REVIEW ON EVALUATION OF HEPATOPROTECTIVE ACTIVITY

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ABSTRACT

Hepatotoxicity is an injury to the liver that is caused by exposure to a drug or other infectious agent. The understanding and treatment of hepatotoxicity has developed rapidly over the last 40 years reducing morbidity and mortality from. Progress had been made by the study of different animal models that shows the clinical, biochemical and histological characteristics of the hepatotoxicity seen in man. This review examines the various approaches to the study of hepatotoxicity in animal models, including both surgical and pharmacological approaches. Hepatotoxicity carries a very high mortality of 80–90% in all over the world. Advances in the field of artificial liver support systems are hindered by the lack of a standard clinically relevant model of hepatotoxicity. For the last 30 years several animal models have been used with limited success. Each model often reflects a particular aspect of hepatotoxicity. Species susceptibility to hepatotoxins and their response to surgical intervention have limited the choice of models. Small animal models have been used extensively for many, but the need for a robust and repeatable larger animal model that can be used to assess potential therapies has not yet been achieved. This review examines the requirements of an animal model, and the techniques that have been used so far.

KEYWORDS: Hepatotoxicity, Animal models, Drug induced hepatotoxicity, Hepatotoxin.

INTRODUCTION: The liver is a vital organ present in all the vertebrae and animals, and is largest organ in body. It plays an important role in transferred and clears all chemicals and is susceptible to the toxicity from these agents. Some pharmaceutical agents, when taken in overdoses and sometimes even when administered within therapeutic ranges, may cause damage to the liver.[1] Chemicals that cause liver injury are called hepatotoxins and chemical-driven liver damage is called Hepatotoxicity.[2] Drug-induced hepatotoxicity represents a
major clinical problem accounting for 50% of all cases of acute liver failure. Although the majority of cases of acute liver failure are due to intentional or unintentional misuse, 16% are idiosyncratic.\textsuperscript{[3]} Some of the inorganic compounds producing hepatotoxicity are arsenic, phosphorus, copper and iron. The organic agents include certain naturally occurring plant toxins such as pyrrolizidine alkaloids and bacterial toxins. In addition, exposure to hepatotoxic compounds may be occupational environmental or domestic that could be accidental, homicidal or suicidal ingestion.\textsuperscript{[1]} Drug-induced liver injury (DILI) categorized the development and utilization of numerous therapeutic compounds, and consequently presents major challenges to the pharmaceutical industry and clinical medicine.\textsuperscript{[4]} Acute hepatic failure (AHF) carries a very high mortality of 80–90%. World-wide viral hepatitis is the most frequent cause, followed increasingly closely by drugs and toxins. Although the care of patients in intensive care facilities and in special liver units has improved and mortality reduced, the definitive treatment is orthotopic liver transplantation. Several types of research are now in progress to help the majority of patients who are either not accepted for transplantation or those awaiting a donor organ.\textsuperscript{[5]}

**HEPATIC BIOTRANSFORMATION**

The liver is located between the absorptive surface of the gastrointestinal tract and drug targets throughout the body, which is central to the metabolism of virtually every foreign substance. Most drugs and xenobiotics are lipophilic in nature, which are able to cross the membranes of intestinal cells. Drugs are rendered more hydrophilic by biochemical processes in the hepatocyte, yielding water-soluble products that are excreted in urine or bile.\textsuperscript{[6]} Oxidative pathways can be carried out by the hepatic biotransformation with the help of the cytochrome P-450 enzyme system.\textsuperscript{[7]} After further metabolic steps, it conjugated with the glucuronide or a sulfate or glutathione, the hydrophilic product is exported into plasma or bile by transport proteins located on the hepatocyte membrane, and it is subsequently excreted by the kidney or the gastrointestinal tract.\textsuperscript{[8]}

**HEPATOTOXICITY INDUCING AGENTS**

Many xenobiotics like chemicals, drugs, house hold things, herbs and environmental chemicals have been induce hepatotoxicity. In xenobiotic-induced hepatic damage, the centrilocubular (zone III) hepatocytes are the primary sites of cytochrome P450 enzyme activity, which frequently makes them most susceptible to xenobiotic-induced liver injury. Carbontetrachloride (CCl4), N-nitrosodiethylamine (NDEA), Acetylaminofluorene (2-AAF),
Galactosamine, d-Galactosamine, Thioacetamide, antitubercular drugs, paracetamol, methotrexate, ethanol and arsenic etc. are used to induce experimental hepatotoxicity in laboratory animals. Following is the list of some chemicals that are responsible for hepatotoxicity.

