Hepatotoxicity: A Systematic Review of Mechanisms, Models, and Biochemical Indicators of Liver Injury.

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❖ Abstract.

The liver is crucial for various physiological functions, such as metabolism, nutrient storage, energy production, and detoxification, which are needed for overall health. The liver, as the body's largest organ, plays a crucial role in the biotransformation of medications, chemicals, and poisons, serving as a protective barrier against dangerous compounds. Nonetheless, exposure to xenobiotic, including pharmaceuticals, alcohol, and environmental toxins, may cause liver damage, resulting in a range of hepatic disorders. Hepatotoxicity denotes liver damage induced by several causes, with significant contributions being drug-induced liver injury (DILI), herbal toxicity, and viral infections. Hepatic illnesses are categorized into stages: hepatitis, fibrosis, cirrhosis, and liver cancer. Globally, liver-related disorders account for around 2 million fatalities each year, with cirrhosis and liver cancer as the predominant causes. Liver injury is generally characterized by cellular necrosis increased liver enzymes (AST, ALT, ALP) raised bilirubin levels, and oxidative stress. Diverse in vitro, in vivo, and investigate hepatic injury and the mechanisms underlying chemical-induced liver damage. Conventional biomarkers, like AST and ALT, are frequently utilized to assess and monitor liver health, while on-going research advancements improve comprehension and therapeutic alternatives for hepatic disorders.

Key words: Hepatotoxicity, Cirrhosis, Hepatitis, Fibrosis, AST, ALT, ALP.

❖ Introduction.

The liver is a vital organ in the body. It performs a fundamental role in the regulating of diverse physiological processes, and its activity is related to different vital functions. The liver, the largest organ in the human body, constitutes 2% of body weight, weighing approximately 1.5 kg in a fully developed adult. The liver participates in biochemical processes related to development, nutritional provision, energy supply, and reproduction. Furthermore, it facilitates the metabolism of carbohydrates and lipids, the secretion of bile, and the storage of vitamins. The liver serves as the locus for drug metabolism and biotransformation, thus playing a protective role in the body against toxic exogenous chemical agents. Consequently, the liver is subjected to varying quantities of medications,

chemicals, and other xenobiotic, ultimately leading to hepatic damage. Numerous liver disorders are attributable to various etiological factors. [1]
Hepatotoxicity is defined as an injury to the liver or impairment of the liver function caused by exposure to xenobiotics such as drugs, food additives, alcohol, chlorinated solvents, peroxidized fatty acids, fungal toxins, radioactive isotopes, environmental toxicants, and even some medicinal plants.[2]

The primary etiological factors of hepatic disease include pathogens (hepatitis viruses A, B, C, Cytomegalovirus, Epstein-Barr virus, and yellow fever virus); conditions associated with metabolic syndrome (obesity-related fatty liver disease, hemochromatosis, and Wilson's disease); xenobiotic (alcohol, pharmaceuticals, and chemicals); genetic hepatic disorders; and autoimmune diseases. Drug-induced liver damage (DILI) is a significant complication linked to the management of several acute and chronic diseases. India and Nigeria have the greatest burden of drug-induced liver injury (DILI), followed by China and South Korea, which experience liver injury generated by herbal and alternative medicines. Herbal drug-induced hepatotoxicity. phases of hepatic impairment. Hepatitis, fibrosis, cirrhosis, and hepatic injury or hepatic carcinoma. Globally, almost 2 million individuals succumb annually to hepatic complications, with 1 million attributed to cirrhosis problems and an additional 500,000 related to liver cancer and viral hepatitis. Currently, cirrhosis is the leading cause of mortality, ranking 11th with 1.16 million fatalities, while liver cancer ranks 16th with 788,000 deaths. Together, they constitute 3.5% of all global deaths. Liver damage is typically associated with cellular necrosis, reduction, and elevation of liver biomarkers including aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total bilirubin (TB), total protein (TP), as well as an increase in tissue lipid peroxidation and oxidative injury. [3]

