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Review Article

EXPERIMENTAL MODELS FOR HEPATOTOXICITY

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ABSTRACT

The liver is among the most complex and important organs in the human body. Hepatotoxicity is a leading cause of morbidity and mortality, and its prevalence is continuously increasing day by day in the industrialized nations. Hepatotoxicity is characterized by nuclear pyknosis and eosinophilic cytoplasm, followed by large excessive hepatic lesion, fatty changes, lipid peroxidation leads to hepatic centrilobular necrosis. Paracetamol, anti-tubercular drugs, alcohol, and azathioprine are considered to be the major risk factors implicated in the progression of hepatotoxicity. Various signaling mechanisms, such as activation of innate immune system like Kupffer cells, natural killer (NK) cells, and NKT, inflammatory mediators, intracellular Ca^{2+} concentration and reactive oxygen species are involved in the pathogenesis of hepatotoxicity. At present, there is no promising therapy is available to treat patients with hepatotoxicity due to lack of understanding of signaling culprits involved in the pathogenesis of hepatotoxicity. Animal models are being developed to better understand the disease pathogenesis and develop drugs for hepatotoxicity. In the present review, we have discussed various experimental models for hepatotoxicity, which may open vistas for developing new drugs to treat hepatotoxicity.

Keywords: Hepatotoxicity, Carbon tetrachloride, Serum glutamic oxalacetic transaminase, Natural killer T, etc.

INTRODUCTION

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 [1]. Hepatotoxicity refers to liver dysfunction, which is associated with certain medicinal drugs and chemicals. Medicinal drugs are converted into chemically reactive metabolites in liver, which have the ability to interconnect with cellular macromolecules such as protein, lipids and nucleic acids, leading to protein dysfunction, lipid per oxidation (LPO), DNA damage and oxidative stress in liver. This damage of cellular function can dismiss in cell death and likely liver damage. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. More than 75% of idiosyncratic drug reactions result in liver transplantation or death [2]. Liver plays a pivotal role in regulating various physiological processes [3]. These hepatotoxic agents activated some enzymes activity in the cytochrome p-450 system such as CYP2E1 also leads to oxidative stress. Injury to hepatocyte and bile duct cells lead to accumulation of bile acid inside liver leads to promotes further liver damage. Damaging hepatocyte results in the activation of innate immune system like Kupffer cells (KC), natural killer (NK) cells, and NKT cells and result in producing pro-inflammatory mediators such as tumor necrosis factor-α, interferon-γ, and interleukin-β produced liver injury. Many chemicals/agents damage mitochondria, an intracellular organelle that produces energy. In mitochondria hepatocellular death is a direct result of drugs acting on these organelles. These programs lead to necrosis or apoptosis; they are mediated through signaling mechanisms arising at the cell membrane (e.g. death receptors) [4]. Various experimental models are employed to induce hepatotoxicity in order to identify the potential pharmacological target for hepatotoxicity. However, the literature for animal model of hepatotoxicity is currently inadequate. This review focuses on various experimentally developed animal models produced hepatotoxicity.

EXPERIMENTAL MODELS FOR HEPATOTOXICITY

Animal models represent a major tool for the study of mechanisms in virtually all of biomedical research [5]. They involve the complexity of the whole animal thus making the monitoring of *in vivo* systems quite difficult. 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 hepato-toxic effects, distribution and clearance. They may be used to elucidate the basic mechanism of xenobiotic activities, which will be useful in understanding their impact on human health. However, the experimental model is a roadmap for discovery of new molecular, noble signaling pathways for the betterment of human race [6,7].

Paracetamol induced hepatotoxicity

Paracetamol, a widely used analgesic and antipyretic drug, produces acute liver damage in high doses. Paracetamol administration causes necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis and eosinophilic cytoplasm, followed by large excessive hepatic lesion. The covalent binding of N-acetyl-P-benzoquinoneimine, an oxidative product of paracetamol to sulfydryl groups of protein, result in lipid peroxidative degradation of glutathione (GSH) level and thereby, produces cell necrosis in the liver [8,9]. Hepatotoxicity was noted after administration of paracetamol (500 mg/kg, orally) for 2 weeks in rats [10].

Galactosamine induced hepatotoxicity

Galactosamine produces diffuse type of liver injury simulating viral hepatitis. It presumably disrupts the synthesis of essential uridylate nucleotides resulting in organelle injury and ultimately cell death. Depletion of those nucleotides would impede the normal synthesis of RNA and consequently would produce a decline in protein synthesis. This mechanism of toxicity brings about an increase in the cell membrane permeability leading to enzyme leakage and eventually cell death. The cholestasis caused by galactosamine may be from its damaging effects on bile ducts or ductules or canalicular membrane of hepatocytes galactosamine decrease the bile flow and its content i.e. bile salts, cholic acid and deoxycholic acid. Galactosamine reduces the number of viable hepatocytes as well as rate of oxygen consumption. Hepatic injury is induced by intraperitoneal single dose injection of D-galactosamine (800 mg/kg) [11].

