Review Article

Diverse Sources of Hepatotoxicity in Rats – A Review

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ABSTRACT

Most of the synthetic drugs available in the market have numerous side effects specifically inducing hepatotoxicity. Recent statistical survey revealed that death toll due to hepatic disorders is increasing in number but there is only limited number of drugs available for the treatment. Currently the researchers across the world are focusing their attention to develop an ideal hepatoprotective agent to treat diseases such as liver cirrhosis, hepatitis B and C infections. Drugs discovered from herbs will give good therapeutic medicine with fewer side effects and lower cost. The virus and reactive oxygen species are the main causes of liver damage, hence an antioxidant may be a useful tool for protecting the liver cells against them. Various *in-vivo* models are available to scrutinize the hepatoprotective potential of the drug on rats. The present review will help to focus on the mechanism responsible for the hepatotoxicity caused by toxicant and its protection by the drug.

Keywords: Antioxidant, Hepatotoxicant, Reactive oxygen species, Herbal drug.

INTRODUCTION

Liver is the largest metabolic organ of the body and is position beneath the diaphragm in the right hypochondrium of the abdominal cavity [1]. It is the major drug-metabolizing and drug-detoxifying organ of the body. It is continuously and widely expose to xenobiotics, hepatotoxins and chemotherapeutic agents that lead to impairment of its functions [2].

Currently, pharmaceutical preparations are serious contributors to liver disease; hepatotoxicity ranking as the most frequent cause for acute liver failure and post commercialization regulatory decisions.

Hepatotoxicity means toxicity to the liver causing damage to hepatic cells. It implies chemical driven hepatic damage associated with impaired liver function caused by exposure to a drug or another agent [3]. Hepatotoxicity may be predictable or unpredictable. Predictable reactions typically are dose related and occur in mostly due to short exposure after some threshold for toxicity was reach. Unpredictable hepatotoxic reactions occur without warning, are unrelated to dose and have variable latency periods, ranging from a few days to one year.

Chemicals that cause liver injury are called as hepatotoxins. More than 900 drugs have been implicated in causing liver injury [4] and it is one of the most common reasons for a drug to be withdrawn from the market [5].

Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. About 75%-80% of blood coming to the liver arrives directly from gastrointestinal organs and then spleen via portal veins which are bring drugs and xenobiotics in concentrated form [6]. Many chemicals damage mitochondria, an intracellular organelle that produces energy. Its dysfunction releases excessive amount of oxidants which in turn damage hepatic cells. Activation of some enzymes in the cytochrome P-450 system, such as CYP2E1, also leads to oxidative stress [7]. Injury to hepatocyte and bile duct cells leads to accumulation of bile acid inside the liver, which promotes further liver damage [8]. Non-parenchymal cells, such as Kupffer cells, fat storing stellate cells and leukocytes (i.e neutrophils and monocyte) also have role in the mechanism.

Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages. Hepatotoxicity is one of very common aliment resulting into serious debilities ranging from severe metabolic disorders to even mortality. Hepatotoxicity in most cases is due to free radical. Free radicals are fundamental to many biochemical processes and represent an essential part of aerobic life and metabolism. Reactive oxygen species mediated oxidative damage to macromolecules such as lipids, proteins and DNA have been implicated in the pathogenecity of major diseases like cancer, rheumatoid arthritis, degeneration process of aging and cardiovascular disease etc. Antioxidants have been reported to prevent oxidative damage caused by free radicals by interfering with the oxidation process through radical scavenging and chelating metal ions [9].

Various factors like plant products, minerals, and some products of bacterial and fungal metabolism lead to hepatotoxicity. Some of the pharmaceutical, chemical products and the waste materials are hepatotoxins, which enter into the human body [10]. Various hepatotoxicant to induced hepatotoxicity are Carbon tetrachloride, Acetaminophen, d-galactosamine, Thioacetamide, Ethanol etc.

