A review on liver disorders and screening models of hepatoprotective agents

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INTRODUCTION

LIVER

The liver is a vital organ present in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis, and production of bio chemicals necessary for digestion. The liver is necessary for survival; there is currently no way to compensate for the absence of liver function long term, although liver dialysis can be used short term. This organ plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It lies below the diaphragm in the abdominal-pelvic region of the abdomen. It produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions [1].

The various functions of the liver are carried out by the liver cells or hepatocytes. Currently, there is no artificial organ or device capable of emulating all the functions of the liver. Some functions can be emulated by liver dialysis, an experimental treatment for liver failure.

FUNCTIONS

Various functions of liver are as follows:
- A large part of amino acid synthesis
- The liver performs several roles in carbohydrate metabolism:
  - Gluconeogenesis (the synthesis of glucose from certain amino acids, lactate or glycerol)
  - Glycogenolysis (the breakdown of glycogen into glucose)
  - Glycogenesis (the formation of glycogen from glucose) (muscle tissues can also do this)
- The liver is responsible for the mainstay of protein metabolism, synthesis as well as degradation
- The liver also performs several roles in lipid metabolism:
  - Cholesterol synthesis
  - Lipogenesis, the production of triglycerides (fats).
- The liver produces coagulation factors I (fibrinogen), II (prothrombin), V, VII, IX, X and XI, as well as protein C, protein S and antithrombin.
- In the first trimester foetus, the liver is the main site of red blood cell production. By the 32nd week of gestation, the bone marrow has almost completely taken over that task.
- The liver produces and excretes bile (a yellowish liquid) required for emulsifying fats. Some of the bile drains directly into the duodenum, and some is stored in the gallbladder.
- The liver also produces insulin-like growth factor 1 (IGF-1), a polypeptide protein hormone that plays an important role in childhood growth and continues to have anabolic effects in adults.
- The liver is a major site of thrombopoietin production. Thrombopoietin is a glycoprotein
hormone that regulates the production of platelets by the bone marrow.

- The liver stores a multitude of substances, including glucose (in the form of glycogen), vitamin A (1–2 years’ supply), vitamin D (1–4 months’ supply), vitamin B12 (1-3 years’ supply), iron, and copper.
- The liver is responsible for immunological effects- the reticuloendothelial system of the liver contains many immunologically active cells, acting as a 'sieve' for antigens carried to it via the portal system.
- The liver produces albumin, the major osmolar component of blood serum.
- The liver synthesizes angiotensinogen, a hormone that is responsible for raising the blood pressure when activated by renin, an enzyme that is released when the kidney senses low blood pressure.

**BREAKDOWN OF CHEMICAL CONSTITUENTS**

- The liver helps in the breakdown of insulin and other hormones.
- It breaks down hemoglobin, creating metabolites that are added to bile as pigment (bilirubin and biliverdin).
- It modifies toxic substances (e.g., methylation) and most medicinal products in a process called drug metabolism. This sometimes results in toxicities, when the metabolite is more toxic than its precursor. Preferably, the toxins are conjugated to avail excretion in bile or urine.
- The liver converts ammonia to urea.

**DISORDERS OF LIVER**

Liver disease (also called hepatic disease) is a broad term describing any single number of diseases affecting the liver. The liver supports almost every organ in the body and is vital for survival. Because of its strategic location and multidimensional functions, the liver is also prone to many diseases.²

- Hepatitis, inflammation of the liver, caused mainly by various viruses but also by some poisons (e.g. alcohol), autoimmune (autoimmune hepatitis) or hereditary conditions. Diagnosis is done by checking levels of Alanine transaminase.
- Non-alcoholic fatty liver disease, a spectrum in disease, associated with obesity and characterized as an abundance of fat in the liver, may lead to hepatitis, i.e. steatohepatitis and/or cirrhosis.
- Cirrhosis is the formation of fibrous tissue in the liver from replacing dead liver cells. The death of the liver cells can be caused by viral hepatitis, alcoholism or contact with other liver-toxic chemicals. Diagnosis is done by checking levels of Alanine transaminase and Aspartate transaminase (SGOT).
- Haemochromatosis, a hereditary disease causing the accumulation of iron in the body, eventually leading to liver damage.
- Cancer of the liver (primary hepatocellular carcinoma or cholangiocarcinoma and metastatic cancers, usually from other parts of the gastrointestinal tract).
- Wilson's disease, a hereditary disease which causes the body to retain copper.
- Primary sclerosing cholangitis, an inflammatory disease of the bile duct, likely autoimmune in nature.
- Primary biliary cirrhosis, autoimmune disease of small bile ducts.
- Budd-Chiari syndrome, obstruction of the hepatic vein.
- Gilbert's syndrome, a genetic disorder of bilirubin metabolism, found in about 5% of the population.
- Glycogen storage disease type II, the build-up of glycogen causes progressive muscle weakness (myopathy) throughout the body and affects various body tissues, particularly in the heart, skeletal muscles, liver and nervous system.

