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A Review On: Diagnosis, Mechanism, Experimental Models, Antioxidant Parameters and Therapeutic Agents of Hepatotoxicity

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ABSTRACT
The liver is the primary organ in charge of regulating the body's internal environment. At this time, there is no mechanism to compensate for the loss of liver function. It has a major influence on nutrient flow and regulates carbohydrate, protein, and fat metabolism. There are several traditional and allopathic treatments that have been shown to provide hepatoprotection, but treating chronic liver disease remains a problem for health care providers. Drugs are a major cause of liver damage. There are over 900 medications, poisons, and plants that have been linked to liver damage. Around 75% of all idiosyncratic medication responses culminate in liver transplantation or death. Acute-dose dependent liver damage, acute fatty infiltration, cholestatic jaundice, liver granulomas, and active liver granulomas are all examples of drug-induced liver disorders.

.**Keywords**: Hepatotoxicity, hepatoprotection, medicinal herbs, drug metabolism, herbal etc.

I. INTRODUCTION

The liver is the body's most vital and biggest organ. Between both the third and fourth weeks of life, it begins to form in the human fetus, and it is the primary location of haematopoiesis during the perinatal era. The normal healthy liver weighs roughly 1500 g and is positioned under the diaphragm in the upper right corner of the belly. [1].

The human liver is made up of four lobes that are uneven in shape and are joined by two hepatic blood veins termed the hepatic artery and hepatic portal vein, both of which are reddish brown in colour. In heapatic lobes, there are two types of cells: parenchymal and nonparenchymal cells. In the hepatic sinusoid, there are primarily two nonparenchymal cells: a sinusoidal endothelial cell and kuffer cells. [2].

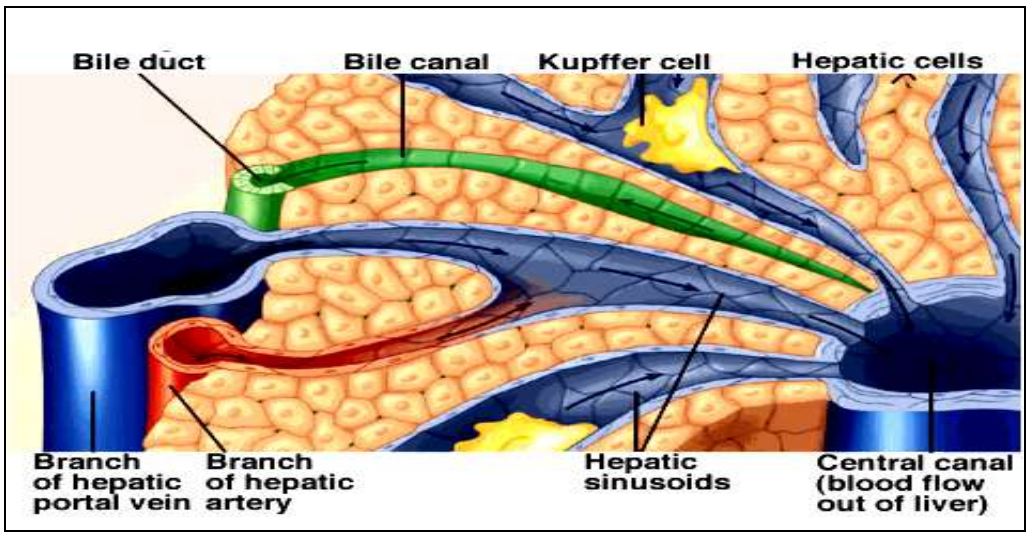

Figure 2: Gross section of human liver
Source; https://basicmedicalkey.com/liver-function

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During bile generation, the liver performs critical processing. Bile is a mixture of water and salts, as well as cholesterol and bilirubin, which are created by Hepatocytes in the liver with the assistance of a hormone called cholecystokinin, which is released by duodenal cell lines during meal digestion. It is primarily responsible for the metabolism of carbohydrates, lipids, and proteins, converting these macromolecules into physiologically useful matter and excreting various potentially harmful compounds from the body. The liver aids in the storage of a variety of critical nutrients, vitamins, and minerals obtained through meals. Several essential protein components of blood plasma are created by the liver, which also aids the body's immune system with the support of Kupffer cells. [2].

