EVALUATION AND REVIEW OF HEPATOPROTECTIVE DRUGS FROM NATURAL RESOURCES

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Summary

The maintenance of a healthy liver is essential for the overall well being of an individual. Liver is the largest organ in the vertebrate body and the site for intense metabolism. Because of the strategic placement in the body, liver is continuously exposed to various xenobiotics and this may result in a variety of liver ailments. Exposure of various toxic chemicals such as certain antibiotic, chemotherapeutic agents, paracetamol, carbon tetrachloride, thioacetamide, excessive alcohol consumption and microbes can cause liver cell injury. Hepatic injury is associated with distortion of the metabolic functions. In absence of reliable liver protective drugs in modern medicine, folk remedies from natural sources are therefore evaluated for their potential hepatoprotective effects against different chemical induced liver damage in experimental animals. The present review is aimed at compiling data on promising phytochemicals from medicinal plants that have been tested in hepatotoxicity models using modern scientific system.

Keywords: Hepatotoxicity, Hepatoprotective activity, Natural products.

Introduction

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. And it functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. The bile secreted by the liver has, among other things, plays an important role in digestion. Therefore, maintenance of a healthy liver is essential for the overall well being of an individual. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide, chronic alcohol consumption and microbes are common. Enhanced lipid per oxidation during metabolism of ethanol may result in development of hepatitis leading to cirrhosis. Since time immemorial, mankind has made the use of plants in the treatment of various ailments. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. But management of liver

disorders by a simple and precise herbal drug is still an intriguing problem. So continuous searching is going on to find the effective and safe hepatoprotective drugs [2]. Evaluation of hepatoproective drugs can be done by using several models but he most prominent models are carbon tetrachloride induced hepatic damage and paracetamol induced hepatotoxicity model.

In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cell [3]. Therefore, due importance has been given globally to develop plant based hepatoprotective drugs effective against a variety of liver disorders. Herbal medicines are in great demand in the developed world for primary health care due their efficacy, safety and lesser side effects [4]. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant-based products. Hence the present review is aimed at collecting and compiling data based on reported works on promising phytochemicals from medicinal plants that have been tested in hepatotoxicity models.

Materials and methods

Animals

Healthy Wistar albino male rats (150 g ± 20) are suitable for hepatoxicity study. They were maintained at standard laboratory conditions and fed with standard food and water *ad libitum*. The experiments are performed following the animal ethics guidelines of Institutional Animals Ethics Committee.

Drugs

Drugs are extracted or separated by using suitable solvent and extraction procedures.

Acute toxicity study

Lethal dose (LD_{50}) is to be determined for choosing the dose of the experiment. One-tenth and one-fifth of the maximum safe dose of the drug tested for acute toxicity were selected as doses for the experiment [5].

Experimental design

Carbon tetrachloride-induced experimental liver damage

After seven days of acclimatization, the rats are divided into five groups of six animals each. Treatment is done for 14 days [6]. Group I served as vehicle control group. Group II-V received CCl_4 in liquid paraffin (1:2) (1.0 ml/kg i.p.) once in every 72 h. Group II is not treated with any drug and served as CCl_4 control. Group III and IV are administered with two doses of experimental drug once daily. Group V received standard drug (generally silymarin; 25 mg/kg). After 24 h of the last dose, blood is collected from retro-orbital plexus or from the heart by cardiac puncture under ether anesthesia. The blood samples are allowed to clot and the serum was separated by centrifugation at 2500 g at 37ºC and is used for biochemical estimation. All the animals are then sacrificed and liver tissues are collected for the evaluation of *in vivo* antioxidant status and histopathological examination.

