REVIEW ARTICLE

LEAD PHYTOMOLECULES FOR HEPATOPROTECTIVE DRUG DEVELOPMENT

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ABSTRACT

Plants are the precious gift of nature to mankind and play a major role in the treatment of various diseased conditons from the ancient times. Functional bioactive compounds of plant origin have been an invaluable source for many human therapeutic drugs and have played a major role in the treatment of diseases around the world. Natural products or their derivatives have led to many existing drugs, offering a chemically diverse space for discovery of hepatoprotective compounds. In order to represent the studies on chemical diversity of phytomolecules with hepatoprotective activity, this review is complied. This review captures a number of isolated phytomolecules having hepatoprotective potential. Phytomolecules as lead compounds for new drug discovery will boost up the researchers to work on it and find effective molecules for the treatment of liver injuries.

Keywords: Hepatoprotective activity, hepatoprotective phytomolecule, liver diseases, biochemical markers

ABBREVIATIONS

CCl_4- Carbon tetrachloride, LPO- Lipid peroxidation, TP- Total protein, ALT- Alanine transaminase, SGPT- Serum glutamic pyurate transaminase, AST- Aspartate transaminase, SGOT- Serum glutamic oxaloacetic transaminase, ALP- Alkanine phosphatase, CAT- Catalase, SOD- Superoxide dismutase, GSH-Px- Glutathione peroxide, TB- Total bilirubin, GSH- Glutathione, ALB- Albumin, MDA- Malondialdehyde, TGs- Triglycerides, γGGT-Gamma glutamyl transpeptidase, LDH- Lactate dehydrogenase, BID- bis in die (twice a day), TID- ter in die (three times in a day), QID- quarter in die (4 times in a day).

INTRODUCTION

Liver diseases (LDs) affect millions of people worldwide. LDs accounts for approximately 2 million deaths per year worldwide, 1 million due to complications of cirrhosis and 1 million due to viral hepatitis and hepatocellular carcinoma. Cirrhosis is currently the 11th most common cause of death globally and liver cancer is the 16th leading cause of death[1]. In contrast, with the improvement in living standards, the prevalence of metabolic LDs including non-alcoholic fatty LD and alcohol-related LD is set to rise, ultimately leading to more cases of end-stage LDs[2].

There are many different types of LDs like cirrhosis, liver cancer and liver failure that can threaten your life. Whether your liver is infected with a virus, injured by chemicals, or under attack from your own immune system, the basic danger is the same that your liver will not work to keep you alive. The main mechanism for the progression of chronic LDs, whatever the cause, is liver inflammation[3]. Hepatocyte necrosis is mainly the result of inflammation that is related to the immune response to target cells. Necroinflammation induces the progression of fibrosis to cirrhosis then hepatocellular carcinoma, causing morbidity and mortality[4]. So, there is urgent need to work on the prevention, diagnosis, appropriate management and treatment of chronic LDs. To reach this goal, research on phytomolecules as lead compounds is also needed.

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Plant derived natural products are relatively nontoxic and also better tolerated, hence they gained attention of modern drug discovery. Various herbal preparations with different philosophies and cultural origins are used by folk medicine practitioners to heal hepatic diseases. Alkaloids, flavonoids, phenolics, tannins, glycosides, gums, resins and oils are such phytochemicals present in root, leaf, flower, stem and bark of the plants and perform several pharmacological functions in human systems. In this review paper important information of phytomolecules such as their source, isolation method, class of compound, structure, and most importantly, hepatoprotective activity results are summarized.

Biochemical markers of liver: The First-Line tools for diagnosis

The prognosis of the chronic liver diseases can be determined by an assessment of the fibrosis stage, based on scores, blood tests, etc. Liver function tests (LFTs) are designed to give information about the state of a patient's liver. The parameters measured include prothrombin time and International normalized ratio (PT/INR), activated clotting time (ACT), albumin, billirubin (direct and indirect) and liver transaminases (AST/ALT-SGOT/SGPT) Several biochemical tests are beneficial in the assesment and management of patients with liver dysfunction. Normal values of different biochemical parameters of liver are given in Table I. Albumin levels are decreased in chronic liver disease, such as cirrhosis. ALT is also called serum glutamic pyruvate transaminase (SGPT) or alanine aminotransferase (ALAT), an enzyme present in cytosol of hepatocytes. When a liver cell is damaged, it leaks this enzyme into the blood, where it is measured. Elevated ALT levels are correlated with the grade of necroinflammation but not with the stage of fibrosis. Although ALT is not specific, it is an alarm, signalling the presence of liver disorders requiring an etiological and prognostic evaluation. Therefore, measurement of ALT should be part of routine blood testing, such as glycaemia or cholesterolaemia[5].

AST, also called serum glutamic oxaloacetic transaminase (SGOT) or aspartate aminotransferase (ASAT), is similar to ALT in that it is another enzyme related with parenchymal hepatocytes. Its level is increased in acute liver damage, but it is also present in RBCs, cardiac, skeletal muscles and is consequently not specific to the liver. The ratio of AST to ALT is sometimes beneficial in discriminating between causes of liver damage[6].

Table I: Normal values of liver biochemical parameters[6-8]

Biochemical parameter	Normal range
Albumin (Alb)	3.5-5.3 mg dL^{-1}
Alanine Transaminase (ALT)	7-56 IU L^{-1}
Aspartate Transaminase (AST)	5-40 IU L^{-1}
Alkaline Phosphatase (ALP)	30-120 IU L^{-1}
Total Bilirubin (TB)	0.2-1.2 mg dL^{-1}
Direct Bilirubin	0.1-0.4 mg dL^{-1}
Gamma Glutamyl Transpeptidase (GGT)	0-42 IU L^{-1}
Catalase (CAT)	107-111 MU L^{-1}

ALP is an enzyme in the cell lining of biliary duct of the liver. ALP concentration in plasma will rise with large bile duct blockage, intra-hepatic cholestasis or infiltrative diseases of the liver. Bilirubin is a breakdown of hemoglobin. Increased TB causes jaundice. A deficiency in bilirubin metabolism (e.g., reduced hepatocyte uptake, impaired conjugation of bilirubin, and reduced hepatocyte secretion of bilirubin) is the sign of cirrhosis and viral hepatitis. If direct bilirubin (conjugated bilirubin) is normal, then the problem is too much of unconjugated bilirubin, and the location of the problem is upstream of bilirubin excretion. Hemolysis, viral hepatitis, or cirrhosis can be suspected. If direct bilirubin is increased, then the liver is conjugating bilirubin normally, but is not able to excrete it. Bile duct blockage by gallstones or cancer should be suspected[7].

GGT is specific to the liver and a more sensitive marker for cholestatic damage than ALP. GGT may be elevated with even minor, sub-clinical levels of liver dysfunction. It can also be helpful in identifying the cause of an isolated elevation in ALP (GGT is raised in chronic alcohol toxicity). Deficiency and malfunctioning of CAT can causes various diseases or disorders like diabetes mellitus, cardiovascular disease, hypertension, anaemia and Alzheimer's disease[8].

