Review Article

A Review on Hepatotoxicity: Diagnosis, Mechanism, Experimental Parameters and Therapeutic Agents

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ABSTRACT

Liver is the principle organ for maintaining the body's internal environment. There is currently no way to compensate for the absence of liver function. Its key impact is on the flow of nutrients and controls the metabolism of carbohydrate, protein and fats. There are many traditional as well as allopathic medicines available which report hepatoprotection but the treatment of chronic liver disease is still a challenge for health care professionals. Drugs are an important cause of liver injury. More than 900 drugs, toxins, and herbs have been reported to cause liver injury. Approximately 75% of the idiosyncratic drug reactions result in liver transplantation or death. Many types of drug induced liver diseases are acute-dose dependent liver damage, acute fatty infiltration, cholestatic jaundice, liver granulomas, active chronic hepatitis, liver cirrhosis, liver tumors etc. In the United States, approximately 2000 cases of acute liver failure occur annually and drugs account for over 50% of them (37% are due to acetaminophen, 13% are idiosyncratic reactions due to other medications). This review light on various drugs which induce hepatotoxicity, their test mechanism of liver damage, hepatoprotective herbs and clinical scenario.

Keywords: Hepatotoxicity, hepatoprotection, medicinal plants, drug metabolism, Ayurveda, mechanism etc.

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INTRODUCTION

The liver is an important and largest organ of the human body. It starts developing in the human embryo between the 3rd and the 4th week of life, and it is the main site of hematopoiesis in the intrauterine period. The healthy human liver has weight around 1500 g, and it is located in the upper right corner of the abdomen and beneath the diaphragm [1].

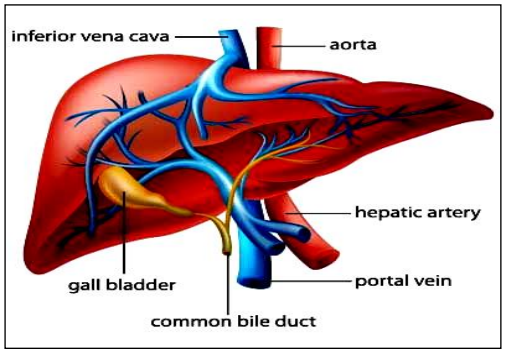


Figure 1: Anatomy of the liver

Source;
https://www.pinterest.com/pin/557813103831383740

The human liver consist of constitutes about 2.4% of total body weight four lobes have unequal in shape and connected with two hepatic blood vessels called as hepatic artery and hepatic portal vein look like reddish brown in color. Two types of cells are present in heapatic lobes i.e. parenchymal and nonparenchymal cells. Liver has mainly two nonparenchymal cells in hepatic sinusoid like a sinusoidal endothelial cell and kuffer cells[2].

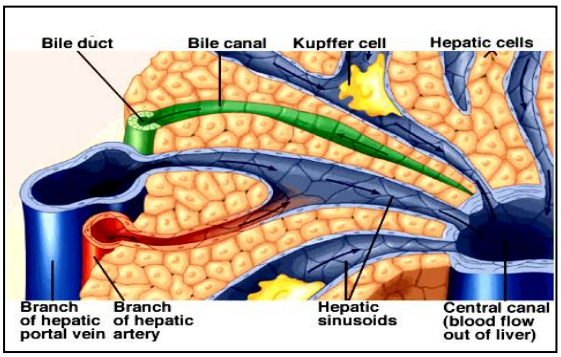


Figure 2: Gross section of human liver

Source; https://basicmedicalkey.com/liver-function

The liver delivers crucial processing during the bile production. Bile is a mixture of water and salts, and additionally also contain cholesterol and bilirubin which produced by Hepatocytes present in the liver, with help of a hormone called cholecystokinin release from duodenum cell lines during the digestion food. It is mainly responsible for carbohydrate, lipids, and proteins metabolism and converts these biomolecules into biologically useful mater and removes several potentially toxic substances from the body via excretion process. The liver facilitates the storage of various important nutrients, vitamins, and minerals

from food sources. Several vital protein components of blood plasma are synthesized through liver mediated process and also enhances the immune process of body with help of Kupffer cells [2].

Epidemiology

Indian population

Liver cirrhosis is newly diagnosed in India affected around 10 lakh people every year. As per the World Health Organization, Liver diseases are the one of the tenth most common cause of death in India. According to the latest WHO data published in may 2014, caused by liver disease in India reached 2.44% of total deaths[3].

