Therefore, attempts to enhance the bioavailability and self-life need to be explored.

5. PICROSIDE AND KUTKOSIDE

A. Introduction

Picroside (**11**) and kutkoside (**12**) are active constituents of roots and rhizomes of *Picrorrhiza kurroa* Royle (Family: Scrophulariaceae), commonly known as "Kutki" or "Kutaki." *P. kurroa* is a low, hairy herb with a perennial woody rhizome. It is endemic to the Himalayan region and grows from Kashmir to Sikkim at an altitude of 3,000–5,000 m. A bitter extract obtained from the rhizomes has been widely used in traditional medicine for the treatment of liver diseases.[92] "Picroliv," a combined formulation of picroside I and kutkoside has been developed as a potent hepatoprotective drug.[93,94]

B. Chemistry

Chemically, these are iridoid glycosides with a common unit known as "catalpol." Picroside I is established as 6′-*O*-cinnamoylcatalpol, while kutkoside is 10-vanilloylcatalpol (Fig. 9).[95,96] The plant is extracted in alcohol, preferably by cold percolation, and further partitioning yields most of the active components in ethylacetate and butanol fractions respectively. Several analytical methods have been developed for the quantitative determination of picroside and kutkoside by TLC,[97] RP-HPLC,[98] LC-MS/MS[99] methods, etc. Picroliv is an enriched iridoid glycoside fraction containing at least 60% of 1:1.5 mixture (w/w) of picroside I, kutkoside and the remainder (40%) being a mixture of iridoid and cucurbitacin glycosides. Systematic bioassay guided fractionation of ethanolic extract of *P. kurroa* showed "Picroliv" as a potential hepatoprotective agent.
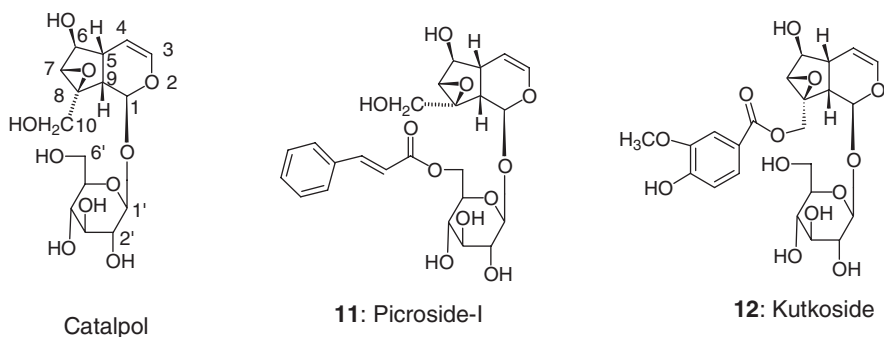

Figure 9. Structures of catalpol (basic unit), picroside I and kutkoside.

C. Biological Activity

Different *in-vivo* studies were done on animal models with hepatic damage induced by various agents to establish the hepatoprotective activity of picroliv. It showed potent dose-dependent (3–12 mg/kg p.o. for 1–2 weeks) activity by significant reversal in the serum and tissue biochemical parameters including histopathology. In these studies picroliv was found to be more active than silymarin.[100] The hepatoprotective activity data of picroliv has been depicted in Table II.

The extract of *P. kurroa* showed hepatoprotective activity when given to patients suffering from jaundice.[114] Picroliv showed curative *in-vitro* activity in primary cultured rat hepatocytes against toxicity induced by thioacetamide, galactosamine, and CCl_4.[103] It resulted in a concentration-dependent restoration of altered viability and biochemical parameters. Picroliv showed dose-dependent choleretic effects in conscious rats and anaesthetized guinea pigs and cats.[109] Picroliv was found potent against viral hepatitis by showing a promising anti-HBsAg-like effect. It is also able to lower serum lipids (total VLDL and LDL cholesterol), triglycerides and phospolipids, in both normal and hyperlipidemic animals.[115] Picroliv has also showed a potent inhibition of hepatocarcinogenesis.[116]