1.1 Epidemiology

1.1.1 Indian population: Hepatic encephalopathy is a recently identified disease in India, affecting roughly 10 lakh individuals each year. Liver illnesses are the tenth most prevalent cause of mortality in India, according to the World Health Organization. According to the most recent WHO data published in May 2014, fatalities due to liver disease accounted for 2.44 percent of all deaths in India. {3}.

1.1.2. Worldwide affected population: In the European Union (EU), around 29 million individuals suffer from chronic liver disease, whereas in the United States, roughly 30 million people suffer from the same ailment. In Europe, liver cirrhosis was predicted to have caused 170,000 deaths in 2013. As a result of alcoholic liver disease (ALD), which produces liver cirrhosis, 493,300 fatalities were documented in 2010. (156,900 female and 336,400 male). In the case of liver cancer, numerous published papers have indicated that it is the most prevalent cause of cancer mortality in today's world of lifestyle. According to a World Health Organization data from 2015, there were 788,000 deaths worldwide due to liver cancer. Between 2014 and 2015, more than 25,000 liver transplants were performed worldwide. [3].

1.2. Metabolic activities of the liver

1.2.1. Lipid metabolism: The liver is important for storing certain triglycerides by breaking down fatty acids into acetylcoenzyme A (oxidation) and converting significant amounts of acetyl coenzyme A into ketone bodies (ketogenesis) [4].

1.2.2. Oxidation of carbohydrates: When blood glucose are high, glycogenolysis, and when blood glucose levels are low, glucogenolysis, the liver helps to maintain blood glucose levels by converting glucose to glycogen. It also aids in blood glucose maintenance during fasting or hunger by converting different amino acids and lactic acid into glucose.[4]

1.2.3. Protein metabolism: Hepatic cells manufacture the majority of increased plasma proteins that are involved in immune response and inflammatory processes.[4].

1.2.4. Hematological function: In conjunction with anti-thrombin, the liver plays a key role in the formation of most blood clotting components. It aids wound healing and immunological regulation by facilitating the inflammatory mediator protein. [4].

1.2.5. Secretion and excretion of bile: Bile is a bile excretory product of the hepatic system, which produces 800-1000ml of bile every day, which contains a variety of salts and compounds that aid digestion. Bile pH ranges from 7.6 to 8.6. Bile is made up of numerous key ions that are involved in digestion-related cellular membrane processing. [4].

1.2.6. Insulin metabolism: The liver is responsible for a substantial percentage of the breakdown of insulin and other hormones. By glucuronidating bilirubin, the liver aids glucuronidation and bile excretion. The liver also breaks down or changes toxic substances..[5].

1.2.7. Other storage functions: The liver aids in the storage of a variety of multidisciplinary substances responsible for basic body building functions such as glucose, vitamins A, D, and B12, as well as metallic nutrients like iron and copper. [5].

1.3. Induced liver disease's causative element

1.3.1. Etiology of hepatic disorder

1.3.2. Infection: Viruses are the most common cause of liver disease, and they can be transmitted by blood or sperm, adulterated food or drink, or nosocomial infections. Hepatitis A, Hepatitis B, and Hepatitis C are some of the most frequent kinds of liver infection that cause hepatitis. [6].

1.3.3. Immunological induced hepatic disorder: There are a number of disorders in which our immune system assaults unknown sections of the body (autoimmune) and disrupts the liver's normal activities. Auto-immune hepatitis is a perfect illustration of the most frequent autoimmune liver disease.[7].

1.3.4. Genetic disorders: Our hereditary system also plays a significant part in the progression of many diseases. An abnormally inherited gene can result in the production of toxic chemicals that

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cause liver damage. Hemochromatosis and Wilson's disease are two well-known hereditary liver illnesses in which the body accumulates too much iron and copper. [8].