Thioacetamide induced hepatotoxicity

Thioacetamide interferes with the movement of RNA from the nucleus to the cytoplasm which may cause membrane injury. A metabolite of thioacetamide (perhaps s-oxide) is responsible for hepatic injury. Thioacetamide reduce the number of viable hepatocytes as well as rate of oxygen consumption. It also decreases the volume of bile and its content, i.e. bile salts, cholic acid and deoxycholic acid. Thioacetamide is oxidized to a reactive metabolite S-oxide which is responsible for the amendment in cell permeability and the concentration of Ca^{2+} increases intracellular in nuclear volume and also obstructs mitochondrial activity which clues to cell death [12]. Administration of thioacetamide (200 mg/kg, i.p) thrice in a weekly for 8 weeks to induced hepatotoxicity [11].

Carbon tetrachloride (CCl_4) induced hepatotoxicity

CCl_4 is metabolized by CYPs in endoplasmic reticulum and mitochondria with the formation of CCl3O-, a reactive oxidative free radical, which initiates lipid peroxidation. Administration of a single dose of CCl_4 to a rat produces, within 24 hrs, a centrilobular necrosis, and fatty changes. The poison reaches its maximum concentration in the liver within 3 hrs of administration. Thereafter, the level falls and by 24 hrs there is no CCl_4 left in the liver. The development of necrosis is associated with leakage of hepatic enzymes into serum [13]. It has been noted that administration of dose (2 ml/kg, S.C.) of CCl_4 for 2 days in rats showed significant increase in serum glutamic pyruvic transaminase (SGPT), serum glutamic oxalacetic transaminase (SGOT) levels which leads to hepatotoxicity [14].

Lead induced hepatotoxicity

Many metals play important roles in the functioning of the enzyme, cell-signaling processes and gene regulation. Lead is a blue-gray and highly toxic divalent metal that occurs naturally in the earth's crust and is spread throughout the environment by various human activities. Lead induced hepatic damage is mostly rooted in LPO and disturbance of the pro-oxidant antioxidant balance by generation of reactive oxygen species (ROS) [15]. Lead toxicity lead to free radical damage by two separate pathway: (1) Generation of ROS, including hydro-peroxides, singlet oxygen, and hydrogen peroxide and, (2) the direct depletion of antioxidant reserves. The cell membrane is the main target of the oxidative damage produced by heavy metals. This is mainly due to changes in polyunsaturated fatty acids having double bonds, largely present in the phospholipids of membranes. Lead is known to produce oxidative damage by enhancing per oxidation of membrane lipids, and LPO is a deleterious process carried out by free radicals. LPO is an outcome of the chain of events involving initiation, propagation, and termination reactions. GSH depletion is another important mechanism of lead toxicity. GSH is a tri-peptide containing cysteine with a reactive –SH group and reductive potency. It can act as a non-enzymatic antioxidant by direct interaction of the –SH group with ROS, or it can be involved in the enzymatic detoxification reaction for ROS as a cofactor. Lead bind exclusively to the –SH group, which decreases the GSH level and can interfere with the antioxidant activity of GSH [16]. Rats administered a single dose (20 mg/kg, i.p.) of lead acetate revealed significant elevations of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP), lactate dehydrogenase, cholesterol, triglyceride and bilirubin which caused hepatotoxicity [17].

Bromobenzene (BB) induced hepatotoxicity

BB, an industrial solvent and an additive in motor oils, causes necrosis in the liver. BB is subjected to biotransformation in the liver and metabolites of BB are highly hepatotoxic. BB is hydrolyzed by monooxygenases CYPs, and inhibitors of CYPs were found to decrease the hepatotoxicity. CYPs mediated epoxidation yields the highly electrophilic BB 3,4-epoxide. The irreversible binding of this very reactive metabolite to proteins like GSH S-transferase (GST), liver fatty acid binding proteins, carbonic anhydrase, is highly correlated with pathological effect. The alternative, more stable, BB-2,3-epoxide was found to covalently bind soluble protein like hemoglobin. Drug metabolizing GSTs catalyze the sequestration of the reactive epoxides through conjugation to GSH. The level of GSH conjugates excreted in the bile correlated with the BB dosage leads to the hepatotoxic effects. The epoxides are also hydrolyzed by the microsomal epoxide hydrolase and CYPs. The resulting bromophenols can be oxidized to hydroquinones and conjugated to GSH. At high doses, conjugation to the metabolites depletes the hepatic GSH pool, and the intracellular protection against ROS and hazardous xenobiotic metabolites is lost. This lead to a number of secondary events that damage cell, like lipid peroxidation, ATP depletion, mitochondrial dysfunction, energy imbalance, and altered intracellular calcium levels [18]. It has been noted that the administration of BB (0.5, 2.0 and 5.0 mm/kg body weight, dissolved in corn oil, 40% v/v) administered orally for 10-12 weeks is responsible for hepatotoxicity in rats [19].