D. Mode of Action

Picroliv antagonizes paracetamol-induced lowering in LDL receptor cell surface expression and increases conjugated dienes in hepatocytes.[117] Its antioxidant effect has been shown to be similar to that of superoxide dismutase,[118] metal-ion chelators, and xanthine oxidase inhibitors. Picroliv restored depleted glutathione levels in rats infected with *P. berghei*, thereby enhancing detoxification and antioxidation. Thus, picroliv maintains a normal oxidation-reduction balance, glutathione metabolism and reduce the increased levels of lipid peroxidation products in the liver.[119] Like silymarin, it showed liver regeneration in rats, possibly via stimulation of nucleic acid and protein synthesis.[120,121] Thus, its hepatoprotective effect appears to result from a combination of membrane-stabilizing, hypolipidemic and antioxidant properties. These properties may also be responsible for the effects on the immune system.

E. Toxicity and Side Effects

Picroliv showed LD_{50} value 2026.9 mg/Kg when administered by the peritoneal route in mice. No mortality was found up to 2.5 g/kg dose in mice or rats through oral route. Long-term toxicity studies

Table II. Hepatoprotective Activity of Picroliv

Toxin	Dosage/kg	%Histopathological Changes	Picroliv dose (mg/kg p.o.)Xdays	% protection with picroliv	Reference
Paracetamol	2g p.o.	35-100	12x7	50-100	101, 102
Carbon tetrachloride	0.7mlx6i.p.	35-120	12x15	70-100	103, 104
Thioacetamide	100mg s.c.	35-170	25x7	50-90	105-107
d-Galactosamine	80mg i.p.	35-890	12x7	50-100	108, 109
Ethyl alcohol	20 ml (40%)x21 p.o.	30-180	6x7	60-90	110-112
Rifampicin	50mgx5 i.p.	15-60	12x6	45-100	113

done on histopathological parameters in rats showed picroliv was non-toxic.[122] A similar experiment done on adult rhesus monkeys showed no abnormality in food intake, daily activities, body weight, hematology, and blood biochemistry.

F. Future Prospects

Picroliv was found to be a safe drug with no reported side effects.[123] Picroliv has successfully completed phase I and phase II clinical trials and waiting for phase III clearance. Being a potent hepatoprotective, this may emerge as a potent hepatoprotective drug in a near future.

6. PHYLLANTHIN AND HYPOPHYLLANTHIN

A. Introduction

Phyllanthin (**13**) and hypophyllanthin (**14**) are potent hepatoprotective lignans found in *Phyllanthus niruri* Linn. (Family: Euphorbiacea). The genus includes more than 600 species of shrubs, trees, and annual/biennial herbs distributed throughout the globe, many of which are used medicinally in different countries such as *P. emblica* L., *P. urinaria* L., *P. reticulates* in Indo-China, *P. niruri* in Brazil and West Indies, *P. elegans* Wall, *P. urinaria* in the Philippines. The plant is commonly known as "Bhuiamliki" in India and "Look Tai Bai" in Thailand. *P. niruri* is a small, erect, annual herb that grows 30–60 cm in height mainly as a weed in both cultivated fields and wastelands. It is a well-known Ayurvedic plant used in folk remedy for jaundice and other liver disorders.

B. Chemistry

Chemically, both phyllanthin (**13**) and hypophyllanthin (**14**) are lignans (Fig. 10).[124,125] Phyllanthin is linked through C8–C8′ of phenyl propanoid units, while hypophyllanthin is additionally linked through C2–C7′ to make a tetrahydronaphthalene ring system. The stereochemistry of phyllanthin was established as 8(S), 8′(R).[126] Phyllanthin and hypophyllanthin have been isolated from the hexane extracts of *P. niruri*.