Histological analysis of liver

Histopathological analysis after the treatment of toxicant is the important parameter for the evaluation of efficacy of test drug under consideration. The liver was sectioned and stained with azocarmine aniline blue (AZAN) dye and evaluated for the development of fibrosis

on the score of I-IV[9]. Histological evaluation parameters of liver are shown in Table II.

Table II: Histological evaluation parameters of liver[9]

Grading	Evaluation parameter
Grade 0	Normal liver histology
Grade I	Tiny and short septa of connective tissue without influence on the structure of hepatic lobules
Grade II	Large septa of connective tissue, penetrating into the parenchyma, tendency to develop nodules
Grade III	Loss of hepatic lobule structure by nodular transformation
Grade IV	Excessive formation and deposition of connective tissue with subdivision of the regenerating lobules and with development of scar

METHOD

In the present study, we have searched and reviewed relevant studies on isolation, characterization and hepatoprotective evaluation of phytomolecules through electronic searches of Pubmed, Science Direct, Wiley, Researchgate, Scopus, and Google Scholar between the years 1982 and 2020. The search includes, the indexing words like 'isolated biomolecule', 'hepatoprotective', 'extraction & isolation' and 'liver disease'. Based on the literature survey, isolated phytomolecules with hepatoprotective potential are discussed in this review.

Plants as indispensable sources for hepatoprotective phytochemicals

Traditional plants are a source of bioactive compounds with diverse scaffolds, well known to treat and manage LDs. But the endeavour for drug discovery from herbal medicines is "experience driven," the search for a therapeutically useful synthetic drug, like "looking for a needle in a haystack," is a daunting task. Herbs used in folk medicines constitute only a small portion of naturally occurring plants.

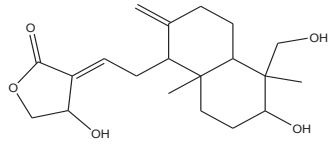
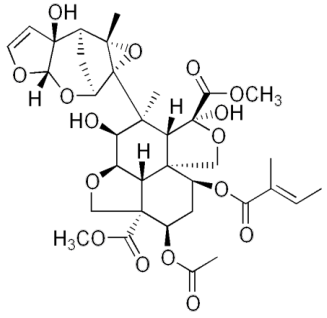
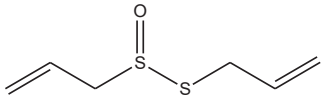
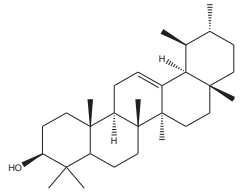
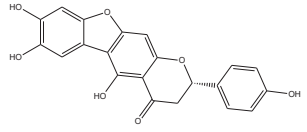
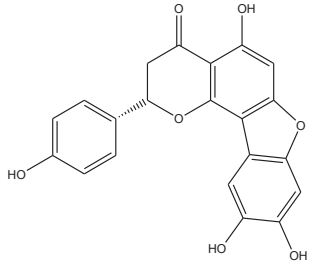
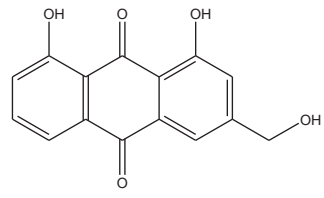
With the advances in analytical technology and biological science, many bioactive chemical entities have been identified in plants through phytochemical and pharmacological studies. The "quasi-drug" stage in drug discovery from herbal medicine includes the preparation of extracts and phytochemical groups from herbs, including the discovery of lead compounds by using modern and conventional research tools. Phytochemical study of extracts of herbal preparations involves isolation, structure/composition elucidation and bioactivity evaluation[10]. Plants contain a number of active ingredients that may be useful for the development of hepatoprotactive agents. Identification and isolation of lead compounds from plant materials are therefore crucial for drug discovery process. The direct approach in lead discovery from plants is to isolate active ingredient(s) from the respective plant extract. Feasibility of this approach mainly depends on the concentration of the bioactive component(s) and the degree of difficulty in purification, so that biological studies can be done precisely[11].

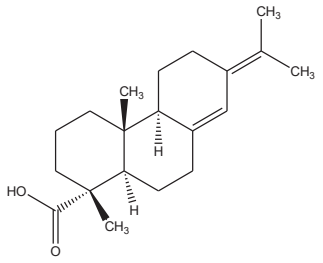
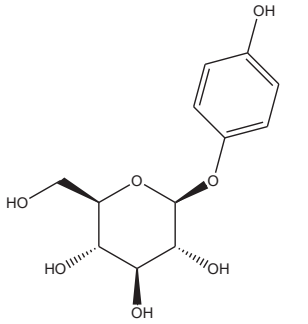
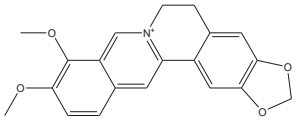
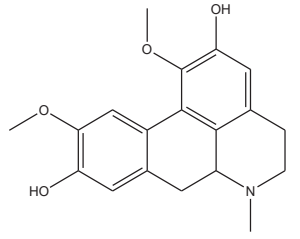
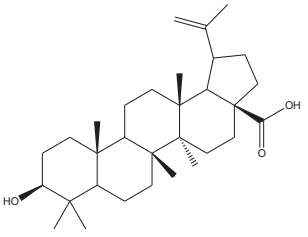
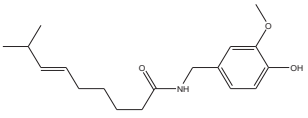
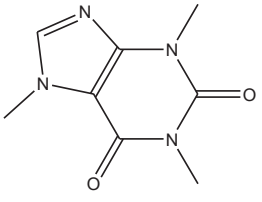
Typical example of drug discovery from plants as hepatoprotective is bicyclol, which was approved in 2001 as a therapeutic agent for hepatitis in China and has obtained patent protection in 15 countries and regions[12]. Schisandrin C, present in *Schisandra chinensis,* has led to the discovery and development of two potent drug derivatives, bifendate and bicyclol[13,14]. Various phytomolecules which showed hepatoprotective potential are summerized in Table III and details of some promising bioactive phytomolecules are discussed here.

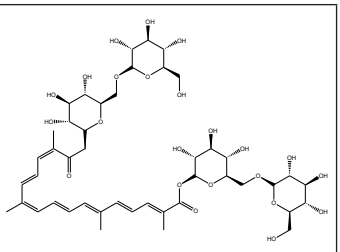
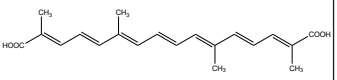
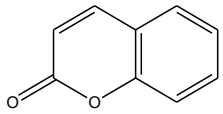
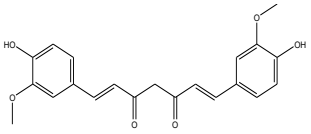
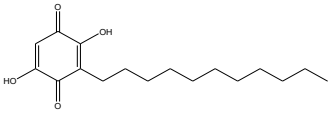
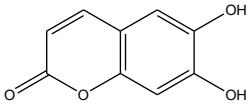
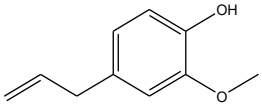
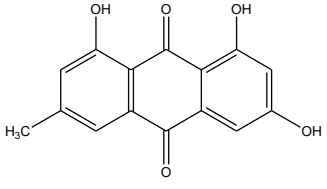
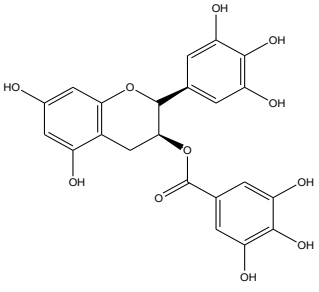
Andrographolide