Alcohol-induced hepatotoxicity

Liver is among the organs most susceptible to the toxic effects of ethanol. Alcohol consumption is known to cause fatty infiltration, hepatitis, and cirrhosis. Fat infiltration is a reversible phenomenon that occurs when alcohol replaces fatty acids in the mitochondria. Hepatitis and cirrhosis may occur because of enhanced lipid peroxidative reaction during the microsomal metabolism of ethanol. Alcohol can induce *in vivo* changes in membrane phospholipid composition and fluidity, because of an increase in hepatic lipid peroxidation, which may eventually affect cellular functions results in loss of membrane structure and integrity. The effects of ethanol can enhance the generation of free radicals during its oxidation in liver. These results in elevated levels of glutamyl transpeptidase, a membrane bound enzyme in serum. Ethanol inhibits GSH peroxidase, decrease the activity of catalase, superoxide dismutase, along with an increase in levels of GSH in liver. The decrease in activity of antioxidant enzymes superoxide dismutase, GSH peroxidase are speculated to be due to the damaging effects of free radicals produced following ethanol exposure or alternatively could be due to a direct effect of acetaldehyde, formed by oxidation of ethanol [20]. It has been observed that the dose of alcohol (5 ml/kg, orally) for a period of 4 weeks and increase in serum levels of ALT, and AST which leads to liver damage in rats [10].

Anti-tubercular drugs induced hepatotoxicity

Drug-induced hepatotoxicity is a potentially serious adverse effect of the currently used anti-tubercular therapeutic regimens containing isoniazid (INH), rifampicin and pyrazinamide. Adverse effects of anti-tubercular therapy are sometimes potentiated by multiple drug regimens. Thus, though INH, rifampicin and pyrazinamide each in itself are potentially hepatotoxic, when given in combination, their toxic effect is enhanced. INH is metabolized to monoacetyl hydrazine, which is further metabolized to a toxic product by CYP450 leading to hepatotoxicity. Patients on concurrent rifampicin therapy have an increased incidence of hepatitis. This has been postulated due to rifampicin-induced CYP450 enzyme-induction, causing an increased production of the toxic metabolites from acetyl hydrazine (AcHz). Rifampicin also increases the metabolism of INH to isonicotinic acid and hydrazine, both of which are hepatotoxic. The plasma half-life of AcHz (metabolite of INH) is shortened by rifampicin and AcHz is quickly converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which is related to the higher incidence of liver necrosis caused by INH and rifampicin in combination. Rifampicin induces hydrolysis pathway of INH metabolism into the hepatotoxic metabolite hydrazine. Pharmacokinetic interactions exist between rifampicin and pyrazinamide in tuberculosis patients, when these drugs are administered concomitantly. Pyrazinamide decreases the blood level of rifampicin by decreasing its bioavailability and increasing its clearance. Pyrazinamide, in combination with INH and rifampicin, appears to be associated with an increased incidence of hepatotoxicity [21]. The combined administration of the INH and rifampicin at the dose (50 mg/kg, orally) for 28 days caused hepatotoxicity in rats [22].

Azathioprine (AZA) induced hepatotoxicity

AZA is an important drug used in the therapy of autoimmune disorder and in preventing graft rejection. The nitro-conjugated double bond of

imidazole ring of AZA is a Michael acceptor. AZA is cleaved *in vitro* to 6-MP non-enzymatically by a nucleophillic attack of sulfhydryl groups primarily GSH, on the b carbon in the activated double bond AZA toxicity to rat hepatocytes was preceded by depletion of GSH. Prior GSH depletion enhanced toxicity while supplemental GSH was protective. In hepatocytes, GSH is consumed during metabolism of AZA to 6-MP. The mechanism of AZA toxicity to mitochondrial injury with profound depletion of ATP and cell death by necrosis. Lipid peroxidation as well as altered levels of some endogenous scavengers is taken as indirect *in vivo* reliable indices for the contribution of free radical generation and in turn oxidative stress [23]. It has been reported that the administration of AZA (15 mg/kg orally) for 4 weeks induced hepatotoxicity in rats [24].

Lithocholic induced hepatotoxicity

The mechanism of the hepatobiliary injury in the lithocholic acid (LCA) progressively used model of cholestatic liver injury. The etiology of LCA induced cholestasis in the rat includes biochemical alterations of the bile canalicular membrane. Due to the poor solubility of LCA the crystalline plugs develop in bile canaliculi and impaired transferring. Administration of LCA can outcome in hepatocellular necrosis with significant reductions in basolateral bile acid uptake and sinusoidal bile acid efflux transporters (Mrp3) increased. These changes in the liver represent an inherent toxicity of accumulating bile acids. The administration of LCA (4 µmol/kg, I.V., single dose) developed hepatotoxicity in rats [25].