A complete spectral studies[127] including single crystal X-ray analysis[128] have left no doubt on the full 3-dimensional structural characterization of phyllanthin and hypophyllanthin. Being important markers of the medicinal plant, several HPTLC methods have also been developed for quantitative estimation of these molecules.[129]

C. Biological Activity

The plant has been effective against infective hepatitis[130,131] and other disorders of liver.[132–134] The hexane fraction of the ethanolic extract showed potent hepatoprotective activity. Its liver protective effects have been established by various *in vitro* and *in vivo* experiments in rats and mice. Human
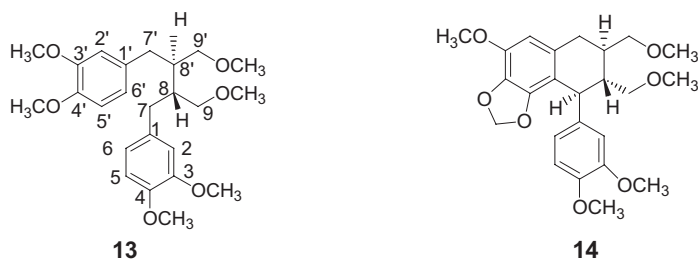

Figure 10. Structures of phyllanthin (**13**) and hypophyllanthin (**14**).

studies also showed its liver protective and detoxifying actions in children with hepatitis and jaundice. In India, it is used as a single drug in the treatment of jaundice in children,[135] and British researchers showed that children treated with *Phyllanthus* extract for acute hepatitis could return the liver function to normal within 5 days. Also, Chinese researchers found its liver protective actions in adults affected with chronic hepatitis.[136,137] Both phyllanthin and hypophyllanthin protect liver against carbon tetrachloride and galactosamine-induced cytotoxicity in primary cultured rat hepatocytes.[138] These lignans also protect liver damage induced by alcohol, and normalized a "fatty liver" condition.

D. Mode of Action

The liver protective effect of phyllanthus extract was due to free radical scavenging activity.[139] It can scavenge superoxides and hydroxyl radicals and hence, inhibit lipid peroxidation.[140] Phyllanthin was reported to exhibit antigenotoxic properties.[141]

E. Toxicity and Side Effects

There are very few published reports regarding the toxicity of phyllanthin and hypophyllanthin. However in a recent report[142] rats fed with the aqueous leaf extract of *P. amarus* showed toxic effects on the hematological and serum biochemical parameters like decrease in RBC count, packed cell volume, Hb concentration and increase in WBC count apart from other effects on liver, testis, kidney, and weight loss. Hence, it was recommended that extreme caution should be exercised in the use of this plant.

F. Future Prospects

Recently, *Phyllanthus* species has gained interest considerably due to good therapeutic potential for many diseases. Extensive phytochemical, pharmacological and clinical studies have been done to establish it as a hepatoprotective agent. However, there are many aspects, which need to be explored like well-controlled double blind clinical trials using large sample size (large number of patients) for their (phyllanthin and hypophyllanthin) efficacy and toxicity, a complete analysis of mode of action, etc.

7. GLYCYRRHIZIN

A. Introduction

Glycyrrhizin (**15**), is a major and active constituent of roots of *Glycyrrhiza glabra* (Family: Leguminacae) commonly known as Indian licorice. It is a most commonly used herb in the traditional medicine system of India, China and other countries. Licorice is an under shrub, usually of 2 m height, erect, perennial plant with light, gracefully spreading pinnate foliage and dark green lanceolate leaflets that hang down at night with violet to lavender color flower. Licorice is used for flavoring, sweetening candies and medical remedies. Licorice is a very sweet (30–50 times as potent as table sugar) herb that detoxifies and protects liver.[143]

B. Chemistry

Chemically, glycyrrhizin (Fig. 11) is a triterpenoid saponin named as (3β, 20β)-20-carboxy-11-oxo-30-norolean-12-en-3-yl-2-*O*-β-D glucopyranosyl-α-D-glucopyranoiduronic acid (**15**). Standardization of licorice is done based on glycyrrhizin content. Enzymatic hydrolysis of glycyrrhizin using glucuronidase yields glycyrrhetinic acid as an aglycone.
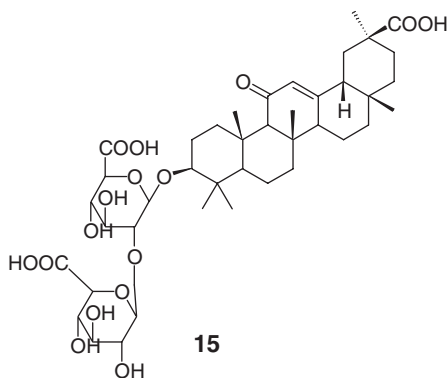
Figure 11. Structure of glycyrrhizin (**15**).