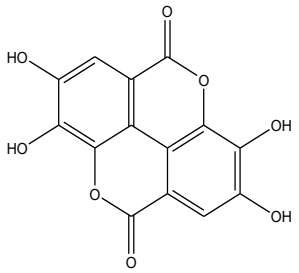
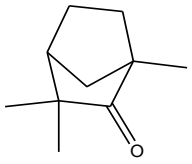
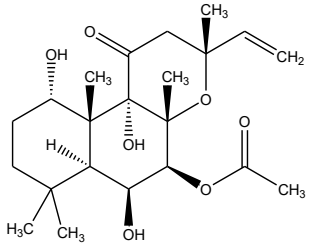
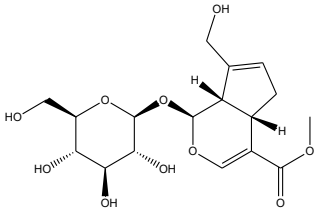
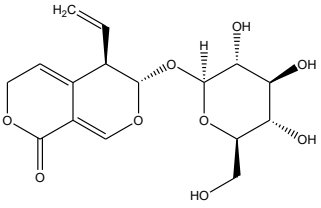
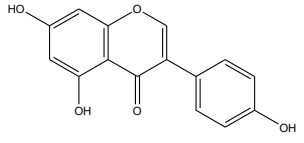
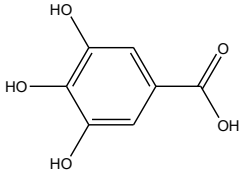
It is a diterpenoid extracted from the plant *Andrographis paniculata* Nees (Family: Acanthaceae). It is an extremely bitter, colorless, crystalline bicyclic compound. Rajani et al. (2000)[15], used cold maceration of three samples of leaf powder (50g each) with three solvents namely, dichloromethane: methanol (1:1), methanol and hydroethanol (95 %). The marc obtained after the methanol and hydroethanol solvents was further extracted with respective solvents. The obtained crystalline mass was washed with toluene to remove the colouring matter and finally dissolved in hot methanol and refrigerated for crystallization. The yield of andrographolide was found to be 1.9-2.0 g. The isolated compound was characterized by TLC ($CHCl_3$:CH_3OH:$C_4H_8O_2$::8:1.5:1; $CHCl_3$:CH_3OH::9:1; $CHCl_3$: $C_4H_8O_2$::6:4; $CHCl_3$: C_3H_6O:HCOOH::7.5:1.65:0.85), UV (λ_{max} 232 nm), FTIR and LCMS studies[15].

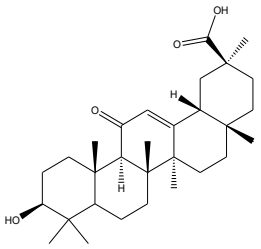
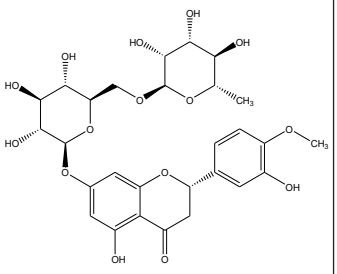
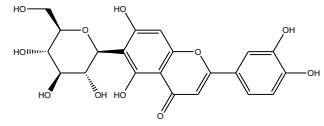
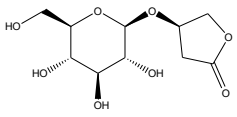
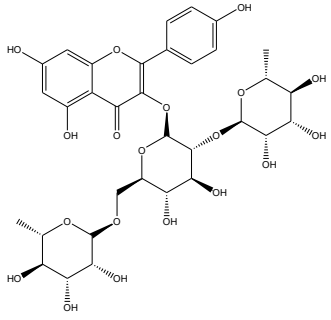
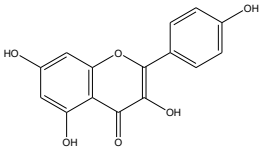
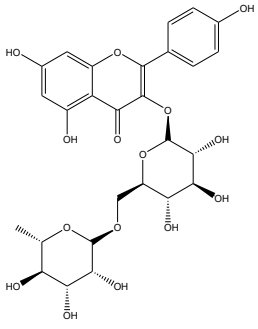
Table III: Hepatoprotective potential of bioactive phytomolecules

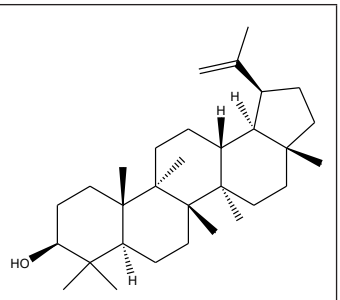
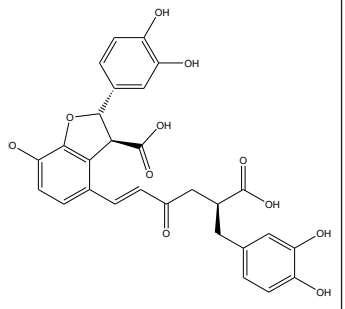
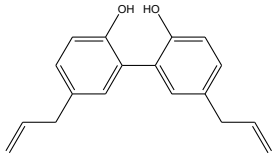
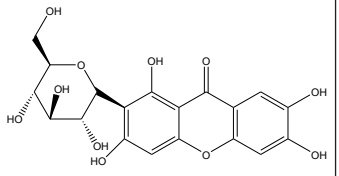
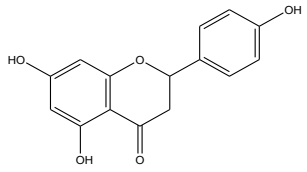
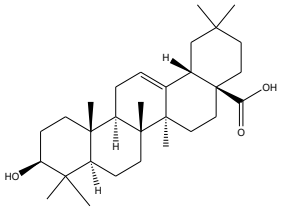
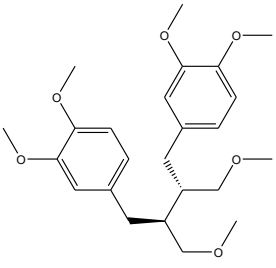
Phytomolecule (Dose)	Class of compound	Animal used	Model of Hepatotoxicity (Dose)	Chemical structure
Andrographolide[17] (5,7 &10 mg kg^{-1})	Labdanedi-terpene	Swiss albino mice	Hexachlorocyclohexane (500 ppm kg^{-1})	
Azadirachtin-A[50] (100 & 200 µg kg^{-1})	Tetranortri -terpenoid	Wistar albino rats	CCl_4 (1 mL kg^{-1})	
Allicin[51] (10 mg kg^{-1})	Organosulfur compound	Mice	Acetaminophen (250 mg kg^{-1})	
α-Amyrin[52] (20 mg kg^{-1})	Pentacyclictri-terpene	Wistar albino rats	CCl_4 (0.2 mL kg^{-1})	
Anastatin A[53] (0,3, 10, 30 & 100 µg mL^{-1})	Flavonoid	Mouse hepatocytes	D-Galactosamine[a]	
Anastatin B[53] (0,3, 10, 30 & 100 µg mL^{-1})	Flavonoid	Mouse hepatocytes	D-Galactosamine[a]	
Aloe-emodin[19] (50 mg kg^{-1})	Antraquinone	Sprague-Dawley male rats	CCl_4 (3 mg kg^{-1})	

