Review Article

Hepatotoxicity Mechanisms and its Biomarkers

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Abstract
The liver may be considered as the most important organ in drug toxicity because it is functionally interposed between the site of absorption and the systemic circulation and is a major site of metabolism and elimination of foreign substances; these feature render it a preferred target for drug toxicity.Drug-induced liver injury (DILI) therefore poses a major clinical problem.DILI is initiated by direct hepatotoxic effects of a drug, or a reactive metabolite of a drug. This review summarizes current mechanistic concepts of DILI in a 3-step model that limits its principle mechanisms, i.e. direct cell stress, direct mitochondrial impairment, and specific immune reactions. Subsequently, initial injury initiates further downstream events, i.e. direct and death receptor-mediated pathways leading to mitochondrial permeability transition, which then results in apoptotic or necrotic cell death. Hepato protective effects of plant drugs and herbal formulations are studied against chemicals (alcohol, CCl_4, β-galactosamine, thioacetamide) and drugs (paracetamol, nimuselide, antitubercular drugs like isoniazid, rifampicin etc.) induced hepatotoxicity in rats and mice as they virtually mimic any form of naturally-occurring liver disease. The level of serum alanine amino transferase (ALT), glutathione-S-transferase alpha (GSTα), and certain enzymatic markers activity reflects damage to hepatocytes and is considered to be a highly sensitive and fairly specific preclinical and clinical biomarker of hepatotoxicity.

Key words: Herbal formulations, paracetamol, serum alanine amino transferase, clinical biomarker.

INTRODUCTION

Hepatotoxicity is an injury to the liver that is associated with impaired liver function caused by exposure to a drug or any other noninfectious agent. In 1989, a panel of 12 European and American experts, defined liver injury as an increase of more than twice the upper limit of the normal range in the levels of serum alanine aminotransferase or conjugated bilirubin, or a combined increase in the levels of aspartate aminotransferase, alkaline phosphatase, and total bilirubin, provided that one of these was more than twice the upper limit of the normal range. Later in the month of February 2001 clinical conference cosponsored by the FDA Center for Drug Evaluation and Research, the Pharmaceutical Research and Manufacturers of America, and the American Association for the Study of Liver Diseases, that an alanine aminotransferase level of more than three times the upper limit of normal and a total bilirubin level of more than twice the upper limit be used as a combined test to define clinically significant abnormalities on liver tests, with further verification through the analysis of additional clinical data. [1]

The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Chemicals that cause liver injury are called hepatotoxins. There are several specific conditions that all fall within the general category of hepatotoxicity. These conditions include: Hepatitis —inflammation of the liver, Hepatic necrosis—death of liver cells, Hepatic steatosis—too much fat in the liver; may be associated with a life-threatening condition called lactic acidosis.[2,3]

Classification of hepatotoxins and mechanism of action of each group of hepatotoxins

DILI can affect both parenchymal and nonparenchymal cells of the liver, leading to a wide variety of pathological conditions, including acute and chronic

hepatocellular hepatitis, fibrosis/cirrhosis, cholestasis, steatosis, as well as sinusoidal and hepatic artery/vein damage.[4] The predominant forms of DILI include acute hepatitis, cholestasis, and a mixed pattern.[5]

Acute hepatitis is defined as a marked increase in aminotransferases coinciding with hepatocellular necrosis. Cholestasis is characterized by jaundice with a concurrent elevation in alkaline phosphatase, conjugated bilirubin, and γ-glutamyl transpeptidase. Mixed-pattern DILI includes clinical manifestations of both hepatocellular and cholestatic injury.

