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A Review on Hepatoprotective Plants

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Abstract

Liver is a vital organ play a major role in metabolism and excretion of xenobiotics from the body. Liver injury or liver dysfunction is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. Liver cell injury caused by various toxic chemicals (certain anti-biotic, chemotherapeutic agents, carbon tetrachloride (CCl_4), thioacetamide (TAA) etc.), excessive alcohol consumption and microbes is well studied. The available synthetic drugs to treat liver disorders in this condition also cause further damage to the liver. Hence, Herbal drugs have become increasingly popular and their use is wide spread. Herbal medicines have been used in the treatment of liver diseases for a long time. A number of herbal preparations are available in the market. 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.

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INTRODUCTION:

Liver is one of the vital organs in human body and principal site for enhanced metabolism and excretion. So it has a superior role in maintenance, performing and regulating homeostasis of the body. It involves in almost all biochemical pathways to

growth, fight against disease, nutrient supply, energy production and reproduction[1].

The liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions. It is exposed to xenobiotics, because of its strategic placement in the body. The toxins absorbed from the intestine tract gain access first to the liver, results in a variety of liver ailments. Thus, liver diseases remain one of the serious health problems. Modern medicines have little to offer for improvement in hepatic disease and it is chiefly the plant based preparations which are employed for the treatment of liver disorders.

Natural products are playing a vital role in health care for decades. Often different sources of natural products, plants have been a source of chemical substance, which serves as drugs in their own right or key ingredients in formulation containing synthetic drugs. The selection of the plant species is a crucial factor for the ultimate success of investigation. Through random selection gives some hint, targeted collection based on chemotaxonomic relationships and ethnomedical information derived from Tradition Medicine are more likely to yield pharmacologically active compounds[2].

Although the advances in modern medicines are significant, there remains an ever increasing demand for herbal medicines. Effective and potent herbal medicines require evaluation by standard scientific methods so as to be validated for the treatment of diseases.

Drug induced liver toxicity is major health problem that challenges not only healthcare professionals but also the pharmaceutical industry and also drug regulatory agencies[3]. The inhibition of free radical generation can serve as facile model for evaluating the activity of hepatoprotective agents[4].

HEPATOTOXICITY INDUCING AGENTS:

Several chemicals have been known to induce hepatotoxicity. Carbon tetrachloride (CCl_4), galactosamine, d-galactosamine/lipopolysachharide (GalN/LPS), thioacetamide, antitubercular drugs, paracetamol, arsenic etc., are used to induce experimental hepatotoxicity in laboratory animals.

Carbon tetrachloride (CCl_4):

Liver injury due to CCl_4 in rats was first reported in 1936[5] and has been widely and successfully used by many investigators[6,7]. Carbon tetrachloride is metabolized by cytochrome P-450 in endoplasmic reticulum and mitochondria with the formation of CCl_3O-, a reactive oxidative free radical, which initiates lipid peroxidation[8,9]. Administration of a single dose of CCl_4 to a rat produces, within 24 hrs, a centrilobular necrosis and fatty changes. The poison reaches its maximum concentration in the liver within 3 hrs of administration. Thereafter, the level falls and by 24 hrs there is no CCl_4 left in the liver[10]. The development of necrosis is associated with leakage of hepatic enzymes into serum. Dose of CCl_4 that induces hepatotoxicity ranges from 0.1 to 3 ml/kg administered intraperitoneally.

Thioacetamide:

Thioacetamide interferes with the movement of RNA from the nucleus to cytoplasm which may cause membrane injury. A metabolite of thioacetamide (perhaps S-oxide) is responsible for hepatic injury. Thioacetamide reduce the number of viable hepatocytes as well as rate of oxygen consumption. It also decreases the volume of bile and its content i.e. bile salts, cholic acid and deoxycholic acid. Dose of thioacetamide is 100 mg/kg, subcutaneous[11].

Paracetamol:

Paracetamol, a widely used analgesic and antipyretic drug, produces acute liver damage in high doses. Paracetamol administration causes necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis and eosinophilic cytoplasm followed by large excessive hepatic lesion. The

covalent binding of Nacetyl-P-benzoquinoneimine, an oxidative product of paracetamol to sulphydryl groups of protein, result in lipid peroxidative degradation of glutathione level and thereby, produces cell necrosis in the liver. Dose of Paracetamol is 1 gm/kg Post oral[12].

