TOXIC RESPONSE OF THE LIVER

Objectives:

- Understand the liver injury.
- Classify the types of liver injury.
- Describes the mechanisms of the liver injury.
- Determines the hepatotoxic compounds.

- The liver is subjected to a number of diseases called liver injury; due to its anatomical proximity to blood supply from digestive tract with ability to concentrate & biotransformed the chemicals make the liver the first organ to encounter the ingested substances (nutrients, drugs, & toxins) in a processes known as first pass effect.

- A basic understanding of hepatotoxicity requires appreciation of the:
  1- Major function of the liver.
  2- Structural organization of the liver.
  3- Processes involved in the excretory function of the liver (bile formation).

<table>
<thead>
<tr>
<th>TYPE OF FUNCTION</th>
<th>EXAMPLES</th>
<th>CONSEQUENCES OF IMPAIRED FUNCTIONS</th>
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</thead>
<tbody>
<tr>
<td>Nutrient homeostasis</td>
<td>Glucose storage and synthesis</td>
<td>Hypoglycemia, confusion</td>
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<td></td>
<td>Cholesterol uptake</td>
<td>Hypercholesterolemia</td>
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<td></td>
<td>Products of intestinal bacteria</td>
<td>Endotoxemia</td>
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<td></td>
<td>(e.g., endotoxin)</td>
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<tr>
<td>Filtration of particulates</td>
<td>Clotting factors</td>
<td>Excess bleeding</td>
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<td></td>
<td>Albumin</td>
<td>Hypoalbuminemia, ascites</td>
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<td></td>
<td>Transport proteins (e.g., very low</td>
<td>Fatty liver</td>
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<tr>
<td></td>
<td>density lipoproteins)</td>
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<tr>
<td>Protein synthesis</td>
<td>Bilirubin and ammonia</td>
<td>Jaundice, hyperammonomia-related coma</td>
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<tr>
<td>Bioactivation and detoxification</td>
<td>Steroid hormones</td>
<td>Loss of secondary male sex characteristics</td>
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<td></td>
<td>Xenobiotics</td>
<td>Diminished drug metabolism</td>
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<tr>
<td>Formation of bile and biliary</td>
<td>Bile acid-dependent uptake of dietary</td>
<td>Fatty diarrhea, malnutrition, Vitamin E deficiency</td>
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<tr>
<td>secretion</td>
<td>lipids and vitamins</td>
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<tr>
<td></td>
<td>Bilirubin and cholesterol</td>
<td>Jaundice, gallstones, hypercholesterolemia</td>
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<td></td>
<td>Metals (e.g., Cu and Mn)</td>
<td>Mn-induced neurotoxicity</td>
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<tr>
<td></td>
<td>Xenobiotics</td>
<td>Delayed drug clearance</td>
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Structural organization:

- Liver was divided into hexagonal lobules oriented around terminal hepatic venules (central veins). The lobule is divided into three zones: the centrilobular, mid/transient, & peripheral zones.
- The functional hepatic unit is the acinus, the basic of acinus is formed by the terminal branches of the portal vein and the hepatic artery.
- The acinus has three regions: zone 1 is closest to the entry of blood, zone 3 about the terminal hepatic vein, and zone 2 is intermediate. The acinus zone is roughly coinciding with three regions of the lobule.

Bile formation:

- Bile is yellow fluid containing bile salts, glutathione, phospholipids, cholesterol, bilirubin and other organic anions, proteins, metals, ions, and xenobiotics.
- The formation of this fluid is a specialized function of the liver. Adequate bile formation is essential for the uptake of lipid nutrient from the small intestine.
- Biliary excretion is important in the homeostasis of metals, notably copper, manganese, cadmium, selenium, gold, silver and arsenic. Inability to export Cu into bile is a central problem in Wilson's disease, a rare genetic disorder characterized by the accumulation of Cu in the liver and then in other tissues e.g. retina.
- Neonates exhibit delayed development of bile salt synthesis and the expression of sinusoidal and canalicular transporters, neonates are more prone to develop Jaundice when treated with drugs that compete with bilirubin for biliary clearance.
Types of the liver injury:

