"Evaluation of Hepatoprotective activity of ethanolic extract of *Polygonum glabrum Willd* on hepatotoxicity induced rats"

Dissertation submitted to

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY

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In partial fulfillment of the requirements for the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

BY

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This is to certify that the dissertation entitled **"Evaluation of Hepatoprotective activity of ethanolic extract of Polygonum glabrum Willd on hepatotoxicity induced rats"** submitted to The Tamilnadu Dr. M.G.R. Medical university, Chennai, in partial fulfillment for the award of degree of Master of Pharmacy in Pharmacology is a bonafide individual research work done by **H. Dinesh Kumar (Reg.No.261226005),** Mohamed Sathak A. J. College of Pharmacy, Chennai, under the guidance and direct supervision of **J.Gunasekaran, M.Pharm, Associate Professor, Department of Pharmacology** during the academic year 2013-2014.

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DECLARATION OF THE CANDIDATE

I hereby declare that the thesis titled **"Evaluation of Hepatoprotective activity of ethanolic extract of *Polygonum glabrum Willd* on hepatotoxicity induced rats"** submitted in partial fulfillment for the award of degree Master of Pharmacy to The Tamilnadu Dr. M.G.R. Medical University and carried out at Mohamed Sathak A.J.College of Pharmacy, Chennai, is my original and independent work done under the direct supervision and guidance of **J.Gunasekaran M.Pharm, Associate Professor, Department of Pharmacology** during the academic year 2013-2014 and this thesis contains no material which has been accepted for the award of any degree or diploma of other Universities.

Place: Chennai

Date: **[H. DINESH KUMAR]**

Dedicated to my

Parents, Teachers

&

Friends

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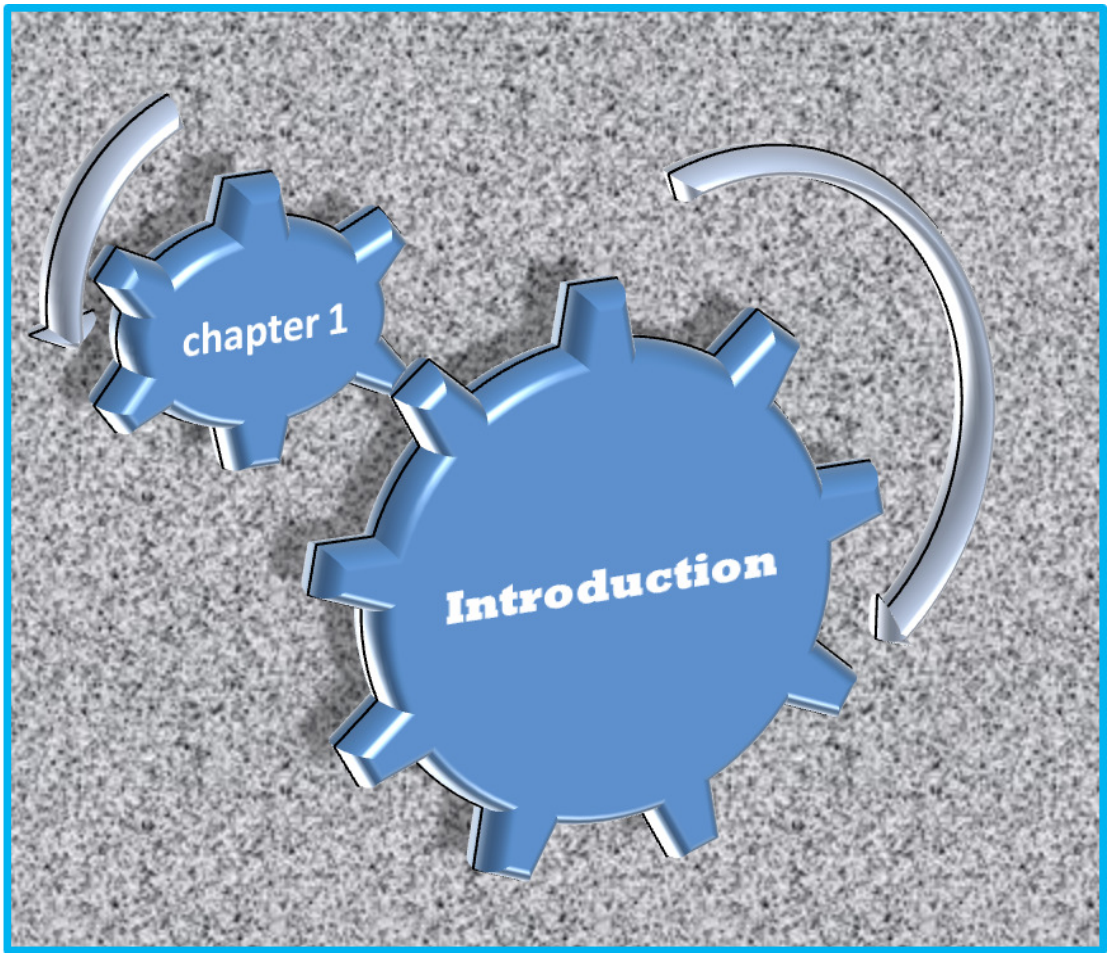
[H.DINESH KUMAR]

LIST OF ABBREVATIONS

- **ALP:** Alkaline phosphatase
- **ALT:** Alanine aminotransferase
- **AST:** Aspartate aminotransferase
- **ANOVA:** Analysis of variance
- **CAT:** Catalase
- **CYP:** Cytochrome p450
- **DILI:** Drug induced liver injury
- **EGF**: Epidermal growth factor
- **GSH**: Gluthione
- **HGF**: Hepatocyte growth factor
- **IUAT**: International union against tuberculosis
- **LTBI**: Latent tuberculosis infection
- **MAH**: Monoacetyl hydrazine
- **MDR**: Multidrug resistant
- **MPG**: Methanolic extract of polygonum glabrum willd
- **NAPQI**: N-acetyl-p-benzoquinone
- **NAT2**: N-acetyl transferase
- **PG**: Polygonum glabrum
- **SOD**: Super oxide dismutase
- **T3**: Triiodothyroxine
- **TBA**: Thiobarbituric acid
- **TB**: Tuberculosis
- **TCA**: Trichloro acetic acid
- **TP**: Total protein
- **USPHS**: U.S. public health service

CONTENTS


chapter 1
Introduction

INTRODUCTION

1.1 LIVER:

Liver is the largest internal organ in the body, constituting about 2.5% of an adult's body weight. During rest, it receives 25% of the cardiac output via the hepatic portal vein and hepatic artery. The hepatic portal vein carries the absorbed nutrients from the GI tract to the liver, which takes up, stores, and distributes nutrients and vitamins. The liver plays an important role in maintaining blood glucose levels. It also regulates the circulating blood lipids by the amount of very low density lipoproteins (VLDLs) it secretes. Many of the circulating plasma proteins are synthesized by the liver. In addition, the liver takes up numerous toxic compounds and drugs from the portal circulation. It is well equipped to deal with the metabolism of drugs and toxic substances. The liver also serves as an excretory organ for bile pigments, cholesterol, and drugs. Finally, it performs important endocrine functions.[1]

1.1.1 Anatomy of the Liver

The liver is almost completely covered by visceral peritoneum and is completely covered by a dense irregular connective tissue layer that lies deep to the peritoneum. The liver is divided into two principal lobes a large right lobe and a smaller left lobe by the falciform ligament, a fold of the mesentery (Figure 24.14a). Although the right lobe is considered by many anatomists to include an inferior quadrate lobe and a posterior caudate lobe, based on internal morphology (primarily the distribution of blood vessels), the quadrate and caudate lobes more appropriately belong to the left lobe. The falciform ligament extends from the undersurface of the diaphragm between the two principal lobes of the liver to the superior surface of the liver, helping to suspend the liver in the abdominal cavity. In the free border of the falciform ligament is the ligamentum teres (round ligament), a remnant of the umbilical vein of the fetus this fibrous cord extends from the liver to the umbilicus. The right and left coronary ligaments are narrow extensions of the parietal peritoneum that suspend the liver from the diaphragm.

1.1.2 The Liver Can Regenerate

Of the solid organs, the liver is the only one that can regenerate. There appears to be a critical ratio between functioning liver mass and body mass. Deviations in this ratio trigger a modulation of either hepatocyte proliferation or apoptosis, in order to maintain the liver's optimal size. Peptide growth factors such as transforming growth

factor- α (TGF- α), hepatocyte growth factor (HGF), and epidermal growth factor (EGF) have been the best-studied stimuli of hepatocyte DNA synthesis. After these peptides bind to their receptors on the remaining hepatocytes and work their way through myriad transcription factors, gene transcription is accelerated, resulting in increased cell number and increased liver mass. Alternatively, a decrease in liver volume is achieved by enhanced hepatocyte apoptosis rates. Apoptosis is a carefully programmed process by which cells kill themselves while maintaining the integrity of their cellular membranes. In contrast, cell death that results from necroinflammatory processes is characterized by a loss of cell membrane integrity and the activation of inflammatory reactions. Liver cell suicide is mediated by proapoptotic signals, such as tumor necrosis factor (TNF).

1.1.3 Histology of the liver.

Histologically, the liver is composed of hepatocytes, bile canaliculi, and hepatic sinusoids as shown in fig 1& 2.

1. **Hepatocytes (hepat- liver; cytes cell).**

Hepatocytes are the major functional cells of the liver and perform a wide array of metabolic, secretory, and endocrine functions. These are specialized epithelial cells with 5 to 12 sides that make up about 80% of the volume of the liver. Hepatocytes form complex three-dimensional arrangements called hepatic laminae.

