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Experimental hepatotoxicity Inducing agents: A Review

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Abstract
Liver is the most important organ that performs vital function of the body. Hepatotoxicity is becoming a leading cause of death world-wide and prevalence is increasing exponentially. There are many traditional as well as allopathic medicines available which impart hepatoprotection but the treatment of chronic liver disease is still a challenge for health care professionals. For this purpose, rodents are routinely being used in the laboratory for induction of hepatotoxicity. Non-invasive methods that includes chemicals (CCL_4, thioacetamide, aflatoxin B1, Acrylamide etc), toxic metals (mercury, lead, arsenic and cadmium), drugs (NSAIDs, antibiotics, chemotherapeutic agents), radiation, high-fat diet and alcohol, are generally used to induce hepatic toxicity. Invasive methods used generally include bile duct ligation and portal vein ligation. This article provides an overview of different types of hepatic toxicants, their doses and time of induction. This review will help researchers in selecting the right model and studying and developing newer hepatoprotective drugs with minimum side effects.
Keywords: Hepatotoxicity, non-invasive methods, invasive method, hepatoprotective drugs, bile duct ligation.

1. Introduction
Liver damage or hepatic toxicity is referred as liver dysfunction which is often associated with exposure to toxins [1], overdosage of medicines or by some therapemutic agents [2]. Some chemicals like CCL_4 [3], PCM [4] used in the laboratory, lead and arsenic used in the industry or natural toxins (microcystins) [5] may cause hepatic toxicity. According to a survey, drug-induced hepatic toxicity accounts for 50 % of the total hospitalization and 50 % of all acute liver failure [6]. Drug-induced hepatotoxicity is one of the most common causes of drug withdrawal from the market. Metabolic syndrome (Diabetes, hypertension, hyperlipidemia, and obesity), insulin resistance, alcohol consumption and oxidative stress also cause liver damage [7]. These hepatotoxic agent damages hepatocytes & causes activation of innate immunity system [8], ultimately produces pro-inflammatory markers such as TNF, IL and gamma interferon as shown in figure 1[9].

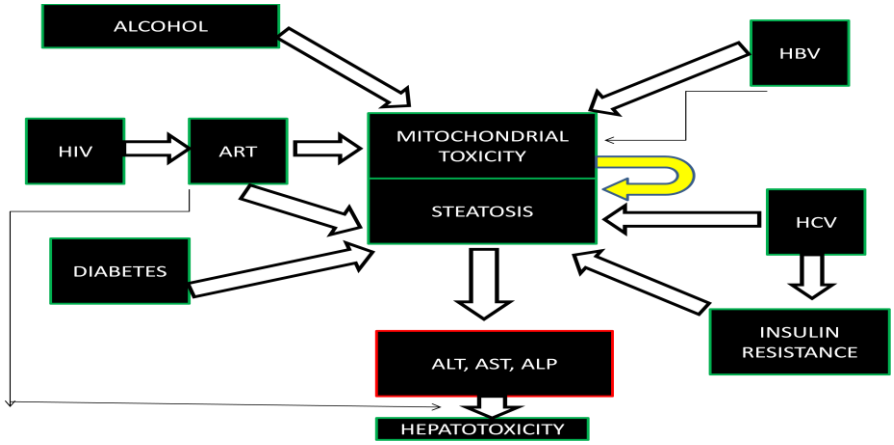

Figure 1: Induction of hepatotoxicity by various agents

Some hepatotoxic agent often damage mitochondria which result in inhibition of electron transport system and induces oxidative stress [10]. Medicinal plant possesses an important role in human health care system. Herbal medicine has a tremendous demand in primary health sector because of their safety, efficacy, and least side effect [11]. It is now established that herbal remedies offer natural healing phenomenon via antagonizing degenerative pathological process [12]. In traditional health system, herbal treatment for the liver disorder is claimed to be safest and effective, hence

development and validation of newer herbal drug is of prime concern. In the development of newer therapeutic agent, animal model play vital role [13]. For the past 30-40 years it is the animal model only that helped in better understanding various liver disorders [14].

In this review, we have tried to summarize the different animal models which are currently being used and discussed the mechanism of induction of hepatotoxicity.