Paracetamol-induced experimental liver damage

The paracetamol (PCM)-induced hepatotoxicity is studied as in Hiroshini [7]. Wistar albino rats of either sex are divided into five groups of 6 animals each. The drugs (two doses) and silymarin (25 mg/kg) are given orally to respective groups once daily for 7 days. On the fifth day, PCM at 2 g/kg was administered orally (p.o.) to all groups

except for vehicle control, 30 min after the respective treatment. PCM control group received only PCM to assist in assessing the severity of toxicity produced by PCM at 2 g/kg body wt. On the seventh day, after 2 h of respective treatments, blood samples are collected from all groups, including control, and serum is separated and analyzed for various biochemical and histopathological parameters as in the case of CCl_4-induced liver damage.

Estimation of biochemical parameters

Serum is analysed for various biochemical parameters like serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) [8] and alkaline phosphatase (ALP) [9] activities. The total protein concentration and total bilirubin are also measured by the method of Lowry et al [10] and Malloy & Evelyn [11] respectively. All the analysis can be performed by using commercially available kits.

Evaluation of antioxidant properties

For assessment of antioxidant activities, immediately after collection of blood the rats are sacrificed and livers are dissected out and washed in ice cold normal saline, blotted dry and weighed. Required quantity of the tissue is weighed and 25% (w/v) of each tissue homogenate is then prepared using KCl solution (1.15% w/v) and centrifuged at 3000 g at 4ºC for 1 h. The supernatant is used for the determination of lipid peroxidation (LPO) [12] and endogenous antioxidant systems such as reduced glutathione (GSH) [13], superoxide dismutase (SOD) [14] and catalase (CAT) [15].

Histopathological observation

For histopathological study, the liver tissues are collected and immediately fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections (4-5 mm) are prepared and then stained with hematoxylin-eosin dye for photomicroscopic observations.

List of hepatoprotective natural drugs

Common name	**Source**	**Chemical constituent**
Milk thistle [16]	Aerial parts of *Silybum marianum* (Compositeae)	Silybin, Silydianin, Silychristine,
Turmeric [16]	Rhizomes of *Curcuma longa* (Zingiberaceae)	Curcumin
Dandelion [17]	Leaves & roots of *Taraxacum officinale* (Asteraceae)	Taraxecerin, Taraxcin
Boldo [16]	Leaves of *Peumus boldus* (Monimiacae)	Laurotetanine, N-methyllaurotetanine, Boldine

Kalmegh [18]	Leaves of *Andrographis paniculata* (Acanthaceae)	Andrographolide, Kalmeghin
Punarnava [16]	Roots of *Boerhaavia diffusa* (Nictaginaceae)	Rotenoids, Boeravinone
Chelidonium [19]	All parts of *Chelidonium majus* (Papaveraceae)	Chelidonine, Protopine, Sanguinarine (alkaloids)
Black nightshade [20]	Fruits of *Solanum nigrum* (Solanaceae)	Solamargine, Andsolasonine
Daruharidra [21]	All parts of *Berberis arista* (Berberidaceae)	Berberine, Berberine chloride, Palmative chloride
Himsra [22]	Roots and bark of *Capparis spinosa* (Capparaceae)	Glucobrassicin, Neoglucobrassicin
Fennel [23]	Leaves, stalks & fruits of *Foeniculam vulgare* (Umbelliferae)	Fenchone, Methylchavicol, limonene, *a*-pinene, Camphene, Camphor
Liquorice [24]	Roots of *Glychyrrhiza glabara* (Leguminosae)	Licorice, Triterpene saponin, Glycyrrhizin, Sulfated polysaccharide
Sharpunkha [25]	Whole plant of *Tephrosia purpurae* (Febaceae)	Tephrosin, Deguelin, Quercetin
Shartarah [26]	Aerial parts of *Fumaria officinale* (Papaveraceae)	Sanguinarine
Eclipta alba [23]	Whole parts of *Eclipta alba* (Compositae)	Ecliptin, Nicotin, Glucoside, Alkaloides
Spirulina [27]	*Spirulina platensis* L. (cyanophyaceae)	C-phycocyanin
Kutki [28]	*Picrorrhiza kurkura* (Scrophulariaceae)	Picroside, Kutkoside, kutkins
Chiretta [29]	Aerial parts of *Swertia chirata* (Gentianaceae)	Alkaloids, Xahthones, Triterpene