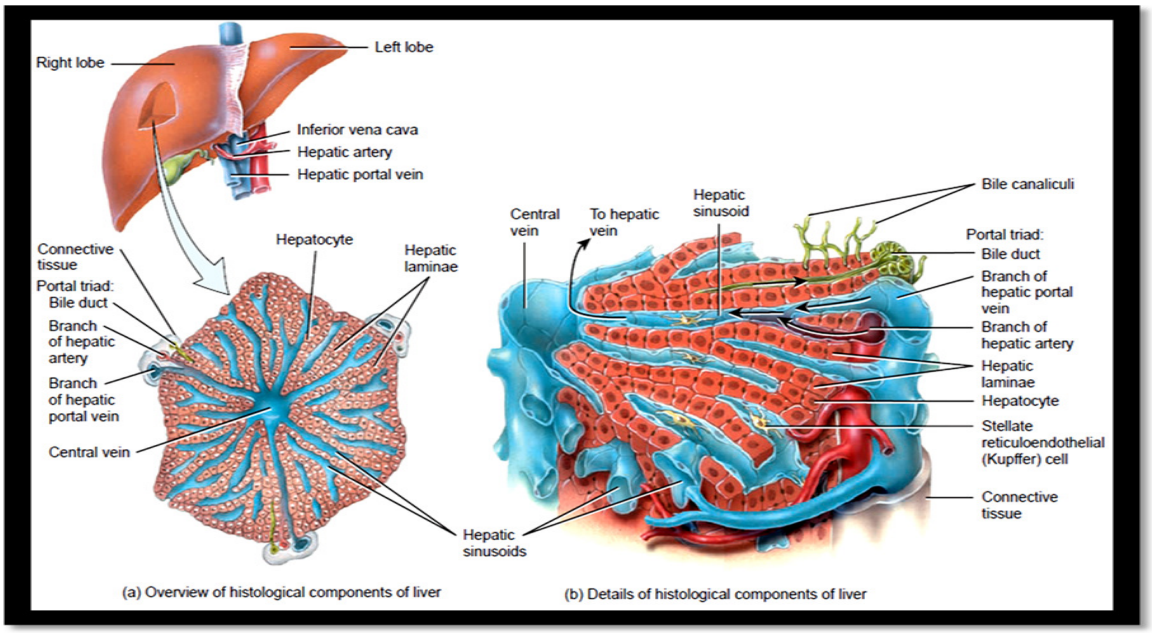


Fig 1: Histological view of the Liver

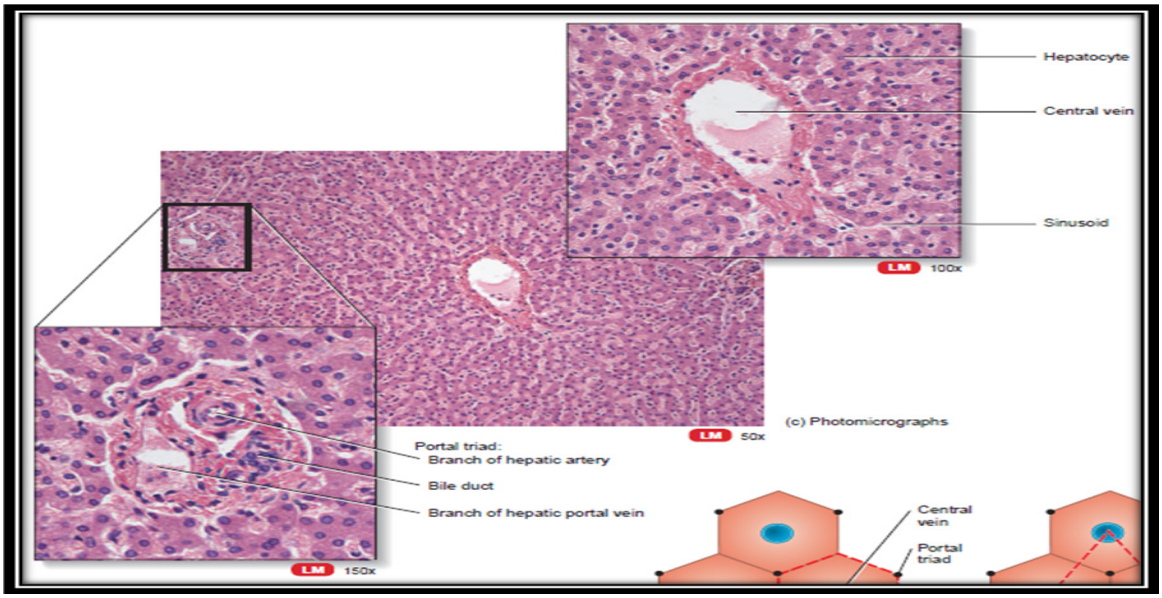
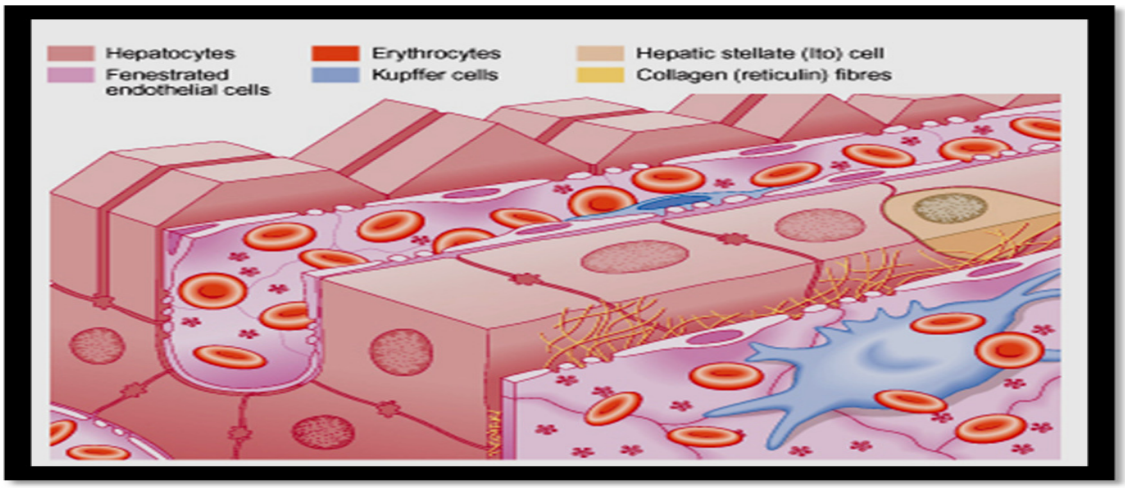


Fig 2: photomicrographs of the liver

The hepatic laminae are plates of hepatocytes one cell thick bordered on either side by the endothelial-lined vascular spaces called hepatic sinusoids. The hepatic laminae are highly branched, irregular structures. Grooves in the cell membranes between neighboring hepatocytes provide spaces for canaliculi (described next) into which the hepatocytes secrete bile. Bile, a yellow, brownish, or olive-green liquid secreted by hepatocytes, serves as both an excretory product and a digestive secretion.



Hepatic stellate cells

Fig 3: The chief cellular features of a hepatic cord, showing hepatocytes, grooved by bile canaliculi. A discontinuous fenestrated endothelium lines the sinusoids, shown containing erythrocytes. Also shown are a Kupffer cell and a hepatic stellate cell. Fine collagen fibres occupy the space of Disse.

Hepatic stellate cells are also known as perisinusoidal lipocytes or Ito cells and are much less numerous than hepatocytes. They are irregular in outline and lie within the hepatic plates, between the bases of hepatocytes. They are thought to be mesenchymal in origin and are characterized by numerous cytoplasmic lipid droplets. These cells secrete most of the intralobular matrix components, including collagen type III (reticular) fibres. They store the fat-soluble vitamin A in their lipid droplets and are a significant source of growth factors active in liver homeostasis and regeneration. Hepatic stellate cells also play a major role in pathological processes. In response to liver damage, they become activated and predominantly myofibroblast-like. They are responsible for the replacement of toxically damaged hepatocytes with collagenous scar tissue - hepatic fibrosis, seen initially in zone 3, around central veins. This can progress to cirrhosis, where the parenchymal architecture and pattern of blood flow are destroyed, with major systemic consequences.

2. Sinusoidal endothelial cells

Hepatic venous sinusoids are generally wider than blood capillaries and are lined by a thin but highly fenestrated endothelium which lacks a basal lamina. The endothelial cells are typically flattened, each with a central nucleus and joined to each other by junctional complexes. The fenestrae are grouped in clusters with a mean diameter of 100nm, allowing plasma direct access to the basal plasma membranes of hepatocytes. Their cytoplasm contains numerous typical transcytotic vesicles.

3. Kupffer cells

Kupffer cells are hepatic macrophages derived from circulating blood monocytes. They are long-term hepatic residents, lying within the sinusoidal lumen, attached to the endothelial surface. They originate in the bone marrow, and form a major part of the mononuclear phagocyte system, responsible for removing cellular and microbial debris from the circulation, and secreting cytokines involved in defence. Kupffer cells remove aged and damaged red cells from the hepatic circulation, a function normally shared with the spleen, but fulfilled entirely by the liver after splenectomy. Kupffer cells are irregular in shape, with long processes extending into the sinusoidal lumen.

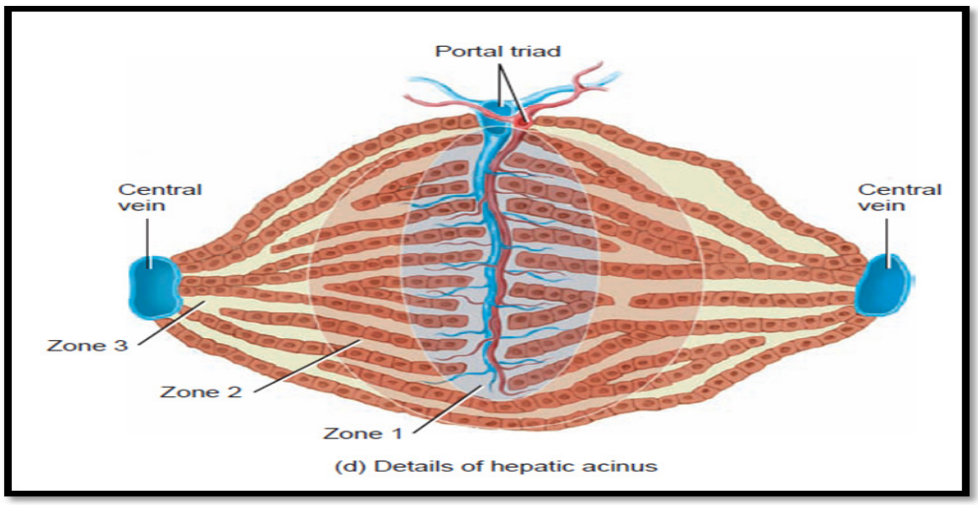


Fig 4: Details of hepatic acinus

4. Bile canaliculi (kan-a-LIK-u- -li small canals).

These are small ducts between hepatocytes that collect bile produced by the hepatocytes. From bile canaliculi, bile passes into bile ductules and then bile ducts. The bile ducts merge and eventually form the larger right and left hepatic ducts, which unite and exit the liver as the common hepatic duct. The common hepatic duct joins the cystic duct (cystic bladder) from the gallbladder to form the common bile duct. From here, bile enters the small intestine to participate in digestion.