Worldwide affected population

In the European (EU) region approximately 29 million people affected from a chronic liver disease and approximately 30 million people are affected from the same condition in America. It was estimated that in 2013, liver cirrhosis resulted in 170,000 deaths in Europe. Alcoholic liver disease (ALD) causes liver cirrhosis as a result in 2010, 493,300 deaths was reported (156,900 female and 336,400 male). In context to Liver cancer many published reports revealed that it is the most common cause of cancer death in current scenario of life style all over the world. In, 2015 as per the report of W.H.O. there are 788,000 deaths occurred due to liver cancer globally. Out of more which more than 25,000 liver transplants were conducted globally in between 2014-2015 and more than 5000 liver transplants were performed each year in Europe only[3].

Hepatic metabolic functions

Carbohydrate metabolism

Liver regulate the blood glucose level to maintain by the help of conversion of glucose to glycogen when blood glucose level is high glycogenolysis and when blood glucose level is low glucogenolysis. It also helps to maintained blood glucose level during fasting or starvation via converting various amino acid and lactic acid in glucose[4].

Lipid metabolism

Liver play crucial role to store some triglycerides via breakdown of fatty acid into acetylcoenzyme A, this process known as oxidation and convert large amount of acetyl co-enzymes A into ketone bodies which is known as ketogenesis[4].

Protein metabolism

The liver plays a crucial role in ATP production via eliminating the amino group, (NH_2) from amino acids. It also play important role in conversion of the toxic ammonia (NH_3) into less toxic urea and excreted out through urine. Most of the blood plasma proteins which are mainly involve in immune response and inflammatory process are synthesized from hepatic cells[4].

Hematological function

Liver plays an important role in the production of most of the blood clotting factors in combination with anti-thrombin. It facilitates the inflammatory mediator protein for wound healing as well as immune modulation[4].

Secretion and excretion of bile

Bile is an excretory product of the hepatic system which secretes 800-1000ml of bile in a day consisting lots of salts and metabolites used for digestion. Bile pH varies between 7.6-8.6 bile mainly consist of several important ions responsible for cellular membrane processing related to digestion [4].

Insulin metabolism

The liver plays an important role in the breakdown of insulin and other hormones. Glucuronidation of bilirubin facilitate by the liver and its excretion into bile. The liver also breakdown or modified toxic substances[5].

Other storage functions

The liver facilitates the storage of various multidisciplinary substances, those are responsible for basic body building functions including glucose, vitamins A, D and B_{12}, metallic nutrients such iron, and copper [5].

Causative agent of induce liver disease

Etiology of hepatic disorder

Infection

Viruses are mainly responsible for causing liver damage and can be spread out through blood or semen, contaminated food or water and via nosocomial infections. There are few common types of liver infection causing hepatitis through viruses are including; Hepatitis A, Hepatitis B, and Hepatitis C[6].

Immune system induced hepatic disorders

There are various diseases in which our own body immune system attacks from unknown parts of the body (autoimmune) and disturb the native functions of liver. The classical example of most common autoimmune liver disease is Auto immune hepatitis[7].

Genetic disorders

Our genetic system is also playing an important role during many disease courses. An abnormal inherited gene may cause serious unwanted synthesis of harmful substances that responsible for liver damage. The commonly known genetic liver diseases are Hemochromatosis and Wilson's disease in which excess iron and copper deposition occur in the body[8].

Cancer induced hepatic disease

Cancer is the most fatal disorder of human body. Liver has also different types of cancer such as, Bile duct cancer, Hepatocellular carcinoma, and cholangio-carcinoma. Many published report suggested that the different types

of liver cancer occurs through viral infections, high dose of low-grade alcohol intake, unaware medicine intake etc.

Other condition

Additionally, there are various common causes of hepatotoxicity including, excessive alcohol intake, unethical drugs intake, Fat accumulation in the liver multiple use of shared injection, chemical dyes, transfusion of blood and its exposure to other people, diabetes and obesity[7].

Fatty liver condition

Fatty liver disease is a condition where neutral fat deposited in large vacuoles of hepatic cells via the process of steatosis. It has multiple causes, in which one of the most common causes is excessive alcohol intake[8].

Jaundice

Jaundice is a hepatic condition in which various symptoms appears including discoloration of skin and sclera to light yellow which is occur due to high level of bilirubin in to the blood. Normally bilirubin level in human blood plasma is 1mg/dL. When the concentration of bilirubin is higher approximately 1.8mg/dL or above it leads to jaundice. There are mainly three types of jaundice conditions, Pre-hepatic (hemolytic jaundice), Hepatocellular (hepatic jaundice) and Post-hepatic jaundice[9].

Liver Cirrhosis

Cirrhosis is a disease condition in which normal liver tissues are replaced by fibrosis process and regenerative nodules that lead towards dysfunction of liver. Main cause of cirrhosis is alcohol, hepatitis viruses, and high fat deposition[10].