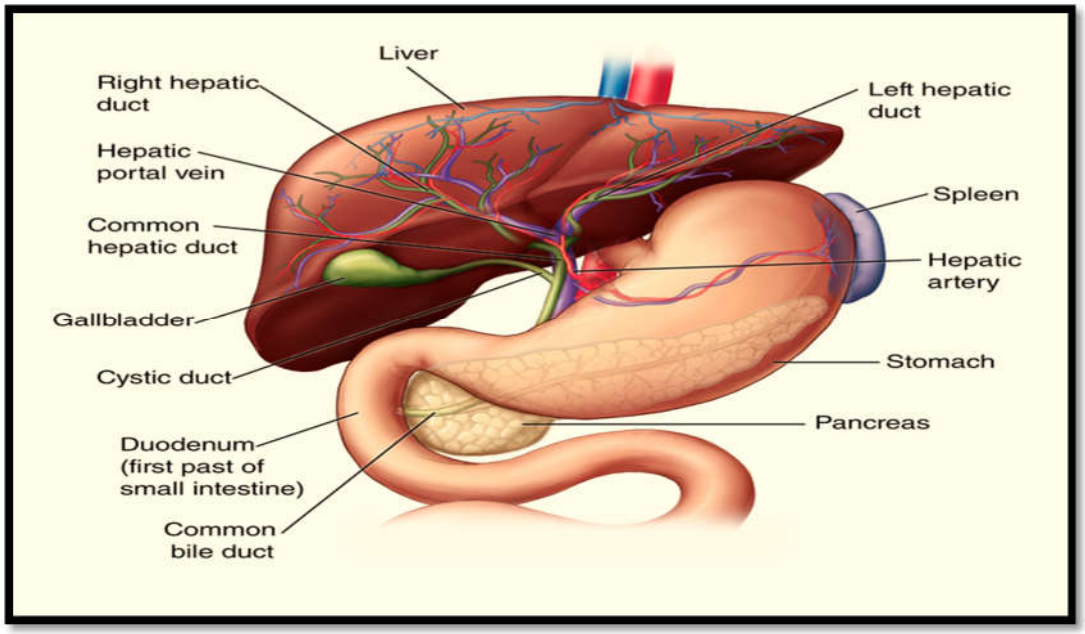

Fig.1 Anatomy of the liver

The liver is situated in the upper right quadrant of the abdominal cavity, beneath the diaphragm and above the stomach, right kidney, and intestines. The liver, like a cone, is a dark reddish-brown organ weighing around 3 pounds. Two separate sources feed blood to the liver, including the following: Oxygenated blood enters via the hepatic artery. Nutrient-dense blood enters via the hepatic portal vein. The liver contains around one pint (13%) of the body's blood volume at any given time. The liver comprises two primary lobes. Each comprises 8 segments, each consisting of 1,000 lobules. The lobules are linked to small channels that converge into bigger ducts, ultimately forming the common hepatic duct. The common hepatic duct conveys bile produced by liver cells to the gallbladder and duodenum through the common bile duct. Roles of the liver the liver manages the majority of chemical levels in the bloodstream and secretes a substance known as bile. Over 500 essential functions have been recognized in the liver. Among the more prominent functions are the following.

• Synthesis of bile.

•Synthesis of certain proteins for blood plasma.

• Synthesis of cholesterol.

• Transformation of surplus glucose into glycogen.

•Regulation of blood amino acid.

• Metabolism of haemoglobin to utilize its iron content (the liver serves as an iron reservoir).

• Conversion of toxic ammonia into urea .

• Detoxification of blood by removing drugs and other harmful substances.

• Regulation of haemostasis.

• Enhancement of immune response.

• Clearance of bilirubin, derived from the breakdown of red blood cells. [4]

Mechanism of Liver Damage

Category	Details
Liver Role in Drug Metabolism	The liver is crucial for drug metabolism. Pathological liver conditions can affect drug efficacy and cause liver damage through metabolic products.
Oxidative and Nitrosative Stress	ROS buildup leads to oxidative stress, damaging cells and DNA, possibly causing cancer. Iron excess worsens oxidative stress via the Fenton reaction. Lipid peroxidation (LPO) is controversial in APAP-induced hepatotoxicity. Peroxynitrite is produced in APAP overdose, damaging mitochondria and leading to cell death.[5]
Mitochondrial Dysfunction	Mitochondrial malfunction leads to necrosis, reduced ATP production, and increased ROS. Mitochondrial permeability transition (MPT) results in the collapse of mitochondrial