Some desirable features in animal models of human disease [15]

Specificity: Model should be specific with respect to the toxicity induced to a particular organ.

Reproducibility: % of animals reaching desired state. Consistent time frame to attain desired state.

Cost: Always an issue. Models which take a long time to develop the desired state can result in expenditure for housing costs.

Size: Large blood volume requirements or need for vascular access may indicate that large animal should be used.

Institutional issues: Model should follow the Animals Ethics Committee guidelines as Animal Investigation Committees vary greatly regarding opinions on which animals or types of models should be allowed.

Reversibility: In certain studies extent of toxicity induced is so high that its reversibility is difficult to study, e.g. fulminant hepatic failure, hepatic encephalopathy.

Feasibility: Whether the laboratory has the expertise, facilities, etc., to generate or handle the model.

2. Classification of hepatotoxic agents used in animal models

In-vitro and *in-vivo* models are routinely used for studying hepatotoxicity. These models provide the information of drug or describe their potency in prevention and cure of various hepatic disorders (induced by hepatotoxic agents). In in-vivo models known concentration of hepatotoxin is administered for a defined period of time and route to induce liver damage. Here test substance (drug) is administered either before or after the toxin treatment. Various chemical agents normally used for inducing animal hepatotoxicity include carbon tetrachloride, paracetamol, acryl amide, adriamycin, alcohol, antitubercular drugs *etc*. These hapatotoxins changes into toxic metabolites and interfere with macromolecules like protein, nucleic acid and lipids. These interferences induce lipid dysfunction, DNA damage and oxidative stress leading to hepatocytes disruption. In in-vitro models hepatic cell lines are treated with hepatotoxin and the effect of the test drugs on the same is evaluated. Depending on the models, the method of induction can be divided as follow.

NON-INVASIVE METHOD

- Chemical-induced hepatotoxicity
- Drug-induced hepatotoxicity
- Metal-induced hepatotoxicity
- Phytotoxin induced hepatotoxicity
- Radiation-induced hepatotoxicity
- Diet-induced hepatotoxicity

INVASIVE METHOD

- Invasive methods generally used are Bile duct ligation and Portal vein ligation.

Since long time, animal model has been used as a tool for biomedical research [16]. An ideal animal model should depict the same mechanism as human do [17]. Animals such as rat, pig, mice, rabbit, guinea pig, cattle, ship and monkey are reliable for conducting hepatotoxic studies [18]. In this review we have limited our discussion only to in-vivo models.

2.1 Chemical-induced hepatotoxicity:

There are several chemicals which cause hepatotoxicity like carbon tetrachloride (CCl_4), thioacetamide (TTA), diethylnitrosamine (DEN). The metabolites of these chemicals lead to various pathological and biological changes. It has been found that glutathione and neutrophils play a critical role in chemically induced hepatotoxicity. Activation and inhibition of signaling kinase, transcription factors, and gene expression profiles may have effects on organelles like mitochondria, cytoskeleton, endoplasmic reticulum, microtubules and nucleus. The resultant deleterious effect may lead to cell death caused by either cell shrinkage, nuclear apoptosis or swelling and necrosis.

CCL_4: Carbon tetrachloride is one of the most common chemical agents used in the laboratory for the study of various liver disorders at acute and chronic condition [19]. CCL_4 alters the plasma membrane, lysosomal membrane and mitochondrial membrane [20]. A metabolite of CCL_4, called trichloromethyl (CCL_3) produced by CYP2E1 isozymes, combines with cellular lipids and proteins to form trichloromethyl peroxy radical which attacks lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical that causes lipid peroxidation and lobular necrosis [20]. A single dose of CCL_4 reaches to its peak plasma concentration within 3 hours and within 24 hours of administration, it causes a change in the histological and biochemical makeup of hepatocytes [21]. Repeated dose of CCL_4 can induce fibrosis and necrosis [22]. It has been shown that subcutaneous dose of 2 ml/kg for 2 days elevates the level of SGPT &

SGOT, however if the dosing continues for 2–4 weeks fibrosis is induced leading to bridging fibrosis in 5-7 weeks and cirrhosis in 8-9 weeks [23]. Details are discussed in table 1 below.