There are also many paediatric liver disease, including biliary atresia, alpha-1 antitrypsin deficiency, alagille syndrome, and progressive familial intrahepatic cholestasis, to name but a few.

**Alcohol-induced Liver Diseases**

An alcohol-induced liver disease, as the name implies, is caused by excessive consumption of alcohol and is a common, but preventable, disease. There are three primary types of alcohol-induced liver disease, including the following.

(a) **Fatty liver**

Fatty liver is excessive accumulation of fat inside the liver cells. Fatty liver is the most common alcohol-induced liver disorder. The liver enlarged, causing upper abdominal discomfort on the right side.
(b) Alcoholic hepatitis
Alcoholic hepatitis is an acute inflammation of the liver, accompanied by the destruction of individual liver cell and scarring. Symptoms may include fever, jaundice, an increased white blood cell count, an enlarged, tender liver, and spider-like veins in the skin.

(c) Alcoholic cirrhosis
Alcoholic cirrhosis is the destruction of normal liver tissue, leaving non-functioning scar tissue. Symptoms may include those of alcoholic hepatitis, in addition to portal hypertension, enlarged spleen, ascites, kidney failure, confusion, or liver cancer. Treatment of alcohol induced cirrhosis includes abstinence from alcohol and intake of an adequate wholesome diet.

Symptoms
- Enlarged liver
- Fever
- Jaundice-yellowing of the skin and eyes
- Increased white blood cell count
- Spider-like veins in the skin
- Portal hypertension
- Enlarged spleen
- As cites - fluid build-up in the abdominal cavity
- Kidney failure
- Confusion

Treatment of Alcohol-induced Liver Diseases
The first treatment of alcohol-induced liver disease is cessation of alcohol consumption. This is the only way to reverse liver damage or prevent liver injury from worsening. Without treatment, most patients with alcohol-induced liver damage will develop liver cirrhosis. Other treatment for alcoholic hepatitis includes nutrients that enhance liver function is recommended. These include carnitine, which will help reverse fatty livers, and vitamin C, which is an antioxidant, aids in collagen synthesis, and increases the production of neurotransmitters such as nor epinephrine and serotonin, as well as supplementing with the nutrients that have been depleted due to the alcohol consumption. Eliminating any food that may be manifesting as intolerance and alkalizing the body is also important. There are some supplements that are recommended to help reduce cravings for alcohol, including choline, glutamine, and vitamin C. As research shows glucose increases the toxicity of centrilobular hepatotoxicants by inhibiting cell division and repair, it is suggested fatty acids are used by the liver instead of glucose as a fuel source to aid in repair; thus, it is recommended the patient consumes a diet high in protein and essential fatty acids, e.g. omega-3. Cessation of alcohol consumption and cigarette smoking, and increasing exercise are lifestyle recommendations to decrease the risk of liver disease caused by alcoholic stress.

(ii) Drugs
Abstinence from alcohol intake and nutritional modification form the backbone in the management of alcohol liver diseases. Symptom treatment can include: corticosteroids for severe cases, anticytokines (infliximab and pentoxifylline), propylthiouracil to modify metabolism and colchicine to inhibit hepatic fibrosis.

(iii) Antioxidants
It is widely believed that alcohol-induced liver damage occurs via generation of oxidants Thus alternative health care practitioners routinely recommend natural antioxidant supplements like milk thistle. Unfortunately, there is no valid clinical data to show that milk thistle truly works.

(iv) Transplant
When all else fails and the liver is severely damaged, the only alternative is a liver transplant. While this is a viable option, liver transplant donors are scarce and usually there is a long waiting list in any given hospital. One of the criteria to become eligible for a liver transplant is to discontinue alcohol consumption for a minimum of six months.

Chronic Liver Disease/cirrhosis
Chronic liver disease is marked by the gradual destruction of liver tissue over time. Several liver
Disease falls under this category, including the following:
(i) Cirrhosis of the liver
(ii) Fibrosis of the liver

Cirrhosis is the eighth leading cause of death in the United States, according to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Because of chronic damage to the liver, scar tissue slowly replaces normal functioning liver tissue, progressively diminishing blood flow through the liver. As the normal liver tissue is lost, nutrients, hormones, drugs, and poisons are not processed effectively by the liver. In addition, protein production and other substances produced by the liver and inhibited.

Fibrosis is the growth of scar tissue due to infection, inflammation injury, or even healing, the over growth of scar tissue can occur in almost any organ. Fibrosis in the liver can inhibit the organ’s proper functioning.