- **Industrial chemical**: CCl₄, Trtra chloroethane Di phenyleoxide Chloroform, Ethylene dichloride, Arsenic, Antimony, Copper, Hydralazines.
- **House hold thing**: Antifreeze Dry cleaning fluids Glue, Stamping Ink Paint Products, Polishes, Paint remover, Wax.
- **Pesticides**: Organochloride, insecticide Herbicide, fungicide Thallium, warfarin Copper Salt, DDT.
- **Pollutant chemical in food and water**: Polychloridated Biphenyls, Polybrominated biphenyls Chloroalkane.
- **Plant Extract**: Pyrrolizidine alkaloids, Pennyroyal, Kava Kava, Broom corn, Bajiaolian, Margosa Oil, Jin Bu Huan, Chaparral.
- **Drugs**: Methotrexate, Paracetamol, Acetophenazine Maleate, Amrinone Lactate, Azacitidine, Asparaginase, Blenoxane, Anabolic steroids.
- **Anti Tuberculosis drug**: Isoniazid, Rifampicin, Rifabutin, Pyrazinamide, Ethionamide, Prothionamide, Para-aminosalicylic acids.

**MECHANISM OF DRUG INDUCED HEPATOTOXICITY**

Liver injury caused by hepatotoxins, such as methotrexate, carbon tetra chloride (CCl4), ethanol and acetaminophen, is characterised by varying degrees of hepatocyte degeneration and cell death via either apoptosis or necrosis. The generation of reactive intermediate metabolites from the metabolism of hepatotoxins and the occurrence of reactive oxygen species (ROS) during the inflammatory reaction, account for a variety of pathophysiologic pathways leading to cell death, such as covalent binding, disordered cytosolic calcium homeostasis, GSH depletion, onset of mitochondrial permeability transition (MPT) and associated lipid peroxidation. The metabolism of hepatotoxins by cytochrome P-450 enzyme subtypes is a key step of the intoxication; therefore, enzyme inhibitors are shown to minimize the hepatotoxin associated liver damage. Oxidant stress and lipid peroxidation are crucial elements leading to hepatotoxin-associated liver injury. In addition to specific treatment for a given hepatotoxin, the general strategy for prevention and treatment of the damage includes reducing the production of reactive metabolites of the hepatotoxins, using anti-oxidative
agents and selectively targeting therapeutics to Kupffer cells or hepatocytes for on-going processes, which play a role in mediating a second phase of the injury.\textsuperscript{[9]}

**Fig: 1**

**EVALUATION OF ANTIHEPATOTOXIC AGENTS**

The therapeutic value, efficacy and toxicity of drugs may be evaluated in animals experimentally made sick, followed by clinical trials. Detailed biochemical and other in vitro assays are obligatory to establish the mechanism of action. Both in vivo and in vitro test systems are employed to assess antihapatotoxic or hepatoprotective activity. These systems measure the ability of the test drug to prevent or cure liver toxicity induced by various hepatotoxins in experimental animal’s rats, mice, rabbits etc.\textsuperscript{[10]}

**EVALUATION OF HEPATOPROTECTIVE ACTIVITY**

Two types of models are used

a) In vitro

b) In vivo

a) In vitro models

Fresh hepatocyte preparations and primary cultured hepatocytes are cultured to study the anti-hepatotoxic activity of drugs. Hepatocytes are treated with hepatotoxin and the effect of the
test drug is evaluated. The level of transaminases released into the medium is determined. An augmented activity of marker transaminases in the medium indicates liver damage. Parameters such as hepatocytes multiplication, morphology, macromolecular synthesis and oxygen consumption are determined.\[^{[11]}\] In an *in vitro* system, compounds affect the cells directly and continuously until the removal of compound containing medium.\[^{[12]}\] These models contribute to the ‘3R’ concept which are refinement, reduction and replacement of animal experimentation which leads to reduction of animal utilization for research purposes.\[^{[13]}\] This system is quite useful for safety evaluation in the early stage of drug discovery as they are helpful in generating sufficient results at a low cost and high speed, and with less use of animals.\[^{[14]}\] Several *in vitro* human and animal liver models are available ranging from short term to long-term cell or tissue culture systems.

**b) In vivo models**

A toxic dose or repeated doses of a known hepatotoxin is administered to animals 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 comparing 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 transaminases (GOT) and alkaline phosphatase in the serum indicates liver damage. Therapeutic efficacy of a drug against diverse hepatotoxins differs especially when their mechanism of action vary. Consequently, the efficacy of each drug has been tested against hepatotoxins which act by varied methods.\[^{[15]}\] Animal models represent a major tool for the studies of mechanisms in virtually all of biomedical research. They involve the complexity of the whole animal thus making the monitoring of *in vivo* systems quite difficult.\[^{[16]}\] An *in vivo* system fully reflects the exposing profile and the cellular function as the compounds are exposed in the successive manner through absorption from the first exposed site followed by metabolism, distribution and elimination. However, it should involve basically the same mechanism as the reactions in humans and the adverse effect must be clinically sufficiently high. Both small animals like rats, mice, rabbits and guinea pigs as well as large animals like pigs, cattle, sheep and monkeys are useful and reliable for studying the hepatotoxicant effects, distribution and clearance.\[^{[17]}\] They may be used to elucidate basic mechanism of xenobiotic activities which will be useful in understanding their impact on human health. However, the relevance of the findings of *in vivo* studies using different animal models to humans may vary due to
differences in drug metabolism and pathobiology in various species. Due to the lack of sufficient data to reliably assess the value of preclinical animal studies to predict hepatotoxicity in humans, the preclinical animal toxicity studies may not be sufficient as the only modelling systems used to predict hepatotoxicity. Further, in order to reduce the use of animal in toxicity studies, there is a need for a long-term in vitro system.[18]