1.3.5. Cancer induced hepatic disease: Cancer is the most lethal disease that affects humans. Bile duct cancer, hepatocellular carcinoma, and cholangio-carcinoma are all cancers that affect the liver. Many published studies have suggested that many forms of liver cancer are caused by viral infections, large doses of low-grade alcohol use, and unwitting pharmaceutical consumption, among other things.

1.3.6. Other circumstances: Heavy alcohol use, unethical drug consumption, fat accumulation in the liver, multiple use of shared injections, chemical dyes, blood transfusion and exposure to other individuals, diabetes, and obesity are all major causes of hepatotoxicity. [7].

1.3.7. Fatty liver condition: Fatty liver disease is a disorder in which neutral fat is formed in vast vacuoles of hepatic cells through the steatosis process. It has a variety of reasons, one of the most prevalent of which being excessive alcohol use. [8].

1.3.8. Jaundice: Jaundice is a hepatic illness characterised by a pale yellow colouring of the skin and sclera, which is caused by a high quantity of bilirubin in the blood. The normal amount of bilirubin in human blood plasma is 1 mg/dL. Jaundice develops when the bilirubin concentration rises to around 1.8 mg/dL or higher. Pre-hepatic (hemolytic jaundice), Hepatocellular (hepatic jaundice), and Post-hepatic jaundice are the three basic kinds of jaundice. [9].

1.3.9. Liver Cirrhosis: Cirrhosis is a disease in which the normal liver tissues are replaced by fibrosis and regenerating nodules, resulting in liver dysfunction. Cirrhosis is caused mostly by alcohol, hepatitis viruses, and excessive fat accumulation.[10].

1.3.10. Hepatitis: Hepatitis is mostly caused by virus-mediated illnesses, in which the liver is inflamed as a result of the body's immunological response. It can either be reversible through self-healing or develop to fibrosis and cirrhosis. Acute and chronic hepatitis are the two types of hepatitis. [11]. It is further divided in to two types.

1.3.11. Infectious hepatitis: Viruses are primarily responsible for the infections that transmit from person to person in this kind of hepatitis. Hepatitis A, B, C, D, E, G, and X are all distinct types of hepatitis caused by different substances.[12].

1.3.12. Non-infection hepatitis: Although basic substances do not cause hepatitis, numerous molecules or drug metabolites may. Excessive consumption of alcohol or other hazardous materials, unethical medicine or chemical ingestion are all more prone to hepatotoxicity, and hepatitis develops as a non-infectious hepatitis. [12].

1.3.13. Hepatocellular carcinoma: It is the most common kind of liver cancer. Hepatic cancer can be caused by a variety of factors, including viral hepatitis, cirrhosis, hereditary genetic disorders, and so on. Primary liver cancer, also known as hepatoma, is a kind of cancer that affects the liver. [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.

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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).

1.4. 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[15].
	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].

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	• 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 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 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].

1.5. Biochemical analysis parameters

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

Blood Bilirubin Test– Bilirubin level increased in many liver diseases.

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Urine Bilirubin–This test is confirms 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

II. MANAGEMENT OF LIVER DISEASE

2.1. Prevention: Preventing and managing chronic liver disease, such as Hepatitis A and B, is a vital strategy that everyone may use to protect themselves. Vaccines, good hygiene, avoiding drinking tap and open water when in a remote area, do not take unethical drugs, cannot share injections, clearly observe the label and precautions of chemicals used in industry and laboratory, practising safest sex, resisting sharing of personal hygiene items such as towel, napkin, trimmer, razors, stop alcohol intake are just a few of the prevention measures provided by various medical organisations.[32].

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

2.3. 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]

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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]

2.4. 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]

III. SCREENING MODEL FOR HEPATO TOXICITY INDUCTION.

Hepatotoxicity is a term that refers to when chemical substances cause liver damage. A significant cause of acute and chronic liver illnesses is drug-induced liver damage. The liver is responsible for converting and removing meals and chemicals in order to reduce the risk of these substances becoming harmful. Hepatotoxins or hepatotoxicants are drugs or naturally occurring compounds that induce liver harm. Exogenous substances of clinical significance include overdoses of some pharmaceutical medications, industrial chemicals, and natural chemicals such as microcystins, herbal treatments, and nutritional supplements. Even when used within the minimal acceptable concentration levels, several medications might cause liver damage. Most of the time reactive metabolites and immune mediated agents are more prone towards the cause hepatotoxicity rather than primary compounds for

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affecting hepatocytes, biliary epithelial cells and or liver vasculature[71].