The roots are extracted in boiling water and on cooling glycyrrhizin get separated into solid compound. It is found up to 4% in the roots. Several analytical methods have been developed to determine glycyrrhizin by HPLC[144,145] and HPTLC.[146]

C. Biological Activity

Glycyrrhizin prevents several forms of experimental liver injury in animals.[147] It has shown hepatoprotective activity in animal models against carbon tetrachloride induced toxicity and hepatitis.[148] One of Japanese formulations of glycyrrhizin, known as stronger neominophagen C (SNMC), combined with, 0.1% cysteine, and 2% glycine has been used for the treatment of chronic liver diseases. Different experiments conducted on SNMC showed its efficacy in the treatment of subacute hepatic failure, chronic hepatitis C,[149] and cirrhosis. However, the effect of glycyrrhizin against the chronic hepatitis B was found to be very poor.[150]

Intravenously administered glycyrrhizin is metabolized in the liver by lysosomal β-D-glucuronidase into 3-mono-glucuronide glycyrrhetinic acid and then excreted with bile into the intestine. It is further metabolized by intestinal bacteria into glycyrrhetinic acid, which can be reabsorbed here (Fig. 12).[151]
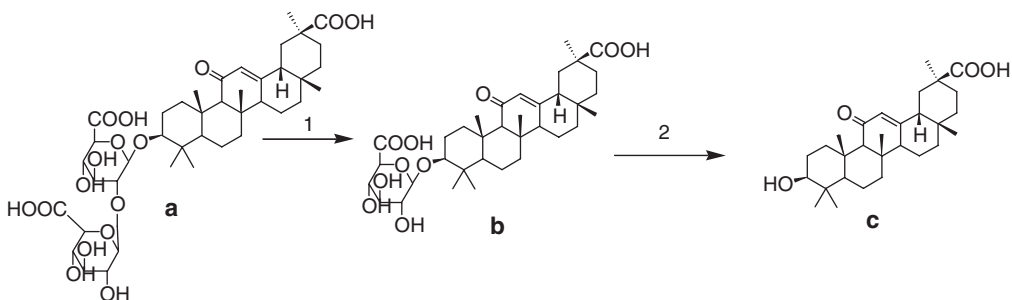

Figure 12. Metabolism of glycyrrhizin after intravenous administration: (1) by lysosomal β-D-glucuronidase in the liver, and (2) by bacteria β-D-glucuronidase in the intestine. **a**. Glycyrrhizin (**b**) 18β-glycyrrhetinic acid mono-β-D-glucuronide (**c**) 18β-glycyrrhetinic acid.

D. Mode of Action

The hepatoprotective activity of glycyrrhizin has been attributed to its lipid peroxidation inhibitory, antioxidant, antiinflammatory, and immunomodulatory activities.[152] Glycyrrhizin enhances hepatic glucuronidation and activates P450 phase I detoxification reactions in animals. Clinical trials conducted on patients with chronic hepatitis showed a decrease in the serum transaminase levels thus, decreasing the chance of developing hepatocellular carcinoma.

E. Toxicity and Side Effects

Consumption of large quantity of glycyrrhizin may cause high blood pressure, salt and water retention, and low potassium levels, which could lead to cardiac problems. Administration of licorice with diuretics or especially with potassium lowering drugs may through potassium into dangerous low levels. In some cases, over-consumption also leads to hormonal disbalances. For pregnant women, it can lead to a risk of preterm labor. LD_{50} for glycyrrhizin in rats was found at 1.94 g/Kg.

F. Future Prospects

Glycyrrhizin has been found more effective in viral hepatitis C. The formulation, stronger neominophagen C (SNMC) is available in market, but treatment with SNMC has inherent side effects like hypertension, sodium and fluid retention and hypokalemia.[153,154] Hence, a better improved formulation and synthesis of milder derivatives of glycyrrhizin is much needed today.

8. SOME RECENT LEADS

There have been several reports regarding various other hepatoprotective agents.