Alma khushk [30]	Leaves of *Phyllanthus emblica* (Euphorbiaceae)	Trigalloyl glucose, Tannin
Bael [31]	Leaves & fruits of *Aegle marmelos* (Rutaceae)	Aegelin, Coumarin
Rub anar shirin [32]	Peels of *Punica granatum* (Punicaceae)	Tannin
Maller [33]	Roots of *Rubia cordifolia* (Rubiaceae)	Alizarin derivative
Chobehini [34]	Roots of *Smilax china* (Liliaceae)	Saponin
Bahera [35]	Fruits of *Terminalia bellirica* (Combretaceae)	Tannin
Papita [36]	Roots of *Carica papaya* (Caricaceae)	Papain, Pseudocarpaine
Bahaman surkh [37]	Whole parts of *Salvia plebelia* (Labiatae)	Sage, Volatile oil
Nirgandi [38]	Seeds of *Vitex negundo* (Verbenaceae)	Alkaloids
Tukhm piaz [39]	Seeds and bark of *Allium cepa* (Liliaceae)	Allylsulphide
Tukhn-i-karats [40]	Fruits of *Apium graveolens* (Umbelliferae)	d-Limonene, d-Selinene, Sesquiterpene
Neem [41]	Leaves of *Azadirachta indica* (Meliaceae)	Desacetylnimbin, Nimbasterol, Glycosides
Asafoetida [42]	Fruits of *Ferual asafetida* (Umbelliferae)	Sesquiterpenes, Sulphur-containing volatile oil
Talmakhana [43]	Roots of *Hygrophila spinosa* (Acanthaceae)	Saponin[21]
Bilai kand [44]	Roots of *Ipomoea turpethum* (Convolvulaceae)	Scopoleptin, Betulin, Lupiol & Beta-sitosterol
Karela [45]	Fruits of *Momordica charantia* (Cucurbitaceae)	Cucurbitacins, cucurbitane

Balchhar [42]	Rhizomes of *Nardostachys jatamansi* (Valerianaceae)	Nardus root, Valerian
Tulsi [46]	Leaves of *Ocimum sanctum* (Labiatae)	Volatile oil
Black piper [42, 47]	Fruits of *Piper nigrum*(Piperaceae)	Volatile oil
Zosima Phil. [48]	Flowering plants of *Zosima absinthifolia* (Vent.) Link (Umbelliferae)	Coumarin derivative: (+)-columbianadin and (−)-deltoin and Flavonoid: Quercetin and Kaempferol
Waterleaf [49]	Whole plant of *Talinum triangulare* (Portulacaceae)	Polysaccharides
East Indian Holly Fern [50]	Rhizomes of *Arachniodes exilis* (Hance) Ching (Dryopteridaceae)	Polyphenols, Flavonoids
Prickly ash [51]	Bark of *Zanthoxylum armatum* DC (Rutaceae)	Berberine, Dictamnine, Xanthoplanine, Armatamid, Asarinin, Fargesin, Lupeol, alpha- and beta-Amyrins
Indian Lettuce [52]	Aerial parts of *Lactuca indica* L. (Compositae)	Quinic acid derivatives and Flavonoids
Kataka-taka [53]	Leaves and bark of *Kalanchoe pinnata* Pers. (Crassulaceae)	Bryophyllol, Bryophollone, Bryophollenone, Bryophynol, Phenanthrene derivatives: 2(9-decenyl)-phenanthrene and 2-(undecenyl)-phenanthrene, Oleanane derivative, Taraxasterol derivative, Amyrin derivative, Epiclerosterol and Ergosterol derivative
Black horehound [54]	Flowering plants of *Ballota glandulosissima* Hub.-Mor & Patzak (Lamiaceae)	Diterpenoids: Hispanolone, Ballonigrine, Dehydrohispanolone Flavonoids: Kumatakenin, Pakipodol, 5-hydroxy-7,3',4' trimethoxy flavone, Velutin, Corymbosin, 5-hydroxy-3,7,4'-trimethoxyflavone retusine, 5-hydroxy-7,4'-dimethoxy flavone, Flindulatin, Ladanein
Lychee [55]	Fruits of *Litchi chinensis* Sonn.	Epicatechin Procyanidin, Anthrocyanin, Quercetin 3-rutinoside (rutin), Quercetin glucoside, vitamin C, Isobutyl acetate, Cis-rose oxide, 2-Geraniol, Isovaleric acid, Guaiacol, Vanillin, 2-Acetyl-2-thiazosine and Trans-cinnamic acid
Fumaria species [56]	Whole plan of *F. cilicica* Hausskn., *F. densiflora* DC., *F. kralikii* Jordan	Flavonoid, Phenolic compound