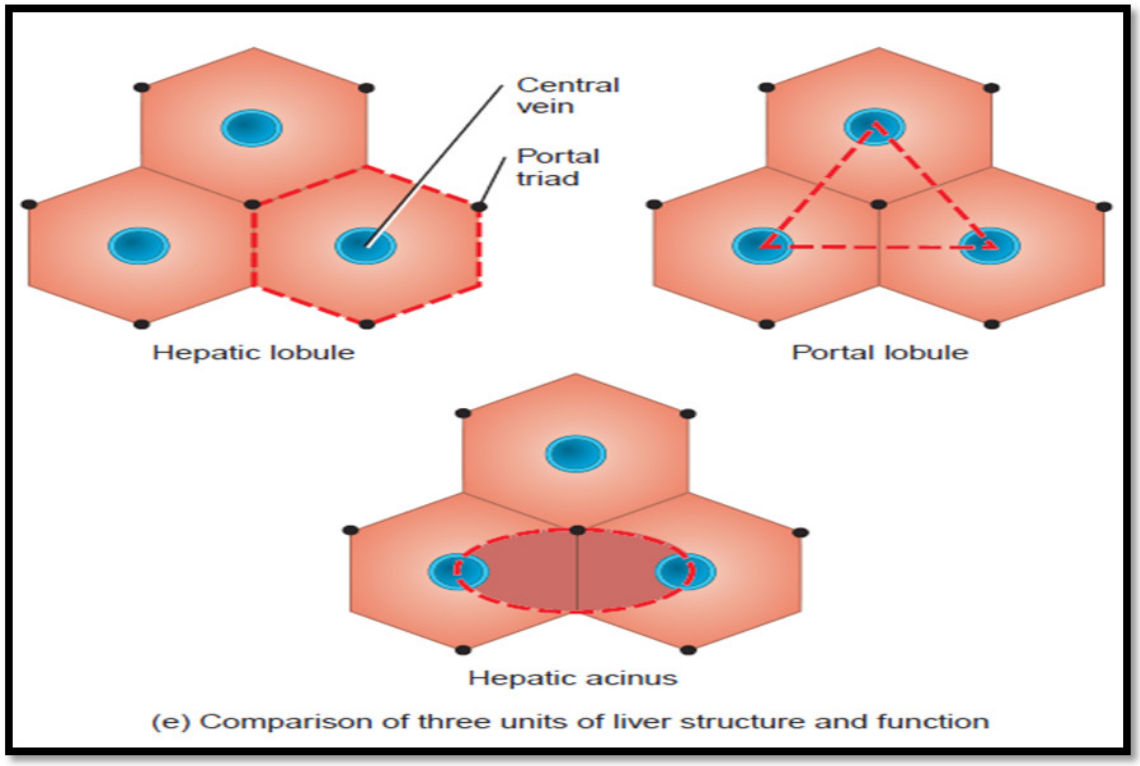


Fig 5: Comparision of three units of liver structure and function

5. Hepatic sinusoids.

These are highly permeable blood capillaries between rows of hepatocytes that receive oxygenated blood from branches of the hepatic artery and nutrient-rich deoxygenated blood from branches of the hepatic portal vein. Recall that the hepatic portal vein brings venous blood from the gastrointestinal organs and spleen into the liver. Hepatic sinusoids converge and deliver blood into a central vein. From central veins the blood flows into the hepatic veins, which drain into the inferior vena cava. In contrast to blood which flows toward a central vein, bile flows in the opposite direction. Also present in the hepatic sinusoids are fixed phagocytes called stellate reticulo endothelial (Kupffer) cells, which destroy worn-out white and red blood cells, bacteria, and other foreign matter in the venous blood draining from the gastrointestinal tract. Together, a bile duct, branch of the hepatic artery, and branch of the hepatic vein are referred to as a portal triad (tri three). The hepatocytes, bile duct system, and hepatic sinusoids can be organized into anatomical and functional units in three different ways:

1. Hepatic lobule. For years, anatomists described the hepatic lobule as the functional unit of the liver. According to this model, each hepatic lobule is shaped like a hexagon (six-sided structure) left at its center is the central vein, and radiating out from it are rows of hepatocytes and hepatic sinusoids. Located at three corners of the hexagon is a portal triad. This model is based on a description of the liver of adult pigs. In the human liver it is difficult to find such well-defined hepatic lobules surrounded by thick layers of connective tissue.

2. Portal lobule. This model emphasized the exocrine function of the liver, that is, bile secretion. Accordingly, the bile duct of a portal triad is taken as the center of the portal lobule. The portal lobule is triangular in shape and is defined by three imaginary straight lines that connect three central veins that are closest to the portal triad. This model has not gained widespread acceptance.

3. Hepatic acinus. In recent years, the preferred structural and functional unit of the liver is the hepatic acinus. Each hepatic acinus is an approximately oval mass that includes portions of two neighboring hepatic lobules. The short axis of the hepatic acinus is defined by branches of the portal triad branches of the hepatic artery, vein, and bile ducts that run along the border of the hepatic lobules. The long axis of the acinus is defined by two imaginary curved lines, which connect the two central veins

closest to the short axis (Figure 24.15e, center). Hepatocytes in the hepatic acinus are arranged in three zones around the short axis, with no sharp boundaries between them. Cells in zone 1 are closest to the branches of the portal triad and the first to receive incoming oxygen, nutrients, and toxins from incoming blood. These cells are the first ones to take up glucose and store it as glycogen after a meal and break down glycogen to glucose during fasting. They are also the first to show morphological changes following bile duct obstruction or exposure to toxic substances. Zone 1 cells are the last ones to die if circulation is impaired and the first ones to regenerate Cells in zone 3 are farthest from branches of the portal triad and are the last to show the effects of bile obstruction or exposure to toxins, the first ones to show the effects of impaired circulation, and the last ones to regenerate. Zone 3 cells also are the first to show evidence of fat accumulation. Cells in zone 2 have structural and functional characteristics intermediate between the cells in zones 1 and 3. The hepatic acinus is the smallest structural and functional unit of the liver. Its popularity and appeal are based on the fact that it provides a logical description and interpretation of (1) patterns of glycogen storage and release and (2) toxic effects, degeneration, and regeneration in the three zones of the hepaticacinus relative to the proximity of the zones to branches of the portal triad.

1.1.4 Role and Composition of Bile

Role and Composition of Bile Each day, hepatocytes secrete 800–1000 mL (about 1 qt) of bile, a yellow, brownish, or olive-green liquid. It has a pH of 7.6–8.6 and consists mostly of water, bile salts, cholesterol, a phospholipid called lecithin, bile pigments, and several ions. The principal bile pigment is bilirubin. The phagocytosis of aged red blood cells liberates iron, globin, and bilirubin (derived from heme). The iron and globin are recycled; the bilirubin is secreted into the bile and is eventually broken down in the intestine. One of its breakdown products stercobilin gives feces their normal brown color. Bile is partially an excretory product and partially a digestive secretion. Bile salts, which are sodium salts and potassium salts of bile acids (mostly chenodeoxycholic acid and cholic acid), play a role in emulsification, the breakdown of large lipid globules into a suspension of small lipid globules. The small lipid globules present a very large surface area that allows pancreatic lipase to more rapidly accomplish digestion of triglycerides. Bile salts also aid in the absorption of lipids following their digestion. Although hepatocytes continually release bile, they increase production and secretion when the portal blood contains more bile acids;

thus, as digestion and absorption continue in the small intestine, bile release increases. Between meals, after most absorption has occurred, bile flows into the gallbladder for storage because the sphincter of the hepatopancreatic ampulla (sphincter of Oddi; see Figure 24.14) closes off the entrance to the duodenum.

1.1.5 Functions of the Liver

In addition to secreting bile, which is needed for absorption of dietary fats, the liver performs many other vital functions:

• ***Carbohydrate metabolism.*** The liver is especially important in maintaining a normal blood glucose level. When blood glucose is low, the liver can break down glycogen to glucose and release the glucose into the bloodstream. The liver can also convert certain amino acids and lactic acid to glucose and it can convert other sugars, such as fructose and galactose, into glucose. When blood glucose is high, as occurs just after eating a meal, the liver converts glucose to glycogen and triglycerides for storage.

• ***Lipid metabolism.*** Hepatocytes store some triglycerides; break down fatty acids to generate ATP; synthesize lipoproteins, which transport fatty acids, triglycerides, and cholesterol to and from body cells; synthesize cholesterol; and use cholesterol to make bile salts.

• ***Protein metabolism.*** Hepatocytes *deaminate* (remove the amino group, NH2, from) amino acids so that the amino acids can be used for ATP production or converted to carbohydrates or fats. The resulting toxic ammonia (NH3) is then converted into the much less toxic urea, which is excreted in urine. Hepatocytes also synthesize most plasma proteins, such as alpha and beta globulins, albumin, prothrombin, and fibrinogen.

• ***Processing of drugs and hormones.*** The liver can detoxify substances such as alcohol and excrete drugs such as penicillin, erythromycin, and sulfonamides into bile. It can also chemically alter or excrete thyroid hormones and steroid hormones such as estrogens and aldosterone.

• ***Excretion of bilirubin.*** As previously noted, bilirubin, derived from the heme of aged red blood cells, is absorbed by the liver from the blood and secreted into bile. Most of the bilirubin in bile is metabolized in the small intestine by bacteria and eliminated in feces.

• ***Synthesis of bile salts.*** Bile salts are used in the small intestine for the emulsification and absorption of lipids.

• ***Storage.*** In addition to glycogen, the liver is a prime storage site for certain vitamins (A, B12, D, E, and K) and minerals (iron and copper), which are released from the liver when needed elsewhere in the body.

• ***Phagocytosis.*** The stellate reticuloendothelial (Kupffer) cells of the liver phagocytize aged red blood cells, white blood cells, and some bacteria.

