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IN - VITRO AND *IN -VIVO* MODELS FOR SCREENING HEPATOPROTECTIVE ACTIVITY

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ABSTRACT

The liver is the key organ of metabolism, secretion and excretion which is continuously and generally exposed to environmental pollutants, xenobiotics and chemo therapeutic agents because of its strategic location in the body. Liver disease is a worldwide problem. Liver injury may be acute and chronic where the acute liver injury can be caused by several factors like drugs, alcohol consumption, poor hygiene and industrial chemicals. This review article enlights the information about the role of liver and its importance. And also the intoxicants for liver to study the hepatoprotective activity on animal model in vivo.

INTRODUCTION

The liver is the key organ of metabolism, secretion and excretion which is continuously and generally exposed to environmental pollutants, xenobiotics and chemo therapeutic agents because of its strategic location in the body. Liver disease is a worldwide problem (Bannasch et al., 1994). Liver injury may be acute and chronic where the acute liver injury can be caused by several factors like drugs, alcohol consumption, poor hygiene and industrial chemicals. The most common causes of chronic liver failure may be due to Hepatitis B, Hepatitis C, Cirrhosis, long term alcohol consumption, malnutrition, and hemochromatosis. Among these causes the most commonly observed are drug induced, alcohol consumption and poor hygiene where drugs account for approximately 20-40% of all instances of hepatic failure and 75% of the idiosyncratic drug reactions lead to liver transplantation or death (Anandakumar et al., 2014).

Drug induced hepatotoxicity: Severe hepatic injuries have been reported by various drugs, industrial chemicals and infections due to viruses. Liver has a major role in many of enzymatic metabolisms and is a primary target for most of the toxicities. Drugs reported for the hepatotoxicity include amoxicillin/clavulanate, isoniazid, and nonsteroidal anti-inflammatory drugs. Several drugs are being drawn out from the marketplace because of delayed discovery of hepatotoxicity which may be due to unpredicted toxicities as a result of differences in the metabolisms in the liver. It is the responsibility of the physicians to be cautious in identifying these drug-related liver injuries as the early revealing can lessen the severity of hepatotoxicity if the drug is discontinued. The materialization of drug-induced hepatotoxicity is highly incoherent, which array from asymptomatic rise of liver enzymes to complete hepatic failure (Michael et al., 2014). The US Food and Drug Administration has withdrawn bromfenac and troglitazone for causing severe liver injury. Bromfenac, a nonsteroidal anti-inflammatory drug (NSAID), was introduced in 1997 as a short-term analgesic for orthopaedic patients and to be dosed for not more than 10 days. But due to its

excess usage more than 50 cases of severe hepatic injury were reported, and the drug was withdrawn in 1998. Antidiadetic drug Troglitazone approved in 1997 as an antidiabetic agent. More than 90 cases of hepatotoxicity were reported within a span of 3 years and it resulted in withdrawal of this drug. Pemoline is also withdrawn as the Food and Drug Administration (FDA) concluded that the overall risk of liver toxicity. In 2005, Abbott and all other generic companies chose to stop sales and marketing of pemoline used for attention deficit disorder and narcolepsy due to its liver toxicity which outweighs the benefits. Other drugs which have significant limitations of use because of their hepatotoxic effects are felbamate an antiepileptic used for complex partial seizures, zileuton indicated for asthma; tolcapone, used for Parkinson disease, trovafloxacin an antibiotic, benoxaprofen an NSAID, and tienilic acid a diuretic (FDA, 2009). A boxed warning which emphasizing the risk of severe liver injury and acute liver failure, have been included for propylthiouracil which is used for hyperthyroidism due to Grave's disease . Methimazole is also another drug which has severe effect on liver and cause liver toxicity. Even deaths are also been reported due to usage of these drugs (Shipp et al., 1955). Severe hepatic injury, including cases of hepatic failure, has been reported in patients taking interferon beta-1a, used in treatment of multiple sclerosis. Asymptomatic elevation of hepatic transaminases has also been reported, and in some patients recurred upon rechallenge. In some cases, these events occurred in the presence of other drugs that has been associated with hepatic injury. The potential risk of interferon beta-1a used in combination with known hepatotoxic drugs or other products (eg, alcohol) should be considered prior to interferon beta-1a administration or when adding new agents to the regimen of patients already on interferon beta-1a (Helen et al., 2004).