There are literally thousands of chemicals that could be toxic to the liver and a few examples of these chemicals that are commonly used in the treatment include:[6]

- Rimadyl (arthritis treatment) in Labradors
- Thiacetarsamide (heart worm treatment)
- Ketoconazole (fungal treatment)
- Tylenol (acetaminophen)
- Glucocorticoids (cortisone)
- Anthelmintics (deforming medication)
- Parasiticides

1. CCl_4 induced hepatotoxicity

Liver injury due to carbon tetrachloride in rats was first reported in1936 and has been widely and successfully used by many investigators. Carbon tetrachloride is metabolized by cytochrome P-450 in endoplasmic reticulum and mitochondria with the formation of CCl_3O^-, a reactive oxidative free radical, which initiates lipid peroxidation. Administration of a single dose of CCl_4 to a rat produces, within 24 h, a centrilobular necrosis and fatty changes. The development of necrosis is associated with leakage of hepatic enzymes into serum.[7]

$$CCl_4 \rightarrow CCl_3O^- + O^-$$

2. Thioacetamide induced hepatotoxicity

Thioacetamide interferes with the movement of RNA from the nucleus to 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. Dose of thioacetamide: 100 mg/kg, S.C. [8]

Thioacetamide, originally used as a fungicide, is a potent hepatotoxicant, bioactivated by cyp450 and/or Flavin-Containing Mono oxygenase (FMO) systems to sulfine (sulfoxide) and sulfene (sulfone) metabolites, which cause centrilobular necrosis. Thioacetamidesulfoxide, a relatively stable intermediate of thioacetamide is the penultimate reactive, is obligatory for the hepatotoxic effects[9].

3) 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 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 it's content i.e. bile salts, cholic acid and deoxycholic acid. Galactosamine reduces the number of viable hepatocytes as well as rate of oxygen consumption. Dose of D-Galactosamine: 400 mg/kg, I.P.[8]

4) 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 enhanced generation of oxyfree radicals during its oxidation in liver. This results in elevated levels of glutamyl transpeptidase, a membrane bound enzyme in serum. Ethanol inhibits glutathione peroxidase, decrease the activity of catalase, superoxide dismutase, along with increase in levels of glutathione in liver. The decrease in activity of antioxidant enzymes superoxide dismutase, glutathione 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[10].

5) 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 sulphydryl groups of protein, result in lipid peroxidative degradation of glutathione level and thereby, produces cell necrosis in the liver. Dose of Paracetamol: 1 gm/kg P.O [11,12].

6) Antitubercular drugs induced hepatotoxicity (Rifampicin, Isoniazid and pyrazinamide)

Drug induced hepatotoxicity is a potentially serious adverse effect of the currently used antitubercular therapeutic regimens containing Isoniazid (INH), Rifampicin and Pyrazinamide. Adverse effects of antitubercular 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 cytochrome P 450 leading to hepatotoxicity.Patients on concurrent rifampicin therapy have an increased incidence of hepatitis. This has been postulated due to rifampicin-induced cytochrome P 450 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 decrease 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.[13]

7) Azathioprine induced hepatic necrosis

AZA is an important drug used in the therapy of autoimmune disorders 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 nucleophilic attack of sulfhydryl groups primarily GSH, on the β 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 hepatocytes involves depletion of GSH leading to mitochondrial injury with profound depletion of ATP and cell death by

necrosis. Lipid peroxidations as well as altered levels of some endogenous scavengers are taken as indirect in vivo reliable indices for the contribution of free radical generation and in turn oxidative stress.[14]

8) Ranitidine induced hepatotoxicity

Liver injury induced by ranitidine is due to its metabolite which may leads to hepatic oxidative damage and one of its metabolite is generating immunoallergic reaction. It also produces a reaction as reflected by infiltration of hepatocytes with ranitidine dose of either 50 or 30 mg/kg. 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 aminotransferases, 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.[15]

DILI accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (APAP, 39%) and idiosyncratic liver injury triggered by other drugs (13%). Because of the significant patient morbidity and mortality associated with DILI, the U.S. Food and Drug Administration (FDA) has removed several drugs from the market, including bromfenac, ebrotidine, and troglitazone. Other hepatotoxic drugs, such as risperidone, trovafloxacin, and nefazodone have been assigned "black box" warnings. DILI is the most common cause for the withdrawal of drugs from the pharmaceutical market.[16]

A GENERAL 3-STEP MODEL FOR DRUG-INDUCED LIVER INJURY

Initial Mechanisms of Toxicity

Direct Cell Stress, Direct Mitochondrial Impairment and Specific Immune Reactions