Cadmium-induced hepatotoxicity

Cadmium metals and metalloids affect almost every organ of the body, including the liver. One such metal is cadmium, which is of concern because of its increasing prevalence as an environmental contaminant [26]. Prolonged exposure to cadmium results in injury to the liver. A large bolus dose of cadmium causes injury to a number of tissues, including the liver [27]. Cadmium induces oxidative damage in different tissues by enhancing per-oxidation of membrane lipids in tissues and altering the antioxidant systems of the cells. The per-oxidative damage to the cell membrane may cause injury to cellular components due to the interaction of metal ions with the cell organelles [28]. Cadmium toxicity leads to enhanced production of ROS such as superoxide ions, hydroxyl radicals, and hydrogen peroxides. These ROS result in increased lipid per-oxidation, hepatic congestion, ischemia and hypoxia [29]. The resultant ischemic hypoxia leads to neutrophil infiltration, KC activation, and inflammation, which could potentially contribute to the widespread hepatocellular apoptosis and necrosis [30]. Cadmium causes increase in serum concentrations of urea, creatinine, glucose, AST, acid phosphatase, alkaline phosphatase, alanine transaminase, aspartate transaminase, and serum bilirubin whereas reducing serum protein and tissue protein concentration. It has been noted that administration of cadmium with dose (1 mg/kg, orally) for 15 days in rats showed increased levels of acid phosphatase, which leads to liver tissue damage [31].

Allyl alcohol-induced hepatotoxicity

The toxicity of allyl alcohol is considered to be mediated via acrolein, which is generated from allyl alcohol by the enzyme alcohol dehydrogenase [32]. Acrolein is a powerful electrophile and reacts with nucleophiles such as sulfydryl groups [33]. The reaction is accelerated by the activity of cytosolic GST to form an aldehyde-GSH adducts, which are metabolized to acrylic acid [34]. GSH is primarily involved in the reaction, which result in a depletion of cellular GSH stores, followed by hepatocellular necrosis [35]. Allyl alcohol induces increase in SGOT, SGPT and total bilirubin, whereas decrease in total protein. The rats treated with allyl alcohol shows necrosis around branches of the central hepatic vein and presence of a large amount of nuclear debris. It has been noted that the administration of a single dose (35 mg/kg, i.p.) of allyl alcohol in rats leads to increased liver weight associated with moderate-to-severe hepatocellular necrosis [36].

Halothane induced hepatotoxicity

Halothane is chemically 2-bromo-2-chloro-1-1-trifluoroethane. It has been used widely as an inhaled anesthetic and as liver toxicant in animal models [37]. It is well established that halothane is metabolized in the liver as a lipophilic xenobiotic to hepatotoxic intermediates by monooxygenases through the CYP450-2E1 system [38]. Thus, halothane anesthesia causes hepatocellular necrosis, destruction of the lipid-protein interactions in human erythrocyte membranes, decrease in activities of membrane enzymes and alteration of cerebral glucose-6-phosphate dehydrogenase activities [39]. Halothane treated rat liver shows extensive centrilobular necrosis and denaturation. Administration of halothane at dose (30 mmol/kg, i.p.) dissolved in 2 ml of olive oil to female, and male rats lead to hepatotoxicity at 12 hrs after the administration of drug [40].

Aflatoxin B1 (AFB1) induced hepatotoxicity

AFB1 is a naturally occurring fungal toxin that causes both acute hepatotoxicity and liver carcinoma in humans and animals. AFB1 produces the hepatotoxicity through the formation of adducts with DNA, observed both *in vitro* and in rat liver [41]. These adducts are derived from highly reactive exo-epoxide metabolites of AFB1, as a result of oxidation reactions within the liver [42]. Several cytochromes P450 have been implicated in this activation and in human these were identified as CYP1A2 and CYP3A4 [43]. Acute toxicity was initially attributed to mainly genotoxic effects of the epoxide; dependent on the formation of DNA adducts, which at high levels lead to cell death. However, a dialdehyde metabolite of AFB1 that rapidly forms from the epoxide, can form adducts with proteins, and these were proposed to contribute to the acute toxicity [44]. In addition, such cellular necrotic damage caused by AFB1 dialdehyde may lead to compensatory liver hyperplasia and by so doing may promote the incorporation of mutations into the DNA of dividing cells and contribute towards carcinogenicity initiated by the AFB1-exo-epoxide [45]. AFB1 increases serum concentrations of SGOT, SGPT, alkaline phosphatase and bilirubin, and decrease in serum cholesterol. The prominent gross pathologic and histopathologic changes in the liver are hemorrhage, necrosis, and massive accumulation of lipid. Rats treated with single dose (1 mg/kg, orally) of aflatoxin developed significant liver damage due to increased activities of SGOT, SGPT and ACP in serum [46].