**Treatment of Liver Cirrhosis**
Treatment depends on the type and stage of the cirrhosis. Its aim is to stop the progress of the cirrhosis, reversing (to whatever extent possible) the damage that has already occurred, and treating complications that are disabling or life-threatening. Stopping or reversing the process requires removal of the cause, as in the following cases:
- In cirrhosis caused by viral hepatitis: use of experimental approaches that include the use of drugs to improve immune responses to viral infection (interferon) or to help destroy the virus (anti-viral compounds).
- In certain types of cirrhosis caused by chronic hepatitis: corticosteroids are indicated.
- In cirrhotic patients with jaundice: use of supplemental fat soluble vitamins may be helpful.
- In Wilson’s disease: removal of excessive copper.
- In hemochromatosis: removal of excess iron.
- For patients with severe cirrhosis, a liver transplant may be life-saving.

**Congenital Liver Defects**
Defects of the liver at birth usually affect the bile ducts. Though rare, some congenital liver defects include the following:

- Biliary Artesia—a condition in which the bile ducts are absent or have developed abnormally
- Choledochal cyst—a malformation of the hepatic duct that can obstruct flow of bile in infants.

Congenital liver defects that affect the flow of bile share some common symptoms. The following are the most common symptoms of congenital liver defect. However, each individual may experience symptoms differently. Symptoms may include:
- Jaundice-yellowing of the skin and eyes.
- Dark urine
- Pale stool

The symptoms of congenital liver defects may resemble other medical conditions or problems. Always consult the physician for a diagnosis.

**Viruses induced liver diseases**
Hepatitis is the inflammation of the liver, resulting in the liver cell damaged and destruction. In autoimmune hepatitis, the body’s own immune system destroys the cells of the liver. It is a chronic inflammatory liver disease with no known cause. It is associated with a disorder called hyper gammaglobulinemia. Hyper gamma globulinemia is a disorder where there are too many circulating protein antibodies in the blood. A chronic infection or certain malignant blood diseases may cause hyper gamma globulinemia. Autoimmune hepatitis may resolve without the treatment in some individual, but, for the majority of individuals, it is chronic and can lead to cirrhosis and liver failure. Autoimmune hepatitis may be classified as type 1 or type 2. Type 1 (classic) is the most common form. It may occur at any age but usually affects young women more than men. Also, other autoimmune disorder can be associated with type 1 such as thyroiditis, Graves’s disease, and ulcerative colitis. Type 2 generally affects girls between the ages of 2-14, but does occur in adults.

**Symptoms**
The following are the most common symptoms of autoimmune hepatitis. However, each individual may experience symptoms differently. Symptoms may include:
- Jaundice – yellowing of the skin and eyes.
- Fatigue
- Abdominal pain
- Severe acne
- Joint pain
- Joint swelling
Cessation of menses
Chest pain
Diarrhea
Fever
Large abdomen due to large liver and spleen
Spider-like blood vessels in the skin
Ascites-fluid build-up in the abdominal cavity

Viruses induced hepatitis
There are six main types of the hepatitis virus that have been identified, including the following:

Hepatitis A
This type of hepatitis is usually spread by fecal-oral contact, or fecal-infected food and water, and may also be spread by blood-borne infection (which is rare). The following is a list of modes of transmission for hepatitis A:
- Consuming food made by someone who touched infected faces
- Drinking water that is contaminated by infected feces (a problem)
- Drinking water that is contaminated by infected feces (a problem in developing countries with poor sewage removal)
- Touching an infected person’s feces, which may occur with poor hand washing
- Outbreaks may occur in large childcare centers, especially when there are children in diapers
- Residents of American Indian reservations or Native Alaskan villages where hepatitis A may be more common
- Sexual contact with an infected person. A vaccine for hepatitis A has been developed and is now available.

Hepatitis B
Hepatitis B has a wide range of clinical presentations. It can be mild, without symptoms, or it may cause chronic hepatitis and, in some cases, can lead to full-blown liver failure and death. Transmission of hepatitis B virus occurs through blood and body fluid exposure such as blood, semen, vaginal secretion, or saliva. Infants may also develop the disease if they are born to a mother who has the virus. Infected children often spread the virus to other children if there is frequent contact or a child has many scrapes or cuts. The following describes persons who are at risk for developing hepatitis B:
- Children born to mother who have hepatitis B (the illness may present up to five years after the child is born)
- Children who are to mother who have immigrated from a country where hepatitis B is widespread such as southeast Asia and China
- Persons who live in long-term care facilities or who are disabled
- Persons who live in households where another member is infected with the virus
- Persons who have a blood clotting disorder such as hemophilia
- Persons who require dialysis for kidney failure
- Persons who may participate in high-risk activities such as intravenous (IV) drug use and/or unprotected heterosexual or homosexual sexual contact
- Persons who have a job that involves contact with human blood
- Persons who received blood transfusions or blood products before the early 1990s
- A vaccine for hepatitis B does exist and is now widely used for routine childhood immunization. The centers for Disease control and prevention (CDC) now recommend that universal infant hepatitis B vaccination should begin at birth except in rare circumstances.