Table 1: List of Hepatotoxicant inducing toxicity in rats

Hepatotoxicant	Dose	Route	Treatment period	Vehicle	Plant (as hepatoprotective)
Carbon-tetrachloride	1 ml/kg bw	s.c.	For 7 days	.....	*Jatropha gossypifolia* [38]
	2 ml/kg bw	s.c.	2nd & 3rd day (Five days)	Liquid paraffin	*Gmelina asiatica* [39]
	3 ml/kg bw	s.c.	3rd day (Five days)	Olive oil	*Khaya senegalensis* [40]
	0.1 ml/kg bw	i.p.	10 days	.....	*Pterocarpus marsupium* [41]
	0.5 ml/kg bw	i.p.	4th & 10th day (Ten days exp period)	Olive oil	*Bauhinia purpurea* [42]
	0.7 ml/kg bw	i.p.	2nd & 3rd days (Five days)	Olive oil	*Clitoria ternatea* [43]
	1 ml/kg bw	i.p.	Once in every 72 hrs for 14 days	Liquid paraffin	*Cucurbita maxima* [44]
	2 ml/kg bw	i.p.	1st & 3rd day (Ten days)	Olive oil	*Cissampelos pareira* [45]
	5 ml/kg bw	i.p.	Once	Liquid paraffin	*Senna surattensis* [46]
	3 ml/kg bw	i.m.	First day (Seven days)	.....	*Zingiber chrysanthum* [47]
	1 ml/kg bw	p.o.	Weekly twice for 8 weeks	Liquid paraffin	*Thuja occidentalis* [48]
	2 ml/kg bw	p.o.	3rd & 6th day (Six days)	Liquid paraffin	*Cajanus cajan,* [49]
Ethanol	4 g/kg bw	p.o.	21 days	.....	*Phyllanthus amarus* [50]
Isonaizid	100 mg/kg bw	i.p.	21 days	Distilled water	*Annona squamosa* [51]
Thioacetamide	100 mg/kg bw	s.c.	On last day	Double distilled water	*Hepax-A polyherbal formulation* [52]
	50 mg/kg bw	.....	.....	.....	*Silybum marianum* [53]
Acetaminophen	500 mg/kg bw	p.o.	After every 72 hrs for 10 days	Distilled water	*Asparagus racemosus* [54]
	2 ml/kg bw	i.p.	On 7th day (Seven days)	.....	*Cassia fistula* [55]
	1 g/kg bw	p.o.	Daily once till 7th day	1% cmc sol'n	*Trichosanthes dioica* [56]
	3 g/kg bw	p.o.	9th day (Ten days)	Rice bran oil	*Boswellia serrata* [57]
	2 g/kg bw	p.o.	Once only	Sucrose solution (40%)	*Psidium guajava* [58]
	2 g/kg bw	p.o.	Single dose	DMSO	*Tridax procumbens* [59]
d-galactosamine	400 mg/kg bw	i.p.	14th day (Fourteen days)	.....	*Calotropis gigantean* [60]
Indomethacin	30 mg/kg bw	s.c.	Once only	.....	*Aronia melanocarpa* [61]
Alfatoxin B1	1 mg/kg bw	p.o.	Once (Seven days)	.....	*Crataeva nurvala* [62]
Bromobenzene	460 mg/kg bw	i.p.	Four times at 12 hr interval on final two days (Seven days)	.....	*Alisma orientale* [63]
Cisplatin	45 mg/kg bw	i.p.	3hrs after the plant extract (Four days)	PBS	*Myristica fragrans* [64]
Rifampicin	1 g/kg bw	p.o.	After every 72 hrs for 10 days	Distilled water	*Anisochilus carnosus* [65]

The *in-vivo* hepatoprotective activity was evaluated on the basis of biochemical parameters viz., SGOT, SGPT, serum albumin and total protein levels, alkaline and acid phosphatase levels, and bilirubin levels and histopathology of the liver tissues. All the serum enzymes were analysed using kits available commercially.

A toxic dose or repeated doses of a known hepatotoxin (carbon tetrachloride CCl_4 paracetamol, thioacetamide, alcohol, d-galactosamine, allyl alcohol etc.) might be administered, to induce liver damage in experimental animals. The test substance is administered along with, prior to and/or after the toxin treatment. Liver damage and recovery from damage are assessed by quantifying serum marker enzymes, bilirubin, bile flow, histopathological changes and biochemical changes in liver. An augmented level of liver marker enzymes such as glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT) and alkaline phosphatase in the serum indicates liver damage. Additionally, hepatotoxicity may result in decline of prothrombin synthesis giving an extended prothrombin time and reduction in clearance of certain substances such as bromsulphthalein may used in the assessment of hepatoprotective action of plants [11].

There are various models, which could be use for the screening of hepatoprotective activity of drug components, and different plant extracts. The various hepatotoxins and their mechanism of action are summarized below:

Carbon tetrachloride

One of the potent hepatotoxic agents is CCl_4; it is circulate to all organs because of its high lipid solubility. On ingestion of toxic doses, it causes blockage in the lipoprotein synthesis and produces fat accumulation in the liver because these lipoproteins carry the triglycerides away from this organ. The synthesis of proteins is reduced and there is a rapid decline in the levels of cytochrome

P450 as well as glucose 6-phosphatase and the sequestration of Ca^{2+} ions by Ca^{2+} ATPase is reduced by the endoplasmic reticulum, as a result, there is an increased intracellular Ca^{2+} concentration [12]. CCl_4 is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical (CCl_3*), and this further reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical (CCl_3OO*) [13, 14, 15]. They form covalent bonds with unsaturated fatty acids, or take a hydrogen atom from the unsaturated fatty acids of membrane lipids, resulting in the production of chloroform and lipid radicals. The lipid radicals react with molecular oxygen, which initiates peroxidative decomposition of phospholipids in the endoplasmic reticulum. The peroxidation process results in the release of soluble products that may affect cell membrane. Cell membrane integrity is broken and the enzymes (such as ALT, AST, etc.) in cell plasma leak out and finally result in cell death [16, 17]. Thus, antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4 induced liver injury [18].

