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A Comprehensive Overview of Models for Hepatoprotective Activity Screening

Ajinkya Chaudhari[1], Payaam Vohra[1] and Akshata Geedh[2]

1. *Department of Pharmacology, KGRDCP, Mumbai 410201, India*
2. *Department of Pharmacology, NIPER MOHALI, Punjab 160062, India*

Abstract: The liver is a crucial imperative organ that is involved in various kinds of metabolic activity and a very stable accessory gland for the digestive system. At this moment, liver dysfunction is a major source of destruction, and its widespread is accentuated in the developed republics. Long-term or persistent inflammation and oxidative stress due to any reason have a substantial impact on the beginning and continuation of chronic diseases such as hepatocellular carcinoma, liver cirrhosis, liver fibrosis, and other hepatic conditions. Liver diseases classified as hepatosis, cirrhosis and acute or chronic hepatitis are one amongst the most severe ailments This review deals with well-established *in vivo*, *in vitro* and *in silico* models for analysing the hepatoprotective activity of extract/drugs. Consequently, animal models are being developed to impressionist hepatic ailments. From several decades, researchers are using distinctive animal models for discovering and understanding the pathogenesis of hepatic ailments. This current cram has been framed to discuss numerous new and traditional experimental models for hepatotoxicity studies. Numerous animal models have evolved to evaluate the pathogenesis and develop drugs for hepatotoxicity. Experimental modes of hepatotoxicity are influential for invention of novel molecular signalling trails for the improvement of human health. This article aims to explore and unfold various possible models available for hepatoprotective models. This miniature review article highlights the hepatopathy models that are being used to observe the activities of liver injuries under hepatotoxic agents.

Key words: Animal model, Hepatotoxicity, *In vivo* screening, *In vitro* assessment, *In silico* examination.

1. Introduction

The liver diseases have become one of the major causes of morbidity and mortality all over world. From among, drug induced liver injury (DILI) is one of the most common causative factor that poses a major clinical and regulatory challenge [1-5].

The manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminate hepatic failure. The liver has more functions than any other human organ and also is a largest part of the body. The blood supply passes through the liver several times a day. The liver has a important role in human metabolism.

The liver produces and secretes bile, it also produce prothrombin and fibrinogen, both blood clotting factors, and heparin. It converts sugar into glycogen. Liver diseases have become one of the major causes of morbidity and mortality in man and animals. Hepatotoxicity due to drugs appears to be the most common factor. Animal models are important tools for determining the pathogenesis or mechanisms of toxicity in biomedical research [6-8]. They have complexity in both *in vivo* and *in vitro*. The creatures as rodents, rabbits, mice, guinea pigs, sheep and monkeys, are used globally conduct an investigation into hepatotoxic footprints. These animals could be utilized to understand the fundamental mechanism of the xenobiotic. However, the experimental animal model is a promising prototype for the drug design and discovery of novel hepatoprotective agents. The most common disease that can affect the liver is ‘viral hepatitis’.

Hepatitis mainly caused by drugs, viruses, bacteria, parasites like amoebas or giardiasis. The use of natural

Corresponding author: Payaam Vohra, Masters, research fields: Pharmacology toxicology, Pharmacy, Hepatoprotective, Pharmacological Models. Email: payaamvohra@gmail.com.

remedies to treat the liver diseases has a long history [9-13].

There are some medicinal plants and their derivatives which still used all over the world in one form or the other, have been tested and found to contain active principles with curative properties against a variety of diseases. Hepatoprotective plants contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes. Recent experience has shown that plant drugs are non-toxic, safe and even free from side effects [14, 15]. The liver, an exuberant organ, acknowledged synchronizing gastrointestinal homeostasis and body function in general. Furthermore, it also serves to abruptly withdraw of toxicants. Consequently, the commanding functioning of the liver is imperative for elegant wellness. Liver diseases result in the lipid peroxidation of liver tissues, depletion in the tissue glutathione (GSH) levels, elevated levels of biochemical markers like serum glutamate oxaloacetate transaminase (SGOT/AST) and serum glutamate pyruvate transaminase (SGPT/ALT) triglycerides, cholesterol, bilirubin and alkaline phosphatase [16-20].