Ranitidine induced hepatotoxicity

Liver injury induced by ranitidine is due to its metabolite which may lead to hepatic oxidative damage, and one of its metabolite is generating the immunoallergic reaction. It also produces a reaction as reflected by infiltration of hepatocytes. Severe inflammatory changes with collagenous septa beginning to form after pronounced centrilobular and bridging necrosis. In the parenchyma, there was focal liver cell necrosis with some accumulation of histocytic elements and slight steatosis and cholestasis. Portal tract shows fibrosis, bile duct proliferation and infiltrate consisting of lymphocytes, plasma cells, polymorphs, and eosinophils. Liver injury is manifested in terms of increase in levels of serum amino transferases, modest hepatic infiltration by both lymphocytes and eosinophils and slight focal hepatocellular necrosis also causes liver cholestasis associated with increased plasma bilirubin and alkaline phosphatase [47]. Administered ranitidine for 24 hrs at dose (30 mg/kg, i.v.) leads to hepatotoxicity in rats increases in serum ALT and serum AST activity. These changes reflect hepatotoxicity in rats [48].

Mercury induced hepatotoxicity

Human activities play a major role in polluting the environment by toxic and carcinogenic metal compounds. These are evidences that these metals by accumulating contaminate waters sources and food chain with their compounds. Mercury and its compounds are widely used in industries, and their hazards to animals have been documented. Mercury is a transition metal, and it promotes the formation of ROS such as hydrogen peroxides. These ROS enhance the peroxides and hydroxyl radicals. These lipid peroxides and hydroxyl radical may cause cell membrane damage and thus destroy the cell. Mercury also inhibits the activities of the free radical quenching enzyme such as catalase, superoxide dismutase, and GSH peroxidase. Mercury causes cell membrane damage like lipid per-oxidation, which leads to the

imbalance between synthesis and degradation of enzyme protein. The excess production of ROS by mercury may be explained by its ability to produce alteration in mitochondria by blocking the permeability transition pore [49]. It has been noted that after the administration of mercuric chloride (5 mg/kg, i.p.) for 20 days and (2 mg/kg, orally) for 30 days induced hepatotoxicity in rats [50].

Hormones induced hepatotoxicity

Although many new agents are now available, androgens are still used in the hormonal manipulation of breast cancer and carry the risk of intrahepatic cholestasis [51]. The chronic use of any 17-alkyl androgen has the potential for the development of hepatic adenocarcinomas [52,53]. Cholestatic hepatitis, likely idiosyncratic, has been reported following the use of the anti-androgen flutamide for prostate cancer [54], megestrol acetate and tamoxifen therapy for breast cancer [55,56]. It has been observed that the rats administered tamoxifen (45 mg/kg/day, i.p.) in 0.1 ml of dimethylsolfoxide and normal saline for 6 days induced hepatotoxicity [57].

Phalloidin induced hepatotoxicity

Phallotoxins such as phalloidin are toxic cyclopeptide compounds produced by the green death cap of mushroom *Amanita phalloides* [58]. Phallotoxins are belonging to the class of bicyclic peptides with a transannular thioether bridge. Their intoxication mechanism in the liver involves a specific binding of the toxins to F-actin that, consequently, prevents the depolymerization equilibrium with G-actin [59]. It induces hepatotoxicity in rats at an intravenous dose of 50 g/100 g body weight phalloidin also induces a cytolytic lesion. Phalloidin causes severe liver damage characterized by marked cholestasis, which is due in part to irreversible polymerization of actin filaments [60].

Acryl amide (AA) induced hepatotoxicity

AA is a water-soluble vinyl monomer used in the production and synthesis of polyacrylamides. Monomeric AA has been shown to cause diverse toxic effects in experimental animals. AA is carcinogenic to laboratory rodents and is described by the International Agency for Research of Cancer as a probable carcinogen to humans. In the human body, AA is oxidized to the epoxide glycidamide (2, 3-epoxypropionamide) via an enzymatic reaction involving CYP4502E1. AA undergoes biotransformation by conjugation with GSH and is probably being the major route of detoxification. Rats were treated daily with AA at dose (6 mg/kg, i.p.) for 15 days leads to hepatotoxicity [61].

Microcystin induced hepatotoxicity

Microcystin-LR, a cyclic heptapeptide synthesized by the blue-green algae, microcystis aeruginosa, is a potent hepatotoxin. Pathological examination of livers from mice and rats that received microcystin-LR revealed severe, peracute, diffuse, centrilobular hepatocellular necrosis, and hemorrhage. Mice receiving sub-lethal doses of microcystin (20 g/kg) for 28 weeks developed neoplastic liver nodules [62].

Adriamycin induced hepatotoxicity

Adriamycin (doxorubicin) is an antibiotic isolated from *Streptomyces peucetius* var Cesius. Adriamycin is considered to be one of the most compelling drugs against a wide range of tumors. However, its clinical potential is contraindicated due to severe cytotoxic side effects Based on *in vitro* model of toxicity using isolated hepatocytes and liver microsomes, adriamycin has been shown to undergo redox cycling between semiquinone and quinone radicals during its oxidative metabolism. It has been noted that a single dose of adriamycin (10 mg/kg) induced hepatotoxicity in rats [63].