• ***Activation of vitamin D.*** The skin, liver, and kidneys participate in synthesizing the active form of vitamin D.[2]

1.1.6 ENDOCRINE FUNCTIONS OF THE LIVER

The liver is important in regulating the endocrine functions of hormones. It can amplify the action of some hormones. It is also the major organ for the removal of peptide hormones.

1.1.7 The Liver Can Modify or Amplify Hormone Action

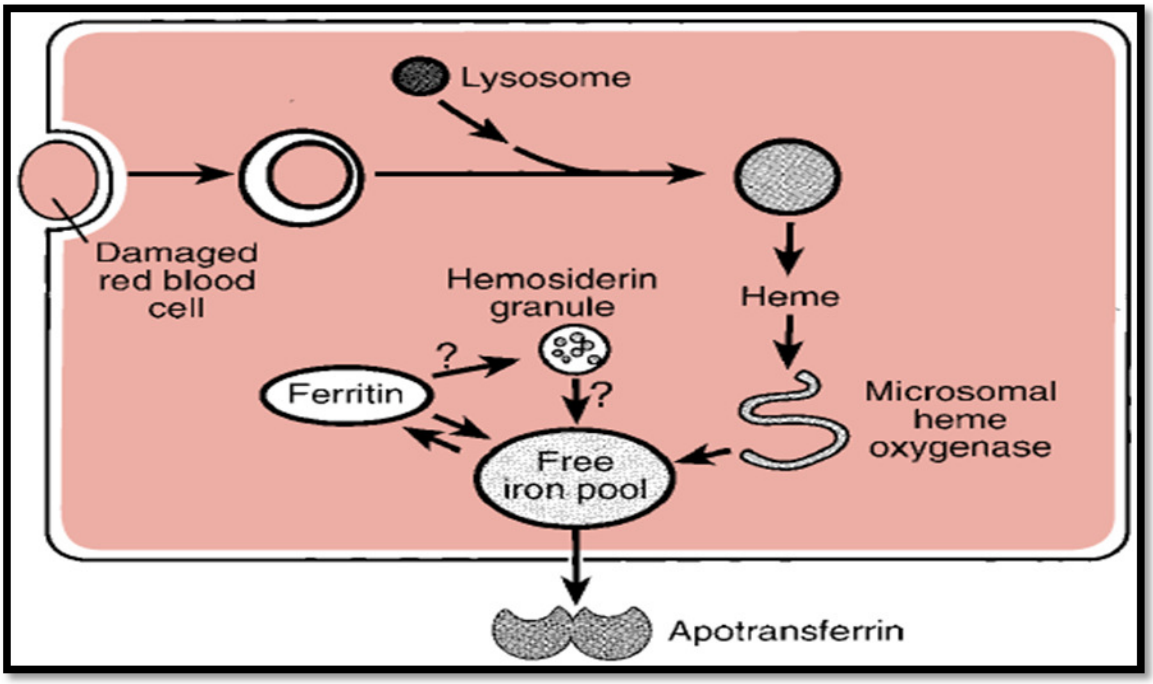
As discussed before, the liver converts vitamin D3 to 25-hydroxy vitamin D3, an essential step before conversion to the active hormone 1,25-hydroxy vitamin D3 in the kidneys. The liver is also a major site of conversion of the thyroid hormone thyroxine (T4) to the biologically more potent hormone triiodothyronine (T3). The regulation of the hepatic T4 to T3 conversion occurs at both the uptake step and the conversion step. Due to the liver's relatively large reserve in converting T4 to T3, hypothyroidism is uncommon in patients with liver disease. In advanced chronic liver disease, however, signs of hypothyroidism may be evident. The liver modifies the function of growth hormone (GH) secreted by the pituitary gland. Some growth hormone actions are mediated by **insulin-like growth factors** made by the liver.

1.1.8 The Liver Removes Circulating Hormones

The liver helps to remove and degrade many circulating hormones. Insulin is degraded in many organs, but the liver and kidneys are by far most important. The presence of insulin receptors on the surface of hepatocytes suggests that the binding of insulin to these receptors results in degradation of some insulin molecules. There is also degradation of insulin by proteases of hepatocytes that do not involve the insulin receptor. Glucagon and growth hormone are degraded mainly by the liver and the kidneys. The liver may also degrade various GI hormones (e.g., gastrin), but the kidneys and other organs probably contribute more significantly to inactivating these hormones.

1.1.9 The Liver Is Important in the Storage and Homeostasis of Iron

The liver is the major site for the synthesis of several proteins involved in iron transport and metabolism. The protein **transferrin** plays a critical role in the transport and homeostasis of iron in the blood. The circulating plasma transferrin level is inversely proportional to the iron load of the body the higher the concentration of ferritin in the hepatocyte, the lower the rate of transferrin synthesis. During iron deficiency, liver synthesis of transferrin is significantly stimulated, enhancing the intestinal absorption of iron. **Haptoglobin**, a large glycoprotein with a molecular weight of 100,000, binds free hemoglobin in the blood. The hemoglobin-haptoglobin complex is rapidly removed by the liver, conserving iron in the body. **Hemopexin** is another protein synthesized by the liver that is involved in the transport of free heme in the blood. It forms a complex with free heme, and the complex is removed rapidly by the liver. The spleen is the organ that removes red blood cells that are slightly altered. Kupffer cells of the liver also have the capacity to remove damaged red blood cells, especially those that are moderately damaged (Fig. 28.8).



The possible pathways following phagocytosis of damaged red blood cells by Kupffer cells.

to release heme. Microsomal **heme oxygenase** releases iron from the heme, which then enters the free iron pool and is stored as ferritin or released into the bloodstream (bound to apotransferrin). Some of the ferritin iron may be converted to **hemosiderin**

granules. It is unclear whether the iron from the hemosiderin granules is exchangeable with the free iron pool.

It was long believed that Kupffer cells were the only cells involved in iron storage, but recent studies suggest that hepatocytes are the major sites of long-term iron storage. Transferrin binds to receptors on the surface of hepatocytes, and the entire transferrin-receptor complex is internalized and processed (Fig. 28.9).

The apotransferrin (not containing iron) is recycled back to the plasma, and the released iron enters a labile iron pool. The iron from transferrin is probably the major source of iron for the hepatocytes, but they also derive iron from haptoglobin-hemoglobin and hemopexin-heme complexes. When hemoglobin is released inside the hepatocytes, it is degraded in the secondary lysosomes, and heme is released. Heme is processed in the smooth ER and free iron released enters the labile iron pool. A significant portion of the free iron in the cytosol probably combines rapidly with apoferritin to form ferritin. Like Kupffer cells, hepatocytes may transfer some of the iron in ferritin to hemosiderin. Iron is absolutely essential for survival, but iron overload can be extremely toxic, especially to the liver where it can cause **hemochromatosis**, a condition characterized by excessive amounts of hemosiderin in the hepatocytes. The hepatocytes in patients with hemochromatosis are defective and fail to perform many normal functions.[3]

1.2 DRUG-INDUCED LIVER INJURY (DILI)

The number of drugs associated with adverse reactions involving the liver is extensive. One of the more common reasons for the withdrawal of a drug from the marketplace is an elevation of liver enzymes.[4] Alcohol-induced liver disease is the most common type of drug-induced liver disease. All other drugs together account for less than 10% of patients hospitalized for elevated liver enzymes.[5] In approximately 75% of these cases liver transplantation is ultimately required for patient survival. The liver's function affects almost every other organ system in the body.

1.2.1 PATTERNS OF DRUG-INDUCED LIVER DISEASE

For some drugs, a genetic or acquired abnormality must exist in a particular metabolic pathway for a toxic reaction to take place (Fig. 38–1). In other cases, the reactions are typically associated with a drug concentration and often respond to simply lowering the dose of the drug. Idiosyncratic reactions tend to occur without association to particular blood concentrations or specifically identified metabolic abnormalities. For example, sulfonylureas like glipizide and antibiotics like ciprofloxacin have caused

severe liver disease, resulting in the need for transplantation in a very small group of patients.[6,7]

1.2.3 ALLERGIC HEPATITIS

Allergic reactions in the liver can be caused by many drugs and result in many different kinds of hepatic damage. Trimethoprim sulfamethoxazole and penicillinase-resistant penicillins such as dicloxacillin induce a reaction typical of hepatic hypersensitivity in a few patients. The reaction usually develops within 4 weeks of the start of therapy.[8,9] It is marked by fever, pruritus, rash, eosinophilia, arthritis, and hemolytic anemia. The formation of granulomas within the liver is often seen on biopsy.[10] The reaction reverses with discontinued therapy and reappears upon rechallenge. Most antibiotics have been associated with this type of reaction, including the fluoroquinolones, macrolides, and β-lactams.3 Allopurinol also has been associated with a number of reports of hypersensitivity reactions involving the liver. The onset of symptoms is 1 to 6 weeks after initiation of therapy. The incidence, like all the allergic liver reactions, is low, estimated at less than 1%. The clinical presentation includes eosinophilia, fever, rash, and arthritis, as previously mentioned. The biopsy may showa pattern of fibrin-ring granulomas similar to those seen in Q fever.[11]