Table 1: Carbon tetrachloride (CCL_4) induced hepatotoxicity

Animal used	Dose (CCL_4)	Route	Duration	References
Male Sprague-Dawley rat	0.5 Ml/kg	I.P.	3 days	[24]
Male Sprague-Dawley rat	0.2 ml/kg	I.P.	2 weeks	[25]
Male Wistar rats	0.5 ml/kg	I.P.	Twice a week for 4 weeks	[26]
Male Wistar rats	0.125 ml/kg	I.P.	7 days	[27]
Wistar strain albino rats	1.0 ml/kg	I.P.	After 72 hours for 10 days	[28]
Male Wistar rats	2 ml/kg	S.C.	After 72 hours for 10 days	[29]

I.P: (intraperitonial) S.C. (Subcutaneously)

Thioacetamide (TAA): Thioacetamide is a white crystalline solid often used to induce fibrosis [19]. Thioacetamide as such is not toxic to the liver but its metabolite, thioacetamide S-oxide (ROS) is heapato-toxic and reduces the number of hepatocytes and oxygen consumption, thus making the hepatocytes oxygen deficient [30]. Thioacetamide also reduces the movement of bile salt and induces cholestasis [31]. Metabolite increases the intracellular concentration of ca++ in nuclear volume that obstructs the activities of mitochondria, causing necrosis and death of hepatocyte in zone 1 and zone 3 [19]. TAA, when administered at low dose, induces the formation of portal-portal or portal central septa & cirrhosis [32]. If we compare with CCl4, the fibrotic lesion is more prominent in TAA-induced cirrhosis [33] and it remains for a longer time (2-3week) after the drug withdrawal [33]. Most of the time TAA is administered as injectables but it can also be given by dissolving in water to produce non-invasive cirrhosis and hepato cellular carcinoma (HCC) [34]. Details of hepato-toxicity induction time, concentration, and route of administration are discussed in table 2 below.

Table 2: Thioacetamide-induced hepatotoxicity

Animal used	Dose Reported (thioacetamide)	Route	Duration	References
Male Wistar rats	200 mg/kg	I.P.	Twice a week for 12 weeks	[35-36]
Male Wistar rats	300 mg/kg	I.P.	14 days	[37]
Male Wistar rats	400 mg/kg	I.P.	2 weeks	[38]

I.P: intraperitonial

Diethyl-nitrosamine (DEN): DEN is well known carcinogenic chemical [39]. In the liver DEN is biotransformed by CYP2E1 (hydroxylation) into ethyldiazonium ion which acts as alkylating agent [40] and reacts with DNA and induce cancer [41]. When the low dose of DEN is administered for several weeks or months, HCC is induced [42]. for this reason, most of the time this model is utterly used to study the progression of HCC from fibrosis [43]. DEN induces hepatotoxicity when animals are treated at a concentration of 50–200 mg/kg, i.p. for 4–12 weeks [44-46].

Aflatoxin B1 (AFB1): Aflatoxin B1 is a natural occurring mycotoxin obtained from *Aspergillus flavus* [47]. AFT is metabolized by cytochrome P450 such as CYP1A2 and CYP3A4 [48] into exo-8, 9-epoxide intermediate which further changes into dialdehyde. This dialdehyde form adducts with hepatic protein and induces hepatic toxicity. Dialdehyde also induces spontaneous mutation at guanine residue [49]. AFB1 increases serum concentrations of biological markers such as SGOT, SGPT, alkaline phosphates, bilirubin and decrease the level of serum cholesterol [1]. The prominent gross pathologic and histopathologic changes in the liver are hemorrhages, necrosis, and massive accumulation of lipid which is induced at different concentrations, i.e. (200 µg/kg–6 mg/kg, p.o. for 2 days–52 weeks) [50-53].