Liver toxicity due to Alcohol: Excess intake of alcohol causes development of large fatty globules (macro vesicular steatosis) throughout the liver and can begin to occur after a few days of heavy drinking and this condition is called Fatty change, or steatosis and these fatty globules are visible under the microscope. Alcohol is metabolized by the alcohol dehydrogenase (ADH) into acetaldehyde, and then metabolized by aldehyde dehydrogenase (ALDH) into acetic acid, which then finally oxidized to carbon dioxide (CO2) and water (H2O). This process generates NADH, thus increasing the NADH/NAD+ ratio which induces fatty acid synthesis. This higher levels of fatty acids signal the liver cells to convert it to glycerol which in turn changes to triglycerides. These triglycerides are accumulated, leading to fatty liver causing steatosis which results in accumulation of fat droplets within the liver cells associated with abnormal liver function tests (Darryl et al., 2004). There may also be the presence of hyperammonia, hypoglycaemia, acidosis and clotting factor deficiency, if left untreated then it leads to acute liver disease which is usually a self limiting episode of liver cell (hepatocytes) inflammation or damage. If the hepatocyte damage is so severe, it affects the whole liver leading to hepatic failure. When permanent structural changes within the liver occur secondary to long standing cell damage with the loss of liver architecture then it leads to Chronic liver disease (Clausen 1987; Roger 2003).

a) Alcoholic hepatitis

Inflammation of hepatocytes is an important characteristic of Alcoholic hepatitis. According to NIAAA, 1993, about 10% to 35% of heavy drinkers develop alcoholic hepatitis. This alcoholic hepatitis is not directly related to the dose of alcohol. Some people are more prone to this reaction which is called alcoholic steato necrosis and the inflammation inclines towards to liver fibrosis. The commencement and continuation of liver injury by inducing apoptosis and necrosis are mainly done by inflammatory cytokines (TNF-α, IL6 and IL8). Intestinal permeability is increased by liver diseases which in turn increases the activity of TNF-α. This eases the endotoxins produced in

intestine to absorb into the portal circulation. Further the Kupffer cells of the liver phagocytise endotoxin, which stimulate the release of TNF-α. TNF-α in turn set off apoptotic pathways through the activation of caspases which result in cell death (Menon et al., 2001).

b Alcoholic liver cirrhosis: Primary biliary cirrhosis is characterised by progressive destruction of the small and intrahepatic bileducts, leading to fibrosis and cirrhosis. There is no cure, and in the long term many patients will require a liver transplantation (Clause, 1987; Roger, 2003) Cirrhosis is the later stage of the serious liver disease manifested by the inflammation, fibrosis and damaged membranes which prevent detoxification of chemicals in the body, ending in scarring and necrosis. As per NIAAA 1990, about 10% to 20% of heavy drinkers will develop cirrhosis of the liver. Alcohol-induced fibrosis is caused due to acetaldehyde which stimulates the deposition of collagen by hepatic stellate cells. The oxidants produced from NADPH oxidase and/or cytochrome P-450 2E1 and acetaldehyde-protein adducts damage the cell membrane (Menon KV et al., 2001). Without total abstinence from alcohol use, will eventually lead to liver failure. Cirrhosis can also result from viral hepatitis. Many viruses affect liver function but only a few are truly infectious to the liver itself, which leads to clinically significant hepatitis. Five human viruses have been identified, including HAV, HBV, HCV, HDV and HEV. All forms have a similar pathology, causing an acute inflammation of the entire liver. Another type of hepatitis called autoimmune hepatitis which is associated with auto-antibodies in the serum, and is usually a chronic, progressive disease causing inflammation, scaring and cirrhosis (Clausen , 1987; Roger, 2003).

3. Liver diseases caused due to poor hygiene

Hepatitis A occurs intermittently and is contagious worldwide, with a tendency of cyclic recurrences. Every year about 1.4 million cases of hepatitis A are reported worldwide. Hepatitis A is a liver disease caused by the hepatitis A virus. It is largely spread due to ingestion of food or water that is contaminated with the faeces of an infected person. The disease is intimately allied with a lack of safe water, insufficient sanitation, poor personal hygiene and also through frozen berries (Simon, 2015).