1.1 Mechanisms of Hepatotoxicity [21-24]

- Pathophysiological Mechanisms of hepatotoxicity are still being discovered and comprise both hepatocellular/extracellular mechanisms.
- Disruption of hepatocyte medications can bound to intracellular proteins by covalent tying which bring about a lessening in adenosine triphosphate (ATP) levels prompting actin interruption. Part of actin fibrils at the surface of the hepatocyte causes blebs and burst of the layer.
- Disruption of transport protein: Bile stream may be interrupted by medications that influence transport proteins at canalicular film. Loss of villous procedures and intrusion of transport pumps, for example, multidrug resistance-related protein 3 forestall discharge of bilirubin bringing about cholestasis.
- Cytolytic T-cell activation: Co-valent tying of medication to Cytochrome P-450 enzyme goes about as an immunogen activating T-cells and cytokines and animating multifaceted immune reactions.
- Apoptosis of hepatocytes: Enhancement of apoptotic pathways by tumor necrosis factor-α receptor of FAS may trigger the course of intercellular caspases, which bring about customized cell death.
- Mitochondrial disruption: A few medications restrain mitochondrial capacity by double impact on both β-oxidation energy productions by hindering the synthesis of nicotinamide adenine dinucleotide and flavin adenine dinucleotide, bringing about diminished ATP generation.
- Bile duct injury: Dangerous metabolites disposed of in bile may bring about harm to bile conduit epithelium.
- Drug Induced Hepatotoxicity.

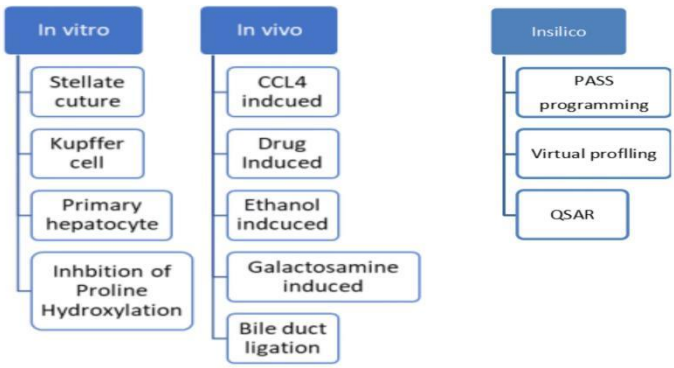

Fig. 1 Types of *In vivo*, *In vitro* and *In silico* Models [3].

2. Materials and Methodology

There are three models for hepatoprotective activity screening (Figures 1 & 2).

2.1 In Vivo Methods

2.1.1 D-galactosamine/lipopolysaccharide-induced fulminant liver failure, D-galactosamine induced hepatotoxicity in mice

- Mechanism of action: The histopathology of the liver gives the evidence for the protection imparted by the herbal mixture and silymarin. The metabolites of D-galactosamine (GaIN), uridine diphosphate amine may deplete several uracil nucleotides such as UDP-lactose, UDP-glucose and uridine triphosphate (UTP), causing reduction of mRNA and glycoprotein synthesis (i.e., reduction of ATP and glycogen synthesis), which leads to cellular membranes alteration. Nevertheless, there is increasing evidence that GaIN causes production of free hydroxyl radicals leading to lipid peroxidation.

2.1.2 Carbon tetrachloride induced hepatotoxicity in rats

- Mechanism of action: CCL4 is widely used experimentally as hepatotoxic which is bio transformed by the cytochrome p-450 system to produce the trichloromethyl free radical which in turn covalently binds to cell membrane and organelles to elicit lipid peroxidation disturbs calcium hemostasis and finally results in death of cell. Lipid peroxidation is a complex and natural deleterious process. The significant increase observed in levels of lipid peroxides in liver of CCL4 intoxicated shows free radical induced liver damage [25-28].

2.1.3 Ethanol

- Mechanism of action: Liver being the major site for detoxification is the primary target for environmental or occupational toxic exposure. The alcoholic liver injury appears to be generated by the effects of ethanol metabolism and toxic effect of acetaldehyde, which may be mediated by acetaldehyde, altered protein. The oxidation of ethanol via the alcohol dehydrogenase pathway results in the production of acetaldehyde with loss of hydrogen ions. NAD (Nicotinamide Adenine dinucleotide) is reduced to NADH. The large amounts of reducing equivalents generated the hepatocytes ability to maintain redox homeostasis and number of disorders as hyper uremia, hyper lipemia, and rise in high-density lipoprotein (HDL). The rise in NADH promotes fatty acid synthesis as a net result hepatic fat accumulation.

Predictive human *in vitro* model and introduced a paradigm of microfluidic culture systems with the goal to mimic the liver with physiologically relevant dimensions, cellular structure, perifusion, and mass transport by taking advantage of micro and nanofabrication technologies.