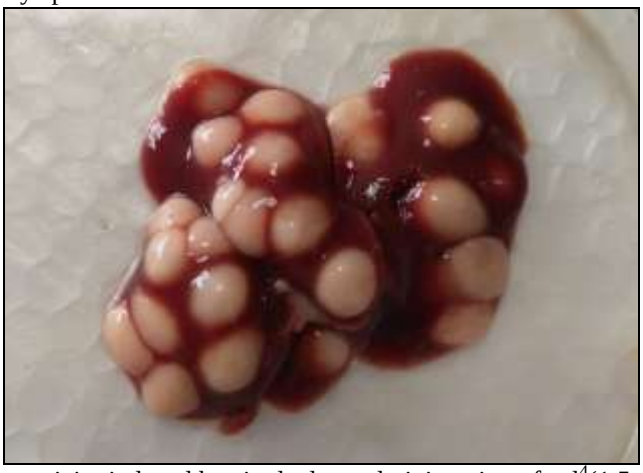

Figure 4: Hepatotoxicity induced by single dose administration of ccl^4(1.5ml/kg body wt.)

3.1.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)r 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)

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3.2. 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].

IV. DIAGNOSIS OF HEPATOTOXICITY

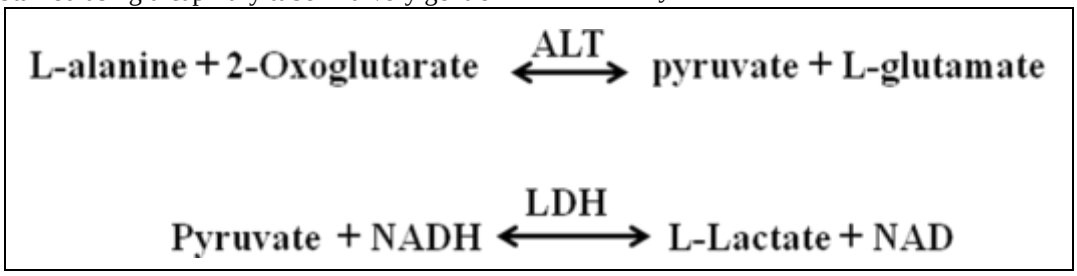
The liver function test is a method used to diagnose liver disease. It includes a number of parameters and their usual ranges; any deviations from this range indicate a problem with the liver. Bilirubin in the blood, bilirubin in the urine, and blood ammonia AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase), ALP (Alkaline Phosphatase), albumin in serum, globulins in blood, and serum prothrombin time are some of the enzymes that may be measured. [73].

4.1 Blood collection: Chloroform and diethyl ether were used to anaesthetize each animal. Blood was obtained using a capillary tube in a very gentle and gradual rupture of the Retro orbital plexus, with 2 mL of blood taken in a blood collection tube. The blood was immediately centrifuged, then allowed to coagulate before being separated by centrifugation at 5000 rpm for 15 minutes. Serum was separated and stored in cuvettes in the freezer at -20°C until analysis.

4.2. Biomarker for hepatotoxicity: All animals were fasted overnight and sacrificed under anaesthesia for biochemical examination at the end of the trial., Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphate (ALP)**,** total bilirubin (TB)**,** and total proteins (TP).