4. Hepatic encephalopathy

It is a neuropsychiatric syndrome that occurs with significant liver dysfunction. The intestinally derived neuroactive and neurotoxic substances such as ammonia pass through the diseased liver or bypass liver through shunts directly to the brain resulting in cerebral dysfunction. Ammonia is thought to increase the permeability of the blood brain barrier, enable other neurotoxins to enter the brain and indirectly alter neurotransmission in brain (Clausen 1987; Roger 2003).

5. Jaundice

Jaundice changes the skin and eyes to turn yellow because of increased levels of bilirubin. When the red blood cells break down, body builds new cells to replace them and the old ones are processed by the liver. If the liver cannot handle the blood cells as they break down, bilirubin builds up in the body and the skin may look yellow. Some causes of jaundice due to poor liver function includes viral hepatitis, medication-induced hepatitis, autoimmune hepatitis, alcoholic liver disease and Gilbert's syndrome and some causes of jaundice due to obstruction includes gallstones, cholestasis of pregnancy, tumours etc. Some causes due to excessive red blood cell destruction caused by hemolysis include malaria, hemolytic anemia and newborn jaundice (Anita et al., 2011).

Anatomy of Liver

The largest gland in the body is liver. It weighs about 1.4 kg averagely in an adult and is situated below the diaphragm in the right hypochondriac and the epigastric regions of abdominopelvic cavity. The liver is covered by visceral peritoneum and a dense irregular connective tissue layer. There are two principal lobes: a right large lobe and a left small lobe and consists of posterior caudate lobe and inferior quadrate lobe. A fold of parietal peritoneum i,e., falciform ligament which

extends from beneath the surface of the diaphragm present between the two principal lobes and helps for the suspension of the liver(Tortora, 2003).

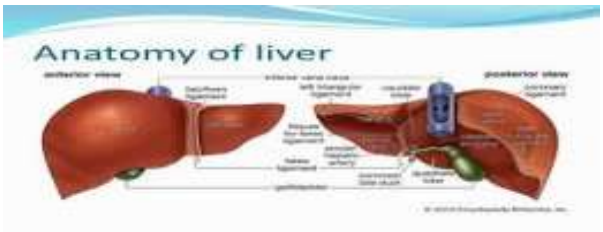

Fig 1. Structure of liver (Encyclopedia Britanica, 2014)

Blood Flow

Portal vein and hepatic artery supply blood to liver. Hepatic artery carries oxygen rich blood and portal vein carries venous blood along with nutrients from the gastrointestinal tract. Blood from the portal vein and hepatic artery are drained to the central vein (Elaine, 2010).

- Hepatic vein – Consists of several small veins that are originating from the lobes• of the liver in the form of small branches that unite to form the hepatic vein. These directly connect to the inferior vena cava, which drains blood from the liver.
- Hepatic artery – It is a blood vessel that supplies 20% of the liver's blood with oxygenated blood.
- Hepatic portal vein – It is a blood vessel that supplies the remaining 80% of the liver's blood and drains venous blood into the liver from the entire gastrointestinal tract.

Microscopic Structure

- Lobules – These are functional units of the liver made up of liver cells which are in hexagonal shape, prearranged in one-cell-thick coat like layers that come from the central vein to the rim of the lobule (Henrey, 1995).
- Hepatocytes – These are the liver cells.
- Sinusoids – These are small blood vessels between the hepatocytes. They receive oxygen-rich blood and nutrients from the hepatic artery and from the intestine. Oxygen and nutrients diffuse through the capillary walls into the liver cells (Steve 1993).
- Portal area – Situated at the corner of each lobule, it is a complex, composed of branches of the hepatic portal vein, hepatic artery, bile duct, and nerve.
- Bile ducts – These are the ducts that carry the bile from the liver. Many small ducts unite to form the hepatic duct in to which bile is drained. The hepatic duct joins the cystic duct, to form the common bile duct that drains in to the duodenum (Arthur Guyton 1995).
- Central vein – It is a blood vessel present in the middle of each lobule which receives blood from the hepatic portal vein and hepatic artery through the sinusoids and drains the blood into the hepatic vein.