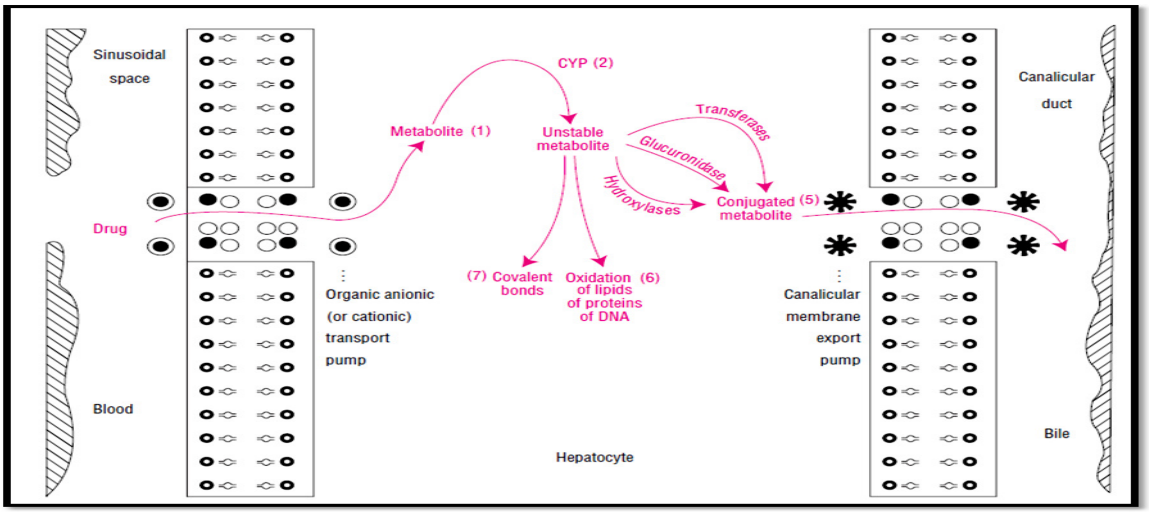
1.2.4 TOXIC HEPATITIS

Toxic reactions are predictable, often dose-related effects in the liver due to specific agents. When taken in overdose, acetaminophen becomes bioactivated to a toxic intermediate known as *N*-acetyl-*p*-benzoquinone imine (NAPQI). NAPQI is very reactive, with a high affinity for sulfhydryl groups. When the liver's glutathione stores are depleted and there are no longer sulfhydryl groups available to detoxify this metabolite, it begins to react directly with the hepatocyte (see Fig. 38–1). Replenishing the liver's sulfhydryl capacity through the administration of N-acetylcysteine early after ingestion of the overdose halts this process.[12] Acetaminophen's toxicity occurs in four stages.[13] Reye's syndrome is an aggressive form of toxic hepatitis often associated with aspirin use in children. Valproate toxicity can also present in this pattern. Early in the process of Reye's syndrome, mitochondrial dysfunction leads to the depletion of acyl coenzyme A and carnitine. Fatty acids accumulate and gluconeogenesis is impaired, resulting in hypoglycemia. A concurrent disruption of the urea cycle occurs, leading to a decrease in the removal of ammonia and a slowing of protein use. A threefold rise in the blood ammonia level

and an increase in the prothrombin time are common findings. In advanced stages of Reye's syndrome, many patients develop intracranial hypertension that can be life threatening and refractory to therapy.[14,15]

1.2.5 Biotransformation of a drug is explained as follows:

1. The drug is actively transported into the hepatocyte by the organic anion transport pump, a transmembrane protein.
2. The metabolite (drug) interacts with one of a number of enzymes, the most common being CYP3A4. The CYPx family of phase I enzymes are regulated by the complementary DNA xenobiotic receptor. The xenobiotic receptor is in turn upregulated by other drugs, changes in cholesterol catabolism, and bile acids.
3. The immediate result of the action of the phase I enzyme is the production of an unstable metabolite.
4. The unstable metabolite then reacts with glucuronidase, various transferases, or hydroxylases to form a conjugated metabolite. The efficacy of these enzymes is affected by the patient's nutritional state and genetic polymorphism, leading to variations in individual risk for toxicity.
5. The conjugated metabolite is removed from the hepatocyte by the canalicular membrane export pump, one of a large family of membrane proteins (other members of this family pump conjugated metabolites back into the blood for excretion by the kidney). These proteins are subject to genetic polymorphism as well, again leading to some patients having an increased risk for toxicity.
6. If unable to form a conjugate, the unstable metabolite can participate in oxidative reactions that damage lipids, proteins, or even DNA.
7. Alternatively the unstable metabolite may form damaging covalent bonds with available anions or cations.



A general diagram of biotransformation of a drug

1.2.6 CHRONIC ACTIVE TOXIC HEPATITIS

Dantrolene, isoniazid, phenytoin, nitrofurantoin, and trazodone have been reported in association with a type of autoimmune mediated disease in the liver.[16,17] Patients experience periods of symptomatic hepatitis followed by periods of convalescence, only to repeat the experience months later. It is a progressive disease with a high mortality rate and is more common in females than males. Antinuclear antibodies appear in most patients. These drugs appear to form antiorganelle antibodies.[18] The exact identification of a causative agent is sometimes difficult since diagnosis requires mu ltiple episodes occurring long after exposure to the offending drug.

1.2.7 TOXIC CIRRHOSIS

The scarring effect of hepatitis in the liver leads to the development of cirrhosis. Some drugs tend to cause such a mild case of hepatitis that it may not be detected. Mild hepatitis can be easily mistaken for a more routine generalized viral infection. If the offending drug or agent is not discontinued, this damage will continue to progress. The patient eventually presents not with hepatitis, but with cirrhosis. Methotrexate causes periportal fibrosis in most patients who experience hepatotoxicity. The lesion results from the action of a bioactivated metabolite produced by cytochrome P450.[19] This process has most commonly been noted in patients treated for psoriasis and arthritis. The extent of damage can be reduced or controlled by increasing the dosage interval to once weekly or by routine use of folic acid supplements.[20]

Vitamin A is normally stored in liver cells, and causes significant hypertrophy and fibrosis when taken for long periods in high doses. Hepatomegaly is a common

finding, along with ascites and portal hypertension. In patients with vitamin A toxicity, gingivitis and dry skin are also very common. This is accelerated by ethanol, which competes with retinol for aldehyde dehydrogenase.[21]

1.3 LIVER VASCULAR DISORDERS

Focal lesions in hepatic venules, sinusoids, and portal veins occur with various drugs. The most commonly associated drugs are the cytotoxic agents used to treat cancer, the pyrrolizidine alkaloids, and the sex hormones. A centralized necrosis often follows and can result in cirrhosis. Azathioprine and herbal teas that contain comfrey (a source of pyrrolizidine alkaloids) are associated with the development of veno-occlusive disease. The exact incidence is rare and may be dose related.3 Peliosis hepatitis is a rare type of hepatic vascular lesion that can be seen as both an acute and a chronic disease. The liver develops large, blood-filled lacunae within the parenchyma. Rupture of the lacunae can lead to severe peritoneal hemorrhage. Peliosis hepatitis has been associated with exposure of the liver to androgens, estrogens, tamoxifen, azathioprine, and danazol. Androgens with a methyl alkylation at the 17 carbon position of the testosterone structure are the most frequently reported agents that cause peliosis hepatitis, usually after at least 6 months of therapy.[22]

1.4 MECHANISMS OF DRUG-INDUCED LIVER DISEASE

1.4.1 CENTROLOBULAR NECROSIS

Centrolobular necrosis is often a dose-related, predictable reaction secondary to drugs such as acetaminophen; however, it also can be associated with idiosyncratic reactions, such as those caused by halothane. Also called *direct* or *metabolite-related hepatotoxicity,* centrolobular necrosis is usually the result of the production of a toxic metabolite (see Fig. 38–1). The damage spreads outward from the middle of a lobe of the liver. Patients suffering from centrolobular necrosis tend to present in one of two ways, depending on the extent of necrosis. Mild drug reactions, involving only small amounts of parenchymal tissue, may be detected as asymptomatic elevations in the serum transaminases. If the reaction is diagnosed at this stage, most of these patients will recover with minimal cirrhosis and thus minimal chronic liver impairment. More severe forms of centrolobular necrosis are accompanied by nausea, vomiting, upper abdominal pain, and jaundice.[23]

1.4.2 STEATOHEPATITIS Steatohepatitis (also known as steatonecrosis) is a specialized type of acute necrosis resulting from the accumulation of fatty acids in the hepatocyte. Drugs or their metabolites that cause steatonecrosis do so by affecting

fatty-acid oxidation within the mitochondria of the hepatocyte (see Fig. 38–1). Hepatic vesicles become engorged with fatty acids, eventually disrupting the homeostasis of the hepatocyte. The liver biopsy is marked by a massive infiltration by polymorphonuclear leukocytes, degeneration of the hepatocytes, and the presence of Mallory bodies.3 Alcohol is the drug that most commonly produces steatonecrotic changes in the liver. When alcohol is converted into acetaldehyde, the synthesis of fatty acids is increased.[24,25] When the hepatocyte has become completely engorged with microvesicular fat, it often breaks open, spilling into the blood. If enough hepatocytes break open, an inflammatory response begins. If the offending agent is withdrawn before significant numbers of hepatocytes become necrotic, the process is completely reversible without long-term sequelae. In nonalcoholic steatohepatitis the same endpoint is often achieved through oxidation of lipid peroxidases.[26] Tetracycline produces steatohepatitis and steatosis.[27] The lesions are characterized by large vesicles of fat found diffused throughout the liver.. The mortality of tetracycline steatohepatitis is very high (70% to 80%), and those who do survive often develop cirrhosis. Sodium valproate also can produce steatonecrosis through the process of bioactivation. Cytochrome P450 converts valproate to D-4-valproic acid, a potent inducer of microvesicular fat accumulation.[28]

1.4.3 PHOSPHOLIPIDOSIS

Phospholipids usually engorge the lysosomal bodies of the hepatocyte.[26] Amiodarone has been associated with this reaction. Patients treated with amiodarone who develop overt hepatic disease tend to have received higher doses of the drug. These patients also have higher amiodarone to N-desethyl-amiodarone ratios, indicating a greater accumulation of the parent compound. Amiodarone and its major metabolite N-desethyl-amiodarone remain in the liver of all patients for several months after therapy is stopped. Usually the phospholipidosis develops in patients treated for more than 1 year. The patient can present with either elevated transaminases or hepatomegaly; jaundice is rare.