Alpha-naphthylisothiocyanate (ANIT) induced hepatotoxicity

ANIT injures bile duct epithelium and hepatic parenchymal cells in rats. It is commonly believed that ANIT undergoes bioactivation by hepatic, CYP450-dependent mixed-function oxidases. Rats administered once with ANIT at dose (75 mg/kg, i.p.) show liver cell damage and biliary cell damage with cholestasis at 24 hrs, but not at 12 hrs, after i.p. administration of ANIT [64].

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REFERENCES

1. Anthea M, Hopkins J, McLaughlin CW, Johnson S, Warner MQ, LaHart D, *et al.* Human Biology and Health. Vol. 98. New Jersey: Prentice Hall; 1993. p. 1176-1.
2. Ostapowicz G, Fontana RJ, Schiødt FV, Larson A, Davern TJ, Han SH, *et al.* Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med 2002;137(12):947-54.
3. Ahsan MR, Islam KM, Bulbul M, Musaddik A, Haque E. Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. Eur J Sci Res 2009;37:302-10.
4. Patel T, Roberts LR, Jones BA, Gores GJ. Dysregulation of apoptosis as a mechanism of liver disease: An overview. Semin Liver Dis 1998;18(2):105-14.
5. Uetrecht J. Role of animal models in the study of drug-induced hypersensitivity reactions. AAPS J 2006;7(4):E914-21.
6. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, *et al.* Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol 2000;32(1):56-67.
7. Dambach DM, Andrews BA, Moulin F. New technologies and screening strategies for hepatotoxicity: Use of *in vitro* models. Toxicol Pathol 2005;33(1):17-26.
8. Handa SS, Sharma A. Hepatoprotective activity of andrographolide from Andrographis paniculata against carbontetrachloride. Indian J Med Res 1990;92:276-83.
9. Kapur V, Pillai KK, Hussain SZ, Balani DK. Hepatoprotective activity of Jigrine on liver damage caused by alcohol-carbon tetrachloride and paracetamol in rats. Indian J Pharmacol 1994;26:35-40.
10. Eliwa HA, El-Denshary ES, Nada SA, Elyamany MF, Omara EA, Asaaf N. Evaluation of the therapeutic effect of whey proteins on the hepatotoxicity induced by paracetamol and alcohol co-administration in rats. IJPRBS 2014;3(2):295-314.
11. Amin ZA, Bilgen M, Alshawsh MA, Ali HM, Hadi AH, Abdulla MA. Protective role of *Phyllanthus niruri* extract against thioacetamide-induced liver cirrhosis in rat model. Evid Based Complement Alternat Med 2012;2012:241583.
12. Ambrose AM, Deeds F, Rather LJ. Further studies on toxicity of thioacetamide in rats. Proc Soc Exp Biol Med 1950;74(1):132-4.
13. Zeashan H, Amresh G, Singh S, Rao CV. Hepatoprotective activity of Amaranthus spinosus in experimental animals. Food Chem Toxicol 2008;46(11):3417-21.
14. Velmurugan V, Arunachalam G. Hepatoprotective activity of methanol extract of stem bark of *Prosopis cineraria* Linn against carbon tetrachloride induced hepatotoxicity. Int J Pharm Sci 2014;6 Suppl 2:491-3.
15. Gurer H, Ercal N. Can antioxidents be beneficial in the treatment of lead poisoning? Free Radic Boil Med 2000;29:927-45.
16. Gürer H, Ozgünes H, Neal R, Spitz DR, Erçal N. Antioxidant effects of N-acetylcysteine and succimer in red blood cells from lead-exposed rats. Toxicology 1998;128:181-9.
17. Suganthi V, Gowri S, Gurusamy K. Hepatoprotective activity of *Cayratia carnosa* on liver damage caused by lead acetate in rats. Sch Res Lib 2013;3(2):76-9.
18. Locke SJ, Brauer M. The response of the rat liver *in situ* to bromobenzene – *In vivo* proton magnetic resonance imaging and 31P magnetic resonance spectroscopy studies. Toxicol Appl Pharmacol 1991;110(3):416-28.
19. Heijne WH, Slitt AL, van Bladeren PJ, Groten JP, Klaassen CD, Stierum RH, *et al*. Bromobenzene-induced hepatotoxicity at the transcriptome level. Toxicol Sci 2004;79(2):411-22.
20. Sandhir R, Gill KD. Hepatoprotective effects of Liv-52 on ethanol induced liver damage in rats. Indian J Exp Biol 1999;37(8):762-6.
21. Padma VV, Suja V, Shyamala Devi CS, Prema. Hepatoprotective effect of Liv-52 on antitubercular drug induced hepatotoxicity in rats. Fitoterapia 1998;69(6):520-2.
22. Issabeagloo E, Taghizadich M. Hepatomodulatory action of *Camellia sinensis* aqueous extract against isoniazid-rifampicin combination