Hepatitis

Hepatitis is mainly caused by virus mediated infections, in which inflammation of the liver occurred due immune response of the body. It may be reversible by self-healing process or can progress to fibrosis and cirrhosis. There are two forms of hepatitis i.e., acute and chronic[11]. It is further divided in to two types.

Infectious hepatitis

In this type of hepatitis mainly viruses are responsible behind the infections which are spread through person to person. There is various type of hepatitis based on different agents such as Hepatitis A, B, C, D, E, G and X[12].

Non-infection hepatitis

Mainly primary compounds itself not cause hepatitis but there are many molecules or drugs metabolites causes liver disease such as excessive intake of alcohol or other toxic material, unethical medication or chemicals consumption are more prone towards hepatotoxicity due to which hepatitis occurs under the non-infectious hepatitis[12].

Hepatocellular carcinoma

It is the abundant form of liver cancer. There are several different causes of hepatic cancer such as viral hepatitis infection or cirrhosis, inherited genetic disorder etc. It is commonly known as primary liver cancer or hepatoma[13].

Table 1: Characterization of liver disorder and it etiologic condition

Liver Disease	Characterization	Etiologic condition
Acute liver Failure	Reduction in liver function	Drugs, toxic chemicals, various liver diseases
Hepatitis (A,B,C,D and E)	Acute or chronic liver damage	Hepatotropic viruses, alcohol assumption, drugs, xenobiotics, auto-immune disease, non-alcoholic fatty liver disease (NAFLD)
Auto immune related hepatitis	Inappropriate immune response against hepatic cells; Development of antibodies against own liver cells	Primary biliary cirrhosis, Primary sclerosingcholangitis, Autoimmune Hepatitis
Genetic disorders	Gene mutations that cause liver injury; Rarely seen	Hemochromatosis, Wilson's disease, deficiency of Alpha-1 antitrypsin.
Liver carcinoma	tumor in the liver	Increased risk of chronic hepatitis, hepatocellular carcinoma (HCC) is most common hepatic tumor.
Hepatic vein Obstruction	Blood clots obstruct blood flow from the liver; Development of symptoms such like jaundice enlarged liver, ascites, and abdominal pain	Hypercoagulable disorders, thrombosis of the hepatic vein, hepatic cancer, parasitic infection.
Cirrhosis	Surface injury of liver tissue that leads to chronic liver damage	Alcoholism, chronic bile duct obstruction, long-term Hepatitis C infection.
Liver Infections	Certain infections that leads to several type of liver damage and blockage of bile ducts	Viral hepatitis (Hepatitis A,B, C, D, and E), some Parasitic infection (yellow fever virus, Herpes viruses).

Hepatotoxicity

Hepatotoxicity is a type of liver dysfunction or damage liver of which is associated with improper uses of antibiotics and potent drugs. Those chemicals which cause liver injury are called hepatotoxins or hepatotoxic agents[13].