1.4.4 GENERALIZED HEPATOCELLULAR NECROSIS

Generalized hepatocellular necrosis mimics the changes associated with the more common viral hepatitis. The onset of symptoms is usually delayed as much as a week or more after exposure to toxin. Bioactivation is often important for toxic hepatitis to develop, but may not be the immediate cause of damage. Many drugs that are associated with toxic hepatitis produce metabolites that are not inherently toxic to the

liver. Instead, they act as haptens, binding to specific cell proteins and inducing an autoimmune reaction (see Fig. 38–1). The rate of bioactivation can vary between males and females and between individuals of the same sex.[29] The cytochrome P450 system (CYP) tends to metabolize lipophilic substrates which are actively pumped into the hepatocyte by an organic anion (or cation) transporting protein. The CYP subspecies 1A, 2B, 3A, and 4A are regulated by the highly inducible xenobiotic receptor on complementary DNA. The receptor is found in the liver, and to a lesser extent in the cells lining the intestinal tract, and is responsible for cholesterol catabolism and bile acid homeostasis. The activity of this receptor is subject to genetic polymorphism as well. This results in a wide variation in the sensitivity of the population to generalized hepatocellular necrosis and other forms of hepatic damage.[23,30] The long-term administration of isoniazid can lead to hepatic dysfunction in 10% to 20% of those receiving the drug. Yet severe toxic hepatitis develops in only 1% or less of this population.[31] The N-acetyltransferase 2 (NAT2) genotype appears to play a role in determining a patient's relative risk. In one study, patients with the slowtype NAT2 genotype had a 28-fold greater risk of developing serum aminotransferase elevations than did patients with the fast-type NAT genotype.[32] Isoniazid is metabolized by several pathways, acetylation being the major pathway. It is acetylated to acetylisoniazid, which, in turn, is hydrolyzed to acetylhydrazine.[33] The acetylhydrazine, and to a lesser extent the acetylisoniazid, are directly toxic to the cellular proteins in the hepatocyte, but rapid acetylators also detoxify acetylhydrazine very rapidly, converting it to diacetylhydrazine (a nontoxic metabolite). Ketoconazole produces generalized hepatocellular necrosis or milder forms of hepatic dysfunction in 1% to 2% of patient's treated for fungal infections. This reaction is fatal in high numbers of patients infected with the human immunodeficiency virus. The onset is usually early in therapy, although it can be delayed until several months into therapy. In immune-compromised patients in whom ketoconazole is used for long periods of time, special care should be taken to watch for changes in liver function.[34]

1.4.5 CHOLESTATIC JAUNDICE

Cholestatic jaundice, or cholestasis, can be classified by the area of the bile canalicular or ductal system that is impaired. Canalicular cholestasis is very often associated with long-term high-dose estrogen therapy. Clinically, these patients are often asymptomatic and present with mild to moderate elevations of serum bilirubin.[35] An intravenous form of vitamin E, α-tocopherol acetate, causes cholestatic jaundice

primarily involving the canalicular duct in premature infants. The incidence of this reaction in those receiving this formulation was high (>10%) and the mortality even higher (>50%).[36] Hepatocellular cholestasis is a much more serious form of cholestatic jaundice that involves both the parenchyma and bile canalicular cells.

1.4.6 MIXED HEPATOCELLULAR NECROSIS AND CHOLESTATIC DISEASE

Patients infrequently present with a purely hepatocellular necrosis or cholestatic damage, but rather with a mixed picture of damage. Flutamide causes a mix of lesions that appear at or about the fortyeighth week of treatment.[37] Niacin in doses greater than 3 g/day, or in doses greater than 1 g/day of sustained-release formulations, cause the same mixed pattern of damage.[38] These patients often presen with only a few signs or symptoms at first, but can progress rapidly to fulminant hepatic failure. Additionally, niacin-induced and other drug-induced mixed hepatocellular disease can be misinterpreted as hepatobiliary cancers.[39]

1.4.7 NEOPLASTIC DISEASE

A large body of the current literature on adverse reactions and the liver addresses the development of neoplasms following drug therapy. Both carcinoma- and sarcoma-like lesions have been identified. Fortunately, hepatic tumors associated with drug therapy are usually benign and remit when drug therapy is discontinued. Except in rare instances, these lesions are associated with long-term exposure to the offending agent.[40] Androgens, estrogens, and other hormonal-related agents are the most frequently associated causes of neoplastic disease. The model for drug-induced hepatic cancer is polyvinyl chloride exposure. Used in the production of many types of plastic products, polyvinyl chloride induces angiosarcoma in exposed workers after as few as 3 years of exposure.[41]

1.5 ASSESSMENT

The best and most important technique for assessing and monitoring drug-induced liver disease is the patient's history. Questions addressing the patient's drug use along with a thorough review of systems are essential. The use of a protocol, such as that proposed by Danan and Benichou, can significantly improve the accuracy of the assessment (Table 38–1).[42] The use of drugs for recreational purposes must not be overlooked. Cocaine has been directly linked to liver disease.[43] Ecstasy, the street name of methylenedioxymethamphetamine, has induced fulminant hepatitis which has led to death in some cases.[44] The more pervasive impact of street drugs on the

incidence of hepatic disease is the concomitant injection or ingestion of adulterants. Many of these adulterants are either directly toxic or serve to enhance the toxicity of the drug. It is also good to try to determine nondrug hepatic disease risk. Arsenic, for example, is known to induce both acute and chronic hepatic reactions. Arsenic in low concentrations is found in insectresistant lumber.[45] Pennyroyal oil, maragosa oil, and clove oil cause a dose-related hepatotoxicity.[46,47] The nutritional status of a patient can be as important to the development of a drug-induced liver disease as the hepatotoxin itself.[48] Patients who are malnourished because of illness or long-term alcohol abuse make up the most troublesome group.[50,49] Low serum levels of vitamins E and C along with lutein and the α- and β-carotenes are associated with asymptomatic elevations in transaminases. Conversely, high serum iron, transferrin, and selenium levels are also associated with asymptomatic elevations of transaminases.[51] All potential drug

reactions should be judged as to the timing of the reaction versus drug administration, pharmacokinetic considerations, Often there is no good clinical test available to determine the exact type of hepatic lesion, short of liver biopsy.[53,52] The specificity of any serum enzyme depends on the distribution of that enzyme in the body. Alkaline phosphatase is found in the bile duct epithelium, bone, and intestinal and kidney cells. 5_-Nucleotidase is more specific for hepatic disease than alkaline phosphatase, because most of the body's store of 5_-nucleotidase is in the liver. Glutamate dehydrogenase is a good indicator of centrolobular necrosis because it is found primarily in centrolobular mitochondria. Most hepatic cells have extremely high concentrations of transaminases. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are commonly measured. Because of their high concentrations and easy liberation from the hepatocyte cytoplasm, AST and ALT are very sensitive indicators of necrotic lesions within the liver. After an acute hepatic lesion is established, it may take weeks for these concentrations to return to normal.[54] Serum bilirubin concentration is a sensitive indicator of most hepatic lesions and has significant prognostic value. High peak bilirubin concentrations are associated with poor survival. Other important findings that indicate poor survival are a peak prothrombin time greater than 40 seconds, elevated serum creatinine, and low arterial pH. The presence of encephalopathy or prolonged jaundice are not good signs for the survival of the patient and are strong indicators for transplantation.[55]

1.6 ISONIAZID AND RIFAMPICIN INDUCED HEPATOTOXICITY:

DILI may occur with all currently recommended regimens for the treatment of latent TB infection (LTBI), including isoniazid for 6 to preferably 9 months, rifampin for 4 months, or isoniazid and rifampin for 4 months [56] This is also true of two-drug regimens of pyrazinamide with either ethambutol or a fluoroquinolone used to treat contacts of multidrug-resistant (MDR) TB cases [57-59] Metabolic idiosyncratic reactions appear to be responsible for most DILI from the first-line anti-TB medications and fluoroquinolones.

1.6.1 Isoniazid

Metabolism. Isoniazid is cleared mostly by the liver, primarily by acetylation by N-acetyl transferase 2 (NAT-2). Acetyl-isoniazid is metabolized mainly to mono-acetyl hydrazine (MAH) and to the nontoxic diacetyl hydrazine, as well as other minor metabolites [60] Interindividual variation in plasma elimination halflife (t1/2), independent of drug dose and concentration, is considerable. Individuals with prolonged t1/2 have extended exposure to the drug. Genetic polymorphisms of NAT-2 correlate with fast, slow, and intermediate acetylation phenotypes [61-63] Microsomal enzymes (e.g., cytochrome P450 2E1) further metabolize isoniazid intermediates through phase 1 pathways.

Acetylator status. In fast acetylators, more than 90% of the drug is excreted as acetyl-isoniazid, whereas in slow acetylators, 67% of the drug is excreted as acetyl-isoniazid and a greater percentage of isoniazid is excreted as unchanged drug into the urine. The influence of acetylation rate on isoniazid hepatotoxicity is controversial. Most studies on this question involved patients on multidrug regimens for TB disease and relied on phenotypic assays of acetylation, which can be imprecise. Fast acetylators may be misidentified as slow if they exhibit delayed drug absorption during blood sampling at limited time points. Early studies suggested that fast acetylators were at higher risk for hepatic injury because they generated more acetyl-isoniazid, which could be further metabolized to other toxic intermediaries [64,65,66] However, fast acetylators clear MAH more rapidly. Slow acetylators may actually have greater cumulative MAH exposure. Increased susceptibility among slow acetylators [67] or a lack of correlation with acetylation rate has been reported [68] NAT-2 genotyping by polymerase chain reaction recently demonstrated that slow acetylators experience transaminase elevations of more than three times the ULN more frequently than rapid acetylators (26 vs. 11%). Slow acetylators also had higher peak ALT than did fast acetylators and, when

rechallenged with isoniazid, more frequently developed transaminase elevation of at least three times the ULN. The significance of these findings awaits further studies.

Mechanism of injury. Reactive metabolites of MAH are probably toxic to tissues through free radical generation. In rats, the free radical scavenger glutathione-related thiols, and antioxidant glutathione peroxidase and catalase activities, are diminished by isoniazid, although glutathione reductase activity is increased [69,70] The antioxidant N-acetyl-cysteine, a substrate for glutathione synthesis, inhibits isoniazid-induced liver injury in pretreated rats, with unknown relevance in humans. Additional metabolic idiosyncratic mechanisms appear to be operative. The isoniazid metabolite acetyl-hydrazine covalently binds to liver macromolecules, a process mediated by microsomal enzymes. Patients with homozygous cytochrome P450 2E1 c1/c1 host gene polymorphism, who have enhanced cytochrome P450 2E1 activity, in one study had a higher risk of hepatotoxicity, particularly in slow acetylators.