Bromobenzene: Bromobenzene (BB) is well known industrial solvent [54]. BB when administered gets biotransformed (hydroxylation) in liver into 3, 4-epoxide [54]. Metabolite binds with glutathione S-transferase (GST). In normal process, GSTs catalyze the sequestration of reactive epoxide and thus protect the hepatocytes. Epoxide further changes into bromophenol and finally hydroquinone are formed which conjugate with GSH and eventually reduce its concentration [54]. Reduction in the level of GSH leads to increase in activities of ROS, lipid peroxidation and mitochondrial dysfunction [54]. Bromobenzene, when administered at a dose of 0.5-5 ml/kg, p.o for 10-12 week, induces hepatotoxicity [55].

Lithocholic acid: Lithocholic acid (LCA) is a common model exclusively used for cholestatic liver injury [56]. Lithocholic acid, when administered, induces a biochemical alteration in bile canalicular membrane. LCA has poor solubility thus it forms a crystalline plug and induces cholestasis [56]. A single dose of LCA (4µmol/100 gram of body weight, i.v) induces cholestatic liver injury [56].

Acryl amide (AA): Acrylamide is regarded as one of the possible causative agent of carcinoma [5]. AA when administered metabolizes into epoxy glycinamide (oxidation) and induces cancer [5]. AA when administered at a dose of 6 mg/kg i.p for 15 days induces hepatoxicity [57].

Acrolein (allyl alcohol): Acrolein generates from allyl alcohol by the action of alcohol dehydrogenase [58]. Acrolein is a strong electrophile and reacts with sulfhydryl group of GSH in hepatocytes [59].The reaction is accelerated by the activity of cytosolic GST to form aldehyde-GSH adducts, which are metabolized to acrylic acid [60]. Acrolein reduces the level of GSH and increases the level of ALT, AST and GGT. Hepatotoxicity of acrolein is seen at a dose of 35 mg/kg, i.p, when given for 8 days [61].

Alpha-Naphthyl isothiocyanate (ANIT): It is a chemical derivative of naphthalene. ANIT damage the bile duct epithelium and hepatic parenchyma cell [62]. ANIT at a dose of 75 mg/kg, i.p, induces cholestasis and bile duct injury with 24 hours [62].

D-Galactosamine (D-Gal): D-Gal is a well-known hepatotoxic chemical used for inducing viral hepatitis with necrosis and inflammation [63]. Hepatotoxicity induced by D-Gal resembles with the drug-induced hepatotoxicity [63]. D-Gal once when consumed, depletes the uridine pool which results in deficiency of RNA and directly affects the protein synthesis [63]. D-Gal reduces the bile flow (cholestasis) & when given at a dose of 800 mg/kg, i.p., for 20 days induces hepatotoxicity [30].

2.2 Drug-induced hepatotoxicity

Drug-induced hepatotoxicity is often life threatening and most of the time it is unpredictable. Some of the drugs causes hepatotoxicity in a dose-dependent manner. Drug-induced hepatotoxicity is classified into two categories. (1) Drug or their metabolite causing direct toxicity by interfering with cellular function. (2) Toxic metabolite inducing toxicity by acting through cytokines or other inflammatory markers.

NSAIDs: It is one of the most frequent prescribed drugs in fever, all grades of pain, inflammation and arthritis [64]. NSAIDs impart toxicity to liver, kidney and the whole of GI [65]. Among all NSAIDs used clinically, paracetamol was found to be the most hepatotoxic and thus it is used in the laboratory to induce hepatotoxicity so that mechanism of hepatocellular injury can be better understood.

Paracetamol: PCM is a widely prescribed analgesic and antipyretic. When consumed, it gets metabolized into N- acetyl-P- benzoquinone imine and this metabolite is detoxified by GSH [66]. When an excess of the metabolite is produced in the body, the level of endogenous GSH depletes. This toxic metabolite binds with nucleophilic macromolecule of hepatocyte and causes necrosis, as shown in figure 1 [67]. PCM, when given at a dose of 500 mg/kg, p.o, for 14 days, induces hepatotoxicity [68].