Functions of Liver (Peach et al., 1959)

- Carbohydrate metabolism – In carbohydrate metabolism the liver performs specific functions like
  1. Storage of glycogen
  2. Conversion of galactose and fructose to glucose
  3. Gluconeogenesis
- Protein metabolism – The most important functions of the liver in protein metabolism include
- 1. Deamination of amino acids.
  2. Formation of urea for removal of ammonia from the body fluids.
  3. Formation of plasma proteins
- Lipid metabolism – Some specific functions of the liver in lipid metabolism are
  1. High rate of ß- oxidation of fatty acids and formation of acetoacetic acid. 2. Synthesis of large quantities of cholesterol and phospholipids
  3. Conversion of large quantities of carbohydrates and proteins to fats.
- Detoxification of drugs and noxious substances – the liver is the major organ involved in the metabolism, detoxification and excretion of various exogenous and endogenous substances such as xenobiotics .
- Metabolism of ethanol
- Inactivation of hormones
- Synthesis of vitamin A from carotene
- Production of heat
- Breakdown of erythrocytes and defense against microbes
- Secretion of bile

- Phagocytosis – The stellate recticulo-endothelial (Kupffer) cells of the liver phagocytize the aged red blood cells, white blood cells and some bacteria.
- Storage of substances like, fat- soluble vitamins A, D, E, K, some water-soluble vitamins riboflavin, niacin, pyridoxine, folic acid, cyanocobalamin, iron and copper.

Hepatotoxicity

There are two main categories of substances that produce hepatotoxicity, one group consists of agents that are intrinsically toxic i.e., their hepatotoxicity is a fundamental property to which most exposed individuals are susceptible. These are called True, Intrinsic or Predictable hepatotoxins. The other group consists of agents that produce hepatic injury only in unusually susceptible humans i.e., their toxic effects results from the special vulnerability of the affected individual. This form of hepatotoxicity is called Non-predictable or Idiosyncratic hepatotoxicity (Nilesh et al., 2005). Different experimental models used to induce hepatotoxicity (Nilesh et al., 2005; William et al., 2003) Experimental hepatotoxicity permits studies of accidental and industrial toxicity, screening of medicinal agents for potential hepatotoxic effects, studies of hepatic physiology, histopathology, regeneration and the development of diagnostic tools.

Types of experimental models

Both In vitro and In vivo models are available for the evaluation of anti-hepatotoxic properties of a drug. In vitro models are employed to elucidate specific aspects of the mechanism of injury. In the In vivo methods, whole animals are used for the demonstration of an injury by an exogenous agent on the liver with its physiological significance.

***IN-VITRO* MODELS**

A number of In vitro models have been developed for the study of hepatotoxicity. These include the perfused liver, liver homogenates and slices, suspension of hepatocytes freshly isolated from the liver or isolated organelles from hepatocytes. The animals are treated with the agent under study prior to their sacrifice and removal of liver for the In vitro studies or by adding the agent under study to the perfusate or to the medium containing hepatic tissue, cells or organelles.

Liver perfusate

Studies of the effect of a number of toxic agents on metabolic activity, bile flow, dye extraction or excretion, and lipid secretion by the isolated liver into the perfusate or into the bile, all have served to elucidate the mechanism of injury. The nature of hepatic injury also has also been studied by observing the effect of hepatotoxic agent on leakage of intracellular enzymes into the perfusate. Liver perfusion has also been useful in the study of effects of drugs on the liver. Anabolic steroids, phenothiazines, erythromycin and other drugs have been demonstrated to interfere with bile flow and BSP (Bromosulfalein Sulfobromo Phthalein) clearance by In vitro perfused rat liver (Esther et al., 2003).

Tissue homogenates and slices

Homogenates of liver tissue have generally served to test some of the adverse effects of toxic agents on functions of liver that cannot be measured directly In vivo. Metabolism of drugs and uptake of labelled aminoacids by these tissue preparations as a measure of protein synthesis serves to demonstrate the effects of toxic injury. Slices of the liver tissue have also been used to measure the inhibition of lipid secretion by the toxin damaged liver and to demonstrate an adverse effect on plasma membrane of the hepatocyte by the study of potassium or enzyme leakage in the medium (William et al., 2003).

Hepatocyte suspension

This method has been developed for the isolation of rat hepatocytes that have retained sufficiently intact metabolic functions to mimic that of the normal liver suspensions of rat hepatocytes. Suspensions of rat hepatocytes and of tissue culture grown cells have been used in studying mechanism of injury induced by phenothiazines, tricylic antidepressants and other drugs. They also have been useful in the mechanism of action of intrinsic toxins like tetracycline, aflatoxins, chlorinated hydrocarbons and galactosamine.