Drug metabolites or less frequently parent drugs cause direct cell stress, target mitochondrial function, or trigger specific immune reactions. The drug metabolizing enzyme system responsible for the creation of hepatotoxic reactive metabolites is the polymorphic cytochrome P450 (CYP450) family that mediates oxidative phase-I drug metabolism. However, conjugative phase-II metabolism may also result in hepatotoxic metabolites, e.g. acyl glucuronides are well known to cause DILI. This Reactive metabolites can exert cell stress initially through a wide range of mechanisms including depletion of glutathione (GSH), or binding to enzymes, lipids, nucleic acids and other cell structures, they may also specifically inhibit other hepatocellular functions such as the apical (canalicular) bile salt efflux pump (BSEP, ABCB11 gene), which causes the intracellular accumulation of substrates may leads to secondary toxic hepatocyte damage. In case of initial targeting of mitochondria, reactive metabolites or parent drugs uncouple or inhibit the mitochondrial respiratory chain causing ATP depletion and increased concentrations of reactive oxygen species (ROS), inhibit β-oxidation leading to steatosis (e.g. after intramitochondrial accumulation of amiodarone).[17]

Hepatic cellular dysfunction and death may initiate immunological reactions, including both innate and adaptive immune responses. Hepatocyte stress or damage release signals that stimulate and activate cells of the innate immune system, including Kupffer cells (KC), natural killer (NK) cells, and NKT cells, which contribute liver injury by producing proinflammatory mediators and secreting chemokines. It has also been noted that various inflamatory cytokines, such as tumor necrosis factor (TNF α), interferon (IFN)-γ, and interleukin (IL)-1β are produced during DILI promotes tissue damage. However, innate immune cells are also the main source of IL-10, IL-6, and certain postglandins, all of which have been shown to play a hepatoprotective role. Thus, it is the delicate balance of inflammatory and hepatoprotective mediators produced after activation of the

innate immune system. In addition to the innate immune responses, clinical features of certain DILI cases strongly suggest that the adaptive immune system is activated and involved in the pathogenesis of liver injury. With regard to the involvement of the adaptive immune system in DILI, our current understanding is based on the hapten hypothesis and the p-i (pharmacological interaction of drugs with immune receptors) concept. Evidence to support these hypotheses is gained by the detection of drug-specific antibodies and T cells in some patients with DILI.[18]

Biomarkers of hepatotoxicity

As liver is a multifunctional organ, a battery of liver function tests is employed to evaluate the effect of drug on liver, which are Non-invasive functional methods:

1) Ascorbic acid content in urine

Measurment of ascorbic acid content of urine is reported as a non-invasive test for screening hepatoprotective agents against CCl_4 induced hepatotoxicity in rats.[(19)] CCl_4, a pharmacological tool to produce liver damage, reduces the excretion of ascorbic acid in rats.

2)Hexobarbitone or zoxazolamine induced sleeping time

Toxic liver prolongs duration of sleeping time for pentobarbitone, hexobarbitone, zoxazolamineetc in mice, rats.

3)Bromosulphthaline clearance test

The liver normally clears bromosulphthalein (BSP), a dye, from the blood. The level of BSP in the blood after intravenous injection of BSP is a sensitive guide to hepatic damage. During the passage of BSP from the plasma to the bile, it undergoes storage, metabolism and excretion by the liver. The abnormal functional effects produced by CCl_4 leads to the retention of BSP in blood.[(20)]

4) Biochemical analysis of blood for SGPT and SGOT

Serum and hepatocyte enzyme AST i.e. Aspartate Transaminase (SGOT), and ALT i.e. Alanine Transaminase (SGPT), are both sensitive markers of hepatocellular injury. When the liver cell is injured or dies, these proteins can leak through the liver cell membrane into the circulation and serum levels will rise. ALT or SGPT is a cytosolic enzyme primarily present in the liver. Its normal serum level is 10-35 Karmel units/ml. ALT reversibly catalyses amino group from alanine to α-ketoglutarate. ALT levels are very high in patients of viral hepatitis and hepatic necrosis, 10 to 200 fold higher in patients of post hepatic jaundice, intrahepatic cholestasis and below 10 fold in patients of metastatic carcinoma, cirrhosis and alcoholic hepatitis. AST or SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscles and kidney. Its normal serum level is 10-40 Karmel units/ml 2 AST reversibly catalyses transfer of amino group from aspartate to α-ketoglutarate.