Table 2: List of hepatotoxic agent and their mechanism behind hepatotoxicity

S. No.	Hepatotoxic agents	Mechanism of hepatotoxicity
1.	**Chemicals** Carbon tetrachloride (CCl4)	-CCL_4 alters the plasma membrane, liposomal membrane and mitochondrial membrane[14].
	Thioacetamide (TTA),	-Its metabolite, thioacetamide S-oxide (ROS) is hepato-toxic and reduces the number of hepatocytes and oxygen consumption[14].
	Diethylnitrosamine (DEN)	-It is carcinogenic chemical, In the liver DEN is biotransformed by CYP2E1 (hydroxylation) into ethyldiazonium ion which acts as alkylating agent and reacts with DNA and induce cancer[14].
	Aflatoxin B1 (AFB1)	-Its dialdehyde form adducts with hepatic protein and induces hepatic toxicity
	Bromobenzene	-Lipid peroxidation and mitochondrial dysfunction[15].
	Lithocholic acid	-Metabolizes into epoxy glycinamide (oxidation) and induces cancer[15].
	Acrolein (allyl alcohol)	-Acrolein reduces the level of GSH and increases the level of ALT, AST and GGT[15]
	Alpha-Naphthyl	-Metabolizes into epoxy glycinamide (oxidation) and induces cancer[5].
	Isothiocyanate (ANIT)	-ANIT damages the bile duct epithelium and hepatic parenchyma cell[16].
2.	**Drugs-** *NSAIDs* Paracetamol Nimesulide Diclofenac Ibuprofen	-AZP metabolize into 6 MP by using sulfhydryl group from GSH, it cause hepatotoxicity[17].
	Anticancer Azathioprine(AZP)	-Metabolites of doxorubicin oxidation are semi quinine & quinine radicals which induces hepatotoxicity[18].
	Adriamycin (Doxorubicin)	-Metabolites of doxorubicin oxidation are semi quinine & quinine radicals which induces hepatotoxicity[18].
	Ranitidine	-A metabolite of ranitidine causes hepatotoxicity via immunological pathway[19].
	Anti-tubercular Isoniazid (INH)	-Metabolize into acetyl-isoniazid in presence of N-acetyl transferase. These intermediates further hydrolyze into acetyl hydrazine and reactive acetyl species which bind with hepatic cell and induces hepato-toxicity[19].
	Rifampicin Pyrozinamide	-Rifampicin when taken in combination with INH, potentiate the hepato-toxicity by enhancing the conversion of acetyl hydrazine into reactive acetyl species[19].
		-Metabolite forms free radical that causes hepatotoxicity[20].
	Antibiotics Erythromycin	-A metabolite of halothane causes hepatocellular necrosis[21].
3.	**Metals** Mercury	-Mercury is a transition metal which promotes the formation of ROSs like H2O2 and induces lipid peroxidation, mitochondrial damage and hepatocellular deterioration[22].
	Cadmium (Cd)	-Cd promotes the formation of ROSs like superoxide and hydroxyl radicals that induces hepatotoxicity[23].
	Lead	-Pb reduces the level of endogenous antioxidants like glutathione and induces organ toxicity, mainly hepatotoxicity[24].
4.	**Phytotoxin** Phallotoxin	-It is binding with F-actin which prevents the depolymerization equilibrium with G-protein and thus induces severe cholestasis[25].
	Microcystine (MCR)	-Induces neoplasia[26].
	Pyrrolizidine alkaloids (mono-crotaline)	-Causes sub-optimal edema and progressive fibrosis which changes into necrosis[27].
5.	**Radiations** Ionizing-radiation (Alpha, Beta, Gamma, X-ray)	-It is inducing lipid peroxidation. Excessive lipid peroxidation results in altered lipid imbalance in the cell membrane (made up of lipid bilayer) and cause hepatic damage[28].

	Non-ionizing radiation (visible light, UV radiation, radio wave)	-Directly associated with metabolic syndrome[29].
6.	**Diet**	
	Alcohol	-Damage the living tissue[30].
	High-fat diet	-Damages the central vein, endothelium & sinusoids[31].

Biochemical analysis parameters

Serum samples were collected and analyzed for hepatic disorders as follow.

Blood Bilirubin Test

Bilirubin level increased in many liver diseases.

Urine Bilirubin

This test is confirming the amount of bilirubin in urine.

Blood Ammonia

Determine the quantity of ammonia in the blood of patient.

AST (Aspartate Aminotransferase)

Determine the amount of AST enzyme in the blood serum.

ALT (Alanine Aminotransferase)

Determine the amount of ALT enzyme in the blood.

ALP (Alkaline Phosphatase)

The ALP test will help to determine the level of enzymes in liver disease. In many cases level of ALP is too high.

Albumin in Serum

Albumin quantity by serum analysis. Albumin is an important protein for drug binding and its transportation. Hence, the level of albumin triggers many metabolic processes in the body.

Globulins in Blood

Globulin are an important protein in mammalian body which play vital role in immune system. Low levels of globulin indicate towards liver dysfunction.

Serum Prothrombin Time

This test is to measure the time of our blood clotting which directly give the sign of liver disease or liver metabolic dysfunction.

Table 3: Normal range of LFT

S. No	Test	Normal range
1.	Bilirubin	5-17µmol/lit
2.	Alkaline phosphatase (ALP)	35-130IU/lit
3.	Aspartate transaminase (AST)	5-40IU/lit
4.	Alanine transaminase (ALT)	5-40IU/lit
5.	Gamma-glutamyl transpeptidase (GGT)	10-48IU/lit
6.	Albumin	35-50g/lit
7.	Prothrombin time (PT)	12-16s

Management of Liver Disease

Prevention

Prevention and management is an important practice through which everyone can evade themselves from chronic liver disease such as Hepatitis A and hepatitis B. There is lots of prevention measures provided from various medical organizations which is mainly contains vaccines, practicing good hygiene, avoiding drinking tap and open water when in remote area, do not take unethical drug, cannot share injections, clearly observe the label and precautions of chemicals using in industry as well as laboratory, practicing safest sex, resist to share of personal hygiene items, such as towel, napkin, trimmer, razors, stop alcohol intake, take proper medicines and injections after exposure to hepatitis A[32].

Allopathic medication

Ursodeoxy cholic acid (Ursodiol), Essential phospholipids, S-adenosyl methionine, ribavirin, lamivudine, steroids, antibiotics[32].

Ayurvedic medications

Table 4: List of some medicinal plants with hepatoprotective chemical constituents/Extract.