	membrane potential, enhancing oxidative stress and DNA damage.[6]
Drug-Induced Liver Injury (DILI)	DILI is rare and unpredictable, usually appearing after weeks or months of use. Incidence varies (1/1000 to 1/200,000). Diagnosis requires a thorough history and exclusion of other liver injury causes. Genetic and environmental factors (e.g., smoking, alcohol) influence susceptibility.[7]
Endoplasmic Reticulum Stress	ROS, calcium changes, and NAPQI production contribute to ER stress in APAP-induced hepatotoxicity. Misfolded proteins and oxidative changes in the microsomes cause ER stress. Mitochondrial dysfunction also contributes to this process.[7]
Chemical-Induced Hepatic Damage	Hazardous chemicals (e.g., antibiotics, chemotherapy agents, CCl_4, aflatoxin) cause liver damage through lipid peroxidation and ROS production.
Direct/Intrinsic/ predictable Drug Reactions	Predictable reactions occur with short-term exposure to certain chemicals (e.g., CCl_4, phosphorus), often with dose-related toxicity. Acetaminophen is an example of a moderately predictable hepatotoxin.[8]
Indirect/Unpredictable Idiosyncratic Drug Reactions	Unpredictable reactions occur without warning, independent of dose, and may take from days to a year to manifest. These reactions have distinct biochemical, clinical, histological, and chronological characteristics.[8]

Specific Mechanisms of Chemical-Induced Damage:

Mechanism	Details
Disruption of Hepatocytes	Covalent binding between chemicals/toxicants and intracellular proteins reduces ATP levels, leading to actin disruption, membrane rupture, and cell death.
Disruption of Transport Proteins	Chemicals affecting canalicular membrane proteins can block bile flow, leading to cholestasis and liver damage.

Cytolytic T-cell Activation	Chemicals can act as immunogens, activating T-cells and triggering an immune response, contributing to liver toxicity.
Apoptosis of Hepatocytes	Tumor necrosis factor-alpha (TNF-α) activates apoptotic pathways, leading to hepatocyte death via caspase activation.
Mitochondrial Disruption	Some chemicals disrupt mitochondrial function, impairing ATP production and energy metabolism.
Bile Duct Injury	Toxic metabolites excreted in bile can damage bile duct epithelial cells, contributing to liver injury.

STAGES OF LIVER DAMAGE.

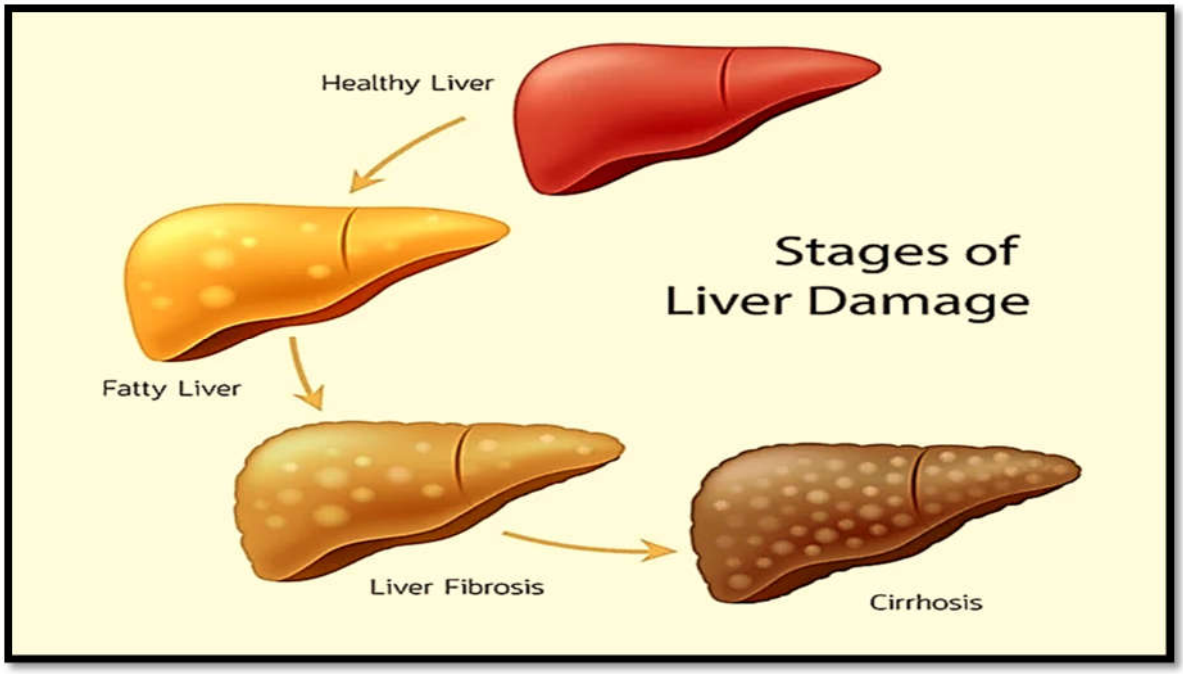

Fig.3 stages of liver damage.