4.3. 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

4.4. 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

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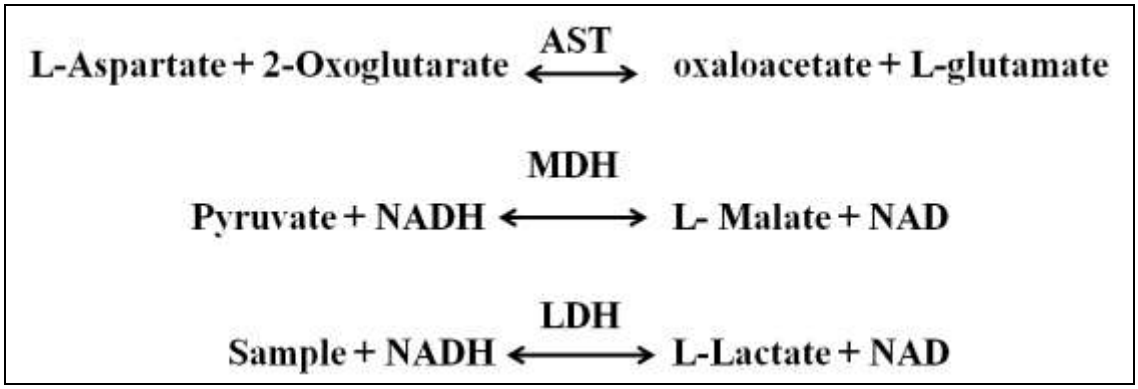
level is 10-40 Karmel units/ml. AST reversibly catalyses transfer of amino group from aspartate to α-ketoglutarate[75].

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

4.5. Estimation of serum alkaline phosphatase (ALP): Many tissues, including bone, liver, gut, placenta, and others, create serum alkaline phosphatase, which is eliminated in bile. An increased serum alkaline phosphatase level, in the absence of bone disease or pregnancy, usually indicates hepatobiliary illness. Hepatic excretion problems or increased ALP synthesis by hepatic parenchymal or duct cells might be the cause of high ALP levels. Alkaline phosphatase measurement is based on a principle. P-nitrophenyl phosphate is hydrolyzed by ALP, resulting in the production of P-nitrophenol and the release of the 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].

4.6. Estimation of serum bilirubin: One of the better liver function tests is total bilirubin, which is a metabolic result of haemoglobin breakdown. An adult's blood normally contains 0.25 mg/dl of conjugated bilirubin. Hepatocyte disorders, blockage to biliary excretion into the duodenum, haemolysis, and deficiencies in hepatic absorption and conjugation of bilirubin therapy, such as Gilbert's disease, cause a rise in bilirubin levels. After the proteins have been precipitated, bilirubin in serum interacts with diazole reagent in the presence of alcohol. [77].

Principle: In an acidic media, bilirubin interacts with diazotized sulphanilic acid to produce a pink colour that shows the presence of azobilirubin, with absorbance proportional to bilirubin content. Because direct bilirubin is water soluble, it reacts quickly in an acidic environment. Indirect and unconjugated bilirubin, on the other hand, is solubilized with a surfactant before reacting similarly to direct bilirubin. At 546/630 nm, absorbance was measured against a blank reagent. [78].

4.7. Estimation of serum total proteins: Albumin, fibrinogen, prothrombin, alpha-1antitrypsin, haptoglobin, ceruloplasmin, transferrin, alpha foetoproteins, and acute phase reactant proteins are all produced by liver cells. Extensive liver damage lowers the blood levels of these plasma proteins.

Principle: In an alkaline solution, peptide bonds react with Cu+2 ions to generate a blue violet complex (Biuret reaction), with each copper ion complexing with 5 or 6 peptide bonds. Iodine is employed to avoid auto reduction of the alkaline copper complex, and tarterate is added as a stabiliser. The colour produced is proportional to the protein content, and absorbance at 546 nm is measured against a blank reagent.

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The livers of rats were homogenised in an ice-cold 10% KCl solution and centrifuged for 10 minutes at 2000 rpm. The supernatant liquid was then collected, and parameters such as catalase, superoxidase, and lipid peroxidation were calculated.

4.8. Antioxidant enzyme evaluation: The liver tissue of each rat was promptly removed, washed in saline, wiped to dryness between filter paper folds, and weighed. The liver was then homogenised in phosphate buffer (pH 7.4), yielding a 10% homogenate. Antioxidant properties include things like, Superoxidase (SOD), Catalase (CAT), Gamma-glutamyl transferase (GGT) and Glutathione-S-transferase (GST) were performed[79].