Hepatitis C
The symptoms of hepatitis C are usually mild and gradual. Children often show no symptoms at all. Transmission of hepatitis C occurs primarily from contact with infected blood, but can also occur from sexual contact or from an infected mother to her baby. Although hepatitis C has milder symptoms initially, it leads to chronic liver disease in a majority of people who are infected. According to the Centre for Disease Control and Prevention (CDC), hepatitis C is the leading indication for liver transplantation. With some cases of hepatitis C, no mode of transmission can be identified. The following describes persons who may be at risk for contracting hepatitis C:
- Children are born to mothers who are infected with the virus.
- Persons who have a blood clotting disorders such as haemophilia and received clotting factors before 1987.
- Persons who require dialysis for kidney failure.
Individuals who received a blood transfusion before 1992.

Persons may participate in high-risk activities such as intravenous drug use and/or homosexual contact.

There is no vaccine for hepatitis C. Persons who are at risk should be checked regularly for signs of chronic hepatitis and failure.

**Hepatitis D**

This form of hepatitis can only occur in the presence of hepatitis B. If an individual has hepatitis B and does not show symptoms, or shows very mild symptoms, infection with D can put that person at risk for full-blown liver failure that progresses rapidly. Hepatitis D can occur at the same time as the initial infection with B, or it may show up much later. Transmission of hepatitis D occurs the same way as hepatitis B, except the transmission from mother to baby is common.

**Hepatitis E**

This form of hepatitis is similar to hepatitis A. transmission occurs through faecal oral contamination. It is a less common than hepatitis A. Hepatitis E is most common in poorly developed countries and rarely seen in the United States. There is no vaccine for hepatitis E this time.

**Hepatitis G**

This is the newest strain of hepatitis and very little is known about it. Transmission is believed to occur through blood and is most commonly seen in drug users, individuals with clotting disorders such as haemophilia, and individuals who require haemodialysis for renal failure. Often hepatitis G shows no clinical symptoms and has not been found to be a cause of acute or chronic hepatitis.

**Drug induced hepatitis**

Hepatitis is the inflammation of the liver resulting in liver cell damage and destruction. Drug hepatitis is rare and caused by toxic exposure to certain medications, vitamins, herbal remedies or food supplement. Usually, the toxicity occurs often taking the causative agent for several months, or from an over dose of a medication such as acetaminophen. Usually if the agent is discontinued once hepatitis is suspected and is rarely restarted unless it is absolutely essential for treatment.

**DISEASE SIGNS**

The classic signs of liver damage include the following:

- Pale stools occur when stercobilin, a brown pigment, is absent from the stool. Stercobilin is derived from bilirubin metabolites produced in the liver.
- Dark urine occurs when bilirubin mixes with urine.
- Bilirubin, when it deposits in skin, causes an intense itch. Itching is the most common complaint by people who have liver failure. Often this itch cannot be relieved by drugs.
- Swelling of the abdomen, ankles and feet occurs because the liver fails to make albumin.
- Excessive fatigue occurs from a generalized loss of nutrients, minerals and vitamins.
- Bruising and easy bleeding are other features of liver disease. The liver makes substances which help prevent bleeding. When liver damage occurs, these substances are no longer present and severe bleeding can occur.\(^3\)

**DIAGNOSIS**

The diagnosis of liver function is made by blood tests. Liver function tests can readily pinpoint the extent of liver damage. If infection is suspected, then other serological tests are done. Sometimes, one may require an ultrasound or a CT scan to produce an image of the liver. Liver diseases may be diagnosed by liver function tests, for example, by production of proteins. Physical examination of the liver is not accurate in determining the extent of liver damage. It can only reveal presence of tenderness or the size of liver, but in all cases, some type of radiological study is required to examine it.\(^4\)

**HEPATOTOXICITY**

Chemicals that cause liver injury are called hepatotoxins. The predominant type of liver disease varies according to country and may be influenced by local factors. The causative factors of liver disorders include virus infection, exposure to or consumption of certain chemicals. The substance that injures the liver cells in some people and results serious harm to the liver caused by drugs and by the combination of drugs and other substances is an important health problem. Among various inorganic compounds producing hepatotoxicity are arsenic, phosphorous, copper.
and iron. The organic agents include certain naturally-occurring plant toxins such as pyrrolizidine alkaloids, mycotoxins and bacterial toxins. The synthetic groups of organic compounds are a large number of medicinal agents. In addition, exposure to hepatotoxic compounds may be occupational, environmental or domestic that could be accidental, homicidal or suicidal ingestion. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Chemicals often cause sub clinical injury to liver which manifests only as abnormal liver enzyme tests. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures.