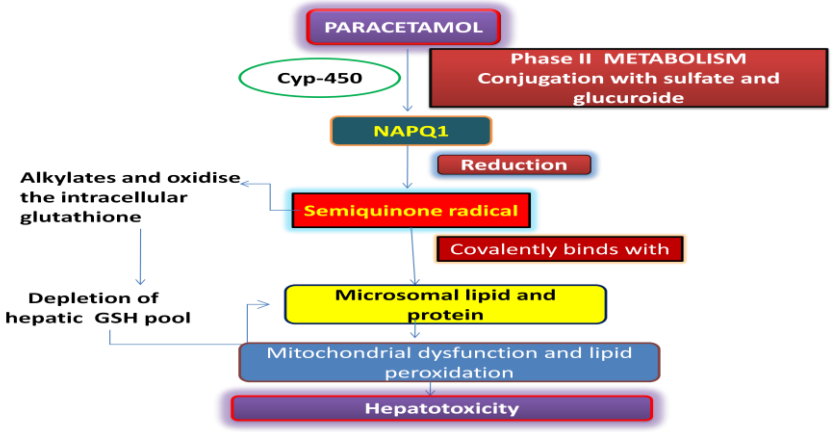

Figure 1: Mechanism of hepatocellular injury by paracetamol

Anticancer: Cancer is a complex disease, causes abnormal cell growth [69]. With advancement in understanding the pathophysiology of cancer, newer drug is being developed with least side effects [70]. Almost all the anticancer drugs cause hepatotoxicity, hence anticancer drugs are used in the laboratory for inducing hepatic injury and understanding its action [71]. Cisplatin at a dose of 3.5-7 mg/kg, i.p, for 1-5 days induces remarkable hepatic injury [72].

Azathioprine (AZP): An important anticancer drug, mostly used as an immunosuppressant drug to prevent graft rejection or autoimmune disorder [73]. Upon administration, AZP metabolize into 6 MP by using sulfhydryl group from GSH, thus hepatotoxicity due to AZP is directly related with GSH. AZP, when given at dose of 15 mg/kg, p.o, for 4 weeks, induces sufficient hepatotoxicity [74].

Adriamycin (Doxorubicin): Obtained from S*treptococcus puicetius varcesius*. Doxorubicin is the most compelling drug used against various types of tumors [75]. Metabolites of doxorubicin oxidation are semi quinine & quinine radicals which induces hepatotoxicity at a dose of 10 mg/kg [75].

Ranitidine: Ranitidine is a H2 antagonist used in peptic ulcer and Gastro intestinal reflux disorder (GERD) to neutralize the acid content in the stomach (duodenum) [76]. A metabolite of ranitidine causes hepatotoxicity via immunological

pathway. It causes steatosis, cholestasis and induces fibrosis in portal track [76]. It also induces proliferation in the bile duct, and sometimes presence of plasma cell, eosinophils and lymphocytes are seen in the long-term use of ranitidine [77]. Ranitidine, when given at a dose of 30-50 mg/kg, i.v, induces hepatotoxicity within 24 hours [77].

Anti-tubercular drugs: Since long time, TB in India remained a challenge for public health care sector. No doubt, complete abolition of TB can be achieved by appropriate dosing schedule or DOTS regimen [78], still a major challenge with the anti-TB drug is the'drug-induced hepatotoxicity' [79]. Isoniazid (INH) upon administration, metabolize into acetyl-isoniazid in presence of N-acetyl transferase. This intermediate further hydrolyze into acetyl hydrazine and reactive acetyl species [80] which bind with hepatic cell and induces hepato-toxicity at the dose of 50-100 mg/kg, p.o or i.p, when given for 28 days [81]. Rifampicin when taken in combination with INH, potentiate the hepato-toxicity by enhancing the conversion of acetyl hydrazine into reactive acetyl species [82]. Rifampicin also increases the metabolism of INH into isonicotinic acid which is again hepato-toxic [82]. It has been found that rifampicin shortens the half-life of acetyl hydrazine that means acetyl hydrazine changes into reactive acetyl species at a much faster rate [82].

Erythromycin: It is one of the potent macrolide (antibiotics) whose metabolite forms free radical that causes hepatotoxicity [83]. Erythromycin stearate at a dose of 100 mg/kg for 14 days and erythromycin esolate at a dose of 800 mg\day for 15 days induces hepatotoxicity [84].