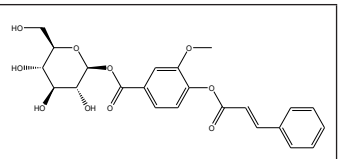
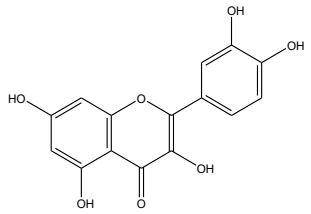
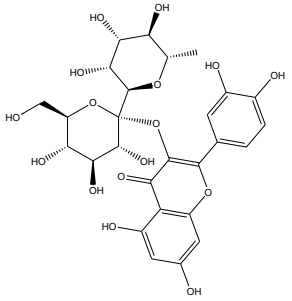
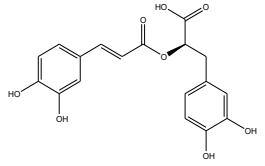
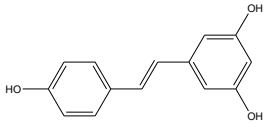
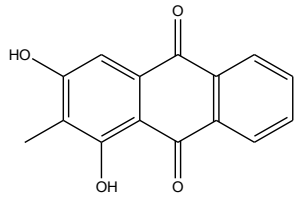
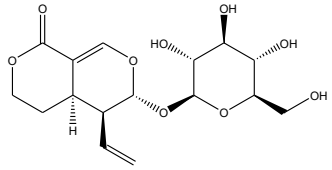
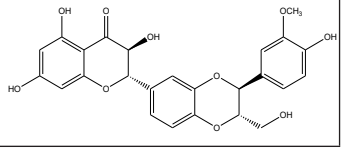
Abietic acid[54] (25, 50, 75 & 100 µg mL^{-1})	Diterpenoid	BALB/c mice	Lipopolysaccharide (1.5 µg 30 g^{-1})	
Arbutin[55] (50, 75 & 250 mg kg^{-1})	Glycosylated hydroquinone	Wistar rats	CCl_4 (1 mL kg^{-1})	
Berberine[56] (80, 120 & 160 mg kg^{-1})	Benzyl-isoquinoline alkaloid	Sprague-Dawley rats	CCl_4 (1 mL kg^{-1})	
Boldine[21] (90 mg kg^{-1})	Alkaloid	Wistar albino rat	Diethylnitrosamine (100 mg kg^{-1})	
Betulinic acid[22] (0.25, 0.5 & 1.0 mg kg^{-1})	Triterpene	Kunming mice	Alcohol (50 %) (10 mL kg^{-1})	
Capsaicin[57] (4 mg kg^{-1})	Homovanillic acid alkaloid	Male albino mice	Lipopolysaccharide (3 mg kg^{-1})	
Caffeine[58] (100 mg kg^{-1})	Purine alkaloid	Female Wistar albino rats	Diethylnitrosamine (200 mg kg^{-1})	

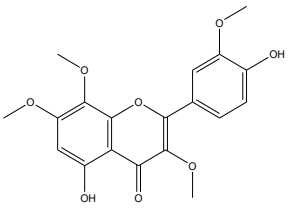
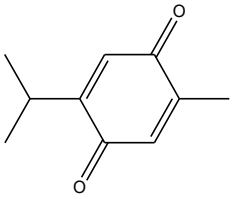
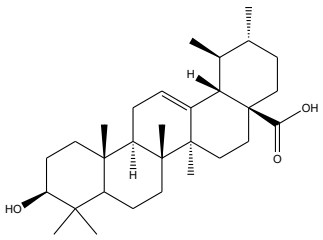
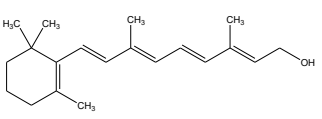
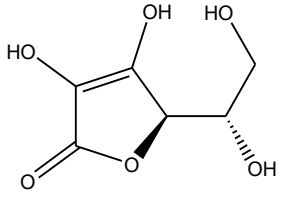
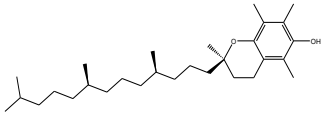
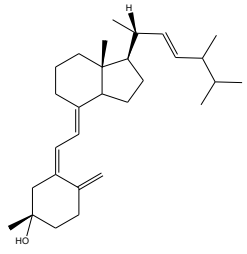
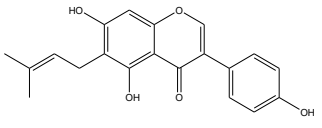
Crocin[59] (50 mg kg^{-1})	Carotenoid	Wistar albino rats	Acrylamide (25 mg kg^{-1})	
Crocetin[27] (140 mg kg^{-1})	Apocarotenoid	Male Kunming mice	CCl_4 (2 mL kg^{-1})	
Coumarin[60] (30 mg kg^{-1})	Benzopyrone	Sprague-Dawley rats	CCl_4 (1.25 mL kg^{-1})	
Curcumin[60] (50 & 100 mg kg^{-1})	Phenolic compound	Swiss albino mice	CCl_4 (1 mL kg^{-1})	
Embelin[35] (50 mg, 100 mg kg^{-1})	Benzoquinone	Male Swiss mice, Wistar albino rats, Sprague-Dawley rats	*N*-Nitrosodiethylamine (1 ppm g^{-1})	
Esculetin[61] (10, 50 & 100 µM)	Coumarin derivative	HepG2 C57BL/6J mice	Ethanol (3 %V/V)	
Eugenol[62] (5 mg kg^{-1})	Monoterpene	Male Wistar rats	Arsenic trioxide (As_2O_3) (4 mg kg^{-1})	
Emodin[63] (20, 40 & 80 mg kg^{-1})	Anthraquinone	Male Sprague-Dawley rats	Lipopolysaccharide (2.8 mg kg^{-1})	
(-)-Epigallocatechin-3-gallate[64] (0.54 %, w/w)	Catechin	Male Sprague-Dawley rats	Acetaminophen (1 g kg^{-1})	

Ellagic acid[60] (50 & 100 mg kg^{-1})	Hydrolyzable tannin	Swiss albino mice	CCl_4 (1 mL kg^{-1})	
Fenchone[65] (0.3 mL kg^{-1})	Volatile essential oil	Sprague-Dawley rats	CCl_4 (1.5 mL kg^{-1})	
Forskolin[66] (5,10,20 & 40 mg kg^{-1})	Labdane diterpene	Male albino rats	CCl_4 (1 mL kg^{-1})	
Geniposide[27] (400 mg kg^{-1})	Iridoid glycoside	Male Kunming mice	CCl_4 (2 mL kg^{-1})	
Gentiopicroside[67] (80, 120 & 150 mg kg^{-1})	Isoprenoids	Male Sprague-Dawley rats	α-Naphthylisothiocyanate (60 mg kg^{-1})	
Genistein[68] (1 mg kg^{-1})	Isoflavone	Female Sprague-Dawley rats	CCl_4 (0.15 mL $100g^{-1}$)	
Gallic acid[37] (100 mg kg^{-1})	Phenolic acid	Wistar rats	*N*-Nitrosodiethylamine (10 mL kg^{-1})	