Name of Botanical Plants	Parts used	Phytoconstituents	Reference
Aphanamixis Polystachya	Leaf root, and bark	Aphanamixoid A	33,34
Acacia Catechu	Heartwood	Catechin, Epicatechin	35-37
Annona Squamosa	Leaf	Ethanolic and aqueous Extracts from leaves	38
Aegle Marmelos	Leaf, Bark	Eugenol	39-41
Abutilon Indicum	whole plant	Abutilin A	42

Adhatoda Vasica	Leaf	Leaf extract	43
Anisochilus Carnosus	Stems	Ethanolic extract	44
Byrsocarpus Coccineus	Leaf	aqueous leaf extract	45,46
Bupleurum Kaoi	Leaves and Root	Polysaccharide enriched fractions, saponin enriched fractions	47,48
Balanites Aegyptiaca	Stem barks	Stem barks extract	49,50
Clerodendrum inerme	Leaves	Ethanolic extract saponin	51
C. opobalsamum	aerial part	Ethanolic extract Eugenol	52
Cordia macleodii	Leaves	methanol	53
Enicostemma Axillare	whole plant	Ethyl acetate extract	54
Ephedra Foliate	whole plant	Crude extract, Ethanolic extracts	55
Ficus glomerata (Ficus racemosa)	Leaves, Bark	Ethanol and methanol extract	56-58
Gentianaolivi Eri	Flower	C glycosyl flavone, isolated from the ethyl acetate fraction	59
Hibiscus sabdariffa	Dried Flowers	Dried flower extract	60,61

Siddha medication

Vilvam, Nilavembu, Aavarai, Pirandai, Karisalai, Nannari, Nellikkai, Manathakkali.

Table 5: List of some siddha medicinal plants with hepatoprotective parts of plant.

S. No.	Botanical name	Name in siddha medicine	Family	Part used	References
1.	*Aegle marmelos*	Vilvam	Rutaceae	Fruit pulp	62
2.	*Andrographis paniculata*	Nilavembu	Acantheceae	Whole plant	63
3.	*Cassia fistula*	Aavarai	Fabaceae	Leaves	64
4.	*Cissas quadrangularis*	Pirandai	Vitaceae	Stems	65
5.	*Eclipta alba*	Karisalai	Asteraceae	Whole plant	66
6.	*Hemidesmus indicus*	Nannari	Apocynaceae	Roots	67
7.	*Phyllanthus emblica*	Nellikki	Euphorbiaceae	Fruits	68
8.	*Solanum nigrum*	Manaththakkali	Solanaceae	Whole plant	69

Homoeopathic medicines: The homeopathic medicines are given below;

1. Scrofoloso 5(S5),
2. Livome,
3. Natrum Sulphuricum 200c (Nat Sulph-200),
4. Natrum Sulphuricum 30c (Nat Sulph-30),
5. Cholesterinum 200c (Chol-200).

Hepatotoxic Model

Hepatotoxicity is a phenomenon which implies as chemical agents drive liver damage. Drug induced liver injuries is a major cause of acute and chronic liver diseases. The liver plays a central role in transforming and clearing the foods and chemicals to minimize the susceptible of these agents as toxic. Those chemicals and naturally driven agents that cause liver injury are called hepatotoxins or hepatotoxicants[70]. Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals, and natural chemicals like microcystins, herbal remedies and dietary supplements. Certain drugs may cause liver injuries when they introduced even within the minimum safety concentration ranges. Most of the time reactive metabolites and immune mediated agents are more prone towards the cause hepatotoxicity rather than primary compounds for affecting hepatocytes, biliary epithelial cells and or liver vasculature[71].

Figure 4: Hepatotoxicity induced by single dose administration of ccl4 (1.5ml/kg body wt.)

Chemicals causing of Hepatotoxicity

Carbon tetrachloride (CCl4), Thioacetamide (TTA), Diethyl nitrosamine (DEN), Aflatoxin B1 (AFB1), Bromobenzene, Lithocholic acid, Acryl amide (AA), Acrolein (allyl alcohol), Alpha-Naphthyl Isothiocyanate (ANIT)[72].