A. Cliv-92

Cliv-92 is presently emerging as a potent hepatoprotective agent[155] isolated from the seeds of *Cleome viscosa* Linne (Family: Capparidaceae). Basically, it is a mixture of three structurally similar coumarinolignoids (Fig. 13), Cleomiscosins A (**16**), B (**17**), and C (**18**)[156,157] and of which **17** is reported to be the most potent one.[153] Cliv-92 was potent against carbon tetrachloride and phalloidin induced liver damage in rats. Its hepatoprotective activity was found to be comparable to silymarin.[158]

B. Oleanolic Acid

Oleanolic acid (**19**, Fig. 14), a triterpenic acid found in weed *Lantana camara* Linn. (Family: Verbenaceae), a native to tropical regions. The plant is commonly known as "Kew bug," "lantana,"
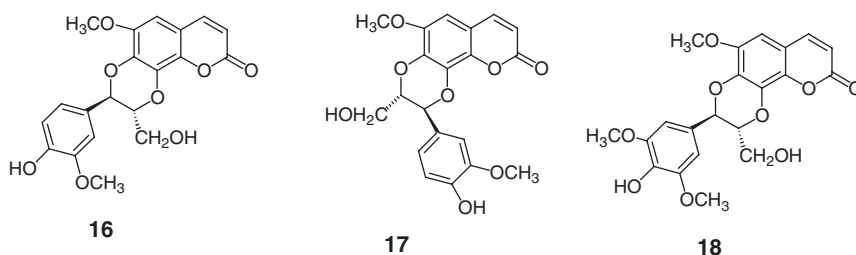

Figure 13. Three main components of Cliv-92: Cleomiscosins A (**16**), B (**17**), and C (**18**).
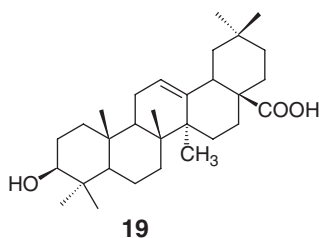
Figure 14. Structure of oleanolic acid (**19**).

"cherry pie," "Tick berry," etc., in different regions. Oleanolic acid has also been reported from several other plants like *Syzygium aromaticum* L., *Ocimum basilicum* L., *Salvia triloba* L., etc. It has been found effective at inhibiting carbon tertrachloride induced liver injury.[159] Its effect is associated with the inhibition of carbon tertrachloride biotransformation by the reduced expression of P450 2E1.[160]

C. Ursolic Acid

Ursolic acid (**20**, Fig. 15) is a common triterpenic acid found in the leaves of *Eucalytus tereticornis*, *Salvia triloba*, *Vinca minor*, *Ocimum basilicum*, etc. It has been reported to have hepatoprotective activity against carbon tetrachloride, ethanol, thiacetamide, and galactosamine damaged liver in rats. Its hepatoprotective action was found to be comparable with silymarin.[159–161]
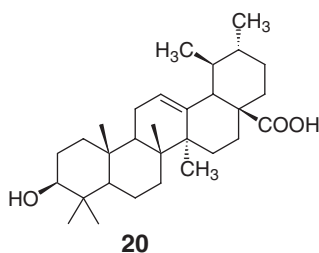
Figure 15. Structure of ursolic acid (**20**).

D. Berberine

Berberine (**21**, Fig. 16) is an isoquinoline alkaloid obtained from the roots, rhizomes and stem bark of *Berberis aristata* DC (Family: Berberidaceae), commonly known as "barberry."[162] The oxidative damage induced in the hepatocytes by *tert*-butyl hydroperoxide (*t*-BHP) was inhibited by berberine probably due to its antioxidant potential.[163,164] In another study, the hepatoprotection activity is also believed to stem from its inhibitory effects on the ion channels of potassium and calcium in the rat hepatocytes.[165]

The other recent leads which are reported as emerging hepatoprotective agents against various hepatotoxins have been described in Table III.

Figure 16. Structure of berberine (**21**).