Organelles (Hewitt et al., 1990; Gharbaran 2014) Organelles isolated from hepatocytes of normal animals or from animals pretreated with hepatotoxic agents, have served to pose some important questions. The adverse effects of hepatotoxic agents such as CCl4, ethionine, phosphorus, thioacetamide, mycotoxins and other agents on the protein-synthesis function of the liver have been studied by measuring the ability of ribosomes isolated from animals pretreated with the respective agent to incorporated labelled aminoacid. Also, the integrity of lysosomes, the functional status and integrity of mitochondria, and the chemical changes in the nucleus and nucleoli, isolated from the liver of animals pretreated with hepatotoxic agents, have been the subject of considerable study by hepatotoxiocologists. The adverse effects of adding hepatotoxic agents In vitro to isolated ribosomes, mitochondria, lysosomes, plasma membrane and Golgi apparatus also have provided information.

Nuclear components (Zimmerman, 1978)
Study of effects of known hepatotoxins on DNA, RNA, nuclei and synthesizing enzymes has provided key information on mechanism of injury. This facet has been approached by examining the various components isolated from the liver after pretreatment of the experimental animals with toxic agents or by isolating the respective compounds from normal animals and examining the effects of the particular toxic agent In vitro on the nucleic acid or enzymes. Thus, demonstration of breaks in the DNA chain or of alkylation of purines or pyrimidines, or of inhibition of nucleic acid synthesizing or repair enzymes has provided important information of hepatotoxic and hepatocarcinogenicity.

Microorganisms - Microorganisms have served as useful models for the study of the mechanism of injury of a number of hepatotoxins. Ethionine, an agent that injures the liver of many species, interferes with the growth of several bacterial and protozoan species by a mechanism that is apparently related to its hepatotoxic effects.

***IN -VIVO* MODELS** (Feroz et al., 2012)
To varying degree, mice, hamsters, guinea pigs, rabbits, dogs, cats, cattle, horse, sheep and several species of birds have been employed, and studies of any particular chemical may include any of these or other species. During recent years, primates have come into use, for the obvious reason of the greater presumed relevance to the disease of humans.

Different In vivo models

- Temporary hepatic ischemia
- Model for direct transhepatic studies in dogs
- Liver cirrhosis and necrosis
- Inhibition of proline hydroxylation
- Influence on collagen synthesis in human skin fibroblasts
- Influence on collagen synthesis in chicken calvaria
- Allyl alcohol induced liver necrosis in rats
- Carbon tetrachloride induced liver fibrosis in rats
- Paracetamol induced hepatotoxicity
- Carbamazepine induced hepatotoxicity
- Tamoxifen induced hepatotoxicity
- Bile duct ligation induced liver fibrosis in rats
- Galactosamine induced liver necrosis

Various Liver Hepatotoxins
Several chemicals are known to cause hepatotoxicity in experimental animals, like carbon tetrachloride (CCl_4), Galactosamine, Lipopolysaccharide,d-Galactosamine/Lipopolysaccharide (GalN/LPS), Thioacetamide, Paracetamol, Alcohol, Anti-tubercular drugs, arsenic and N-nitrosodiethylamine (NDEA).

Parameters of hepatotoxicity: Measures of hepatotoxicity includes lethality, histologic changes seen by light and electron microscopy, chemical changes seen in the liver, and physiologic and biochemical tests that measure the functional status or that reflect the type of intensity of hepatic injury.

Lethality Death as a measure of hepatotoxic potency is applicable mainly to known hepatotoxins. Employment of the LD50 or other measures of lethal potency permits comparison of hepatotoxic agents.