Halothane: It is among the commonly used general anesthetics (inhaled) [85]. A metabolite of halothane causes hepatocellular necrosis [85] within 12 hours when given at a dose of 300 mmol/kg, i.p, in 2 ml of olive oil [86].

Tamoxifen: Tamoxifen is selective estrogen receptor antagonist modulator (SERMs) [87]. SERMs are the drug of choice in all stages of hormone-responsive breast cancer [87]. Upon metabolism, it changes into 4-hydroxy-N-desdimethyltamoxifen, 4-hydroxytamoxifen & -des dimethyltamoxifen [88]. SERM when administered above the therapeutic window become carcinogenic and produces free radicals [88]. 45 mg/kg/day of tamoxifen when given in 0.1 ml of dimethyl sulfide & normal saline for 6 days, induces hepatotoxicity [89].

2.3 Phytotoxin-induced hepatotoxicity

Phallotoxin: It is a cyclopeptide compound obtained from green death cap of mushroom (*Amanita phalloides*) [90]. Hepatotoxic mechanism of phallotoxin comprises of binding with F-actin which prevents the depolymerization equilibrium with G-protein and thus induces severe cholestasis [91]. Hepatotoxic dose of phallotoxin is 50-100 gram/body weight, i.v. [92].

Microcystine (MCR): It is a cyclic heptapeptide synthesized by blue-green algae (M*icrocystis aeruginosa*) [5]. MCR at a dose of 20 µg/kg, when given for 28 days, induces neoplasia [5].

Pyrrolizidine alkaloids (mono-crotaline): This group of alkaloids causes sub-optimal edema and progressive fibrosis which sometime changes into necrosis [93].

Radiation: There are two types of radiations (1) Ionizing radiation (alpha, beta, gamma, and X-ray) which damage the living tissue [94], (2) Non-ionizing radiation (visible light, UV radiation & radio wave). Ionizing radiation damages the central vein, endothelium & sinusoids [95]. Details of radiation-induced hepatotoxicity are shown in table 3.

Table 3: Radiation-induced hepatotoxicity

ANIMAL USED	DOSE	DURATION	References
Male Wistar albino rats	Single dose of gamma rays (6 Gy)	15 consecutive days	[96]
Male Sprague-Dawley rats	5 Gy of c-radiation	2 days	[97]
Male Wistar albino rats	the acute single dose level of 3 or 6 Gy	7 days	[98]

2.4 Metal- induced hepatotoxicity

Mercury: Mercury is used randomly in industries and is one of the common causes of water pollution and health hazard.[99-100]. Mercury is a transition metal which promotes the formation of ROSs like H_2O_2 and induces lipid peroxidation, mitochondrial damage and hepatocellular deterioration [101]. Mercury also diminishes the activities of natural antioxidants like GSH, SOD and Catalase [101]. Mercury, when given at a dose of 5 mg/kg, i.p, for 20 days and 3 mg/kg for 30 days, induces hepatotoxicity [102].

Cadmium (Cd): It is another metallic toxin of global concern due to its capacity to impart lipid peroxidation, mitochondrial injury and hepatocellular damage [103]. Cd promotes the formation of ROSs like superoxide and hydroxyl radicals that induces hepatotoxicity [104]. Cd induces inflammation and congestion in bile canaliculi and sinusoids and elevates the level of biological markers like ALT, AST & GGT [105]. Cd, when given at the dose of 1 mg/kg p.o for 15 days, induces marked hepatotoxicity [106].

Lead: Lead (Pb) is natural metallic toxin found under the earth crust [107]. Pb reduces the level of endogenous antioxidants like glutathione and induces organ toxicity, mainly hepatotoxicity [108] at the dose of 50 mg/kg, when given for 40 days [109], whereas rats administered with a single dose (20 mg/kg, i.p.) of lead acetate revealed significant

elevation of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP), lactate dehydrogenase, cholesterol, triglyceride and bilirubin [110].