	and *F. parviflora* Lam. (Fumariaceae)	
Dahipalas [57]	Leaves of *Cordia macleodii* (Boraginaceae)	Flavonoids and Triterpenoids
Desert hyacinth [58]	Fresh stems of *Cistanche tubulosa* (Orobanchaceae)	Acylated phenylethanoid oligoglycosides
Spiny Amaranth [59]	*Amaranthus spinosus* Linn. (Amaranthaceae)	alkaloids, flavonoids, phenolic acids, steroids, amino acids, terpenoids, lipids, saponins, Betalains, Beta-sitosterol, Stigmasterol, Linoleic acid, Rutin, Catechuic tannins, Carotenoids Amaranthine, Isoamaranthine, Hydroxycinnamates, Quercetin and Kaempferol glycosides
Jangli amla [60]	Aerial part of *Phyllanthus amarus* Schum. et. Thonn. (PA) (Euphorbiaceae)	Phenolic compounds: Phyllanthin and Hypophyllanthin, Flavonoids: Quercetin and Astragalin; Amarinic acid, Amarin and Phyllanthisiin D
Spade Flower [61]	Whole plant of *Hybanthus enneaspermus* (L.) F. Muell (Violaceae)	Flavanoids: aurantiamide acetate, isoarborinol, b-sitosterol and triterpene
Schouwia thebica webb. [62]	Aerial parts of *Schouwia thebica* webb. (Cruciferae)	chrysoeriol-7-O-xylosoide (1-2)-arabinofuranoside, chrysoeriol, quercetin, quercetin-7-Orhamnoside, and kaempferol-3-O-b-D-glycoside
Hog weed [63]	Leaves of *Boerhaavia diffusa* Linn. (Nynctaginaceae)	Vit-C, Flavonoid: Campesterol, Phenolic compound: Quercetin, Kaemferol and its derivative
Field Milkwort [64]	Leaves of *Polygala arvensis* Willd (Polygalaceae)	Polyarvin, Polygalitol, Rhoifolin
Nirgudi [65]	Leaves of *Vitex negundo* (Verbenaceae)	Iridoid glycoside, Flavonoid, Vi-C, Caroene
Laurustinus [66]	Leaves of *Viburnum tinus* L. (Adoxaceae)	Iridoid glucosides: viburtinoside A and B, Coumarin diglucoside: Scopoletin 7-O-b-D-sophoroside, Dinicotinic acid ester 2,6-di-C-methyl-nicotinic acid 3,5-diethyl ester, Bidesmosidic saponins, Hexamethoxy-flavone, Flavonol glycosides, Suspensolide A and oleanolic acid
Spurred Gentian [67]	whole plant of *Halenia elliptica* (Gentianaceae)	Phenolic compounds: xanthones, Flavonoids
Yellow Autumn crocus [68]	Bulbs of *Sternbergia fisheriana* (Herbert) Rupr. (Amaryllidaceae)	Lycorine