Table 1. VARIOUS LIVER INTOXICANTS AND THEIR MECHANISM OF ACTION

S.No	Liver Intoxicant	Mechanism of action	Dose for hepatotoxicity	References
1	Carbontetrachloride (CCl_4)	It is metabolized in endoplasmic reticulum and mitochondria with the formation of CCl_3O^- a reactive oxidative free radical, which initials lipid peroxidation. $CCl_4 \rightarrow CCl_3O^- + O^-$ Within 24hrs of administration centrilobular necrosis and fatty changes are observed. The max. conc is achieved within 3rs of administration after which the level falls with almost no CCl_4 left in 24hrs	0.1 to 3 ml/Kg body weight , intraperitoneally	Zimmerman et al., 1976; Agarwalet al, 1983; Handaet al., 2008.
2	Galactosamine	Liver injury as in Viral hepatitis. It disturbs the synthesis of essential uridylate nucleotides resulting in organelle injury and ultimate death. A histologic change includes necrosis and inflammatory infilteration of periportal areas, mitoses and cell proliferation, appearance of Councilman bodies, and an increased no. of Kupper cells. Depletion in these nucleotids affects the normal synthesis of RNA and thus protein synthesis. This further causes increase in cell permeability leading to enzyme leakage and finally cell death. The oxygen consumption and the no. of viable hepatocytes is reduced.	400mg/Kg body weight, intraperitoneally	Saraswatet al., 1996. Keppleret al.,1968
3	Thioacetamide	Its metabolite (s-oxide) causes hepatic injury. Interferes with the movement of RNA from the nucleus to the cytoplasm causing membrane injury. Decreases the volume of bile and its contents, and also the no. of viable hepatocytes.	100mg/Kg body weight, subcutaneously	
4	Paracetamol	Its oxidative product, acetyl-P-benzoquinoneimine, binds with suphydryl groups of protein resulting in cell necrosis in the liver. It causes necrosis of centrilobular hepatocytes followed by large hepatic lesions.	1gm/Kg body weight P.O	Handaet al., 2008.
5	Lipopolysaccharides	It releases several inflammatory mediators like tumor necrosis factor alpha, prostaglandins nitric oxide, interleukins, &leukotrienescausis hepatocellular damage and apoptosis. d-GalN mainly inhibits hepatic protein synthesis. When co-administered with LPS, d-GalN inhibits hepatic cytoprotective protein synthesis.		Saraswatet al., 1996.

6	Alcohol	Causes an increase in hepatic lipid peroxidation which causes alterations in membrane lipid composition. Enhances the generation of oxy free radicals during its oxidation in liver, which further results in loss in membrane integrity and structure. Ethanol inhibits glutathione peroxidase, decrease activity of superoxide dismutase, catalases, with the increase in glutathione levels in liver. Ethanol exposure causes damaging effects due to free radical generation or may be due to acetaldehyde formed by its oxidation.		Paronettoet al.,1993
7	Antitubercular drugs	Isoniazide, Rifampicin and Pyrazinamide are potentially hepatotoxic. They are potentially hepatotoxic when given alone or in combination. INH is metabolized to monoacetyl hydrazine, which is further metabolized by cytochrome P_{450} to a toxic product leading to hepatotoxicity. Rifampicin also increases the metabolism of INH to isonicotinic acid and hydrazine, both of which are hepatotoxic. Pyrazinamide decreases the bioavilability of rifampicin and increases its clearance. Pyrazinamide in combination with INH and rifampicin causes increased incidences of hepatotoxicity.		Padma et al., 1998
8.	Arsenic	Arsenic forms strong complexes with sulfhydryl groups and generates ROS (reactive oxygen species) such as superoxide, hydroxyl and peroxyl radicals during its metabolism in cells. Its exposure causes depression in antioxidant defense systems leading to various oxidative damage		Nevinet al., 2005
9	NDEA/DEN (N-nitrosodiethyl amine)	It forms DNA carcinogen adducts in the liver and induces hepatocellular carcinomas without cirrhosis through the development of putative pre neoplastic enzyme-altered hepatocellular focal lesions.		

Histopathology (Sultana et al., 2005) Liver samples were excised after draining the blood, washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. 5 mm thickness Paraffin sections were taken, processed in alcohol–xylene series and were stained with alum hematoxylin and eosin. The Liver sections were examined microscopically for histopathological changes. Toxic hepatic injury can be categorized by using light microscopy, electron microscopy and scanning electron microscopy.

Light microscopy (LM) (Kus et al., 2005) The traditional method for demonstrating toxic hepatic injury and categorizing its type is study by LM. It provides the yardstick against which other abnormalities can be measured.

However, LM provides only a crude estimation for the quantization of the degree of injury.

Electron microscopy (EM) (Nadia et al., 2014) This method provides a much earlier demonstration of hepatocyte injury and it also permits the better recognition of %damage of liver when compared to light microscopy. It is also useful in differentiating lesions that appears to be similar in light microscopy. It provides evidence of injury and may yield information regarding the mechanisms of injury.