Table 4: Metal-induced hepatotoxicity

Animal used	Dose reported	Route	Duration	References
Male albino rats	5 mg/kg (HgCl2)	s.c.	on the 7th day of experiments	[111]
Male Wistar rats	80 mg/l (HgCl2)	oral	4 weeks	[112]

s.c: subcuteniously, $HgCl_2$: Mercuric chloride

Alcohol: Alcohol is well known for causing hepatotoxicity (ALD, fatty infiltration, hepatitis & cirrhosis). Alcohol-induced fatty infiltration is reversible and subsides when consumption is withdrawn [113]. Alcohol causes hepatitis and cirrhosis by inducing lipid peroxidation. Excessive lipid peroxidation results in altered lipid imbalance in the cell membrane (made up of lipid bilayer) that causes loss of integrity of cell membrane and finally there is an elevation in glutamyl transpeptidase. GluTr further reduces the level of endogenous antioxidants like GSH & SOD [114]. 20 % of ethanol (5ml in forenoon and 5 ml in afternoon for 6 months) and 30 % v/v p.o for 20 days induces hepatotoxicity [115]. Further experimental details for alcohol-induced hepatotoxicity are shown in Table 5 below.

Table 5 Alcohol-induced hepatotoxicity

Animal Used	Dose (Ethanol)	Route	Duration For Induction	References
Wistar albino rats	2.0 ml/l00 g	p.o.	21 days	[116]
Wistar female rats	3.76 gm/kg	p.o.	twice a day for 25 days	[117]
Male Wistar albino rats	5 g/kg/day	p.o.	60 days	[118]
Male Wistar albino rat	7.9 g/kg	p.o.	45 days	[119]

p.o: oral

High-fat diet: NAFLD is directly associated with metabolic syndrome (obesity, diabetes, hypertension, and hyperlipidemia) [120]. Sometimes nutritional factor like high-fat diet also induces the progression of steatosis to NASH and NAFLD [121]. High fat induced liver disorder is among the best-accepted models for understanding the etiology of liver disease and association with metabolic syndrome [121].

2.5 Invasive model for hepatotoxicity

Bile duct ligation (BDL): BDL is one of the most common invasive animal models for secondary biliary fibrosis [122]. Rats are used in this model because they don't have gall bladder [123]. Liver consist of two types of epithelial cells (a) hepatocytes (b) cholangiocytes. Cholangiocyte's function is to modify the bile composition which is then secreted into the canalicular membrane, obstructing the biliary flow thus inducing severe cholestasis that leads to necrosis and apoptosis [123]. BDL includes the incision of mid abdomen, isolation of common bile duct and incorporation of two ligatures at the proximal and the distal part of bile duct [123]. BDL thus stimulates the proliferation of biliary epithelial cells, inflammation, and fibrosis [123].

Portal vein ligation (PVL): PVL is another invasive animal model for studying the pathogenesis of liver cirrhosis. Shunting of portal vein system reduces the portal blood supply and causes liver cirrhosis [124]. PVL is induced by first performing laparotomy with upper abdomen incision [125]. 10-20 gauge needles & 2-3 silk ligature are tied along the side of the portal vein and then abdomen is closed. This ligation then induces edema and inflammation followed by tissue damage leading to cirrhosis [125]

3. Conclusion

Hepatotoxicity is mainly related to the generation of free radicals, presence of inflammatory markers and environmental factors. All these factors contribute to the pathological abnormalities in hepatocytes and results in acute liver disease and if remained undiagnosed shift to chronic liver disease (CLD). CLD is characterized by regular destruction and regeneration of hepatic parenchymal cells that leads to fibrosis and cirrhosis. Most of the time CLD is associated with portal hypertension and liver failure. There is always a high probability for development of HCC from fibrosis and cirrhosis. HBV, HCV, NAFLDs, and ALD are most common causes of CLD, hence there is a need to understand the pathogenesis of CLD so that newer and novel diagnostic-therapeutic tools can be developed. Till date no animal models have been developed that can that can mimic all attributes of human liver disorders, so it becomes very important to select appropriate models. Rodents, mainly mice are preferred animal model as they have short life span, easy to breed and their genetic make-up is very much similar to humans. Non-invasive methods are commonly used whereas invasive models are less commonly used as operation is bit complex and used when non-invasive methods are not suitable.

Conflict of interest: None

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