18β-Glycyrrhetinic acid[69] (50 &100 mg kg^{-1})	Triterpene	Wistar rats	Triptolide (2.4 mg kg^{-1})	
Hesperidin[70] (200 mg kg^{-1})	Flavonoid	Wistar rats	Isoniazid (27 mg kg^{-1}), Rifampicin (54 mg kg^{-1}) Pyrazinamide (135 mg kg^{-1})	
Isoorientin[71] (or homoorientin) (15 mg kg^{-1})	Flavone	Wistar rats	CCl_4 (1 mL kg^{-1})	
Kinsenoside[72] (500 mg kg^{-1})	Glycoside	Male ICR mice	CCl_4 (0.1 mL)	
Kaempferol[73] (4.5 mg)	Flavonoid	Male ddY mice	CCl_4 (30 µL)	
Kaempferol[74] (10 & 20 mg kg^{-1})	Flavonoid	Kunming mice	Alcohol (2, 4, 6, 8 &10 g kg^{-1})	
Kaempferol 3-*O*-rutinoside and Kaempferol 3-*O*-glucoside (Astragalin)[38] (200 & 400 mg kg^{-1})	Flavonoid	Male Kunming mice	CCl_4 (10 mL kg^{-1})	

Lupeol[75] (25 mg kg^{-1})	Triterpene	Swiss albino mice	7,12-Dimethylbenz(a) anthracene (DMBA) (50 mg kg^{-1})	
Lithospermic acid[76] (100 mg kg^{-1})	2-Arylbenzofuran flavonoid	Male BALB/c mice	CCl_4 (10 mL kg^{-1})	
Magnolol[77] (0.01, 0.1 & 1 µg mL^{-1})	Lignan	Sprague-Dawley rats	Acetaminophen (500 mg kg^{-1})	
Mangiferin[78, b]	Xanthone-glucoside	Rats	D-Galactosamine (400 mg kg^{-1})	
Naringenin[79] (50 mg kg^{-1})	Trihydroxy-flavanone	Swiss mice	CCl_4 (1 mL kg^{-1})	
Oleanolic acid[80] (100 & 200 µg $mouse^{-1}$ day^{-1})	Triterpenoid	Male BALB/c mice	Rifampicin (10 mg kg^{-1}) Isoniazid (10 mg kg^{-1}) Pyrazinamide (30 mg kg^{-1})	
Phyllanthin[81] (1, 2, 3 & 4 µg mL^{-1})	Lignan	Rat Hepatocytes	Ethanol (80 µL mL^{-1})	

Picroliv[60] (or Kutkin) (50 mg kg^{-1})	Iridoid glycoside	Swiss albino mice	CCl_4 (1 mL kg^{-1})	
Quercetin[82] (100 mg kg^{-1})	Flavonoid	Albino rats	Thioacetamide (100 mg kg^{-1})	
Rutin[83] (10, 50 & 150 mg kg^{-1})	Flavonoid	BALB/cN mice	CCl_4 (2 mL kg^{-1})	
Rosmarinic acid[47] (10, 25 & 50 mg kg^{-1})	Coumaric acid derivative	Wistar albino rats	Acetaminophen (600 mg kg^{-1})	
Resveratrol[84] (200 mg kg^{-1})	Polyphenol	HepG2 C57BL/6J mice	Ethanol (200 mg kg^{-1})	
Rubiadin[85] (50 & 200 mg kg^{-1})	Anthraquinone-glycoside	Wistar albino rats	CCl_4 (2 mL kg^{-1})	
Sweroside[86] (200 & 400 mg kg^{-1})	Secoiridoid	ICR male mice	CCl_4 (10 mL kg^{-1})	
Silibinin[87] (200 mg kg^{-1})	Polyphenolic	Sprague-Dawley rats	Lieber–DeCarli standard liquid high-fat diet[a]	

Ternatin[88] (25 & 50 mg kg^{-1})	Flavone	Mice	Acetaminophen (300 mg kg^{-1})	
Thymoquinone[89] (1 mM)	Monoterpene	Male Sprague-Dawley rats	CCl_4 (5 mM)	
Ursolic acid[80] (100&200 µg $mouse^{-1}$ day^{-1})	Pentacyclictri-terpenoid	Male BALB/c mice	Rifampicin (10 mg kg^{-1}) Isoniazid (10 mg kg^{-1}) Pyrazinamide (30 mg kg^{-1})	
Vitamin A[90] (Retinol) (400 IU $kg^{-1}day^{-1}$)	Fat soluble vitamin	Wistar albino rats	Gasoline Vapors (17.8 $cm^3h^{-1}m^{-3}$)	
Vitamin C[91] (Ascorbic acid) (200 mg kg^{-1})	Water soluble vitamin	Male Wistar albino rats	Acelofenac (120 mg kg^{-1}) Diclofenac (120 mg kg^{-1})	
Vitamin E[91] (α-Tocopherol) (200 mg kg^{-1})	Fat soluble vitamin	Male Wistar albino rats	Acelofenac (120 mg kg^{-1}) Diclofenac (120 mg kg^{-1})	
Vitamin D[92] (40 mg kg^{-1})	Fat soluble vitamin	Albino rats	Streptozotocin (65 mg kg^{-1})	
Wighteone[93] (25 mg kg^{-1})	Flavonoid	Albino rats	CCl_4 (2 mL kg^{-1}) D-galactosamine	

a, Data not explored in research paper; b, Data incomplete (to be obtained from the Abstract)

Another expeditious procedure for the isolation and quantification of andrographolide has been reported by Syukri et al. (2016)[16]. The finely ground *A. paniculata* Nees was extracted twice with ethanol at interval of 24 h. Then, the extract was evaporated under reduced pressure and fractioned using *n*-hexane. The insoluble fraction was again fractionated with ethyl acetate. The various fractions were subjected to quantification by different analytical techniques like column chromatography (CH_3OH: $CHCl_3$::1:9), TLC ($C_4H_8O_2$: C_3H_6O::7:3, $CHCl_3$: CH_3OH::9:1, and $CHCl_3$: $C_4H_8O_2$::7:3), HPLC (solvent: methanol:water::6:4, detector: photometric diode array (PDA), λ=200-400 nm) and FTIR. The purity of andrographolide was found to be 95.74±0.29 %[16]. The hepatoprotective activity of andrographolide was studied by Trivedi et al. (2007) against the hexachlorocyclohexane induced oxidative liver damage. The results of the study showed increase in GSH, GR, GSH-Px, SOD and CAT levels while γ-GTP and GST levels showed decrease[17].