Kakora [69]	Leaves of *Momordica dioica* Roxb.	Flavonoid
Jatropha [23]	Leaves of *Jatropha curcas* Linn (Euphorbiaceae)	Apigenin, Vitexin, Isovitexin, Stigmasterol, α -D-sitosterol and α -D-glucoside
Mangosteen [23]	Fruits of *Garcinia mangostana* Linn. (Guttiferae)	Xanthone: garcinone E, Isoflavones, Tannin and Flavonoids

Discussion

The liver plays an astonishing array of vital functions in the maintenance and performance of the body. Some of these major functions include carbohydrate, protein, and fat metabolism, detoxification, and secretion of bile. Therefore, the maintenance of a healthy liver is vital to overall health and well being. Unfortunately, the liver is often abused by environmental toxins, poor eating habits, alcohol, and prescription and over-the-counter drug use, which can damage and weaken the liver and eventually lead to hepatitis, cirrhosis, and alcoholic liver disease.

Liver damage induced by CCl_4 is a commonly/widely used model for the screening of hepatoprotective drugs [70]. CCl_4 is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical ($CCl_3^{\cdot}$), and this further reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical ($CCl_3OO^{\ddagger}$) by cytochrome P450 $2E_1$ enzyme [71]. These free radicals in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, also disturbs Ca^{2+} homeostasis, and finally result in cell death and the necrosis of hepatocytes [72-74].

Acetaminophen (Paracetamol, N-acetyl-P-aminophenol (APAP)) is one of the most common pharmaceuticals associated with both intentional and accidental poisoning and causes liver failure. Acetaminophen is rapidly absorbed from the stomach and small intestine and metabolized by the conjugation in the liver to non-toxic agents. In acute overdose or when the maximum daily dose is exceeded over a prolonged period, the normal conjugative pathway of metabolism becomes saturated. Excess APAP is then oxidatively metabolized in the liver via the mixed function oxidase P450 system to a toxic metabolite N-acetyl-P-benzoquinoneimine (NAPQI). NAPQI has an extremely short half-life and is rapidly conjugated with glutathione, a sulphydryl donor. Under conditions of excessive NAPQI formation or reduced glutathione store, NAPQI covalently binds to vital proteins and the lipid bilayer of hepatocyte membranes. The result is hepatocellular death and centrilobular liver necrosis [75, 76].

Estimating the activities of serum marker enzymes, like SGOT, SGPT, ALP and total protein, bilirubin can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol, are released in to the blood stream. Generally, SGOT, SGPT, ALP, LPO, bilirubin levels are increased and CAT, SOD, reduced GSH & total protein levels are decreased in case of liver damage. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage which can be confirmed by histopathological study [77].

Popularity of herbal remedies is increasing globally and at least one quarter of patients with liver diseases use ethno botanicals. This approach will help exploring the real therapeutic value of these natural pharmacotherapeutic agents and standardized the dosage regimen on evidence based findings to become more than a fashionable trend. Many herbals are on the market to support health, relieve symptoms and cure diseases. However, most of these products lack scientific pharmacological validation. In experimental hepatotoxicity models in laboratory or higher animals, several herbals exerted hepatoprotective and curative effects that warrants their clinical testing. Due to lack of scientific-based pharmacological data, most of the herbal formulations cannot be recommended for the treatment of liver diseases.

Conclusion

The present study reveals plant extracts with hepatoprotective properties against toxic chemicals that cause liver injury, seeming to validate their use in folk medicine. These plants may offer new alternatives to the limited therapeutic options that exist at present in the treatment of liver diseases or their symptoms, and they should be considered for future studies. The present review suggests that biologically active molecules derived from natural resources especially herbal extracts may serve as suitable primary compounds for effective and targeted hepatoprotective drugs. In this review, effort has been taken to collect and compile the details regarding a few hepatoprotective natural products, which will be useful to the society to venture into a field of alternative systems of medicine.

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