induced oxidative stress in rat. ABR 2012;3(3):18-27.
23. Raza M, Ahmad M, Gado A, Al-Shabanah OA. A comparison of hepatoprotective activities of aminoguanidine and N-acetylcysteine in rat against the toxic damage induced by azathioprine. Comp Biochem Physiol C Toxicol Pharmacol 2003;134(4):451-6.
24. Talpur M, Shaikh S, Shah SA, Memon AR, Khohazo HK. Zinc sulphate in azathioprine induced hepatotoxicity - An experimental study. WJBMS 2014;1(4):72-80.
25. Bonvicini F, Gautier A, Gardiol D, Borel GA. Cholesterol in acute cholestasis induced by taurolithocholic acid. A cytochemical study in transmission and scanning electron microscopy. Lab Invest 1978;38(4):487-95.
26. Järup L, Berglund M, Elinder CG, Nordberg G, Vahter M. Health effects of cadmium exposure – A review of the literature and a risk estimate. Scand J Work Environ Health 1998;24 Suppl 1:1-51.
27. Dudley RE, Svoboda DJ, Klaassen C. Acute exposure to cadmium causes severe liver injury in rats. Toxicol Appl Pharmacol 1982;65(2):302-13.
28. Sarkar S, Yadav P, Trivedi R, Bansal AK, Bhatnagar D. Cadmium-induced lipid peroxidation and the status of the antioxidant system in rat tissues. J Trace Elem Med Biol 1995;9(3):144-9.
29. Stohs SJ, Bagchi D, Hassoun E, Bagchi M. Oxidative mechanisms in the toxicity of chromium and cadmium ions. J Environ Pathol Toxicol Oncol 2000;19(3):201-13.
30. Rikans LE, Yamano T. Mechanisms of cadmium-mediated acute hepatotoxicity. J Biochem Mol Toxicol 2000;14(2):110-7.
31. Singh N, Rani P, Gupta M, Goel N, Tandan N. Effects of aqueous extract of camellia sinensis on liver markers of cadmium treated rats. E3 J Biotechnol Pharm Res 2013;4(5):89-93.
32. Serafini-Cessi F. Conversion of allyl alcohol into acrolein by rat liver. Biochem J 1972;128(5):1103-7.
33. Esterbauer H, Zollner H, Scholz N. Reaction of glutathione with conjugated carbonyls. Z Naturforsch C 1975;30(4):466-73.
34. Boyland E, Chasseaud LF. Enzyme-catalysed conjugations of glutathione with unsaturated compounds. Biochem J 1967;104(1):95-102.
35. Jaeschke H, Kleinwaechter C, Wendel A. The role of acrolein in allyl alcohol-induced lipid peroxidation and liver cell damage in mice. Biochem Pharmacol 1987;36(1):51-7.
36. Starckx S, Batheja A, Verheyen GR, Jonghe SD, Steemans K, Dijck BV, *et al.* Evaluation of miR-122 and other biomarkers in distinct acute liver injury in rats. Toxicol Pathol 2013;41(5):795-804.
37. McLain GE, Sipes IG, Brown BR Jr. An animal model of halothane hepatotoxicity: Roles of enzyme induction and hypoxia. Anesthesiology 1979;51(4):321-6.
38. Spracklin DK, Hankins DC, Fisher JM, Thummel KE, Kharasch ED. Cytochrome P450 2E1 is the principal catalyst of human oxidative halothane metabolism *in vitro*. J Pharmacol Exp Ther 1997;281(1):400-11.
39. Trulson ME, Ulissey MJ. Halothane anesthesia alters cerebral enzymes: A histochemical study in the rat. Acta Anat (Basel) 1987;130(2):163-7.
40. You Q, Cheng L, Reilly TP, Wegmann D, Ju C. Role of neutrophils in a mouse model of halothane-induced liver injury. Hepatology 2006;44(6):1421-31.
41. Essigmann JM, Croy RG, Nadzan AM, Busby WF Jr, Reinhold VN, Büchi G, *et al*. Structural identification of the major DNA adduct formed by aflatoxin B1 *in vitro*. Proc Natl Acad Sci U S A 1977;74(5):1870-4.
42. Swenson DH, Miller EC, Miller JA. Aflatoxin B1-2,3-oxide: Evidence for its formation in rat liver *in vivo* and by human liver microsomes *in vitro*. Biochem Biophys Res Commun 1974;60(3):1036-43.
43. Gallagher EP, Wienkers LC, Stapleton PL, Kunze KL, Eaton DL. Role of human microsomal and human complementary DNA-expressed cytochromes P4501A2 and P4503A4 in the bioactivation of aflatoxin B1. Cancer Res 1994;54(1):101-8.