AST levels are 10 to 200-fold elevated in patients with acute hepatic necrosis, viral hepatitis, CCl_4 and drug induced poisoning.

Alkaline phosphatase

Serum alkaline phosphatase is produced by many tissues, especially bone, liver, intestine and placenta and is excreted in the bile. In the absence of bone disease and pregnancy, an elevated serum alkaline phosphatase levels generally reflect hepatobiliary disease. The mechanism of elevated ALP levels may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchymal or duct cells. Principle involved in estimation of alkaline phosphatase: ALP hydrolyses substrate P-nitrophenyl phosphate with the formation of P-nitrophenol and liberation of phosphate ion.[21]

Serum Bilirubin

Estimation of bilirubin, metabolic product of the breakdown of heme is one of the better liver function tests. Normally, 0.25 mg/dl of conjugated bilirubin is present in the blood of an adult. Bilirubin level rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum, in hemolysis and defects of hepatic uptake and conjugation of bilirubin treatment such as Gilberts disease.[3] Bilirubin in serum

reacts with diazo reagent in the presence of alcohol, after the proteins had been removed by precipitation.[22]

Serum Protein

Liver cells synthesize albumin, fibrinogen, prothrombin, alpha-1-antitrypsin, haptoglobin, ceruloplasmin, transferrin, alpha foeto proteins and acute phase reactant proteins. The blood levels of these plasma proteins are decreased in extensive liver damage.[3]

6)Hepatocyte Viability and Oxygen Uptake tests

Hepatotoxicants reduce the viability of hepatic cells as assessed by trypan blue exclusion and oxygen uptake tests[23]. In liver, CCl 4 is metabolised to CCl 3 O - by cytochrome P-450 and the reactive oxidative free radical intermediate generated, O - causes further damage. Utilisation of oxygen by hepatocytes gets reduced; therefore the viability of hepatocytes is reduced.[24]

7) Free radical scavengers

Free radicals are reactive molecules involved in many physiological processes and human diseases such as cancer, aging, arthritis, Parkinson syndrome, ischaemia, toxin induced reaction, alcoholism, liver injury etc. The damage to hepatic parenchymal cells, leading to hepatic injury, is due to oxidative stress within the cells caused by partially reduced free oxygen (PRFO) species such as O_2 (Superoxide anion), H_2 , O_2 , and OH (hydroxy free radical). The elevation of free radical levels seen during the liver damage is due to enhanced production of free radicals and decreased scavenging potential of the cells. A variety of intrinsic antioxidants (reduced glutathione, superoxide dismutase, glutathione-S-transferase etc.) are present in the organism, which protect them from oxidative stress. Technically, the estimation of free radicals directly is not possible due to the transient nature of the free radicals. Thus estimations are usually done indirectly by measuring the "Antioxidant defense status" of the liver microsomes. Hepatoprotection by enzymatic free quenching is brought about by elevating the levels of antioxidant enzymes in tissues such as the Superoxide dismutase (SOD), Peroxidase and Catalase.

Free radical generation

The three partially reduced intermediate species between O_2 to H_2O are derived from enzymatic and nonenzymatic reaction as under:

A. Superoxide (O_2-)

superoxide anion O2 - may be generated by direct auto oxidation of O_2 during mitochondrial electron transport reaction. Alternatively O_2 is produced enzymatically by xanthine oxidase and cytochrome P 450 in the mitochondria or cytosol. O_2 so formed is catabolised to produce H_2O_2 by superoxide dismutase.

B. **Hydrogen peroxide (H_2O_2)**

H_2O_2 is reduced to water enzymatically by catalase (in the peroxisomes) and glutathione peroxidase GSH (both in the cytosol and mitochondria).

C. **Hydroxyl radical**

OH - radical is formed by two ways in biologic processes-by radiolysis of water and by reaction of H2O2 with ferrous (Fe^{++}) ions, the latter process is termed as Fenton reaction.