Chronic liver disease progresses in roughly four stages:

Stage 1: Hepatitis

Hepatitis means inflammation in your liver tissues. Inflammation is your liver's response to injury or toxicity. It's an attempt to purge infections and start the healing process. Acute hepatitis (an immediate and temporary response) often accomplishes this. But when the injury or toxicity continues, so does the inflammation. Chronic hepatitis causes hyperactive healing that eventually results in scarring (fibrosis).

Stage 2: Fibrosis

Fibrosis is a gradual stiffening of your liver as thin bands of scar tissue gradually add up. Scar tissue reduces blood flow through your liver, which reduces its access to oxygen and nutrients. This is how your liver's vitality begins to gradually decline. Remarkably, some amount of fibrosis is reversible. Your liver cells can regenerate, and scarring can diminish if the damage slows down enough for it to recover.

Stage 3: Cirrhosis

Cirrhosis is severe, permanent scarring in your liver. This is the stage where fibrosis is no longer reversible. When your liver no longer has enough healthy cells left to work with, its tissues can no longer regenerate. But you can still slow or stop the damage at this stage. Cirrhosis will begin to affect your liver function, but your body will attempt to compensate for the loss, so you might not notice at first.

Stage 4: Liver failure

Liver failure begins when your liver can no longer function adequately for your body's needs. This is also called decompensated cirrhosis your body can no longer compensate for the losses. As liver functions begin to break down, you'll begin to feel the effects throughout your body. Chronic liver failure is a gradual process, but it is eventually fatal without a livertransplant. You need a liver to live.[14]

- **IN VITRO MODELS**

Immortalized cell lines.

The majority of immortalized cell lines derived from livers now accessible exhibit a deficiency in the phenotypic characteristics of liver tissue. The immortalized liver-derived cell lines HepG2, Hep3B, Plc./PRFs Huh7, HBG, HepaRG, and Fa2N-4 are commonly utilized. The HepG2 cell line, established in the 1970s, expresses several liver-specific genes. Nonetheless, it has been shown that the expression profiles of genes associated with phase I and phase II metabolism vary over passages, complicating the assessment of results from different laboratories and passages. The HepaRG human hepatoma cell line retains the expression of various liver-specific activities, including cytochrome P450s, nuclear receptors, membrane transporters, and phase II enzymes. The karyotype of HepaRG cells is stable, and they can differentiate into hepatocytes.[16, 17, 18]

Novel liver-derived cell culture systems.

In certain toxicity assessments, primary hepatocytes and hepatocyte-like cells have limited utility under standard culture conditions. The sustained functionality of primary hepatocytes

and hepatocyte-like cells in culture presents an on-going challenge, and evaluating the effects of chemical exposure in primary hepatocyte cultures is further complicated by inadequate absorption, distribution, metabolism, and excretion (ADME) characteristics resulting from cellular disconnection from the circulatory and other organ systems. New culture techniques are being developed to reverse the dedifferentiation process, establish a more heterotypical environment, and generate more precise in vitro liver toxicity models. This section will provide an in-depth analysis of four tactics: co-cultures, bio artificial livers, three-dimensional culture systems, and stem cell models.[21, 22]

❖ IN VIVO

Carbon Tetrachloride

The toxicity of carbon tetrachloride (CCl4) is contingent upon the duration of exposure and the amount administered. Low-dose effects encompass lipid peroxidation, cytokine release, disruption of Ca2+ homeostasis, and the potential for apoptosis, subsequently leading to cellular regeneration. Elevated dosages or extended exposure may result in more severe adverse effects and induce harm over an extended duration. The patient may develop cirrhosis, fibrosis, or maybe malignancy. The cytochrome P450-dependent monooxygenases metabolize CCl4 primarily through the CYP2E1 isoform located in the mitochondria and endoplasmic reticulum. The highly reactive trichloromethyl radical (CCl3) is generated, resulting in hepatotoxicity. The body's antioxidant defense system may be undermined by these radicals, which can interact with proteins, assault unsaturated fatty acids, induce lipid peroxidation, and diminish cytochrome P450 levels. This leads to dysfunction, reduced protein levels, triglyceride accumulation (fatty liver), and alters the balance of water and electrolytes by elevating hepatic enzyme levels in plasma.[23]

Acetaminophen.