Table 7: Different animals model for hepatotoxicity

Sr. No.	Animal Name & Age	Name of chemical, induced N.D.	Dose & Duration	References
1	Albino Wistar rats (3-month-old)	Carbon tetra chloride (CCl_4)	1.5 ml/kg i.p. for single dose	Cheng J-S, et al., (2010)
2	Male Swiss albino mice (3-month-old)	Thioacetamide	200 mg/kg i.p. twice a week for 12 weeks	Shirin H, et al., (2012)
3	Male Brown Norway Rats (6-week-old)	Thioacetamide	400 mg/kg i.p. for 2 weeks	Kabiri N, et al., (2013)
4	Male Wistar rats (3-month-old)	Mercury chloride (HgCl2)	(80 mg/l) as drinking water for 4 weeks	Haouem S, et al., (2014)
5	Male Wistar rats (3-month-old)	Mercury chloride (HgCl2)	5 mg/kg s/c injection of mercury (Hg) in the form of mercuric chloride on the 7th day of experiments	Oda SS, et al (2012)
6	Male albino Wistar (3-month-old) rat	Ethanol	Ethanol 2.0 ml/l00 g p.o. for 21 days	Sharma A, et al (2012)
7	Male albino Wistar (3-month-old) rat	Ethanol	Ethanol 3.76 gm/kg twice a day p.o. for 25 days	Modi H, et al., (2012)
8	Male albino Wistar rats (3-month-old)	Aspirin	200mg/kg/BW, twice a day(i.p.)	Ravnskov, U., *et al.*, (2005)

CCl_4 induced hepatotoxicity

Carbon tetrachloride is one of the most common chemical agents used in the laboratory for the experimental study of various liver disorders at acute and chronic condition. 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. A single dose of CCl_4 reaches to its peak plasma concentration within 3 - 24 hours of administration and causes change in the histological and biochemical makeup of hepatocytes. Repeated dose of CCl_4 can induce fibrosis and necrosis. Various literature reports 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[73].

Diagnosis of Hepatotoxicity

The liver function test is the diagnosis procedure for liver disease. It include various parameters and its normal rang if any changes in its normal value that means some abnormality in the liver Blood bilirubin, urine bilirubin, blood ammonia AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase), ALP (Alkaline Phosphatase), albumin in serum, globulins in blood, and serum prothrombin time [73].

Blood collection

Each animal were anaesthetized with chloroform and diethyl ether. The blood collection was carried out with the help of capillary tube in a very gently and slowly rupture of Retro orbital plexus and 2 ml blood collected in blood collection tube. The collected blood was shifted immediately to centrifugation, allowed to clot and serum was separated by centrifugation at 5000 rpm for 15 min. Serum was separated and then preserved in the cuvettes at -20°C in the freezer until analysis.

Biomarker for hepatotoxicity

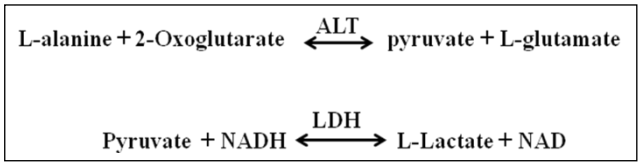
At the end of the study all animals were fasted overnight and sacrificed under anesthesia for biochemical analysis such as, Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphate (ALP), total bilirubin (TB), and total proteins (TP).

Estimation of Serum AST or SGPT (UV- Kinetic method)

Aspartate Transaminase (SGOT), and (ALT) Alanine Transaminase (SGPT), both are very important and sensitive markers of hepatocellular injury. If the liver cell is injured or dies, these proteins can leak out through the liver cell membrane into the circulation and serum levels will rise. The normal serum level is 10-35 Karmel units/ml. ALT reversibly catalyses amino group from alanine to α-ketoglutarate[74].

Principle

SGPT catalyses the transfer of amino group from L-Alanine to 2 oxoglutarate with the formation of pyruvate and L-glutamate. The pyruvate so formed is allowed to react with NADH to produce L-lactate. The rate of this reaction is monitored by an indicator reaction coupled with LDL in the presence of NADH (nicotinamide adenine dinucleotide). The oxidation of NADH in this reaction is measured as a decreasing in the absorbance of NADH at 340 nm, which is proportional to SGPT activity[74].



Where;

ALT: Alanine amino transferase

LDH: Lactate dehydrogenase

NAD: Nicotinamide adenine dinucleotidez

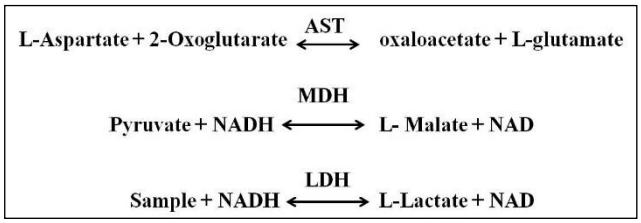
NADH: Nicotinamide adenine dinucleotide hydrogen

Estimation of serum ALT or SGOT (UV- kinetic method)

The levels of ALT are very high in patients of viral hepatitis and hepatic necrosis. There is 10-to-200-fold higher level of ALT 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. The normal serum level is 10-40 Karmel units/ml. AST reversibly catalyses transfer of amino group from aspartate to α-ketoglutarate[75].