Aloe-emodin

It is an anthraquinone glycoside that was extracted from the leaves of *Cassia tora* (Family: Caesalpiniaceae) by Maity et al. (2003)[18]. The dried leaves were extracted with methanol by cold percolation method. The dried methanolic extract was mixed with water and extracted with petroleum ether and 0.5 N potassium hydroxide. The potassium hydroxide extract was acidified with dilute hydrochloric acid and again extracted with solvent ether. The ether layer was subjected to TLC (benzene: methanol:: 90:10) that revealed one major fluorescent spot under UV (λ_{max} 225 nm). The resulting compound was subjected to characterization by column chromatography (benzene:methanol::9:1) and mass spectroscopy[18]. Dong et al. (2009) studied the hepatoprotective effect of emodin against the fibrogenesis induced by CCl_4. The fibrogenesis was inhibited by the activation of hepatic stellate cell (HSC) which was assessed by reverse transcription-polymerase chain reaction (RT-PCR)[19].

Boldine

It is the most abundant alkaloid present in *Peumus boldus* (Family: Monimiaceae). The boldine was extracted from the dried powder of leaves and stem by solid-liquid extraction process, using 80 mL of hydroethanol solvent (70%), by Lara-Fernández et al. (2013)[20]. Then, the mixture was refluxed at different temperatures and monitored continuously every 2 h. The extracted boldine was characterized by using RP-HPLC (mobile phase: methanol: water:: 70:30, flow rate: 0.3 mL min^{-1}, detector PDA at 280 nm) method[20]. The hepatoprotective activity of boldine was studied by Subramaniam et al. (2019) against diethylnitrosamine - induced liver carcinogenesis. The results of the study revealed that boldine modulate the enzymatic and nonenzymatic antioxidant activities, like messenger RNA and protein expressions of Bcl-2, Bax, and cleaved caspase. The histopathological studies also showed normal architecture with intact round nuclei after treatment with boldine[21].

Betulinic acid

It is a pentacycliclupane-type triterpene which was extracted and isolated from the bark of *Betula papyrifera* (Family: Betulaceae) by Yi et al. (2014)[22]. The dried bark was refluxed with methanol. After drying, the methanol extract was dissolved in dichloromethane and 2 M NaOH and the lower layer of liquid filtered. The residue was mixed with ether and then water, the upper layer of this liquid was collected and subjected to column chromatography (hexane:ethyl acetate::6:1), UV (λ_{max} 210 nm). The obtained betulin was oxidized by Jones reagent to give betulonic acid, subsequent reduction by sodium borohydride in tetrahydrofuran gave a mixture of 3α and 3β hydroxyl product. Crystallization of the mixture from methanol resulted in 3β-hydroxyl betulinic acid. The structure of betulinic acid was confirmed by comparing the results of MS, ^{1}H-NMR and ^{13}C-NMR. The percentage purity of betulinic acid was found to be 96.5 %. The isolated betulinic acid was subject to hepatoprotective activity against alcohol-induced liver injury. Various biochemical and histopathological parameters were evaluated during the study. The results of the study revealed that betulinic acid acid significantly decreased the levels of CAT, GSH, GSH-Px and MDA[22,23].

Berberine

It is an alkaloid, which was isolated by Pradhan et al. (2013)[24] from fresh roots of *Berberis vulgaris* (Family: Berberidaceae) and Nampoothiri et al. (2017)[25] from the powdered rhizomes of *Alpinia calcarata* and *Alpinia galangal* (Family: Zingiberaceae) with water. The obtained extract was treated with 1.0 N hydrochloric acid and sodium hydroxide and further extracted with diethyl ether. The aqueous layer was further extracted with chloroform and concentrated to give a yellow coloured solution. This solution was purified by column chromatography using different ratio of solvents. The $CHCl_3$: MeOH (90:10) fraction yielded a single peak in TLC. The purified berberine

was quantified by UV-Visible spectroscopy, FTIR and ^{1}H NMR and LCMS techniques[24,25]. The hepatoprotective activity of berberine was studied by Mehrzadi et al. (2018) against methotrexate-induced liver toxicity. Various parameters like ALT, AST, ALP, MDA, GSH, CAT, SOD and GSH-Px were analysed. The results of the study showed that berberine significantly decreased the level of ALT, AST, MDA and ALP while GSH, SOD, GSH-Px and CAT activity was increased[26].

Crocetin

It is a natural apocarotenoid dicarboxylic acid that was extracted from the dried fruits of *Gardenia jasminoides* (Family: Rubiaceae) by Chen et al. (2016)[27]. The dried coarse powder of fruit was subjected to cold percolation with ethanol (40 %). The dried extract was subjected to column chromatography with water and increasing amount of ethanol (0, 25, 40, 60 % V/V). The residue obtained after 25 % ethanol extraction was again eluted in column with water and ethanol (10, 25, 35% V/V). The 60 % eluted fraction was separated with column chromatography with ethyl acetate and increasing amount of methanol-water (10, 20, 30, 50% V/V in ratio 16:13 V/V). Finally, the crocetin was isolated with 30 % methanol-water fraction by treatment with 10 % potassium hydroxide. The structure of crocetin was confirmed by TLC, HPLC-UV, LC-MS and NMR techniques. The isolated crocetin was evaluated for hepatoprotective activity against CCl_4- induced liver injury. The results of the study showed that crocetin significantly decreased the level of ALT, AST and ALP; it also increased the activity of SOD and CAT[27].

Coumarin

Bourgaud et al. (1994)[28] isolated coumarin from the leaves of *Melilotus officinalis* (Family: Leguminosae). The powdered material was macerated with hydroalcoholic (80 %V/V) solvent, filtered and evaporated at low temperature. Obtained residue was dissolved in acetate buffer, hydrolysed with emulsion and acidified with hydrochloric acid (1 N). The resultant was quantified by HPLC method[28]. Coumarin was also extracted from the aerial part of *Melilotus officinalis* (Family: Fabaceae) by Al-Ani W.M.K. et al. (2014)[29] using Soxhlet apparatus (80 % hydroethanol). Then, the extract was mixed with water and partitioned with petroleum ether. Finally, the aqueous layer was extracted with ether. The ether layers were dried by using anhydrous sodium sulphate and purified by column chromatography (stationary phase: silica gel and mobile phase: dichloromethane). The result of the preparative TLC (R_f 0.64, toluene:acetone:water::4:5:1) was found to be comparable with the standard value (R_f 0.65). The structure of coumarin was elucidated and confimed by GC-MS, TLC, HPTLC and UV spectroscopy[29]. The seeds of *Malus domestica* (Family: Rosaceae) were processed by Soxhlet extraction and kinetic maceration. Powdered drug was successively extracted by different solvents of decreasing and increasing polarity separately using Soxhlet apparatus by Mustafa et al. (2018). In kinetic maceration process, the powdered drug was placed in a beaker containing water, methanol, chloroform or *n*-hexane solvent using a shaker. The coumarin was isolated from the chloroform extract by treatment with 1.0 N sodium hydroxide and hydrochloric acid. Then, the crystals were filtered and identified by TLC ($CHCl_3$: acetone::4:1), column chromatography (ethyl acetate:ether::1:9 to 9:1), FTIR, ^{1}H-NMR and ^{13}C-NMR spectra[30].