**In vivo models**

The study of animal models of hepatotoxicity has followed three general approaches, surgical, pharmacological and other procedures. Surgical techniques include hepatic resections and devascularization of the liver. Pharmacological manipulation includes the administration of hepatotoxins such as paracetamol, D-galactosamine, and thioacetamide.

![Diagram of different models of hepatotoxicity]

**Fig: 2 Different models of hepatotoxicity**

1) **Surgical models**

The surgical models can be divided into three groups; variations of partial heptectomy, total heptectomy and a series of studies looking at partial and complete devascularization of the liver. It is well recognized that intense regeneration and almost 100% survival will follow a partial heptectomy involving a 70% resection in rats and pigs. The altered physiological
state leading to increased mortality is thought to be a reflection of increased total portal blood flow through the remnant tissue.\textsuperscript{[19,20]} This increased ‘portal flow per unit of liver tissue’ induces flow injury and damage to sinusoidal endothelial cells leading to Kupffer cell activation and release of cytokines inducing liver damage.\textsuperscript{[21]} Increased portal flow per unit of liver tissue will also increase the endotoxaemic load from the gut.\textsuperscript{[22]}

<table>
<thead>
<tr>
<th>SR. No</th>
<th>MODEL</th>
<th>ANIMAL USED</th>
<th>RESULT</th>
<th>REFERENCES</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Partial hepatectomy</td>
<td>Rats</td>
<td>Decrease survival &gt; 85% resections</td>
<td>[23]</td>
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<tr>
<td>2</td>
<td>Resection/Ligation Model</td>
<td>Rats</td>
<td>Encephalopathy III, late hypoglycaemia. Increased NH3, lactate, prothrombin time.</td>
<td>[24]</td>
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<td>3</td>
<td>Total hepatectomy</td>
<td>Pigs</td>
<td>Survival 15–26 h Pre-terminal encephalopathy, Hypoglycaemia and AST rise.</td>
<td>[25,26]</td>
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<tr>
<td>4</td>
<td>Partial devascularization</td>
<td>Pigs</td>
<td>Minimal AST rise, late hypoglycaemia 75 min clamp – 50% survival</td>
<td>[27]</td>
</tr>
<tr>
<td>5</td>
<td>Total devascularization</td>
<td>Pigs</td>
<td>Increasing encephalopathy, AST</td>
<td>[28]</td>
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2) Pharmacological models

<table>
<thead>
<tr>
<th>MODELS USED</th>
<th>ANIMAL USED</th>
<th>RESULT</th>
<th>REFERENCES</th>
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<tbody>
<tr>
<td>1 ETHANOL INDUCED HEPATOTOXICITY</td>
<td>Wistar albino rats</td>
<td>Elevation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (TB). &amp; decreased levels of catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase</td>
<td>[29]</td>
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<tr>
<td></td>
<td>Male Wistar Rats</td>
<td>Alcoholic liver diseases (ALD), Increases in ALT, AST, ALP enzyme</td>
<td>[30]</td>
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<tr>
<td>2 ACETAMINOPHEN (PARACETAMOL)</td>
<td>Swiss albino mice</td>
<td>Haemorrhages, fatty changes and necrosis</td>
<td>[31]</td>
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<td></td>
<td>Dogs</td>
<td>Encephalopathy and coma 6–8 h. (Phenobarbitone induction and sub cutaneous administration of</td>
<td>[32]</td>
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<tr>
<td>Experimental Model</td>
<td>Hepatotoxicity Description</td>
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<tr>
<td>Rats</td>
<td>Elevation In Serum Enzymes &amp; Hepatic Injury [33]</td>
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<td>Albino mice</td>
<td>Oxidative stress, hepatic injury [34]</td>
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<tr>
<td>Wistar rats</td>
<td>Focal necrosis, oxidative stress [35]</td>
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<td>Dogs</td>
<td>No changes [36]</td>
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<tr>
<td>Rats</td>
<td>Encephalopathy, increased AST, PT, NH3, ICP. Hepatic necrosis, cirrhosis [37]</td>
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### References