4.8.1. Superoxidase (SOD): The suppression of photoreduction of nitroblue tetrazolium (NBT) by SOD enzyme was used to measure superoxide dismutase activity. In a final volume of 3.0 mL, the reaction mixture comprised 50 mM sodium phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 mM sodium carbonate, 12 mM L-methionine, 50 mM NBT, 10 mM riboflavin, and 100 mL crude extract. A reaction without crude extract was used as a control. At room temperature, the SOD reaction was carried out by exposing the reaction mixture to white light for 15 minutes. A spectrophotometer was used to measure absorbance at 560 nm after 15 minutes of incubation. The quantity of enzyme producing 50% inhibition of photochemical reduction of NBT was defined as one unit (U) of SOD activity. [79].

4.8.2. Catalase (CAT): At room temperature, catalase activity was determined spectrophotometrically by measuring the drop in absorbance at 240 nm caused by the breakdown of H2O2. The activity of catalase was determined using the Aebi technique. Under test circumstances, one unit (U) of catalase activity was defined as the quantity of enzyme that generated an absorbance change of 0.001 per minute. In a total volume of 3.0 ml, the reaction mixture comprised 100 mM sodium phosphate buffer (pH 7.0), 30 mM H2O2, and 100 L crude extract. [79].

4.8.3. Gamma-glutamyl transferase (GGT): Using an extinction value of 9.6 mM-1 cm-1, the specific activity of GST was calculated as mol GSHCDNB (1-chloro-2.4-dinitrobenzene) conjugate formed/min/mg protein. Using Elman's reagent, the lowered GSH levels in the tissue homogenates were quantified spectrophotometrically. The enzyme activity and GSH were computed using the Folin-phenol reagent to quantify the protein content of the 10,000 g supernatant fraction, which was assessed using bovine serum albumin as the standard. [80].

4.8.4. Glutathione-S-transferase (GST): The GST levels in the tissues were determined using the Habig et al., 1974 technique. The tissues (50 mg) were homogenised in 50 mM Tris–HCl buffer (pH 7.4) with 0.2 M sucrose and centrifuged at 16,000g for 45 minutes at 4°C. The pellet was discarded, and the enzyme source was taken from the supernatant. The enzyme supply was 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 in a 3 mL reaction mixture. Glutathione was the catalyst for the reaction. The absorbances were measured against a reagent blank at 340 nm. The results were given in milliseconds per milligramme of protein. The levels of GST were determined using spectrophotometry. [80].

4.9. Physiological parameter: Wet liver weight of liver/100 gm and body weight of experimental animals.

4.9.1. 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.

4.9.2. 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.

V. HISTOPATHOLOGY OF LIVER:

5.1. 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].

5.2. 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.

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The sections were allowed to remain in an oven at 60^{o}C for 1 hour. Paraffin melts and egg albumin denatures, there by fixing of tissue to slide.

5.3. 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.

5.4. Histopathological parameters

Figure 6 illustrates the histopathological profile of liver tissues, which exhibits normal hepatic cells with well-preserved cytoplasm, conspicuous nucleus and nucleolus, and well-defined central vein. In CCl4-intoxicated rats, histopathological analysis revealed fatty degeneration of hepatocytes, hepatic cell necrosis, portal tract fibrosis, and the formation of a fatty cyst. The liver's sinusoids were clogged, and the globule's principal vein was constricted. Normal lobular pattern with a low degree of lipid alteration, lack of necrosis, and lymphocyte infiltration indicate liver defence against the harmful chemical. However, in the hepatic parenchyma of animals treated with hepatoprotective medications, buildup of fatty lobules (steatosis), necrosis, and dispersed lymph mononuclear (LMN) cell infiltration were seen..