Atmaca et al. (2011)[31] studied the heaptoprotective activity of coumarin and its derivatives against CCl_4-induced hepatic injury. For oxidative stress, the levels of LPO, MDA, SOD and CAT were evaluated and for hepatic injury, GGT and LDH levels were detected. The results of the study revealed that chemical structure of coumarin significantly protected from oxidative stress[31].

Calotropagenin

The ethanol extract of the *Calotropis procera* leaves has been extracted and the hepatocytotoxical potential of HepG2 has been examined by Al-Taweel et al*.,* (2017)[32]. The cell viability test technique was used to examine the results. Calotropagenin's IC50 was found to be 10.40±0.98 µg mL^{-1}. Calotropagenin has a significant anti-heptocytotoxic capability when compared to ethanol leaf extract (IC_{50} 27.40±1.65 µg mL^{-1}), according to the findings of the study[32].

Colchicine

Colchicine belongs to a class of lipid-soluble tricyclic alkaloids used to treat a variety of diseases including, gout, pericarditis and rheumatoid arthritis. It is found in *Calotropis procera* leaves. The preventive effect of colchicine against induced injury to the liver by CCl_4 (0.5 mL $100g^{-1}$) by blocking the cytochrome p-450 action has been investigated in Martinez et al*.,* (1995)[34]. At a dosage of 10 µg/animal/day, colchicine was administered. Other bio-chemical studies were conducted to assess the preventive effects of colchicine, as well as the cytochrome p-450 effect, such as ALT, AST, GGT, LPO,

MDA and p-nitroanisole o-demethylase level. Colchicine substantially lowered cyctochrome p-450 levels and p-nitroanisole-o-demethylase activity, according to the results of the study[33, 34].

Embelin

It belongs to the class of alkyl benoquinones. It was extracted and isolated from the fruits of *Embelia robusta* (Family: Primulaceae) by Poojari et al. (2011)[35]. Embelin was extracted by Soxhlet extraction by using *n*-hexane. After 36 h of extraction, the crude embelin was precipitated and recrystallized by ice-cold absolute ethanol. The glistening orange crystals of embelin were charaterized by UV-spectroscopy (λ_{max}-225nm) and reversed phase HPLC fingerprinting [(solvent A: 0.01 M KH_2PO_4: MeOH (90:10) and solvent B: MeOH: 0.01 M KH_2PO_4 (90:10); (Rt 6.72 min for embelin)]. The isolated embelin was explored for its potential as hepatoprotective agent against CCl_4 and *N*-nitrosodiethylamine-induced liver injury models. The results showed decreased levels of SGOT, SGPT, ALP, GGT, TB, ALB and TGs[35].

Gallic Acid

It is a phenolic acid that was extracted and isolated from the fruit pulp of *Terminalia chebula* (Family: Combretaceae). Genwali et al. (2013)[36] reported the procedure for extraction and isolation of gallic acid from the ethyl acetate soluble fraction of methanolic extract. The dried fruit was extracted with methanol by using Soxhlet apparatus and then fractionated with ethyl acetate. The ethyl acetate fraction was subjected to column chromatography (toluene: ethylacetate: formic acid:: 6:6:1), TLC (toluene: ethylacetate: formic acid::6: 6:1), UV (λ_{max} 220 & 270 nm) and FTIR analysis[36]. Latief et al. (2016) studied the hepatoprotective activity of gallic acid against nitrosodiethylamine-induced liver inflammation. Various biochemical parameters were studied to evaluate the potential of gallic acid. The results of the study showed that gallic acid significantly decreases the LPO, SOD and ATPases level. The histopathology results showed significant reduction in inflammatory cell infiltration and degeneration of hepatocytes[37].

Kaempferol

Kaempferol-3-*O*-rutinoside and Kaempferol 3-*O*-glucoside are flavonols that are widely distributed in nature. Wang et al. (2015)[38] extracted and isolated the derivative of kaempferol from the dried flowers of *Carthamus tinctorius* (Family: Asteraceae). The dried flowers were subjectd to extraction with hydroethanol (70 %V/V) at room temperature. The extract was dried and dissolved in water, then subjected to resin column Separation (or: fractionation) with increasing concentration of ethanol (20-80 %V/V) successively. The 50 % V/V hydroethanol fraction was dried and subjected to polyamide column elution from hydroethanol (10-80 %V/V). Kaempferol-3-*O*-rutinoside was precipitated from the 10 %V/V hydroethanol fraction. The 50 %V/V hydroethanol fraction was again purified with 50 %V/V methanol using the Sephadex LH-20 column that gave kaempferol-3-*O*-glucoside. The yields were 3.5 g and 2.4 g of kaempferol-3-O-rutinoside and kaempferol-3-O-glucoside, respectively from 2000 g of air dried material. The structures of isolated compounds were elucidated by MS and ^{1}H-NMR spectroscopy. In later phase of experiment, the isolated compounds were subjected to hepatoprotective activity against CCl_4 - induced hepatic injury in mice. The results of the study revealed that both the compounds significantly decreased the levels of AST, ALT, ALP and MDA[38].

Phytol

Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol) is a naturally occurring chemical. It is generated by nearly all photosynthetic organisms, especially algae, plants, and bacteria, since it is a component of the chlorophyll molecule (cyanobacteria). In ruminant animals, it is also produced as an essential intermediate in catabolism. Phytol is therefore regarded in the biology of our planet to be the most abundant acyclic isoprenoid. Gut digestion of ingested plant materials in ruminants produces phytol, which is transformed into phytanic acid and subsequently deposited in fat depositions. The hepatoprotective effect of phytol on ethanol (3.76 g kg^{-1}) induced liver damage in Wistar rats has been investigated by Gupta et al., in (2019). Dosages of 100 mg kg^{-1} and 200 mg kg^{-1} have been used to study several biochemical parameters such as SGPT, SGOT, ALP, TB, TP, SOD, CAT and GSH. The study found that the levels of biochemical parameters decreased considerably, which means phytol causes increase in levels of TP, SOD, CAT and GSH[39-44].

Pinoresinol

This is a lignan family member, usually connected to the two polypropanoid units. Pinoresinol is a chemically produced form of lignin called tetrahydro-1*H*,3*H*-furo[3,4-c] furan, isolated from *Calotropis procera* leaves. In 2010 Kim et al., studied pinoresinol's hepatoprotective

ability against liver damage produced by CCl_4 in mice. Pinoresinol was administered before CCl_4 at a dosage of 20 µL kg^{-1} at the dosages of 25, 50, 100 and 200 mg kg^{-1}. The study showed that pinoresinol cures acute liver lesions considerably from oxidative stress, as well as inflammatory mediator suppression, through NF-kB and AP-1 pathways[45,46].

Rosmarinic acid

Lucarini et al. (2014) extracted and isolated it from the leaves of *Rosmarinus officinalis* (Family: Lamiaceae). The dried leaves were macerated with hydroethanol (80 %V/V) at room temperature. The rosmarinic acid was isolated from the extract by dissolving it in methanol/ water (1:1 V/V) and chromatographed over preparative RP-HPLC. The chemical structure was elucidated and confirmed by UV, ^{1}H-NMR and ^{13}C-NMR techniques. The hepatoprotective activity of isolated rosmarinic acid was evaluated against acetaminophen induced liver injury. The results of the study revealed that rosmarinic acid significantly decreased the ALT, AST and ALP levels. The histopathology was scored on the scale of 0-4 and showed decreased in inflammatory cell infiltration and necrosis in Wistar albino rats pretreated with rosmarinic acid[47].