**LIST OF HEPATOTOXIC COMPOUNDS**

Various chemical agents that are hepatotoxic are as follows

### Table 1. CHEMICAL AGENTS

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INORGANIC AGENTS</strong></td>
<td>Metals and metalloids: antimony, arsenic, beryllium, bismuth, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, gold, phosphorous, selenium, tellurium, thallium, zinc, hydrazine derivative, iodides&lt;sup&gt;[5, 6]&lt;/sup&gt;.</td>
</tr>
<tr>
<td><strong>ORGANIC AGENTS</strong></td>
<td>Natural : Plant toxins Albitocin, cycasin, nutmeg, tannic acid, icterogenin, pyrrolidizines, saferole, indospicine.</td>
</tr>
<tr>
<td></td>
<td>Mycotoxins: Aflatoxins, cyclochlorotine, ethanol, luteoskyrin, griseofulvin, sporidesmin, tetracycline, and other antibiotics.</td>
</tr>
<tr>
<td></td>
<td>Bacterial toxins: Exotoxins (C.diphtheria, Clostridium botulinus), endotoxins, ethionine.</td>
</tr>
<tr>
<td></td>
<td>Synthetic: Non-medicinal compound, organic amines, Azo compounds. Phenol and derivatives, various other organic compounds</td>
</tr>
</tbody>
</table>

Various medicinal agents that are hepatotoxic are listed as follows:

### Table 2. MEDICINAL AGENTS

<table>
<thead>
<tr>
<th>Category of drugs</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Neuropsychotropics</td>
<td>Hydrazine, tranylcypromine anticonvulsants, antidepressants.</td>
</tr>
<tr>
<td>2) Anti-inflammatory and anti-muscle spasm agents</td>
<td>Cinchopen, cholchicine, ibuprofen, salicylates, indomethacin.</td>
</tr>
<tr>
<td>3) Hormonal derivatives and other drugs used in endocrine disease</td>
<td>Acetohexamide, Azepinamide, Carbutamide, Tolbutamide.</td>
</tr>
<tr>
<td>4) Antimicrobials</td>
<td>Clindamycin, novobiocin, penicillin, tetracycline, sulfonamide, amodiaquine, isoniazid, rifampin.</td>
</tr>
<tr>
<td>5) Antineoplastic</td>
<td>L-Asparaginase, azacytidine, methotrexate, 6-mercaptopurine, chlorambucil, clavicin&lt;sup&gt;[7]&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Various hepatotoxins can be classified according to their mechanism of action as follows:
Table 3. CLASSIFICATION OF HEPATOTOXINS AND MECHANISM OF ACTION OF EACH GROUP

<table>
<thead>
<tr>
<th>Category of agents</th>
<th>Mechanism of action</th>
<th>Histological lesion</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intrinsic Toxicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct</td>
<td>Direct Physiochemical destruction by peroxidation of hepatocytes.</td>
<td>Necrosis and/or steatosis</td>
<td>CCL₄, phosphorus</td>
</tr>
<tr>
<td>Indirect cytotoxic</td>
<td>Interference with hepatocellular metabolic pathways</td>
<td>Steatosis or necrosis</td>
<td>Ethionine, ethyl alcohol, tetracycline[⁸]</td>
</tr>
<tr>
<td>Cholestatic</td>
<td>Interference with bile excretory pathways</td>
<td>Cholestasis duel destruction</td>
<td>Methylene dianiline, anabolic and contraceptive steroids[⁹]</td>
</tr>
<tr>
<td><strong>Host Idiosyncrasy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>Drug allergy</td>
<td>Necrosis or cholestasis</td>
<td>Chlorpromazine, phenytoin, sulfonamides.</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Production of hepatotoxic metabolites</td>
<td>Necrosis or cholestasis</td>
<td>Isoniazid, valproic acid [¹⁰]</td>
</tr>
</tbody>
</table>

Table 4: PATTERNS OF DRUG INDUCED CELL INJURY

<table>
<thead>
<tr>
<th>Type of injury:</th>
<th>Hepatocellular</th>
<th>Homeostatic</th>
<th>Mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>≥ Twofold rise</td>
<td>Normal</td>
<td>≥ Twofold rise</td>
</tr>
<tr>
<td>ALP</td>
<td>Normal</td>
<td>≥ Twofold rise</td>
<td>≥ Twofold rise</td>
</tr>
<tr>
<td>ALT: ALP ratio</td>
<td>High, ≥5</td>
<td>Low, ≤2</td>
<td>2-5</td>
</tr>
</tbody>
</table>

**Examples** [¹¹]
- Acetaminophen
- Allopurinol
- Amiodarone
- HAART
- NSAID
- Anabolic steroid
- Chlorpromazine
- Clopidogrel
- Erythromycin
- Hormonal contraception

Chemicals produce a wide variety of clinical and pathological hepatic injury. Biochemical markers (e.g. alanine transferase, alkaline phosphatase and bilirubin) are often used to indicate liver damage.