Histopathology. Nonspecific changes resemble those of viral hepatitis with nonzonal necrosis, and are massive in up to 10% of severe cases. Subacute hepatic necrosis can be seen in 30% of cases [71]

Drug interactions. Isoniazid inhibits the activity of several cytochrome P450 2E and 2C enzymes, potentially increasing the plasma concentrations of other potentially hepatotoxic drugs, such as phenytoin and carbamazepine [72-74] Rifampin appears to enhance a metabolic hepatocellular idiosyncratic reaction in patients receiving isoniazid, perhaps by promoting the formation of toxic isoniazid metabolites [75,76]

Hepatic adaptation. Up to 20% of individuals treated with isoniazid alone for LTBI may experience low-grade, transient, asymptomatic transaminase elevation, most of which represents hepatic adaptation [77]

Clinical presentation of hepatotoxicity. Some individuals may be asymptomatic, whereas others may experience symptomatic hepatotoxicity at varying serum transaminase concentrations. Constitutional symptoms may be seen early in severe hepatotoxicity, and may last from days to weeks. Nausea, vomiting, and abdominal pain are seen in 50 to 75% of patients with severe illness, whereas fever is noted in 10% and rash in 5% of patients. Overt jaundice, dark urine, and clay-colored stools are late signs of clinical worsening. Coagulopathy, hypoalbuminemia, and hypoglycemia signify life-threatening hepatic dysfunction. The regression of isoniazid hepatotoxicity usually takes weeks. Recovery is complete in most after discontinuation of isoniazid.

Overall rates of hepatotoxicity. Initial experience with isoniazid up to the 1960s indicated the rates of treatment-limiting adverse events were similar in placebo- and isoniazid-treated groups, except for gastrointestinal complaints [78] with hepatitis occurring relatively rarely. In the late 1960s, isoniazid's ability to cause asymptomatic elevations in hepatic transaminases and clinically significant hepatitis was recognized. In 1970, 19 of 2,321 Capitol Hill workers treated with isoniazid developed clinical signs of liver disease and two died of resulting complications [79] The U.S. Public Health Service (USPHS) surveillance study [80] of 14,000 isoniazid-treated individuals found an overall rate of significant, probable isoniazid hepatitis of 1%, with a cluster of seven of eight reported deaths in one city. A subsequent study, using passive detection, by the International Union Against Tuberculosis (IUAT), found the overall rate of hepatitis in patients receiving up to 12 months of isoniazid was 0.5 versus 0.1% receiving placebo [81] From the 1970s to the 1990s, isoniazid-related hospitalization rates declined from as much as 5.0 per 1,000 treatment initiations to 0.1 to 0.2 (median, 0.15), and mortality rates fell from as high as 1.0 per 1,000 to 0–0.3 per 1,000 (median, 0.04) [82,83] These declines may have been related to careful patient selection, education, and active monitoring for adverse reactions to isoniazid [85] A study of isoniazid for treatment of LTBI, involving more than 11,000 patients in Seattle–King County, Washington, reported that symptomatic transaminase elevation of more than five times the ULN occurred in 0.1% of treatment initiations [86] Routine follow-up transaminase monitoring of asymptomatic individuals was not done in this clinic, which the authors estimated could have raised the incidence of significant transaminase elevation into the range of older studies, to approximately 0.6%. Hepatotoxicity rates could also have been higher if based on those patients actually taking medication, rather than on treatment initiations. The study generally demonstrated a relatively low risk of isoniazid hepatotoxicity within the context of a TB program providing patient education, specific instructions about adverse events, and monthly clinical observations. A subsequent study of 3,788 patients treated for LTBI with isoniazid in San Diego, California, reported that transaminase elevations of three times the ULN in symptomatic individuals and five times the ULN in asymptomatic individuals occurred in 0.3% of cases [87]

Age. Most isoniazid-associated hepatotoxicity is age associated. The Seattle study of symptomatic transaminase elevation showed ranges from 0% in those younger than 14 years to 0.28% in those older than 65. The San Diego study reported a trend

toward age-related hepatotoxicity, with only 15% of the study population aged 35 years or older. The Tennessee study reported that age-specific AST elevation more than five times the ULN ranged from 0.44% in those younger than 35 years to 2.08% for those older than 49 years [88] a statistically significant difference. In comparing these studies, 20% of the more than 11,000 patients in the Seattle study population were at least 35 years old, compared to 59% in the USPHS and 54.6% in the Tennessee studies. Sample sizes for this age group were comparable in the Seattle (n _ 2,228) and the Tennessee studies (n _ 1844). Differences in the findings among these studies may be attributed to differing definitions of hepatotoxicity, patient selection, and in ability to exclude confounding causes of hepatotoxicity. The severity of isoniazid-related hepatitis has been reported to also increase with age, with higher mortality in those older than 50 years [89,90]

Racial differences. In theUSPHS study, African-American males appeared to have less risk of DILI than white males, but there was no difference for women of any race. Asian males appeared to have nearly double the rate of probable isoniazid hepatitis than white males and nearly 14 times that of black males. In the Seattle–King County study, there was a nonsignificant trend toward higher hepatotoxicity in white individuals, without other significant racial differences. The Memphis, Tennessee, study found no associations among racial groups or demographic subgroups and hepatotoxicity. There do not appear to be consistent racially based risks for high-grade hepatotoxocity.

Sex. There is currently no clear evidence to point to an overall sex-related difference in the incidence of hepatotoxicity. Pregnant women in the third trimester and in the first 3 months of the postpartum period may be at higher risk for the development of hepatitis [91] In the USPHS study, there was no overall difference between women and men in rates of probable isoniazid hepatotoxicity. The Seattle–King County study found a nonsignificant trend toward higher isoniazid-related hepatotoxicity in women compared with men, although the incidence of severe hepatotoxicity was relatively low in both men and women. The Memphis and San Diego studies found no significant associations between sex and hepatotoxicity

Deaths. Several retrospective studies and reviews with methodologic limitations suggest that the severity of isoniazidinduced hepatotoxicity, when it does occur, may be worse in women. In the USPHS study there were 8 deaths among 13,838 enrolled subjects (0.57 per 1,000 treated), 5 of which were in African-American women, with

7 of 8 deaths occurring in Baltimore, Maryland. Most of those who died had potential cofactors for hepatotoxicity, including severe alcoholism or ingestion of other hepatotoxic drugs. Another cofactor may have been involved in the observed clustering, as a subsequent review of death certificates showed a surge in cirrhosis-related deaths in Baltimore and surrounding counties during the time period of this study [92] In the IUAT study, there were three deaths with a death rate of 0.14 of 1,000 treated. A review of probable and possible isoniazid hepatitis cases from 1970 to 1992 suggested a case fatality rate of 0.042 per 1,000 persons beginning therapy and a rate of no greater than 0.07 per 1,000 persons completing therapy. This review included some of the previously discussed fatalities. There were 62 probable and possible isoniazid hepatitis deaths, 50 (81%) of the patients were female, and 49 (79%) were non-Hispanic black or Hispanic. Although most individuals who died were older than 35 years, a surprising 31% were younger. Although these are numerator data only, they indicate that no age group is free of risk. Another review of fatal cases also suggested that women may be at higher risk for death from isoniazid-related hepatitis.

Cofactors. In the USPHS surveillance study, alcohol consumption appeared to more than double the rate of probable isoniazid hepatitis, with daily consumption increasing the rate more than four times. Transaminase elevation may be, in some cases, related to chronic ethanol use. Hepatotoxicity during concomitant administration of other hepatotoxic drugs, such as acetaminophen [93], methotrexate [94], sulfasalazine [95], or carbamazepine [95], as well as others, has been reported.

HIV-infected individuals. HIV-infected individuals appear to experience isoniazid-related hepatotoxicity in the same range as HIV-uninfected individuals [96], although no direct comparisons through clinical trials have been done.

Hepatitis C. Two studies showed no independent isoniazid hepatotoxicity risk associated with hepatitis C infection. In Baltimore, Maryland, a cohort of 146 tuberculin skin test–positive injection-drug users, 95% of whom were infected with hepatitis C, with baseline serum transaminase concentrations less than three times the ULN, and 25% of whom were HIV infected, received isoniazid for LTBI. Observed with monthly blood tests, 32 patients (22%) had increased transaminase concentrations to more than five times the ULN. Abnormal results were associated with alcohol use, but not with race, age, chronic hepatitis B infection, or HIV infection. Both the rate of hepatitis and the rate of isoniazid discontinuation were within the historical range for populations with a low prevalence of hepatitis C

infection (10 to 22% and 0.1 to 10%, respectively). A second study in Spain found that only excessive alcohol consumption and a high baseline ALT concentration were independently associate with isoniazid hepatotoxicity. The presence of hepatitis C virus (HCV) antibody was associated with hepatotoxicity only on univariate analysis in this study.

Elevated baseline transaminases. The Tennessee retrospective study found that a baseline AST greater than the ULN was a risk factor for developing transaminase elevation greater than five times the ULN, as did another study among intravenous drug users.

Other factors increasing frequency or severity of hepatotoxicity.

Concomitant treatmentwith rifampin,malnutrition, prior isoniazidrelated hepatotoxicity, and continued use of isoniazid while symptomatic have been described to contribute to higher-grade isoniazid hepatotoxicity.

1.6.2 Rifampin

Rifampin, and similarly rifapentine, may occasionally cause dosedependent interference with bilirubin uptake, resulting in subclinical, unconjugated hyperbilirubinemia or jaundice without hepatocellular damage. This may be transient and occur early in treatment or in some individuals with preexisting liver disease [90-92] Rifampin occasionally can cause hepatocellular injury and potentiate hepatotoxicities of other anti-TB medications [93,94] In a study of patients with brucellosis treated with the combination of rifampin and minocycline, rifampin-attributed ALT increases of at least 250 IU/L were seen in approximately 5% of patients [95]. In two small series of patients with primary biliary cirrhosis, in whom baseline transaminases were significantly elevated, clinically significant hepatitis was attributed to rifampin in 7.3 and 12.5% of patients [96,97].

Mechanisms of hepatotoxicity. Conjugated hyperbilirubinemia probably is caused by rifampin inhibiting the major bile salt exporter pump [98]. Asymptomatic elevated bilirubin may also result from dose-dependent competition with bilirubin for clearance at the sinusoidal membrane or from impeded secretion at the canalicular level [99,100]. Rare hepatocellular injury appears to be a hypersensitivity reaction, and it may be more common with large, intermittent doses [101]. Hypersensitivity reactions have been reported in combination with renal dysfunction, hemolytic anemia, or "flulike syndrome" [102,103].