Scanning electron microscopy (SEM) (Dujovne et al., 1978) This method of study reveals the information regarding the structural changes induced by toxic agents. Studies of cholestatic effects of hepatotoxins utilizing the scanning technique provided the data base for a new hypothesis for the development of cholestasis.

Liver function assessment parameters

Blood Panel: A Complete blood count should be run especially if they have any of the symptoms of liver disease. The CBP might show a decrease in the number of red blood cells (RBC's). This decrease in RBC's is called anemia. The white blood cell count (WBC) might be elevated (leukocytosis), normal or decreased (leucopenia), mostly depending on the cause of the liver problem and how long it has been present and consequently the following tests must be performed to assess the liver function**.**

Alkaline Phosphatase (ALP): (Kaplan et al., 1983) This enzyme is found primarily in the cells of the biliary system. It is also found in white blood cells, bone, kidney, placenta and intestine. An elevation in the level of serum alkaline phosphatase suggests disease of bile ducts and an indication of liver disease.

Acid phosphatase (ACP): (Bull et al., 2002) Acid phosphatase is found in small amounts in a number of tissues such as bones, kidneys, spleen, liver and a large amount is present in prostate gland. In case of certain liver and bone diseases, excessive destruction of platelets and prostate cancer leads to elevated acid phosphatase activity.

Serum glutamate pyruvate transaminase (SGPT) (Moss et al., 1994) This enzyme is also called as ALT (Alanine aminotransferase) and is found primarily in the cytoplasm of the liver cell. It is also found in small amounts in the heart, kidneys, and muscles. This enzyme leaks into the bloodstream whenever there is damage to the membrane of the liver cell. It is a measure of the integrity of the hepatocytes, and it correlates to the number of hepatocytes affected.

Serum glutamic oxaloacetate transferase (SGOT) (Moss et al., 1994) This enzyme is also called as AST (Aspartate amino transferase). It is practically found in every tissue of the body, including RBC. It is in high concentration particularly in case of cardiac muscle and kidney. The measurement of the serum AST level is helpful for the diagnosis of myocardial infarction, hepatocellular disease and skeletal muscle disorders.

Lactate dehydrogenase (LDH) (Paguthariv u et al., 2015) It is localized in the cytoplasm of cells and is extruded into the serum when cells are damaged or necrotic.

Albumin (Doumass et al., 1971) Albumin is the major protein that circulates in the blood stream. It is synthesized by the liver and secreted into the blood. Low serum albumin concentrations indicate poor liver functions. The serum albumin concentration is usually normal in chronic liver diseases until cirrhosis and significant liver damage is present. Albumin levels can be low in conditions other than liver diseases including malnutrition, some kidney diseases and other rarer conditions

Bilirubin (Willard et al., 1982) In liver disease the amount of bilirubin in the bloodstream is elevated either by enhanced red blood cell destruction or by increased production or by decreased secretion of bile or

Block in bile ducts.

Liver biopsy

This is a very valuable test in the diagnosis of liver disease. A sample of the liver can be obtained during an exploratory surgery or during an ultrasound procedure. The pathologist can look at the hepatocytes

microscopically and determine if disease is present and what the cause is.

Hepatoprotection

Liver disease remains one of the serious health problems. In the absence of consistent liver protective drugs in allopathic medical practices, herbs play an important role in the management of various liver disorders (Jain et al., 2005). The liver is an important organ because a person's nutritional level is not only determined by what he or she eats, but by what the liver processes. The invention of liver tonics, which act upon liver for protecting against toxins, poisons and pathogens, stimulates regeneration of liver cells and protects against inflammation (Buettner 2000). The interest in hepatoprotective activity kindled after the publication of the report on isolation of silymarin, a flavonolignan, from Silybum marianum and its efficacy as a hepatoprotective agent. The discovery drew the attention of the research workers throughout the world towards medicinal plants to search for hepatoprotective agent among them. Cinchorium intybus, Tinospora cordifolia, Wedelia calendulacea, Boerhavia diffusa, Andrographics paniculata, Phyllanthus niruri, glycryrrhiza glabara, picrorhiza kurrooa, Beta vulgaris, Daucus carota, Garcina kola, Solanum nigrum and Raphanus sativus etc., have been tested and reported as excellent hepatoprotectives (Panda et al., 2003;)

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