44. Neal GE, Judah DJ, Stirpe F, Patterson DS. The formation of 2,3-dihydroxy-2,3-dihydro-aflatoxin B1 by the metabolism of aflatoxin B1 by liver microsomes isolated from certain avian and mammalian species and the possible role of this metabolite in the acute toxicity of aflatoxin B1. Toxicol Appl Pharmacol 1981;58(3):431-7.
45. Roebuck BD. Hyperplasia, partial hepatectomy, and the carcinogenicity of aflatoxin B1. J Cell Biochem 2004;91(2):243-9.
46. Sharmila Banu G, Kumar G, Murugesan AG. Effect of ethanolic leaf extract of *Trianthema portulacastrum* L. On aflatoxin induced hepatic damage in rats. Indian J Clin Biochem 2009;24(4):414-8.
47. Hemieda FA, Abdel-Hady el-SK, Elnga MA. Biochemical and histological studies on H2-receptor antagonist ranitidine-induced hepatotoxicity in rats. Indian J Exp Biol 2005;43(9):782-5.
48. Maddox JF, Luyendyk JP, Cosma GN, Breau AP, Bible RH Jr, Harrigan GG, *et al*. Metabonomic evaluation of idiosyncrasy-like liver injury in rats cotreated with ranitidine and lipopolysaccharide. Toxicol Appl Pharmacol 2006;212(1):35-44.
49. Nicolli A, Basso E, Petronilli V, Wenger RM, Bernardi P. Interactions of cyclophilin with the mitochondrial inner membrane and regulation of the permeability transition pore, and cyclosporin A-sensitive channel. J Biol Chem 1996;271(4):2185-92.
50. Jagadeesan G, Sankarsami Pillai S. Hepatoprotective effects of taurine against mercury induced toxicity in rats. J Environ Biol 2007;28(4):753-6.
51. Werner SC, Hanger FM, Kritzler RA. Jaundice during methyl testosterone therapy. Am J Med 1950;8(3):325-31.
52. Bernstein MS, Hunter RL, Yachnin S. Hepatoma and peliosis hepatis developing in a patient with Fanconi's anemia. N Engl J Med 1971;284(20):1135-6.
53. Henderson JT, Richmond J, Sumerling MD. Androgenic-anabolic steroid therapy and hepatocellular carcinoma. Lancet 1973;1(7809):934.
54. Rosman AS, Frissora-Rodeo C, Marshall AT, Reiter BP, Paronetto F. Cholestatic hepatitis following flutamide. Dig Dis Sci 1993;38(9):1756-9.
55. Foitl DR, Hyman G, Lefkowitch JH. Jaundice and intrahepatic cholestasis following high-dose megestrol acetate for breast cancer. Cancer 1989;63(3):438-9.
56. Pinto HC, Baptista A, Camilo ME, de Costa EB, Valente A, de Moura MC. Tamoxifen-associated steatohepatitis – Report of three cases. J Hepatol 1995;23(1):95-7.
57. Oh JM, Jung YS, Jeon BS, Yoon BI, Lee KS, Kim BH, *et al.* Evaluation of hepatotoxicity and oxidative stress in rats treated with tert-butyl hydroperoxide. Food Chem Toxicol 2012;50(5):1215-21.
58. Anderl J, Echner H, Faulstich H. Chemical modification allows phallotoxins and amatoxins to be used as tools in cell biology. Beilstein J Org Chem 2012;8:2072-84.
59. Zanotti G, Falcigno L, Saviano M, D'Auria G, Bruno BM, Campanile T, *et al*. Solid state and solution conformation of [Ala7]-phalloidin: A synthetic phallotoxin analogue. Chemistry 2001;7(7):1479-85.
60. Herraez E, Macias RI, Vazquez-Tato J, Hierro C, Monte MJ, Marin JJ. Protective effect of bile acid derivatives in phalloidin-induced rat liver toxicity. Toxicol Appl Pharmacol 2009;239(1):21-8.
61. Clark SP, Davis MA, Ryan TP, Searfoss GH, Hooser SB. Hepatic gene expression changes in mice associated with prolonged sublethal microcystin exposure. Toxicol Pathol 2007;35(4):594-605.
62. Khan MR, Afzaal M, Saeed N, Shabbir M. Protective potential of methanol extract of *Digera muricata* on acrylamide induced hepatotoxicity in rats. Afr J Biotechnol 2011;10:8456-64.
63. Sakr SA, Abo-El-Yazid SM. Effect of fenugreek seed extract on adriamycin-induced hepatotoxicity and oxidative stress in albino rats. Toxicol Ind Health 2012;28(10):876-85.
64. Ohta Y, Kongo-Nishimura M, Hayashi T, Kitagawa A, Matsura T, Yamada K. Saikokeishito extract exerts a therapeutic effect on alpha-naphthylisothiocyanate-induced liver injury in rats through attenuation of enhanced neutrophil infiltration and oxidative stress in the liver tissue. J Clin Biochem Nutr 2007;40(1):31-41.