This drug functions as an antipyretic analgesic. Excessive consumption leads to acute liver damage, resulting in hepatocyte necrosis. It serves as a prevalent experimental model for drug-induced liver injury with therapeutic relevance. The majority is transformed into glucuronic or sulfated derivatives and expelled at therapeutic levels; the remainder undergoes intermediate processes, and then removed by conjugation with glutathione. The cytochrome P450, primarily the CYP2E1 isoform, oxidizes excess N-acetyl-p-benzoquinone (NAPQI) during overdoses and swiftly attaches to glutathione. Excessive NAPQI and depletion of glutathione can result in oxidative stress; adduct formation, a covalent bond between metabolites and proteins, and mitochondrial dysfunction. The result is hepatocellular death or necrosis.[23]

Ethanol

The liver is the organ most susceptible to the detrimental effects of ethanol. The CYP2E1 isoform of cytochrome P450 metabolizes ethanol, leading to oxidative stress, the generation of reactive oxygen species, and an elevation in lipid peroxidation, so altering the phospholipid composition of the cellular membrane. Peroxidation leads to the degradation of membrane lipid structure and stability, resulting in elevated blood levels of the membrane-

associated enzyme glutamyl-transpeptidase. Ethanol inhibits glutathione peroxidase and diminishes the activity of catalase and superoxide dismutase. The adverse effects of free radicals produced upon exposure are believed to contribute to the reduction in the activity of antioxidant enzymes, specifically superoxide dismutase and glutathione peroxidase.

D-galactosamine.
This hepatotoxin induces morphologic and functional damage akin to that resulting from viral hepatitis. Hepatocellular necrosis and steatosis may occur following a single dose. It depletes uracil nucleotides, hence obstructing RNA synthesis and subsequently protein production. The toxicity process leads to cellular death through the loss of ion pump activity and increased cellular membrane permeability, resulting in enzyme release and elevated intracellular Ca2+ concentration.[23]

Thioacetamide.
This sulfur-containing organic compound, once utilized as a fungicide, is today employed in laboratories, the paper and textile sectors, and for leather treatment. It influences the production of proteins, DNA, and RNA, as well as the activity of glutamyl transpeptidase (GGT), potentially resulting in both acute and chronic liver damage. The CYP450 and/or flavin-containing monooxygenase system bioactivates thioacetamide, converting it into sulfine, a sulfoxide, and subsequently into sulfone molecules. Sulfine induces modifications in cellular permeability, mitochondrial dysfunction, an elevation in intracellular Ca+2 concentration, nuclear enlargement, and an increase in nuclear volume. The release of nitric oxide synthase and the nuclear factor is induced by sulfone-type compounds.[23]

TRADITIONAL BIOMARKER FOR LIVER INJURY

Serum glutamate oxaloacetate transaminase (SGOT) test:
Injury to liver, heart, kidney, muscle, or brain cells results in the release of the SGOT enzyme. At 37°C, the normal serum level is 46 IU/L. Acute liver necrosis; viral hepatitis, carbon tetrachloride (CCl4), and drug-induced toxicity all result in a 10–200-fold increase in this enzyme's levels. Furthermore, people with intrahepatic cholestasis, post-hepatic jaundice, and alcoholic and hepatic steatosis exhibited a tenfold increase.[24]

Test for serum glutamate pyruvate transaminase (SGPT):
Injured hepatic cells secrete the SGPT enzyme. The standard serum SGPT value at 37°C is 49 IU/L. Individuals with hepatic necrosis and viral hepatitis exhibit markedly elevated SGPT values. Moreover, intrahepatic cholestasis is 10 to 200 times more prevalent in individuals with post-hepatic jaundice, although it is less than 10 in those with cirrhosis, alcoholic hepatitis, and metastatic cancer.[25]

Serum total protein and albumin test:
Total protein typically varies from 5.5 to 8 g/dl with regular assessment. Significant hepatic damage leads to a decrease in blood plasma protein concentrations. Albumin synthesis in the liver often varies from 3.5 to 5.0 g/dl. Chronic liver disease is marked by reduced serum

albumin concentrations. Hypoalbuminemia may result from significant hepatocyte loss associated with liver disorders. Hyperglobulinemia may be induced by chronic inflammatory diseases, including cirrhosis and chronic hepatitis. [26]

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) catalyzes the conversion of superoxide radicals into hydrogen peroxide and oxygen [55]. Dismutation refers to the conversion of a singular reactant into two separate products. Superoxide dismutase is a vital enzyme that catalyzes the conversion of superoxide radicals into molecular oxygen and hydrogen peroxide.[27]

Glutathione S-transferase alpha.