- The liver injury caused by chemicals (hepatotoxicity) is not a single entity, thus the lesion observed does not depend only on the chemical agent involved, but also on the nature of exposure (acute or chronic, reversible or irreversible), mechanism of toxicity, number & type of the cells affected & localization within liver (periportal, mid or transient & centrilobular zones).
- Accordingly, injuries divided into the following major types:
  1. Steatosis (fatty liver),
  2. Necrosis,
  3. Apoptosis,
  4. Fibrosis,
  5. Cirrhosis,
  6. Cholestasis,
  7. Hepatitis,
  8. Carcinogenesis.

1. Steatosis (fatty liver):

- Is accumulation of abnormal amount of lipids (mainly triglycerides) as vacuoles & droplets within hepatocytes; as a result of imbalance between rate of synthesis & release of these lipids by liver cells into circulation.
- In general, chemicals-induced steatosis is often reversible & doesn't necessary lead to hepatocytes death and it is an acute response to many but not all hepatotoxicants.
2. Necrosis (cell death):

- Is an acute response of liver injury associated with cell swelling, leakage of nuclear material and influx of inflammatory cells as a result of plasma membrane damage, alteration in calcium homeostasis, lipid peroxidation, disruption to cytoskeleton and damage to cell organelles. It occurs in focal, zonal or massive pattern.

3. Apoptosis (cell death):

- Characterized by cell shrinkage, nuclear fragmentation, formation of apoptotic bodies, & lack of inflammation.
- Apoptosis is more difficult to be detected histologically because of the rapid removal of affected cells.
4. **Fibrosis:**

- Chronic type of liver injury, usually results from long-term & multiple exposures to a toxic chemical that cause destruction to the hepatic cells with replacement by collagen fibers. With continuing collagen deposition, the architecture of the liver is disrupted by interconnecting fibrous scars.

5. **Cirrhosis:**

- Cirrhosis is an irreversible damage result mainly from alcohol induced fatty liver & has a poor prognosis of survival.
6. **Cholestasis:**

- Characterized by decreased volume of bile formed or impaired secretion of specific solute into bile leads to increase level of cpds in blood that are normally concentrated in bile, particularly bile acids & bilirubin due to either hepatic disorders (Canalicular) or biliary duct damage (cholangiodestructive).
- Cholestasis could be acute or chronic response, & it’s result from many different types of chemicals that cause cholestasis.

7. **Hepatitis:**

- Is inflammation of liver caused by many factors including drugs, toxins, bacteria & viruses. It is characterized by emigration of neutrophils, lymphocytes & other inflammatory cells into region of damaged hepatocytes.
- Immunoresponse of liver cells to some types of chemical agents can resulted after their biotransformation, binding with liver proteins & leak out of injured hepatocytes serve as an antigen that stimulate the production of antibodies.
- Hepatitis could be an acute or chronic type.

8. **Carcinogenesis:**
• Tumor of hepatic tissue caused by chemicals that undergo metabolic conversion to more toxic intermediates before they exert their carcinogenicity by interaction with nuclear materials, mainly the DNA, results in alterations in the genetic machinery causing mutations & initiation of a chain of events that result in cancer formation. It’s a chronic type of liver injury.

Mechanisms of Liver Injury:
• Liver damage occurs due to its location and its high capacity for converting chemicals to reactive cpds. In general balance between phase I and phase II enzymes systems determine whether a reactive metabolite will initiate liver cell injury or be detoxified safely.
• Hepatotoxicity forms are either hepatocellular (disruption of cytoskeleton), hepatobiliary (cholestasis) or hepatic mitochondrial damage.