2.1.4 Ferrous sulphate induced hepatotoxicity in rats

- Mechanism of action: Iron toxicity results when too much iron is injected or less often when too much is given orally. Iron overload is associated with liver damage, characterized by massive Iron deposition in hepatic parenchymal cells, leading fibrosis and eventually, to Hepatic necrosis. Lipid peroxidation (LPO) had been proposed to be the major factor in iron Toxicity, including iron induced hepatotoxicity.

Drug-Induced Toxicity: Isoniazid and Paracetamol are widely used drugs. Sometimes Anticancer drug Doxorubicin and Hormonal drug Ethinyl Estradiol are also used to induce Cholestasis [26, 27].

2.2 In Silico Models

In silico experiment to predict the activity spectra for substances (PASS). This experiment predicts a compound's activity spectrum as probable activity (Pa) or probable inactivity (Pi). The results are based on a

structure–activity relationship (SAR) analysis of the training set, which consists of more than 180,000 compounds showing greater than 3,678 types of biological activities. The values of Pa and Pi lie within the range 0.000 to 1.000. When Pa is greater than Pi, the compound is thought to be experimentally active. Pa > 0.7 indicates the probability of pharmacological potential is rich with values 0.5 < Pa < 0.7 reflecting considerable pharmacological effects experimentally. Pa < 0.5 shows less pharmacological activity [28-30].

Drug-likeness calculation and prediction of biological activity the targeted compounds were assessed for their drug-likeness characteristics based on the Lipinski rule of five and shall be assessed for potential bioactivity by calculating their activity scores as GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor inhibitors, and enzyme inhibitors. All the parameters were checked with the aid of the software Molinspiration (www.molinspiration.com). Calculated drug-likeness scores of each compound were compared with the specific activity of each compound and were compared with the standard drug.

Molecular Dynamic Simulation: MD simulation looks at the stability of inhibitor–target complexes, structural specifics, and orientational flexibilities, as well as the accuracy of inhibitor–target binding affinities.

Statistical analysis: Data shall be expressed as mean ± SEM (Standard error of the mean) of five animals. For statistical analysis, analysis of variance (ANOVA) must be followed by post hoc Dunnett's test for multiple comparisons. The outcomes are considered to be significant at the $p < 0.05$ level. The statistical analysis must be carried out using either the statistical software package for social science (SPSS, version 20.0, IBM Corporation), Prism version 7.0a (GraphPad Software Inc.), or Microsoft® Excel.

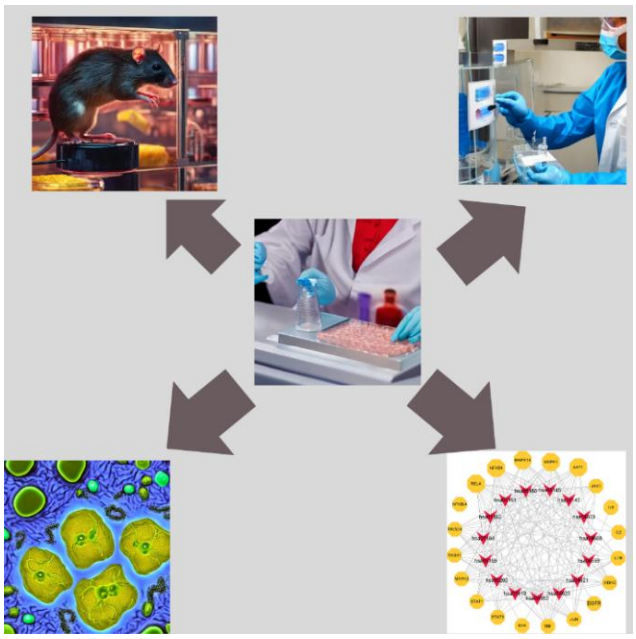

Fig. 2 Linking of *In vivo*, *In vitro* and *In silico* Pharmacological screening [16].

2.3 In Vitro Methods

2.3.1 Kupffer cell culture

Co-cultures using Kupffer cells are powerful new *in vitro* tools for modelling the liver. Hepatocyte monocultures have served as the standard *in vitro* model for ADME/Tox-related research, including metabolism and drug-drug interactions [31, 32].

Kupffer cells play an active role in the remodeling and maintenance of liver extracellular matrix.

Kupffer cells secrete potent mediators of the inflammatory response that control liver inflammation.