Principle

SGOT catalyses the transfer of amino group from L-Aspartate to 2-oxoglutarate with the formation of oxaloacetate and L-glutamate. The rate of this reaction is monitored by an indicator reaction coupled with malate dehydrogenase (MDL) in which the oxaloacetate formed is converted to malate ion in presence of NADH. The oxidation of NADH in this reaction is measured as a decreasing in the absorbance of NADH at 340 nm, which is proportional to SGOT activity[75].



Where,

AST: Aspartate amino transferase

MDH: Malate dehydrogenase

LDH: Lactate dehydrogenase

NAD: Nicotinamide adenine dinucleotide

NADH: Nicotinamide adenine dinucleotide hydrogen

Estimation of serum alkaline phosphatase (ALP)

The serum alkaline phosphatase is produced by many tissues, especially bone, liver, intestine placenta etc. and 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[76].

Principle

Estimation of serum alkaline phosphatase hydrolyses p-nitro phenyl phosphate in the presence of oxidizing agent $Mg^{+}2$. This reaction is measured as absorbance is proportional to the ALP activity. P nitro phenyl phosphate is used as a working reagent with water and 20 µl sample[76].

Estimation of serum bilirubin

The total bilirubin is a metabolic product of the breakdown of hemoglobin 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 haemolysis and defects of hepatic uptake and conjugation of bilirubin treatment such as Gilbert's disease. Bilirubin in serum reacts with diazole reagent in the presence of alcohol, after the proteins had been removed by precipitation[77].

Principle

Bilirubin reacts with diazotized sulphanilic acid in acidic medium to form a pink coloured which indicates the presence of azobilirubin with absorbance directly proportional to bilirubin concentration. Direct bilirubin, being water soluble directly reacted in acidic medium. However, indirect and unconjugated bilirubin is solubilized using a surfactant and then it reacts similar to direct bilirubin. Absorbance were taken at 546/630 nm against blank reagent[78].

Estimation of serum total proteins

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

Principle

The peptide bond of proteins reacts with Cu+2 ions in alkaline solution to form a blue violet complex (Biuret reaction), each copper ion complexing with 5 or 6 peptide bonds. Tarterate is added as stabilizer while iodine is used to prevent auto reduction of alkaline copper complex. The color formed is proportional to the protein concentration and absorbance is measured at 546 nm against blank regent.

Rats liver were homogenized in ice-chilled 10% KCl solution and centrifuge at 2000 rpm for 10 minutes. Then collected the supernatant liquid and estimate the parameters like catalase, super oxidase and lipid peroxidation.

Antioxidant enzyme evaluation

The liver tissue of each rat were removed immediately, washed in saline, blotted between filter paper fold to dryness and weighed. Then the liver was homogenized in phosphate buffer (pH – 7.4) to give a 10% homogenate. Antioxidant properties like, Superoxidase (SOD), Catalase (CAT), Gamma-glutamyl transferase (GGT) and Glutathione-S-transferase (GST) were performed[79].

Superoxidase (SOD)

Superoxide dismutase activity was determined by measuring the inhibition in photoreduction of nitroblue tetrazolium (NBT) by SOD enzyme. The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 mM sodium carbonate, 12 mM L-methionine, 50 µM NBT, 10 µM riboflavin and 100 µL of crude extract in a final volume of 3.0 mL. A control reaction was performed without crude extract. The SOD reaction was carried out by exposing the reaction mixture to white light for 15 min at room temperature. After 15 min incubation, absorbance was recorded at 560 nm using a spectrophotometer. One unit (U) of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT[79].

Catalase (CAT)

Catalase activity was measured spectrophotometrically at room temperature by monitoring the decrease in absorbance at 240 nm resulting from the decomposition of H_2O_2. Catalase activity was measured according to the method of Aebi. One unit (U) of catalase activity was defined as the amount of enzyme that caused an absorbance change of 0.001 per min under assay conditions. The reaction mixture contained 100 mM sodium phosphate buffer (pH 7.0), 30 mM H_2O_2 and 100 µL of crude extract in a total volume of 3.0 ml[79].

Gamma-glutamyl transferase (GGT)

The specific activity of GST was expressed as µmol GSHCDNB (1-chloro-2.4-dinitrobenzene) conjugate formed/min/mg protein using an extinction coefficient of 9.6 mM^{-1} cm^{-1}. The reduced GSH levels of the tissue homogenates were measured spectrophotometrically using Elman's reagent. The activity of the enzymes and GSH were calculated to 1 g protein content of the 10,000 g supernatant fraction, which was determined by Folin-phenol reagent with bovine serum albumin as the standard[80].