HEPATOPROTECTIVE PLANTS:

Herbal based therapeutics for liver disorders has been in use in India for a long time and has been popularized world over by leading pharmaceuticals. Despite the significant popularity of several herbal medicines in general, and for liver diseases in particular, they are still unacceptable treatment modalities for liver diseases. The limiting factors that contribute to this eventuality are (i) Lack of standardization of the herbal drugs; (ii) Lack of identification of active ingredient(s)/principle(s); (iii) Lack of randomized controlled clinical trials (RCTs) and (iv) Lack of toxicological evaluation[13]. The use of natural remedies for the treatment of liver diseases has a long history, starting with the Ayurvedic treatment, and extending to the Chinese, European and other systems of traditional medicines. A large number of plants and formulations have been claimed to have hepatoprotective activity. Nearly 160 phytoconstituents from 101 plants have been claimed to possess liver protecting activity. In India, more than 87 plants are used in 33 patented and proprietary multi-ingredient plant formulations[14]. In spite of the tremendous advances made, no significant and safe hepatoprotective agents are available in modern therapeutics. Therefore, due importance has been given globally to develop plant based hepatoprotective drugs effective against a variety of liver disorders. The present review is aimed at compiling data based on reported works on promising phytochemicals from medicinal plants that have been tested in hepatotoxicity models. The hepatoprotective activities of *Andrographis paniculata, Anoectochilus formosanus, Azadirachta indica, Cassia roxburghii*, *Coccinia grandis, Colchicum autumnale*, *Flacourtia indica, Foeniculum vulgare, Indigofera tinctoria*, *Lepidium sativum, Orthosiphon stamineus*, *Prostechea michuacana, Rubia cordifolia, Scutellaria rivularis*, *Solanum nigrum* and *Terminalia catappa*.

Andrographis paniculata

Andrographolide active constituent of *Andrographis paniculata* (Family of Acanthaceae) antagonized the toxic effects of paracetamol on certain enzymes (SGOT, SGPT and ALP) in serum as well as in isolated hepatic cells as tested by trypan blue exclusion and oxygen uptake tests, in a significant dose dependent (0.75-12 mg/kg p.o. x 7days) manner[15]. Neoandrographolide increase GSH, glutathione 5-transferase, glutathione peroxidase, SOD and LPO level[16].

Anoectochilus formosanus

Aqueous Extracts (AFEW-2) of fresh whole plant of *Anoectochilus formosanus* (Family of Orchidaceae) at dose 130 mg/kg showed inhibition of chronic hepatitis (induced by CCl_4) in mice by reducing SGPT and hepatic hydroxyproline level. It also diminished the hypoalbuminemia and splenomegaly. In an *in vitro* study, the LD_{50} values for H_2O_2 induced cytotoxicity in normal liver cells were significantly higher after kinsenoside (isolated from AFEW-2) pretreatment at the dose 20-40 ug/ml[17].

Azadirachta indica

Effect of *Azadirachta indica* leaf (Family of Meliaceae) extract on serum enzyme levels (glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, acid phosphatase and alkaline phosphatase) elevated by paracetamol in rats was studied with a view to observe any possible hepatoprotective effect of this plant. It is stipulated that the extract treated group was protected from hepatic cell damage caused by paracetamol induction. The findings were further confirmed by histopathological study of liver. The antihepatotoxic

action of picroliv seems likely due to an alteration in the biotransformation of the toxic substances resulting in decreased formation of reactive metabolites[18].

Cassia roxburghii

Seeds of *Cassia roxburghii* DC (Family of Fabaceae) had been used in ethnomedicine for various liver disorders for its hepatoprotective activity. The methanolic extract of *Cassia roxburghii* reversed the toxicity produced by ethanol CCl_4 combination in dose dependent manner in rats. The extract at the doses of 250 mg/kg and 500 mg/kg are comparable to the effect produced by Liv-52®, a well established plants based hepatoprotective formulation against hepatotoxins[19].