1. Lipid peroxidation (affect directly) ex: CCL4
2. Abnormal metabolite formation (affect directly) ex: Paracetamol, Allyl alcohol, Bromobenzen, Halothane, Beryllium.
3. Protein synthesis inhibition (affect indirectly) ex: uncouplers -Aspirin or analogs formation –Methionine & Ethionine; Glucose/Galactose & Galactosamine.
4. Lipid accumulation (affect indirectly) ex: Ethanol, Tetraeycline.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hepatocellular</th>
<th>Hepatobiliary</th>
<th>Mitochondrial</th>
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<tbody>
<tr>
<td>alanine aminotransferase (ALT)</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>aspartate aminotransferase (AST)</td>
<td>X</td>
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<tr>
<td>sorbitol dehydrogenase (SDH)</td>
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<td>glutamate dehydrogenase (GLDH)</td>
<td>X</td>
<td>X</td>
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<td>total bile acids (TBA)</td>
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<tr>
<td>alkaline phosphatase (ALP)</td>
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<td>gamma glutamyltransferase (GGT)</td>
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<tr>
<td>5'-nucleotidase (5-NT)</td>
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<tr>
<td>total bilirubin (TBILI)</td>
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<td>X</td>
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<tr>
<td>Potential ancillary markers</td>
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<tr>
<td>lactate</td>
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<tr>
<td>lactate dehydrogenase (LDH)</td>
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<tr>
<td>ornithine carbamyltransferase (OCT)</td>
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<td>X</td>
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<tr>
<td>unconjugated bilirubin (UBILI)</td>
<td>X</td>
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</table>
Types of Hepatotoxic Compounds:

- Chemicals that cause liver injuries could be drugs, toxins, industrial pollutants, cleaning agents…etc. The important field is drug-induced liver diseases (DILD), because drugs can induce almost all forms of acute or chronic liver diseases. They are considered the main chemicals that enter the body & some of it can produce more than one type of liver injury.
- Some of hepatotoxic cpds are used experimentally such as Ethanol, Galactosamine & Carbon tetrachloride for research purposes to investigate drug-induced liver toxicity. Carbon tetrachloride is the mostly used compound because it is widely studied & has more than one approved mechanism of liver toxicity. Its wildly used in induction of liver injury as a hepatotoxic model for scientific researches.

1. Carbon tetrachloride (CCl4):
   - Colorless liquid with characteristic odor, insoluble in water, miscible with organic solvents such as alcohol & fats. Acute exposure & poisoning may occur from any route such as inhalation, skin absorption & ingestion.
   - Two major pathways of liver damage caused by CCl4 are:
     1. Covalent binding of CCl3• to macromolecules & DNA cause genetic alterations ended by liver cancer.
     2. Lipid peroxidation lead to excessive necrosis ended by cirrhosis.
   - Also CCl4 can induce hepatitis & cholestasis as minor pathways of toxicity in liver; so that is why CCl4 used as hepatotoxic model because it can induce almost all types of liver injury.
   - Effect of CCl4 is dose dependant; at small dose cause fatty degeneration ( inhibit protein synthesis by affecting ribosomes only) while at larger dose cause necrosis ( rupture cell membrane & mitochondria).
CCl4 toxicity enhanced by:
1. CP450 inducers (Phenobarbital, Ethanol).
2. Vitamins deficiency.
3. Any condition or cpds that increase Ketons & Lactons (DM, Alloxan, Streptozotocin).
4. Hypoxia.

Protection from CCl4:
• Avoid exposure.
• Agents reverse CCl4 action. e.g.
  1. Free radical scavengers (GSH, Cysteine).
  3. CP450 inhibitors (SKF-525, Cobalt chloride).
  4. Reduce Ca++ entrance (EDTA, Chlorpromazine).