Glutathione-S-transferase (GST)

The GST levels were analyzed in the tissues using the method of Habig et al., 1974. The tissues (50mg) were homogenized in 50 mM Tris–HCl buffer, pH 7.4, and containing 0.2 M sucrose and centrifuged at 16,000g for 45 min at 4°C. The pellet was discarded and the supernatant was used as the enzyme source. The reaction mixture in a volume of 3 mL contained 2.4 mL of 0.3 M potassium phosphate buffer (pH 6.9), 0.1 mL of 30 mM CDNB and 0.1 mL of 30 mM GSH, as enzyme source. The reaction was initiated by glutathione. The absorbances were read at 340 nm against the reagent blank. The results were expressed as µM/min/mg protein. The GST levels were measured using spectrophotometrically[80].

Physiological parameter

Wet liver weight of liver/100 gm and body weight of experimental animals.

Determination of body weight

Animals were weighed at the start of experiment and their final body weight using an electronic balance at the end of experiment.

Determination of wet liver weight

Animals were sacrificed and livers were isolated and washed with saline and weight determined by using an electronic balance. The liver weights were expressed with respect to its body weight i.e. gm/100gm.

Histopathology of Liver

Processing of isolated liver

The animals were sacrificed and the liver of each animals were isolated and cut into small pieces, preserved and fixed in 10% formalin for two days. Then the liver pieces were washed in running water for about 12 hours to remove the formalin and were followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hours each. Then finally dehydration is done using absolute alcohol with three changes for 12 hours each. After paraffin infiltration the liver pieces were subjected to automatic tissue processing unit. Embedding in paraffin vacuum hard paraffin was melted and the hot paraffin was poured into L-shaped blocks. The liver pieces were then dropped into the molten paraffin quickly and allowed to cool[81].

Biopsy of liver tissue

The blocks were cut using microtome to get sections of thickness of 5µ. The sections were taken on a micro slide on which egg albumin i.e., sticking substance was applied. The sections were allowed to remain in an oven at 60°C for 1 hour. Paraffin melts and egg albumin denatures, there by fixing of tissue to slide.

Staining

Eosin is an acid stain, hence it stains all the cell constituents pink which are basic in nature i.e., cytoplasm. Hematoxylin, a basic stain which stains all the acidic cell components blue i.e.: DNA in the nucleus.

Histopathological parameters

Histopathological profile of the liver tissues shows in Figure 6 normal hepatic cells each with well-preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein. Histopathological profile of liver in CCl_4 intoxicated rats has shown the fatty degeneration of

hepatocytes, hepatic cell necrosis, portal tract fibrosis and presence of fatty cyst. The sinusoids of liver were congested and the central vein of globule was constricted. Liver protection against the toxic substance as evident by normal lobular pattern with a mild degree of fatty change, absence of necrosis and lymphocyte infiltration. However, accumulation of fatty lobules (steatosis), necrosis and scattered lymph mononuclear (LMN) cell infiltrate in hepatic parenchyma were noticed in the section of animal treated with hepatoprotective drugs.

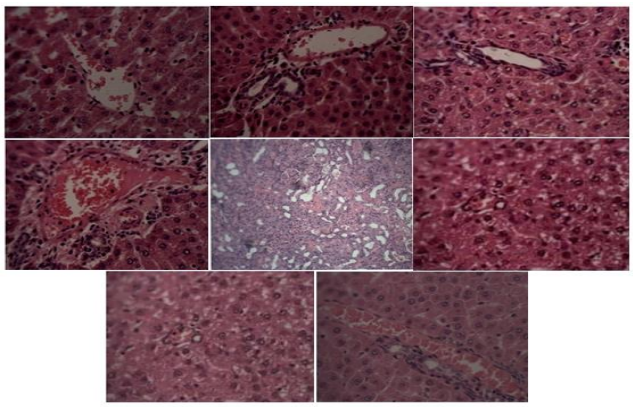


Figure 6: Histopathological section of the albino Wister rat liver shows the normal hepatic cells well preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein. Also shows the fatty degeneration of hepatocytes, hepatic cell necrosis and globule was constricted.

CONCLUSION

The present review is directed towards investigate the Hepatotoxicity is the foremost health difficulties over all worlds with the confluences of liver cirrhosis, chronic liver problems and drug induced liver injury which is leading cause of death in western and developing countries. The traditional system of medicines; Ayurveda, Unani, Siddha etc. can provide us valuable guidelines to the selection, preparation and application of herbal formulation for hepatic dysfunction. A large amount of medicinal plants used traditionally for immunomodulation and hepatoprotection. There is urgent need of the health professionals and scholars working in the field of pharmacology to develop an alternative medicine or diagnostic aids to cure different kinds of liver diseases spreaded in worldwide. This review also provides some of the *in vivo* and *in vitro* experimental models to evaluate new drugs, compounds and formulate some of the important hepatoprotective plants that can be further validated using the modern scientific methodology.

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