Coccinia grandis

Alcoholic extract of the fruits of *Coccinia grandis* Linn (Family of Cucurbitaceae) was evaluated in CCl_4 induced hepatotoxicity in rats and levels of AST, ALT, ALP, total proteins, total and direct bilirubin were evaluated. At a dose level of 250 mg/kg, the alcoholic extract significantly ($p<0.05$) decreased the activities of serum enzymes (AST, ALT and ALP) and bilirubin which were comparable to that of silymarin[20] revealing its hepatoprotective effect.

Colchicum autumnale

Colchicine, the major alkaloid in *Colchicum autumnale* (Family of Colchicaceae) protects the liver of experimental animals against several hepatotoxins i.e., D-galactosamine and paracetamol by its ability to bind microtubule protein. A colchicine derivative, trimethylcolchicinic acid (TMCA) that does not bind tubulin i.e., tested on chronic liver damage induced by CCl_4 and by bile duct ligation (BDL). So, both compounds were equally potent but that TMCA could be administered at larger doses than colchicines without side effects and better hepatoprotective actions[21].

Flacourtia indica

The extracts of the aerial parts of *Flacourtia indica* (Burm. F.) Merr. (Family of Salicaceae) were evaluated for hepatoprotective properties. In paracetamol induced hepatic necrosis in rat models, all extracts were found to reduce serum aspartate transaminase (AST), serum alanine transaminase (ALT) and serum alkaline phosphatase (ALP). The most significant reduction of the serum level of AST and ALT were exhibited by petroleum ether and ethyl acetate extracts at a single oral of dose of 1.5g/kg of body weight with a reduction of 29.0% AST and 24.0% ALT level by petroleum ether extract, and 10.57% AST and 6.7% ALT level by ethyl acetate extract compared to paracetamol (3 g/kg of body weight) treated animals. Histopathological examination also showed good recovery of paracetamol induced necrosis by petroleum ether and ethyl acetate extracts. On the other hand, the methanol extract did not show any remarkable effect on paracetamol induced hepatic necrosis. The hepatoprotective effects exhibited by petroleum ether and ethyl acetate extract might be mediated through the inhibition of microsomal drug metabolizing enzymes[22]. But, in this study the dose they have used is too high and it is not successful or rationale for human dose.

Foeniculum vulgare

Foeniculum vulgare Mill. (Family of Umbelliferae) is an annual, biennial or perennial aromatic herb, depending on the variety, which has been known since antiquity in Europe and Asia Minor. The leaves, stalks and seeds (fruits) of the plant are edible. *Foeniculum vulgare* is an aromatic herb whose fruits are oblong, ellipsoid or cylindrical, straight or slightly curved and greenish or yellowish brown in colour[23]. Volatile components of fennel seed extracts by chromatographic analysis include transanethole, fenchone, methylchavicol, limonene, α-pinene, camphene, β-pinene, β-myrcene, α-phellandrene, 3-carene, camphor and cisanethole[24,25]. Hepatoprotective activity of

Foeniculum vulgare (fennel) essential oil was studied using a carbon tetrachloride induced liver fibrosis model in rats. The hepatotoxicity produced by chronic carbon tetrachloride administration was found to be inhibited by *Foeniculum vulgare* essential oil with evidence of decreased levels of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin[26].

Indigofera tinctoria

A bioactive fraction, indigtone (12.5-100mg/kg p.o) characterized as trans-tetracos-15-enoic acid (TCA), obtained by fractionation of a petroleum ether extract of the aerial parts of *Indigofera tinctoria* (Family of Fabaceae), showed significant dose dependent hepatoprotective activity against paracetamol (200mg/kg i.p) and CCl_4 (0.5ml/kg p.o mixed with liquid paraffin 1:1) induced liver injury in rats and mice. Pretreatment reduced Hexobarbitone induced sleep time, and zoxazolamine induced paralysis time. Pre and post treatment reduced levels of transaminases, bilirubin, TG, LPO and restored the depleted GSH in serum[27].

Lepidium sativum

The role hepatoprotective of methanolic extract of *Lepidium sativum* (Family of Brassicaceae) at a dose of 200 and 400 mg/kg was investigated in CCl_4 induced liver damage in rats. Significant reduction in all biochemical parameters were found in groups treated with *Lepidium sativum*. The severe fatty changes in the livers of rats caused by CCl_4 were insignificant in the *Lepidium sativum* treated groups[28].