Ethanol

Alcohol treatment results in increase in the release of endotoxin from gut bacteria and membrane permeability of the gut to endotoxin, or both. Elevated levels of endotoxin activate kupffer cells to release substances such as eicosanoids, TNF-alpha and free radicals. Prostaglandins increase oxygen uptake and most likely are responsible for the hypermetabolic state in the liver. The increase in oxygen demand leads to hypoxia in the liver, and on reperfusion, alpha-hydroxyethyl free radicals are formed which lead to tissue damage in oxygen poor pericentral regions of the liver lobule [19].

In chronic alcoholics, ethanol produces hepatomegaly. In this case, water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass [20] and also produces significant elevation in TBARS, GSH, SOD, CAT, GPx, Vitamin E and C and reduced Iron and Copper levels indicating all impaired liver function and these parameters have been reported to sensitive indicator of liver injury [21].

Acetaminophen

It is a most commonly used analgesics, it effectively reduces fever and mild-to moderate pain, is considered to be safe at therapeutic doses. However, acetaminophen overdose causes severe hepatotoxicity that leads to liver failure in both humans and experimental animals [22].

Paracetamol produces an experimental damage to the liver cells. The covalent binding of N- n-acetyl-p-benzoquinoneimine, an oxidation product of paracetamol, to sulfhydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity [23].

Paracetamol had mainly metabolized by glucuronide and sulphate conjugation. A small amount of paracetamol is metabolized by cytochrome P-450 to a highly reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI) and is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid [24].

The cells have normally protected from injury by conjugation of this toxic metabolite with glutathione. As the dose of paracetamol increases, the glutathione content of hepatocytes gets exhausted and the hepatocytes become vulnerable to the noxious effects of the metabolite resulting in liver cell necrosis [25].

GSH is one of the most abundant tripeptide present in the liver and its functions are removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals and maintenance of membrane protein thiols and as a substrate for glutathione peroxidase and glutathione-s-transferase. This GSH protects hepatocytes by combining with reactive metabolite of paracetamol thus preventing their covalent binding to liver proteins [26]. NAPQI binds covalently to tissue macromolecules were lead to mitochondrial dysfunction followed by acute hepatic necrosis through lipid peroxidation induced by decreasing GSH in the liver. Damage to the structural integrity of liver is reflected by an increase in the levels of serum transaminases AST, ALT and ALP, because they are cytoplasmic in location and were released into the circulation after cellular damage. Elevation of serum levels of these enzymes are consider as an index of liver damage [27].

Thioacetamide

It is a typical hepatotoxin that causes centrolobular necrosis when interferes with the movement of RNA from the nucleus to the cytoplasm, which may cause membrane injury. A metabolite of TAA (S-oxide) is responsible for hepatic injury [28]. The mechanism behind thioacetamide toxicity is thought to be associated with its metabolite (s-oxide). It interferes with the movement of RNA from the nucleus to cytoplasm that may cause membrane injury. It reduces the number of viable hepatocytes as well as rate of oxygen consumption and decrease the volume of bile and its content, i.e., bile salts, cholic acid, and deoxycholic acid [29].

d-Galactosamine

Exogenous administration of d-galactosamine has been found to induce liver damage, which closely resembles human viral hepatitis [30]. The toxicity of d-galactosamine results from inhibition of RNA and protein synthesis in the liver[31, 32]. The metabolism of d -galactosamine may deplete several uracil nu-cleotides including UDP-glucose, UDP-galactose and UTP [33] which are trapped in the formation of uridine-diphospho-galactosamine. Accumulation of UDP-sugar nucleotides [34] may contribute to the changes in the rough endoplasmic reticulum and to the disturbance of protein metabolism.

Further, intense galactosamination of membrane structures is thought to be responsible for loss in the activity of ionic pumps. The impairment in the calcium

pump, with consequent increase in the intracellular calcium is considered to be responsible for cell death. An evidence of hepatic injury is leakage of cellular enzymes into the plasma. When liver cell plasma membrane is damaged, varieties of enzymes normally located in the cytosols are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage.

Isoniazid

During the metabolism of INH, hydrazine is produced directly (from INH) or indirectly (from acetyl hydrazine). From earlier study it is evident that hydrazine play a role in INH-induced liver damage in rats [35]. The combination of INH and RIF was reported to result in higher rate of inhibition of biliary secretion and an increase in liver cell lipid peroxidation, and cytochrome P450 was thought to be involved the synergistic effect of RIF on INH [36]. However, its role in INH-induced hepatotoxicity is unclarified, as INH itself is an inducer of CYP2E1 [37].

CONCLUSION

Health related problems are on high today. The use of plants, plant-extract or plant derived pure chemicals to treat disease is a therapeutic modality, which has stood the need of time. A lot of herbal drugs and formulations are on the market to support health, relieve symptoms and cure diseases. However, most of these products lack scientific pharmacological validation. More efforts need to be emphasised towards methodological scientific evaluation for their safety and efficacy by subjecting to vigorous pre-clinical studies followed by clinical trials to put forward. This approach will help to evaluate the genuine therapeutic value of these pharmacotherapeutic agents and standardized the dosage regimen on evidence-based findings. In this review, other than a brief introduction about the mechanism of hepatotoxicity, an attempt has been made to compile some reported hepatoprotective plants may be useful to develop evidence based alternative medicine to cure different kinds of liver diseases.

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