Liver injury is defined as rise in either
(a) ALT level more than three times of upper limit of normal (ULN),
(b) ALP level more than twice ULN, or
(c) Total bilirubin level more than twice ULN when associated with increased ALT or ALP [¹²]

Liver damage is further characterized into hepatocellular (predominantly initial Alanine transferase elevation) and cholestatic (initial alkaline phosphatase rise) types. However they are not mutually exclusive and mixed type of injuries are often encountered. Specific histo-pathological patterns of liver injury from drug induced damage are discussed below

**Zonal Necrosis**
This is the most common type of drug induced liver cell necrosis where the injury is largely confined to a particular zone of the liver lobule. It may manifest as very high level of ALT and severe disturbance of liver function leading to acute liver failure. Causes include: Paracetamol, carbon tetrachloride
Hepatitis
In this pattern hepatocellular necrosis is associated with infiltration of inflammatory cells. There can be three types of drug induced hepatitis:
(A) Viral hepatitis type picture is the commonest, where histological features are similar to acute viral hepatitis.
(B) In the focal or non specific hepatitis scattered foci of cell necrosis may accompany lymphocyte infiltrate.
(C) Chronic hepatitis type is very similar to autoimmune hepatitis clinically, serologically as well as histologically.

Causes
(a) Viral hepatitis like: Halothane, isoniazid, phenytoin
(b) Focal hepatitis: Aspirin
(c) Chronic hepatitis: Methyldopa, diclofenac

Cholestasis
Liver injury leads to impairment of bile flow and clinical picture is predominated by itching and jaundice. Histology may show inflammation (cholestatic hepatitis) or it can be bland without any parenchyma inflammation. In rare occasions it can produce features similar to primary biliary cirrhosis due to progressive destruction of small bile ducts (Vanishing duct syndrome).

Causes:
(a) Bland: Oral contraceptive pills, anabolic steroid, androgens
(b) Inflammatory: Allopurinol, co-amoxiclav, carbamazepine
(c) Ductal: Chlorpromazine, flucloxacillin

Steatosis
Hepatotoxicity may manifest as triglyceride accumulation which leads to either small droplet (micro vesicular) or large droplet (macro vesicular) fatty liver. There is a separate type of steatosis where phospholipids accumulation leads to a pattern similar to the diseases with inherited phospholipids metabolism defects (e.g. Tay-Sachs disease)

Causes
(a) Micro vesicular: Aspirin (Reye's syndrome), ketoprofen, tetracycline
(b) Macro vesicular: Acetaminophens, methotrexate
(c) Phospholipidosis: Amiodarone, total parenteral nutrition

Granola
Drug induced hepatic granulomas are usually associated with granulomas in other tissues and patients typically have features of systemic vasculitis and hypersensitivity. More than 50 drugs have been implicated.

Causes: Allopurinol, phenytoin, isoniazid, quinine, penicillin, quinidine

Vascular lesions
They result from injury to the vascular endothelium.

Causes: Venoocclusive disease: Chemotherapeutic agents, bush tea
Peliosishepatis: anabolic steroid
Hepatic vein thrombosis: Oral contraceptives

Neoplasm
Neoplasms have been described with prolonged exposure to some medications or toxins. Hepatocellular carcinoma, angiosarcoma and liver adenomas are the ones usually reported.

Causes: Vinyl chloride, combined oral contraceptive pill, anabolic steroid, arsenic, thorotrast

SCREENING MODELS FOR HEPATOTOXIC STUDIES

Various models needed for the screening of hepatoprotectives can be classified as follows:
• In vivo models
• In vitro studies

These are briefly described as:

(a) In vivo models
(i) Toxic Chemicals-induced liver damage
A toxic dose or repeated doses of a known hepatotoxin (carbon tetrachloride, paracetamol, thioacetamide, isoniazid, alcohol, D-galactosamine, allylalcohol, etc.) is administrated, to induce liver damage in experimental animals. The test substance is administered along with, prior to and/or after the toxin treatment. If the hepatotoxicity is prevented or reduced the test substance is effective.
Liver damage and recovery from damage are assessed by measuring serum marker enzymes, bilirubin, histopathological changes in the liver, biochemical changes in liver (E.g.: hydroxyproline, lipid etc.) and bile flow. When liver is damaged
liver enzymes such as glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT) and alkaline phosphatase enter into the circulation. An increase in the levels of these marker enzymes in the serum is an indication of liver damage. Other effects of induced liver damage such as reduction of prothrombin synthesis giving an extended prothrombin time and reduction in clearance of certain substances such as bromsulphthalein can be used in the evaluation of hepatoprotective plants. The hepatoprotective effect of a drug against different hepatotoxins differs especially when the mechanism of action of toxins are different. Therefore, the efficacy of each drug has to be tested against hepatotoxin which acts by different methods [13].