Table III. Some More Hepatoprotective Leads and Their Activity Against Hepatotoxins

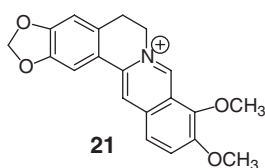| S. N o.	Name of the lead molecule	Basic structure	Plant origin	Hepatoprotective activity	Experimental animal	Reference
1	Schisandrin B	dibenzocyclooctadiene derivative	*Schisandra chinensis*	CCl_4 and drug induced	mice & rats	[166]
2	Kahweol and Cafestol	diterpenes	*Coffea arabica, C. robustica.*	CCl_4	mice	[167]
3	Quercetin	flavonoid	*Oenothera biennis, Podophyllum spp.* etc.	ethanol induced	human hepatocytes	[168]
4	Lupeol	pentacyclic triterpene	*Crataeva nurvala*	aflatoxin B_1	rats	[169]
5	Rubiadin	anthraquinone derivative	*Rubia cordifolia*	against CCl_4	rats	[170]
6	Caffeic acid	phenolic acid	*Ipomoea purga, Ocimum basilicum* etc.	against CCl_4 and paracetamol	rodents	[171]
7	Bergenin	C-glucoside of 4-*O*-methyl gallic acid	*Mallotus japonicus*	CCl_4 and D-galactosamine	primary cultured rat hepatocytes	[172]
8	Tiliroside	flavanol glycoside	*Magnolia fargesii*	D-galactosamine induced	mice	[173]
9	Kolaviron	biflavonoid	*Garcinia kola*	CCl_4	rats	[174]
10	Thymoquinon	benzoquinone	*Nigelle sativa*	t-butyl hydroperoxide and CCl_4	rat hepatocytes and mice	[175]
11	Bupleurosides III, VI, IX, & XIII	triterpenic saponins	*Bupleurum scorzonerifolium*	against D-galactosamine	primary cultured rat hepatocytes	[176]
12	Emodin	anthraquinone derivative	*Ventilago leiocarpa*	CCl_4 and D-galactosamine	rats	[177]
13	Myristicin	phenolic derivative	*Myristica fragrans*	lipopoly-saccharide and D-galactosamine	mice	[178]
14	Trans-Tetracos-15-enoic acid	unsaturated fatty acid	*Indigofera tinctoria*	CCl_4 and paracetamol	rats and mice	[179]

9. CONCLUSION

Herbs have recently attracted attention as health beneficial food and as source materials for drug development. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, with relatively little knowledge regarding their modes of action. During the last few years, the use of herbal supplements was increased from 2.5% to 12%. Natural compounds that reduce chemically activated enzymes, such as cytochrome P450 2E1, could be considered as good protective candidates against chemically induced toxicity for their role in the activation of many chemicals to comabat toxic and carcinogenic agents. There are several herbal preparations available based on these leads in the market. Many a times, these herbal extracts or preparations are used as complementary and alternative medicines (CAM) for liver diseases. Clinical trials to evaluate the hepatoprotective efficacy and toxicity of herbs are difficult due to heterogenous formulations and dosage, but these studies are possible in the case of pure compounds. Despite the tremendous advances made in medicine, so far no effective hepatoprotective agent is available in the market. With the revolution of the natural sciences and evidence-based medicine there is no doubt that herbal products contain chemically defined components that can protect the liver from various injuries. Although additive effects may be lost, the active molecules must be isolated and tested through well designed experiments and finally in randomized, placebo-controlled studies to enable rational clinical use of the agents. Thus, biologically active molecules derived from herbal extracts may serve as suitable primary compounds for effective and targeted hepatoprotective drugs (Table IV).

Table IV. Hepatoprotective Leads at a Glance

S.No.	Lead molecule	Basic skeleton	Mode of action	Potent action against
1.	Silymarin	flavanolignoids	antioxidant	alcoholic liver diseases, acute and chronic viral hepatitis, toxin induced liver diseases.
2.	Andrographolide, neoandrographolide	diterpenic lactones	free radical scavenging	paracetamol induced liver damage,
3.	Curcumin	phenolic	antioxidant	liver damage by alcohol and drugs
4.	Picroside, kutkoside	irridoid glycosides	free radical scavenging	liver damage by drugs and other toxins
5.	Phyllanthin, hypophyllanthin	lignans	free radical scavenging	chronic hepatitis B virus
6.	Glycyrrhizin	triterpenic glycoside	antioxidant/antiinflammatory	chronic hepatitis C