Kupffer cell cytokine mediators control hepatocyte metabolic rates through direct interactions with phase I and phase II enzymes.

Zymosan particles are phagocytic stimuli and promote superoxide dismutase.

2.3.2 Stellate cell culture

These cultures are isolated from rat liver by collagenase digestion.

Hepatic Stellate cells (HSCs) are the main effector cells of liver fibrosis. Following a fibro genic stimulus, these cells transdifferentiate from a quiescent to a myofibroblast-like phenotype leading to increased proliferation, migration and contractility HSCs are resident perisinusoidal cells in the subendothelial space between hepatocytes and sinusoidal endothelial cells (SEC) that contribute to hepatic development, regeneration, immune responses, angiogenesis, and storage of vitamin A HSCs express glial fibrillar acidic protein, while activated HSCs express smooth muscle α-actin. Vimentin is detected in HSCs from normal and cirrhotic livers, and although desmin is expressed in quiescent cells, desmin expression increases throughout the transdifferentiation process.

2.3.3 Primary hepatocyte cell culture

Fresh hepatocyte preparations and primary cultured hepatocytes are used.

The basic method: Isolation of hepatocytes by perfusion of liver with collagenase or utilization primary cultured hepatocytes. Determination of the viability of the hepatocytes. Incubation of the cell culture with hepatotoxin and with or without the test drugs. Determination of the activity of transaminases released into the medium by the hepatocytes.

Hepatotoxins: CCI4, paracetamol, d-galactosamine, etc.

2.3.4 Inhibition of Proline Hydroxylation

The thermal stability of triple collagen of alpha helix of collagen is depended on intramolecular hydrogen bond synthesized by enzyme prolyl 4 hydroxylase. Prolyl 4 hydroxylase is inhibited in order to provide a suitable model for hepatoprotective activity.

Liver cirrhosis and necrosis: Excessive formation of connective tissue with collagen over production reduces hepatic blood flow. Collagen is formed as a response to chronic injury. The collagenous fibers consist of triple helical molecules. Their formation depends on the presence of hydrogen bonds. If the number of hydrogen bonds is reduced the resulting collagen cannot form the triple helix and is degraded instead of being deposited in the extracellular matrix.

The aim of fibro suppressive compounds is to reduce only the excessive formation of insoluble collagen. Fibro suppressive effects by inhibition of proline hydroxylation can be screened with *in vitro* methods, however, the desired organ specificity has to be tested in models of liver cirrhosis and fibrosis in viva [33, 34].

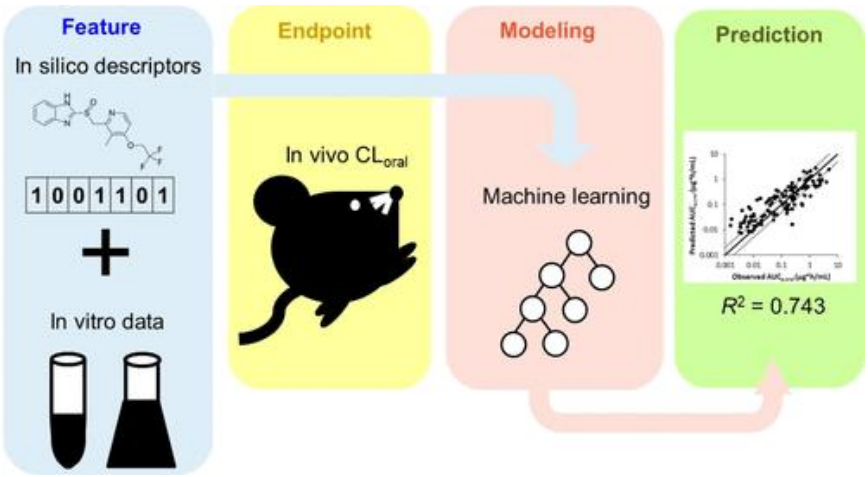

Fig. 3 Outline for pharmacological screening [35].

3. Conclusions

The application of *in vitro* and *in vivo* experimental hepatotoxic models in liver research is essential to understand and assess mechanisms of liver toxicity and to study potential hepatoprotective drugs. Due to an increase in cases, liver disease or hepatotoxicity is a popular subject of growing concern. Thus, under light of above points and literature review one can say in nutshell that there has been a significant development in pharmacological screening (Figure 3) for hepatoprotective substances via development in *in vitro*, *in silico* and *in vivo* progress.

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