Free radicals may produce membrane damage by the following mechanisms

A. Lipid peroxidation

Polyunsaturated fatty acids (PUFA) of membrane are attacked repeatedly and severely by oxygen derived free radicals to yield highly destructive PUFA radicals,lipidhydroperoxy radicals and lipid hydroperoxidation. The lipid peroxidase is decomposed by transition metals such as iron. Lipid peroxidation is propagated to other sites causing widespread membrane damage and destruction of organelles.

B. Oxidation of proteins

Oxygen-derived free radicals cause cell injury by oxidation of protein macromolecules of the cells, cross linking of labile amino acids as well as by fragmentation of polypeptides directly. The end result is degradation of cytosolic neutral proteases and cell destruction.

C. DNA damage

Free radicals cause breaks in the single strands of the nuclear and mitochondrial DNA. This results in cell injury; it may also cause malignant transformation of cells.

D. Cytoskeletal damage

Reactive oxygen species are also known to interact with cytoskeletal elements and interfere in mitochondrial aerobic phosphorylation and thus cause ATP depletion.

8. Superoxide dismutase (SOD)

SOD is a ubiquitous cellular enzyme, which dismutates superoxide radical to hydrogen peroxide and oxygen.[25]Dismutation is a reaction in which a single reactant is converted into two different products. Superoxide dismutase, one of the chief cellular defense mechanisms, scavenges superoxide radicals by catalyzing the conversion of two of these radicals into hydrogen peroxide and molecular oxygen.

9. Catalase

The hydrogen peroxide formed by superoxide dismutase and other processes is scavenged by catalase, a ubiquitous heme protein that catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen.[26]

10. Glutathione (GSH)

GSH is a tripeptide of glycine, glutamic acid and cysteine. Glutathione is an important naturally occurring antioxidant as it prevents the hydrogen of sulfydryl group to be abstracted instead of methylene hydrogen of unsaturated lipids. Therefore, levels of glutathione are of critical importance in tissue injury caused by toxic substances. The antioxidant enzymes superoxide dismutase and glutathione form the first line of defense against free radical induced damage, offer protection against free radicals and thereby, maintain low levels of lipid peroxides[10]. The primary biological function of glutathione is to act as a non-enzymatic reducing agent to help keep cysteine thiol side chains in a reduced state on the surface of proteins. Glutathione is also used to prevent oxidative stress in most cells and helps to trap free radicals that can damage DNA and RNA.

11. Lipid peroxidation

Lipid peroxidation can be defined as the oxidative deterioration of lipids. Lipid hydroperoxides are non-radical intermediates derived from unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol itself. These are formed in enzymatic or non-enzymatic reactions involving free radical[27].

12. Glutathione peroxidase

Glutathione peroxidase (GPx) is an enzyme which catalyzes the reduction of hydroperoxides, including hydrogen peroxides, and functions to protect the cell from peroxidative damage. It is a seleniumcontaining enzyme and reduces H_2O_2 to H_2O by oxidizing glutathione (GSH) Rereduction of the oxidized form of glutathione (GSSG) is then catalysed by glutathione reductase. GPx activity can be measured indirectly by a coupled reaction with glutathione reductase. Oxidized glutathione (GSSG) produced upon reduction of an organic hydroperoxide by GPx, is recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 340 nm. The rate of decrease in the A340 is directly proportional to the GPx activity in the sample[28].

REFERENCES

1. Victor J, Navarro MD and John R. Drug-Related Hepatotoxicity N Engl J Med 2006;354:731-9.
2. Lee MK, Yeo H, Kim J, and Kim YCJ. Pharm. Pharmacol. 2000;52(3): 341-5
3. Harshmohan. The liver, biliary tract and exocrine pancreas. In: *Text book of pathology,* 4th Ed, Jaypee Brothers Medical Publishers (P) Ltd. New Delhi. 2002; 22-4 and 569-630.
4. LarreyD . Drug-induced liver diseases. J Hepatol . 2000 ; 32 : 77 – 88(from moa of ht)
5. GunawanB ,Kaplowitz N . Clinical perspectives on xenobiotic induced