Silymarin

It is a mixture of flavonolignans (silychristin, silydianin, silybin A, silybin B, isosilybin A and isosilybin B) that is obtained from the fruits of *Silybum marianum* (Family: Asteraceae). Sameh Abou Zid (2012)[48] described the extraction procedure of silymarin. The fruit oil was removed by cold pressing the dried fruit, the compressed mass was broken up, the pressed residue was extracted with ethyl acetate and the ethyl acetate extract was evaporated and characterized by TLC (chloroform-acetone-formic acid::75:16.5:8.5), UV (λ_{max}-365nm), HPLC and UPLC techniques[48]. Freitag et al. (2015)[49] evaluated the hepatoprotective potential of silymarin against the acetaminophen-induced liver injury in spontaneously hypertensive rats. The results of the study revealed that silymarin significantly decreased the levels of ALT, AST, ALP and γGGT[49].

Ursolic acid

The ursolic acid (3-β-hydroxy-urs-12-ene-28-oic acid) belongs to the chemical class of pentacyclic triterpenoid carboxylic acids. Many studies to assess its hepatoprotective properties have been carried out. It is the active ingredient in the leaves of *Calotropis procera*.

Kishen et al., undertook a further *in vitro* investigation to assess the hepatoprotective efficacy of ursolic acid toxicity (1 %) in cell lines for HepG2. MTT assay was used to determine the percentage of cell vitality. The results of the investigation indicated that the percentage cell viability was 85 % at full dosage (i.e. 100 µM) of ursolic acid[94,95].

A further investigation was carried out by Ali et al., on *N*-diethylnitrosamine (200 mg kg^{-1}) inducing hepatocarcinogenesis with isolated ursolic acid for chemical preventative effects. For the *in vitro* activity, hepG2 cell lines were employed and Wistar albino rats were used for *in vivo* investigation. Concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 0.78 and 1.56 Ug mL^{-1} utilising an MTT test technique were assessed for the *in vitro* chemo-preventative potential of ursolic acid. On male Wistar albino rats, the *in vivo* potential was tested at 500 mg kg^{-1} dosage. This research showed that the high levels of serum biochemicals and hepatocyte architecture are restored considerably by ursolic acid[96].

Some marketed herbal formulations

Various herbal formulations are available in the market for the treatment of liver ailments. The most widely used formulation is Liv52, available in tablet and syrup form in the market since decades. The common ingredients in most of the formulations are *Andrographis paniculata* (Kalmegh), *Eclipta alba* (Bhringraj), *Terminalia arjuna* (Arjuna), *Phyllanthus niruri* (Bhuiaonla) and *Picrorhiza kurroa* (Kutki)[98,99]. Apart from these plants, many other plant extracts are also used in the different marketed formulations, as shown in Table IV, having hepatoprotective activity[100,101].

CONCLUSION

Liver disease is almost entirely preventable with the major risk factors, namely, alcohol, obesity and hepatitis B and C accounting for up to 90 % of cases. The world is moving towards natural products due to their low cost and reliability over side effects resulting from existing drugs. Researchers are intensifying their efforts for the development of phytopharmaceuticals against LDs. Herbal medicine as a source of new compounds for drugs is going to become a global trend in the pharmaceutical industry. It is well known that the medicinal value of plants depends on the presence of bioactive molecule(s)

Table IV: Hepatoprotective herbal marketed formulations in India[100,101]

Brand Name	Plant used in formulation	Dose	Manufacturer
Liv 52	*Achillea millefolium, Capparis spinosa, Cassia occidentalis, Cichorium intybus, Solanum nigrum, Tamarix gallica, Terminalia arjuna*	2-3 teaspoons BID/TID	Himalaya Drug Co.
Livergen	*Andrographis paniculata, Apium graveolens, Asteracantha longifolia, Cassia angustifolia, Trachyspermum ammi, Trigonella foenum-graecum*	2-4 teaspoons BID	Standard Pharmaceuticals
Livokin	*Andrographis paniculata, Apium graveolens, Berberis lycium, Carum copticum, Cichorium intybus, Cyperus rotundus, Eclipta alba, Ipomoea turpethum, Oldenlandia corymbosa, Picrrorhiza kurroa, Hygrophila spinosa, Plumbago zeylanica, Solanum nigrum, Tephrosia purpurea, Terminalia arjuna, Terminalia chebula, Trigonella foenum-graecum*	1-2 teaspoons BID/TID	Herbo-Med
Stimuliv	*Andrographis paniculata, Eclipta alba, Phyllanthus niruri, Justicia procumbens*	1-2 teaspoons BID/TID	Franco-Indian Pharmaceuticals Pvt. Ltd.
Octagen	*Arogyavardhini rasa, Phyllanthus niruri*	As directed by physician	Plethico Pharmaceuticals Ltd.
Tefroliv	*Andrographis paniculata, Eclipta alba, Ocimum sanctum, Phyllanthus niruri, Picrrorhiza kurroa, Piper longum, Solanum nigrum, Tephrosia purpurea, Terminalia chebula*	1 teaspoons TID	TTK Pharma Pvt. Ltd.
Adliv Forte	*Andrographis paniculata, Picrrorhiza kurroa, Eclipta alba, Phyllanthus niruri*	As directed by physician	Albert David Ltd.
Jaundex	*Tinospora cordifolia, Tecomella undulata, Phyllanthus niruri, Picrorhiza kurroa, Terminalia chebula*	2 teaspoons BID	Sandu Pharmaceuticals Ltd.
Amlycure DS Syrup	*Eclipta alba, Phyllanthus emblica, Terminalia arjuna, Terminalia bellirica, Berberis aristata, Plumbago zeylanica, Raphanus sativus, Boerhavia diffusa, Terminalia chebula, Tinospora cardifolia, Solanum nigrum, Hordeum vulgare, Trachyspermum ammi, Coriandrum sativum, Withania somnifera, Rubia cordifolia, Andrographis paniculata, Ocimum sanctum*	2-3 teaspoons TID/QID	Aimil Pharmaceutical India Ltd.
Livomap	*Boerhaavia diffusa, Melia azadirachta, Trichosanthes cucumerina, Zingiber officinale, Picrorhiza kurroa, Tinospora cordifolia, Cedrus deodara, Terminalia chebula, Crataeva religiosa, Moringa oleifera, Berberis aristata, Artemisia absinthium, Tephrosia purpurea, Phyllanthus niruri*	1-2 Tablets BID or as directed by physician	Maharishi Ayurveda Products Pvt. Ltd.

with drug-like properties. Varieties of phytomolecules have been isolated, characterized and evaluated for hepatoprotective activity by the investigators. However, extracts and phytomolecules need to be appropriately formulated to facilitate their physiological target to give more precise hepatoprotective results. Factors such as low permeability and solubility could affect the absorption and delivery of bioactive molecules[97]. Finally, to produce more effective plant-based hepatoprotective drugs, it will be necessary to carry out further studies on the structural modifications of the active principles derived from herbal extracts using computational chemistry techniques.

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