2. Acetaminophen (In Europe called Paracetamol):
• Its hepatotoxicity not occur with typical therapeutic dose, because most of it will be glucuronidated or sulfated with little drug bioactivation.
• Injury after large doses of acetaminophen is enhanced by fasting and other conditions that deplete GSH and is minimized by treatment with N- acetylcysteine that enhance hepatocyte synthesis of GSH.
• Alcohol increases hepatotoxic effect of acetaminophen at dose within the high therapeutic range, due to accelerate bioactivation of acetaminophen to electrophilic N-Acetyl-P-benzoQuinoneImine (NAPQI), reactive intermediate metabolite react with cellular nucleophiles & arylate hepatocellular proteins.
• NAPQI also induces massive oxidative stress, which is even enhanced because GSH is being consumed. This leads to changes in the calcium homeostasis & induces damage to DNA.
• Finally, NAPQI also damages mitochondria by forming covalent adducts to mitochondrial proteins & posing an oxidative stress in mitochondria. This leads to a rapid fall in cellular ATP level. Thus, although apoptotic stimuli will be present at several levels including release of pro-apoptotic factors from mitochondria & signals from the cytosol, the liver parenchyma will primarily be subject to necrotic cell death, because the energy crisis in mitochondria will largely block the ATP-requiring pathways of apoptosis.
Acetaminophen toxicity is very dangerous since Most pts who have taken an overdose of acetaminophen will initially be asymptomatic (clinical evidence of end-organ toxicity often does not manifest until 24-48 hrs after an acute ingestion) where necrosis will be occur. In addition, the antidote therapy (NAC) is most effective only when initiated within 8 hrs after an ingestion.

Knowledge of maximum recommended & minimum toxic doses, as well as underlying conditions that increase susceptibility, can help clinician to determine risk of toxicity. In addition, history should include any co-ingestants e.g. Salicylates or medications may delay acetaminophen absorption (e.g., Anticholinergics or Opioids).

Initial appropriate supportive care is essential & includes:
1. Immediate assessment of pt's airway, breathing, and fluid status is critical.
2. Assessing for other potential life-threatening co-ingestions (e.g., Salicylate) is very important.

3. Uncouplers e.g. Aspirin:
   - Uncouplers are compounds prevent coupling between phosphate moiety & ADP, thus affecting mitochondrial respiration & ATP synthesis; resulting in:
     1. Reduce enzymes synthesis.
     2. Reduce protein synthesis.
     3. Cell membrane dysfunction leading to edema.
   - All these events lead to degeneration & necrosis of the liver.
4. Analogue formation:
   • It is mean introducing harmful compound structurally similar to useful compound needed by the body, resulting in loss of its function & induce toxicity. E.g.:
     1. Ethionine vs. Methionine.
        Where Methionine is essential for the body activity, protein synthesis, & conjugation of macromolecules while Ethionine cause inactivation of all these functions & fatty degeneration by trapping Adenin.
     2. Galactosamine vs. glucose /Galactose.
        Where Galactosamine trapping uridine lead to reduce protein synthesis & change cell membrane permeability resulting in edema & massive necrosis.

5. Ethanol: (fatty degeneration, necrosis & cirrhosis)
   Ethanol is bioactivated by alcohol dehydrogenase to acetaldehyde, a reactive aldehyde, which subsequently is detoxified to acetate by aldehyde dehydrogenase. Both enzymes exhibit genetic polymorphisms that result in higher concentration of acetaldehyde. Alcohol consumption by people with this slow polymorphism leads to uncomfortable systems of flushing and nausea caused by high systemic levels of acetaldehyde.

![Ethanol metabolism diagram]

Figure 13-6. Three pathways of alcohol oxidation: ADH, MEOS, and Catalase.
6. Cholestasis:
- There are Five potential mechanisms for cholestasis involving:
  1- inhibited uptake.
  2- diminished transcytosis.
  3- impaired secretion.
  4- diminished contractility of canaliculus.
  5- leakiness of junctions that seal canalicular lumen from the blood.

Strategy in Hepatoprotection against Liver Injury
The goal of treatment of hepatotoxicity is to stop further damage & managing the present injury as early as possible. In acute liver diseases, most cases have fewer treatment options available due to limitation impact of underlying management, while in chronic condition the disease cannot be cured. In most cases, liver transplantation considered the treatment of choice when other treatments failed.

It is imperative to prevent further exacerbation; protection rather than treatment is the better way to avoid the disease, the basic preventive measures are listed below in chronic liver diseases as follow:
1. Complete abstinence from alcohol.
2. Vaccination against hepatitis types.
3. Avoidance of medications with hepatotoxic side effects.
4. Vitamins & herbal remedies support for their safety use.
5. Low fat diet.
6. Avoidance of iron supplement unless anemia is present.