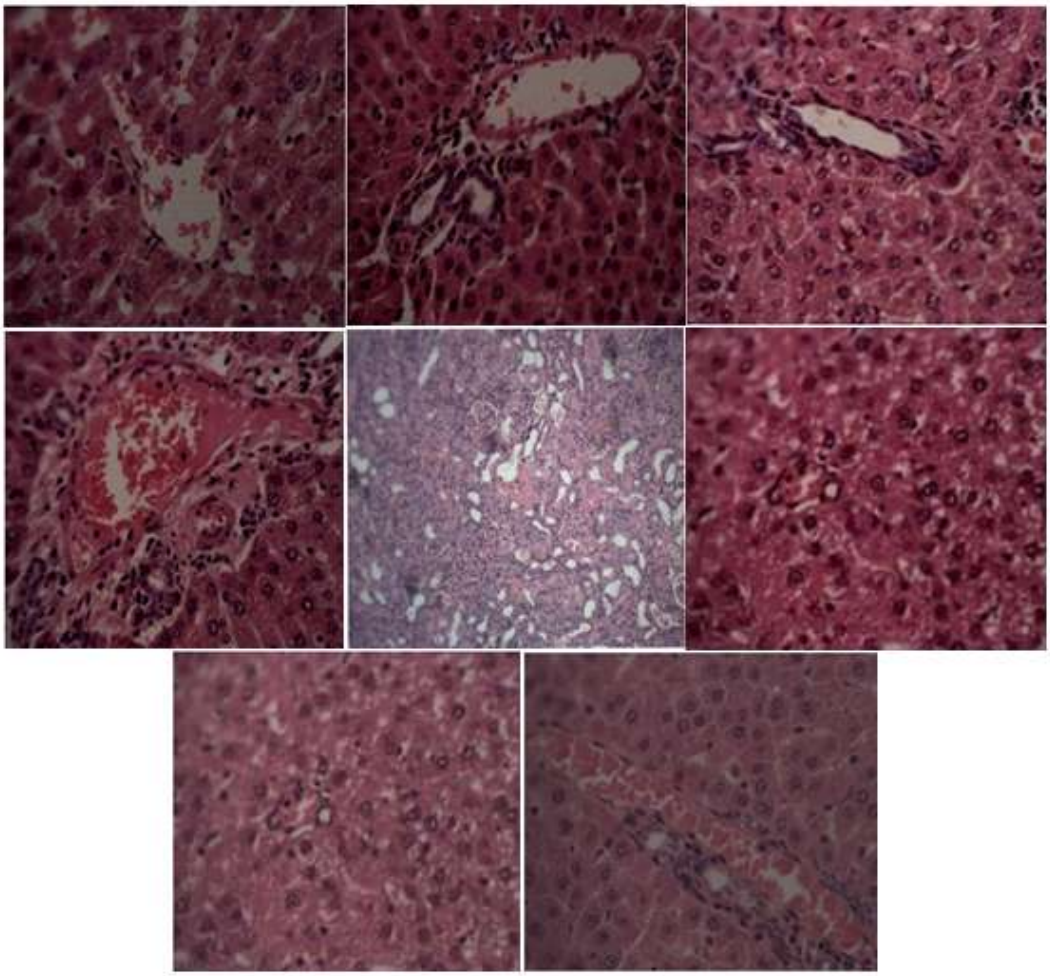

Figure 6: Histopathological section of the albino Wister rat liver shows the normal hepatic cells well preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein. Also shows the fatty degeneration of hepatocytes, hepatic cell necrosis and globule was constricted.

VI. CONCLUSION

Hepatotoxicity is the main cause of mortality in both developed and developing nations, with the confluences of liver cirrhosis, chronic liver issues, and drug-induced liver damage being the major cause of death in both developed and developing countries. Traditional medical systems such as Ayurveda, Unani, Siddha, and

others can offer us with useful instructions for selecting, preparing, and using herbal formulations for hepatic dysfunction. A significant number of medicinal plants have been employed for immunomodulation and hepatoprotection in the past. Health experts and academics working in the field of pharmacology are urgently needed to produce alternative medicines or diagnostic tools to treat the many types of liver disorders that are prevalent across the world. This study also includes in vivo and in vitro experimental approaches for evaluating novel medications, chemicals, and formulations of significant hepatoprotective plants, which may be further confirmed using current scientific techniques.

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