The enzyme alpha-glutathione S-transferase (α-GST) facilitates the elimination of harmful substances from cells. This marker is a more precise indicator of hepatocellular injury than conventional biochemical liver function assays, as it is ubiquitous throughout the liver, has substantial cytosolic content, and possesses a short half-life in plasma. Immunohistochemical studies indicate that α-GST is solely found in hepatocytes. In comparison to aminotransferases, its serum activity is considered a more reliable indicator of hepatic damage.[27]

The enzyme glutamate dehydrogenase.

Liver lobules are the primary sites of glutamate dehydrogenase (GLDH) presence and production. Additionally, the kidneys, pancreas, brain, and intestines possess it, but in reduced quantities. Muscle tissue was minimal. GLDH is one of the most essential enzymes in the mitochondrial matrix. The liver has numerous matrix-dense mitochondria, whereas muscle tissue is characterized by an abundance of cristae-rich mitochondria. Research indicates that GLDH action is limited beyond the liver. GLDH levels are not raised in persons with muscle diseases when compared to ALT levels.[28]

THE FUTURE OF BIOMARKERS FOR LIVER INJURY.

In the last decade, there has been a significant surge in interest about the development of new biomarkers for liver injury. This has been chiefly influenced by three factors: The necessity for sensitive, non-invasive biomarkers to identify innovative drugs that could induce idiosyncratic hepatotoxicity in a wider population during first pharmacological trials. The necessity for biomarkers to predict outcomes in clinical scenarios involving substantial liver damage.The necessity for non-invasive biomarkers to translate pathophysiological pathways from rodents to humans. The acetaminophen (APAP) overdose model is the most often employed model for identifying novel biomarkers for all three aims. [29]

Biomarkers of mitochondrial damage.

Mitochondrial impairment and dysfunction are considered significant contributors to drug-induced hepatotoxicity. They hold particular significance in APAP toxicity. Multiple markers of mitochondrial damage have been identified or suggested. Research indicates that after an acetaminophen overdose and hypoxic hepatitis, levels of the mitochondrial enzyme glutamate dehydrogenase (GLDH) and mitochondrial DNA (mtDNA) in human circulation are increased, and these markers are specific to injuries involving mitochondrial damage. Recent

studies have identified acylcarnitine and the mitochondrial matrix enzymes ornithine carbamoyl transferase and carbamoyl phosphate synthetase-1 (CPS1).

Biomarkers of DNA damage.

DNA damage can be assessed through smearing or laddering on an agarose gel, or by employing immunoassays to identify nucleosomes. The aforementioned approach has been employed to evaluate nuclear DNA fragments in serum and plasma from people experiencing acute liver injury due to various etiologies, including acetaminophen-induced liver injury. These pieces frequently exceed those observed in healthy controls. Despite low sensitivity and specificity, these metrics appear to be elevated in non-survivors compared to survivors.

Histopathological studies

The livers of the experimental mice were excised, minced into small fragments, and stored in a 10% aldehyde solution overnight prior to dehydration. Paraffin was utilized to embed desiccated tissues. A microtome was employed to produce 4 μm sections. After a 5-minute wash with distilled water, liver slices were dewaxed in xylene and subsequently rehydrated in various grades of alcohol. The liver slices were counterstained with eosin for 20 seconds following a 40-second staining with haematoxylin. The slides were examined at 100X and 400X magnification using a Nikon ECLIPSE E200 microscope, following appropriate staining, to identify damage including necrosis, portal inflammation, vascular congestion, fatty infiltration, vacuolar degeneration, leukocyte infiltration, and structural loss of hepatic nodules.[3O]

❖ Conclusion

The liver is essential for overall health, as it regulates critical physiological functions like metabolism, nutrient storage, energy production, and detoxification. Nonetheless, its persistent exposure to deleterious chemicals, such as narcotics, alcohol, and environmental contaminants, renders it vulnerable to hepatotoxicity and a range of liver illnesses. Conditions such as drug-induced liver injury, viral infections, and herbal-induced toxicity can result in significant liver damage, advancing through stages of hepatitis, fibrosis, cirrhosis, and ultimately liver failure. The worldwide prevalence of liver-related disorders, particularly cirrhosis and liver cancer, underscores the necessity for enhanced diagnostic instruments and treatment methodologies. Conventional biomarkers, such as ALP, AST, ALT, and Bilirubin, are pivotal in evaluating liver health; nonetheless, further research is crucial to enhance our comprehension of liver pathology and to formulate more efficacious treatments for hepatotoxicity and the advancement of liver disease.

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