ACETAMINOPHEN-INDUCED HEPATOTOXICITY [14]
The analgesic acetaminophen causes a potentially fatal, hepatic centrilobular necrosis when taken in overdose. The initial phases of toxicity indicated that acetaminophen was metabolically activated by cytochrome P450 enzymes to a reactive metabolite that depleted glutathione (GSH) and covalently bound to protein. It was shown that repletion of GSH prevented the toxicity. This finding led to the development of the currently used antidote N-acetylcysteine. The reactive metabolite was subsequently identified to be N-acetyl-p-benzoquinone imine (NAPQI) [15]. It is detoxified by glutathione (GSH) to form an acetaminophen-GSH conjugate. After a toxic dose of acetaminophen, total hepatic GSH is depleted by as much as 90%, and as a result, the metabolite covalently binds to cysteine groups on protein, forming acetaminophen-protein adducts. This mechanism is shown below

![Schematic representation depicting the role of metabolism in acetaminophen toxicity](image-url)

**Oxidative stress**
Oxidative stress is another mechanism that has been postulated to be important in the development of acetaminophen toxicity. Thus, increased formation of superoxide would lead to hydrogen peroxide and peroxidation reactions by Fenton-type mechanisms. It has been shown that NAPQI reacts very rapidly with GSH \((k_1 = 3.2 \times 10^4 \text{ M}^{-1} \text{s}^{-1} \text{ at pH } 7.0)\) [16], and there are a number of potential mechanisms that have been suggested to play a role. Under conditions of NAPQI formation following toxic acetaminophen doses, GSH concentrations may be very low in the centrilobular cells, and the major peroxide detoxification enzyme, GSH peroxidase, which functions very inefficiently under conditions of GSH depletion [17], is expected to be inhibited. In addition, during formation of NAPQI by cytochrome P450, the superoxide anion is formed, with dismutation leading to hydrogen peroxide formation [18]. Also, it has been suggested that peroxidation of acetaminophen to the semi Quinone free radical would lead to redox cycling between the acetaminophen and the semi Quinone. This mechanism may lead to increased superoxide and toxicity [19]. However, it was found that the semi Quinone reacted rapidly to form polymers and no evidence for reaction of oxygen was observed [20]. A significant amount of evidence has pointed to the potential involvement of oxidative stress in
Acetaminophen toxicity. Nakae et al. (1990) reported that administration of encapsulated superoxide dismutase decreased the toxicity of acetaminophen in the rat. Moreover, the iron chelator, deferoxamine, has been shown to decrease toxicity in rats [21]. It was showed that deferoxamine caused a delay in the rate of development of acetaminophen toxicity in mice, but after 24 h, the relative amount of toxicity was not affected [22].

**Role of Kupffer Cells**

Several laboratories have studied the role of macrophage activation [22] in acetaminophen toxicity. Kupffer cells are the phagocytic macrophages of the liver. When activated, Kupffer cells release numerous signalling molecules, including hydrolytic enzymes, eicosanoids, nitric oxide, and superoxide. Kupffer cells may also release a number of inflammatory cytokines, including IL-1, IL-6, and TNF-α and multiple cytokines are released in acetaminophen toxicity. Hogaboam et al., (1999a, 2000) Bourdi et al., (2002a, b) Laskin et al. (1995) examined the role of Kupffer cells in acetaminophen hepatotoxicity by pre-treating rats with compounds that suppress Kupffer cell function (gadolinium chloride and dextran sulphate). These investigators reported that rats pre-treated with these compounds were less sensitive to the toxic effects of acetaminophen.

Similar effects were reported in the mouse. Goldin et al. (1996) [23] showed that treatment of mice with liposome’s containing dichloro methylene di phosphonate to knock out the Kupffer cells also decreased acetaminophen toxicity. Similarly, pre-treatment of mice with gadolinium chloride or dextran sulphate decreased the toxic effects of acetaminophen [24]. These studies suggested a critical role for Kupffer cells in the development of acetaminophen hepatotoxicity.

**CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY**

Toxic injury of the liver induced by carbon tetrachloride is a model system of hepatotoxicity. Injury produced by CCl₄ seems to be mediated by reactive metabolite – trichloromethyl free radical (CCl₃) formed by the hemolytic cleavage of CCl₄ or by an even more reactive species trichloromethylperoxy free radical (Cl₃ COO) formed by the reaction of CCl₃ with O₂. This biotransformation is catalyzed by a cytochrome p450 dependent monoxygenase. CCl₄ is reductively converted by P450 to the trichloromethyl radical the fate of this radical is of interest. First the radical add covalently to unsaturated fatty acids, trichloromethyl fatty acids, particularly of membrane phospholipids.

Recently these substituted fatty acids have been noted to be partially resistant to replace from endoplasmic reticular phospholipase A₂. This seems to be result of cross linking of trichloromethyl fatty acid radical, which adds to double bond of other adjacent fatty acids (link et. al). The physiologic significance of this cross linking on membrane structure and function may be of great importance, particularly if these phospholipids are transformed to other critical sites in the cell. Besides covalent binding to lipid, the cells can abstract an electron from unsaturated fatty acids, yielding CHCl₂ and or fatty acid radical. Either the trichloromethyl fatty acid radical or the fatty acid radical can react with oxygen to form peroxy radical, which initiates the lipid peroxidation chain reaction.