hepatotoxicity. DrugMetabRev .2004 ; 36 : 301–12.
6. Zimmerman HJ, Ishak KG. “Hepatotoxin injury due to drugs and toxins” London Churchill Livingstone 2002; 4: 622-24.
7. Handa SS and Sharma A. Hepatoprotective activity of Andrographolide from *Andrographispaniculata* against carbon tetrachloride. Ind. J. Med. Res 1990; 92: 276-92.
8. Saraswat B, Visen PKS, Dayal R, Agarwal DP and Patnaik GK. Protective action of ursolic acid against chemical induced hepatotoxicity in rats, Ind. J. Pharmacol.1996; 28: 232-39
9. Shashi K, Ramaiah, Apte U, Mehendale HM. Cytochrome P4502E1 induction increases ethanol liver injury in diet restricted rats, Drug Meta &Dispo 2001; 29(8):1088-1095.
10. Sandhir R and Gill KD. Hepatoprotective effects of Liv-52 on ethanol induced liver damage in rats. Ind . J. Expt. Biol. 1999; 37:762-66.
11. Kapur V, Pillai KK, Hussain SZ and Balani DK. Hepatoprotective activity of Jigrine on liver damage caused by alcohol-carbon tetrachloride and paracetamol in rats. Ind. J. Pharmacol. 1994; 26:35-40.
12. Handa SS and Sharma A. Hepatoprotective activity of Andrographolide from *Andrographis paniculata* againstCCl_4. Ind. J. Med. Res .1990; 92: 276 92.
13. Padma VV, Suja V, Shyamala DCS and Prema. Hepatoprotective effect of Liv-52 on antitubercular drug-induced hepatotoxicity in rats. Fitoterapia. 1998; 69(6): 520-22.
14. M Raza, M Ahmad, AGado, OA Al-Shabanah. A comparison of hepatoprotective activities of amino guanidine and N-acetyl cysteine in rat against the toxic damage induced by azathioprine. Comparative BiochemPhysiol 2003; 134: 451–56.
15. HemiedaFaried AE, Abdel Hady El-Sayed. Biochemical and histological studies on H2- receptor antagonist ranitidine-induced hepatotoxicity in rats. Ind J Exp Bio 2005;43:782-85.
16. Michael P, Holt, Cynthia Ju. Mechanisms of drug-induced liver injury. The AAPS Journal. 2006;8(1).
17. Russmann S, Kullak-Ublick GA, Grattagliano I. Current concepts of mechanisms in drug-induced hepatotoxicity. Curr Med Chem. 2009;16(23):3041-53.
18. Michael P, Holt, Cynthia J. Mechanisms of drug-induced liver injury. The AAPS Journal. 2006;8(1)
19. Visweswarm D, Rao PR and Satyanarayana S, A noninvasive method for evaluating hepatoprotective drugs in CCl_4 induced hepatotoxicity. Ind. J. Pharmacol. 1994;26(4): 301-03.
20. Klaassen CD and Plaa GL. Effect of CCl_4 on the Metabolism, Storage and Excretion of Sufobromophthalein. Toxicol . Appl. Pharmacol. 1968; 12: 132-9.
21. Kind PRN and King EJ. Estimation of plasma phosphates by determination of hydrolysed Phenol with Antipyrine. J. Clin. Pathol.1954; 322-30.
22. Malloy HT and Evelyn KA. The Determination of bilirubin with the photoelectric colorimeter. J. Biol. Chem . 1936;119: 481-90.
23. Lee WM . Drug-induced hepatotoxicity. N Engl J Med .2003 ; 349 : 474 - 485 .
24. Zimmerman MD and Hayman J. Function and integrity of the liver, In: Clinical diagnosis andmanagement by laboratory methods In: Clinical diagnosis and management by laboratory methods , 17 th Ed. 1976; 217-50.
25. Handa SS. In: Manual, TCDC International workshop cum training on herbal drugs. 1997;70-9.
26. Berg JM, Tynaoczko JL, Stryer L. In: *Biochemistry,* 5 thedition, W.H. Freeman and Company, New York . 2000; 506-07.
27. Gilbert DL, Oxygen and living processes: an inter-disciplinary approach, 1981; Springer, NY. 107-21.
28. HalliwellB J. Neurochem. 1995; 59:1609-23