Orthosiphon stamineus

The hepatoprotective activity of the methanol extract of *Orthosiphon stamineus* (Family of Lamiaceae) was assessed in paracetamol induced hepatotoxicity rat model. Change in the levels of biochemical markers such as AST, ALT, ALP and lipid peroxides were assayed in both paracetamol treated and control (untreated) groups. Treatment with the methanolic extract of *Orthosiphon stamineus* leaves (200 mg/kg) has accelerated the return of the altered levels of biochemical markers to the near normal profile in the dose dependent manner[29].

Prostechea michuacana

Methanol, hexane and chloroform extracts of *Prostechea michuacana* were studied against CCl_4 induced hepatic injury in albino rats. Pretreatment with methanolic extract reduced biochemical markers of hepatic injury levels demonstrated dose dependant reduction in the *in vivo* peroxidation induced by CCl_4. Likewise, pretreatment with extracts of *Prostechea michuacana* on paracetamol induced hepatotoxicity and the possible mechanism involved in this protection were also investigated in rats after administering the extracts of *Prostechea michuacana* at 200, 400 and 600mg/kg. The degree of protection was measured by monitoring the blood biochemical profiles. The methanolic extract of orchid produced significant hepatoprotective effect as reflected by reduction in the increased activity of serum enzymes, and bilirubin. These results suggested that methanolic extract of *Prostechea michuacana* could protect paracetamol induced lipid peroxidetion thereby eliminating the deleterious effects of toxic metabolites of paracetamol. This hepatoprotective activity was comparable with sylmarin. Hexane and chloroform extracts did not show any apparent effect. The findings indicated that the methanolic extract of *Prostechea michuacana* can be a potential source of natural hepatoprotective agent[30].

Rubia cordifolia

Rubiadin isolated from *Rubia cordifolia* Linn, (Family of Rubiaceae) at a dose of 50, 100 and 200 mg/kg was administered orally once daily for 14 days in rats. The substantially elevated serum enzymatic activities of serum GOT, GPT, ALP and GGT; decreased activities of glutathione S-transferase and glutathione reductase were restored

towards normalization in dose dependent manner which were induce by CCl_4 treatment in rats. It also significantly prevents the elevation of hepatic MDA formation and depletion of reduced GSH content in the liver[31].

Scutellaria rivularis

Baicalein, Baicalin and Wogonin three major components isolated from entire plant of *Scutellaria rivularis* Benth (Family of labiatae); Wogonin (5 mg/kg i.p), exhibit best effect in CCl_4 and D-GalN treated rats. Baicalein and Baicalin at the dose 20 mg/kg i.p in D-GalN and APAP; at dose 10 mg/kg i.p in CCl_4 treated rats exhibit best effect. Protective effects were seen by comparing the serum GOT, GPT and histopathologic examination (hepatic lesions)[32].

Solanum nigrum

The effects of *Solanum nigrum* (Family of Solanaceae) extract (SNE) was evaluated on thioacetamide (TAA) induced liver fibrosis in mice. Mice in the three TAA groups were treated daily with distilled water and SNE (0.2 or 1.0 g/kg) via gastrogavage throughout the experimental period. SNE reduced the hepatic hydroxyproline and α-smooth muscle actin protein levels in TAA treated mice. SNE inhibited TAA induced collagen (α1) (I), transforming growth factor-β1 (TGF-β1) and mRNA levels in the liver. Histological examination also confirmed that SNE reduced the degree of fibrosis caused by TAA treatment. Oral administration of SNE significantly reduces TAA induced hepatic fibrosis in mice, probably through the reduction of TGF-β1 secretion[33].

Terminalia catappa

Punicalagin and Punicalin isolated from the leaves of *Terminalia catappa* L. (Family of Combretaceae) reduced hepatitis by reducing levels of AST and ALT which increased by APAP administration in rats[34].

DISCUSSION:

Popularity of herbal remedies is increasing globally and at least one quarter of patients with liver diseases use ethnobotanicals. More efforts need to be directed towards methodological scientific evaluation for their safety and efficacy by subjecting to vigorous preclinical studies followed by clinical trials to unravel the mysteries hidden in the plants. 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/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[35].

CONCLUSION:

In this review article, 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 in to a field of alternative systems of medicine. A more thorough review on various herbal products available in India and abroad as a hepatoprotectant is in near future.

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