The hepatoprotective study was carried out in adult male Wistar rats (150-175 g). They were housed in clean polypropylene cages and fed with commercial rat chow and water ad libitum.
A total of 30 animals were equally divided into 5 groups: Group I (normal control) and Group II (CCl4-treated control) were given 0.9% saline (5 ml/kg, b.w.) for 9 days. Group III and Group IV were pre-treated with AV (100 and 200 mg/kg, p.o., respectively) for 9 days, while Group V was pre-treated with silymarin (25 mg/kg, p.o.) for 9 days. Liver damage was induced in these rats, except Group I, with 1:1 (v/v) mixture of CCl4 and olive oil (1ml/kg, s.c.) at Day 7 while, olive oil (0.5 ml/kg, s.c.) was injected to Group I. The doses of the test drug were selected on the basis of earlier work in our laboratory.9 After 48 h of CCl4 treatment, i.e., on the 9th day the animals were sacrificed under anesthesia and blood was collected for the assay of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase(SGPT) and serum alkaline phosphatase (ALP).25

THIOACETAMIDE INDUCED HEPATO TOXICITY26

The ameliorative activity of aqueous extract of the flesh of dates (Phoenix dactylifera L.) and ascorbic acid on thioacetamide-induced hepatotoxicity was studied in rats. Sixty male rats were divided into six equal groups of 10. Two groups were controls, one treated with thioacetamide and one with only distilled water. Two groups received extract of flesh Phoenix dactylifera and intraperitoneal (IP) thioacetamide (400 mg/kg) either before or after administration of flesh extract. Two groups received ascorbic acid and intraperitoneal (IP) thioacetamide (400 mg/kg b.w.t.) either before or after administration of ascorbic acid. Treatment with aqueous extract of date flesh or by ascorbic acid significantly reduced thioacetamide-induced elevation in plasma bilirubin concentration and enzymes.

Thioacetamide (TA), originally used as a fungicide, is a potent hepatotoxin which has been much studied since the first report of its toxic properties.27-32 Earlier literature33-34 suggests that an obligatory intermediate metabolite of TA that binds to proteins with the formation of acetylimidolysine derivatives is responsible for TA-induced hepatotoxic effects (Fig. 1). TA stimulates DNA synthesis and mitosis in the liver of rats at doses that produce limited necrosis36-37. The rise in DNA synthesis induced by TA follows a time sequence, which is similar to that seen after partial hepatectomy38. Rats treated with 50 mg TA/kg body weight undergo hepatocellular proliferation as suggested by 3H-thymidine (3H-T) incorporation, which peaks 36 hr after administration.
Isoniazid and its metabolites acetyl isoniazid, hydrazine and monoacetylhydrazine were investigated for generation of oxygen free radicals during incubation with rat liver slices. Lipid peroxidation was assessed by the thiobarbituric acid reactive substances test using malonaldehyde as the external standard, while hepatotoxicity was assessed by histopathology studies. Malonaldehyde formed in liver slices after 10 hours of incubation with the drugs was 1.28 ± 0.24 nmol/mg for isoniazid (control 1.12 ± 0.17 nmol/mg); 0.88 ± 0.45 nmol/mg for acetyl isoniazid (control 0.84 ± 0.42 nmol/mg); 1.43 ± 0.14 nmol/mg for monoacetylhydrazine (control 1.10 ± 0.12 nmol/mg) and 1.36 ± 0.02 nmol/mg for hydrazine (control 1.13 ± 0.04 nmol/mg). Histologically, all slices exhibited hepatic necrosis by 4 hours. However, hydrazine-induced hepatotoxicity was characterized by nuclear hyperchromasia, karyolysis and karyohexis while monoacetylhydrazine exhibited hydropickaryomegaly only. Isoniazid and acetylisoniazid cytotoxicity exhibited a mixture of the above features such that it could be attributed to the two metabolites, hydrazine and monoacetylhydrazine. In conclusion, there was no evidence implicating oxygen free radicals in isoniazid-induced hepatotoxicity\(\text{[40]}\).

(ii) Anti-hepatitis Virus activity
At present, simple in vivo test systems are not available to determine anti-hepatitis virus activity in rodent models. However, duck and monkey models have been introduced to test anti-hepatitis B activity\(\text{[42]}\).

(iii) Choleretic activity
Techniques are available to collect bile by cannulising the bile duct, in anaesthetised as well as conscious animals, to study the effect of drugs on the secretion.

(iv) Regeneration of hepatocytes
The effect of drugs on hepatocytes regeneration can be tested by surgical removal of a portion of the liver in experimental animals.

(b) In vitro studies
Fresh hepatocyte preparations and primary cultured hepatocytes are used to study direct anti-hepatotoxic activity of drugs. Hepatocytes are treated with hepatotoxic and the effect of the plant drug on the same is evaluated. The activities of the transaminases released into the medium are determined. An increase in the activity in the medium indicates liver damage. Parameters such as hepatocyte multiplication, morphology, macromolecular synthesis and oxygen consumption are determined.
REFERENCES


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