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Dr. Arvind Singh Negi *was born on January 1, 1970 in Uttarakhand (India). He did B.Sc. from Lucknow Christian Degree College in 1989 and did M.Sc. in Organic Chemistry from University of Lucknow in 1991. In February 1992, he joined at Central Drug Research Institute, Lucknow, India as a CSIR-Junior Research Fellow for Ph.D. program and awarded in 1999. In 1995, he joined Indian Grassland and Fodder Research Institute, Jhansi (IGFRI-ICAR) as Scientist and afterwards in 2000, he joined Central Institute of Medicinal and Aromatic Plants (CIMAP-CSIR), Lucknow, as Scientist-C. Presently as a Scientist E-I, he is working on structural modification of natural products for biologically active entities.*

Dr. Kotesh Kumar Jonnala *was born in 1969 in Warangal, India. In 1993 he obtained his MS degree in organic chemistry from Chanda Kanthaiah Memorial College (Kakatiya University, Warangal, India). He obtained his Ph.D. degree in natural product chemistry in 1999 from the Kakatiya University, Department of Chemistry, under the direction of the retired Professor Srinivasa Rao Poruri. In the year 2000, he joined Institute of Himalayan Bioresource Technology, a premier laboratory of Council of Scientific and Industrial Research (CSIR), Palampur, India. In 2004, he moved to Central Institute of Medicinal and Aromatic Plants (CSIR) at Lucknow, India, where he was promoted as In-charge NMR division. His research interest includes development of application of NMR experiments for structure elucidation of small and complex natural products, semi synthesis leading to value addition.*

Dr. Suaib Luqman *was born in 1975 at Allahabad (Uttar Pradesh), India. In 1995 and 1997, he obtained his B.Sc. (Biology) and M.Sc. (Biochemistry) degree from Ewing Christian College (An Autonomous College of University of Allahabad) and University of Allahabad, Allahabad respectively. He obtained his Ph.D. degree in Science (Biochemistry) in 2003 from the University of Allahabad under the supervision and guidance of Dr. Syed Ibrahim Rizvi. During his Ph.D. degree, he availed Fellowships of University Grants Commission (UGC) and Council of Scientific and Industrial Research (CSIR), New Delhi. In 2004, he joined the Genetic Resources and Biotechnology Division of Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, as a Scientist.*

His research interest includes bioprospection of molecules and genes, molecular targets including enzymes and proteins.

Dr. Karuna Shanker *was born in 1971 in Uttar Pradesh, India. In 1993 he obtained his M.Sc. in organic chemistry from Rohilkhand University, Bareilly. He did his Ph.D. in Chemistry from Dayalbagh Educational Institute (Deemed University), Dayalbagh, Agra. In 1999, he joined CCRAS, Department of AYUSH Government of India/CRI, Lucknow as a Research Officer. Thereafter, he joined Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, as a Scientist in 2004. His area of interest is plant drug research.*

Dr. Madan Mohan Gupta *was born on July 1, 1956. He did his B.Sc. from Gorakhpur University in 1974 and M.Sc. in Organic Chemistry from the same university in 1976. He did his Ph.D. from RML Avadh University Faizabad in 1982. He has been working on medicinal plants since 1977 after joining at Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow. His main research interest is in plant drug chemistry. Presently, he is Senior Scientist and Head, Analytical Chemistry Division at CIMAP, Lucknow.*

Dr. Suman Preet Singh Khanuja *was born on August 13, 1958. He did his B.Sc. (Hons) Agriculture from G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand) in 1980. He did his M. Sc. Agriculture in Genetics and Plant Breeding from the same university in 1982. Afterwards, he moved to Indian Agricultural Research Institute (IARI), New Delhi to pursue Ph.D. in Genetics, and awarded with a doctorate degree in 1987. He started his professional career as Scientist in the Biotechnology Centre at Indian Agricultural Research Institute (IARI), New Delhi in 1986. In April 1996, he joined Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, as Scientist E-II and Head Genetic Resources and Biotechnology Division. In May 2001, he became Director of the Institute. Presently, he is actively involved in the genetic research of medicinal and aromatic plants. His main area of interest is bioprospection of medicinal and aromatic plants for novel drugs and agrochemicals, variety development, DNA fingerprinting, Genomics and Plant Molecular Biology, etc.*