Drug interactions. Rifampin activates hepatotocyte pregnane X receptors, leading to induction of cytochromes. Rifampin also induces uridine diphosphate-glucuronosyl-transferases and P-glycoprotein transport, which are involved in the metabolism of other drugs[104-108] Rifampin interacts with numerous drugs metabolized by these and other hepatic enzymes, including warfarin, prednisone, digitoxin, quinidine, ketoconazole, itraconazole, propranolol, clofibrate, sulfonylureas, phenytoin, HIV protease inhibitors, and HIV nonnucleoside reverse transcriptase inhibitors[109].

Clinical characteristics of hepatotoxicity. Cholestasis may be insidious. Idiosyncratic hypersensitivity reaction to rifampin, manifested as anorexia, nausea, vomiting, malaise, fever, mildly elevated ALT, and elevated bilirubin, usually occurs in the first month of treatment initiation[110].

Overall hepatotoxicity. Four published TB-related studies have assessed rifampin alone for treatment of LTBI. In a study by the Hong Kong Chest Service, transaminase elevations above the ULN were more common among patients receiving isoniazid- containing regimens than they were among the 77 of 172 patients treated with rifampin alone who had follow-up liver enzyme analyses. There was no significant difference between the geometric means of serum ALT for the placebo and rifampin groups. In the second study[111], none of the 49 individuals, 20% of whom used alcohol and 8% of whom used injection drugs, treated with rifampin for 6 months had symptomatic liver injury. There was no assessment for asymptomatic transaminase elevations. Among 157 adolescents treated with rifampin, 4 (2.5%) developed ALT elevations at least two times the ULN, for which treatment was permanently discontinued in one[112,113]. A randomized study of isoniazid versus rifampin for treatment of LTBI in Montreal, Canada, found that, among 53 patients who completed 80% of a 4-month course of rifampin for LTBI, none experienced significant transaminase elevation. The apparent low rate of hepatotoxicity observed in these limited studies awaits confirmation in larger prospective studies.

Isoniazid and Rifampin

A Canadian study found that rates of hepatitis were similar for patients treated with intermittent isoniazid and rifampin compared with historical control subjects receiving daily isoniazid for 12 months. The rate of symptomatic hepatitis with the combination of isoniazid and rifampin has been estimated at 2.55% in a meta-analysis that included patients with TB disease, a higher incidence than in regimens containing one or the other drug[114].


Chapter 2
Aims &
Objectives

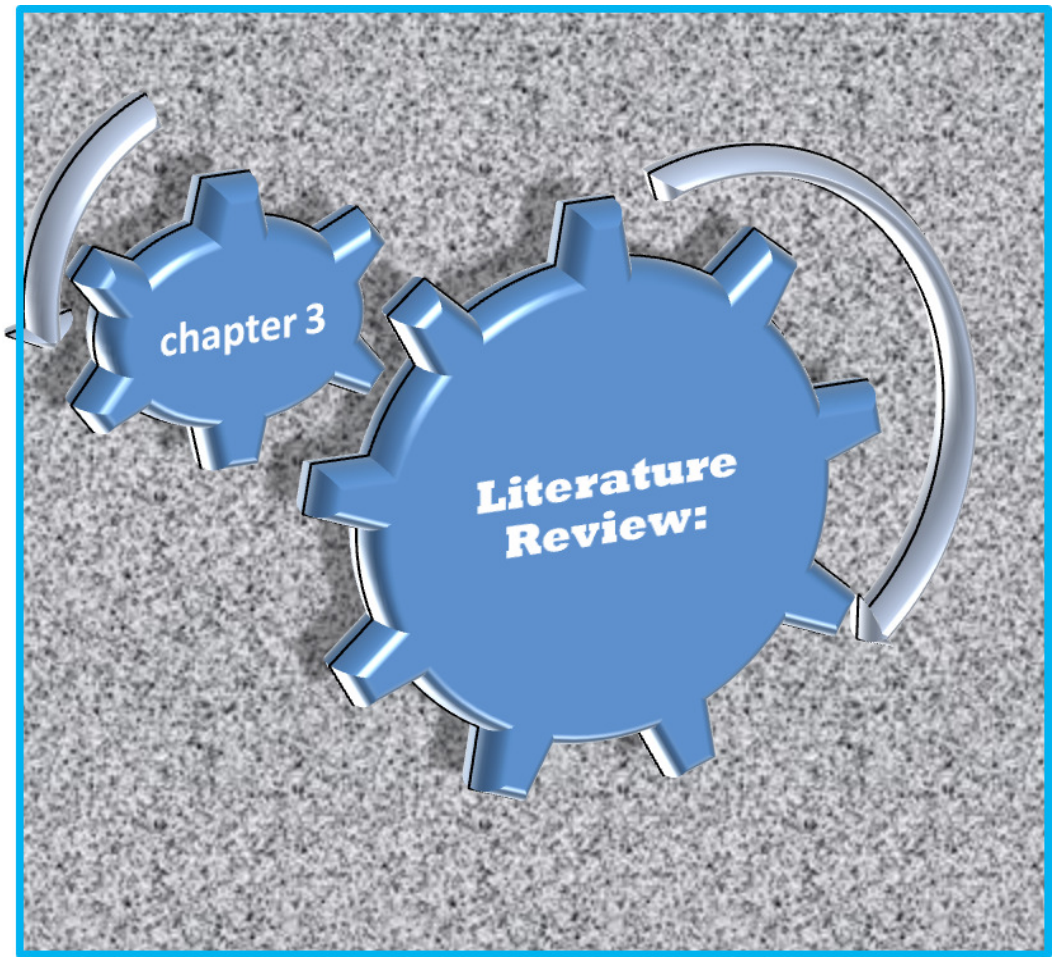
AIM &OBJECTIVE

Aims: Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits[-116]. Therefore present study was designed to Evaluate Hepatoprotective activity of ethanolic extract of polygonum glabrum on hepatoxicity induced by anti-tubercular drugs like INH and rifampicin in rats.

2.2 OBJECTIVES OF THE STUDY:

1. To prepare ethanolic extract of *Polygonum glabrum*
2. To study the phytochemical screening of ethanolic extract *Polygonum glabrum*
3. To study the acute oral toxicity ethanolic extraction of *Polygonum glabrum*
4. To study the hepatoprotective activity of ethanolic extract of *Polygonum glabrum in* rats

5. To carry out histopathological studies.



REVIEW OF LITERATURE

Muddathir AK,et.al., Reported Anthelmintic properties of Polygonum glabrum.
They isolated a pure anthelmintic substance (PGA) from the methanol-aqueous extract of the leaf of Polygonum glabrum Willd., a semi-aquatic Sudanese species of the family Polygonaceae. The antiparasitic in-vitro activity of several fractions isolated from the plant, has been examined comparatively with that of PGA. PGA also showed molluscicidal activity against Biomphalaria glabrata and Limnea truncatula Müll. Structural determination of PGA was attempted following data analysis of UV, IR, 13C NMR, 1H NMR and MS spectra and suggests that PGA is a terpenoid.

Sinha F,et.al.,Reported Pharmacognostic studies on leaf of Polygonum glabrum Willd. has been carried out along with its numerical values, fluorescence characteristics and ash and extractive values.

Muddathir AK, Balansard G, Timon-David P, Babadjamian A, Yogoub AK, Julien MJ. Anthelmintic properties of Polygonum glabrum.J Pharm Pharmacol. 1987 Apr;39(4):296-300.

SinhaP, KumarA, WahiSP.Pharmacognostical studies on leaf of polygonum glabrum willd. Anc Sci Life. 1986 Jul;6(1):13-9.

PLANT PROFILE

Plant Profile

Botanical Name**:** ***Polygonum glabrum* Willd**

Scientific Classification

Kingdom **:** Plantae

Subkingdom : Tracheobionta

Class **:** Magnoliopsida

Order **:** Polygonales

Family **:** Polygonaceae

Genus **:** *Polygonum* L.

Species **:***Polygonum glabrum* Willd.

Synonyms :

Polygonum densiflorum, Polygonum portoricense, Persicaria glabra

Common Name :

English **:** Common Marsh Buckwheat

Vernacular Name:

India (Santal): Sauri arak, Jioti.

Balrampur, Oudh : Larboma, Bih langani bonka.

Assam: Patharua,

Bombay: Rakta rohia,

Tamil: Actalaree, Sivappu Kumbakodaali,

Mangalore **:** Neeru kanagilu[1],

Malayalam**:** Chuvanna mudela mukkum,

Bengali: Bihagni, Sada Kukri.

Habit: An annual herb.

Habitat and Distribution:

Common, grows gregariously in marshy areas, Plains from the coast to 1400m. India, Nepal, Sri Lanka, Pakistan, Java, South China, Africa, Afghanistan, South Japan, Malaysia, Sylhet, Chittagong; in ditches and swamps. In India: Karnataka, Gujarat, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, Sikkim, Uttar Pradesh and West Bengal.

Description:

Polygonum glabrum Willd - An erect annual, with reddish stem, 0.6-1.5 m long procumbent and usually reddish below, shrubby, ochreas membranous, glabrous, lowers in terminal and axillary spike like racemes, perianth rose or red. Nuts are dark brown and shiny. Leaves are of 7.5-23 cm long, lanceolate, finely acuminate; stipules 2.2-3.2 mm long, those on the old stem torn, when young completely sheathing the stem, the mouth truncate, not ciliate. Flowers are pink, small, in paniculate slender racemes. The plant mostly found in river banks, stream sides and marshy areas. It is found as dense clumps and is sub shrubs and growing up to 2.5 cm. The young shoots and roots are cooked with vegetables.

Chemical constituents:

The phytochemical screenings of chloroform extract shows the presence of chemical compounds like alkaloid, carbohydrates, flavonoids and methanol extract shows the presence of alkaloids, flavonoids, and phenolic compounds. The chromatographic studies shows various spots (Paper/Hptlc) with chloroform extract may confirm the presence of alkaloid contents in the plant. The spectral studies like U.V and FTIR are the preliminary work for the detection of some alkaloidal contents in the plant. Aerial parts contain a number of biologically active terpenoids and flavones. Leaves contain flavonoids like quercetin, rhamnetin, quercitrin, avicularin and rutin. Stem and seeds contain vanillic acid, syringic acid, p-hydroxybenzoic acid, protocatechuic acid, Gallic acid and coumaric acid, kaempferol-3-methyl ester, quercetin and hyperin

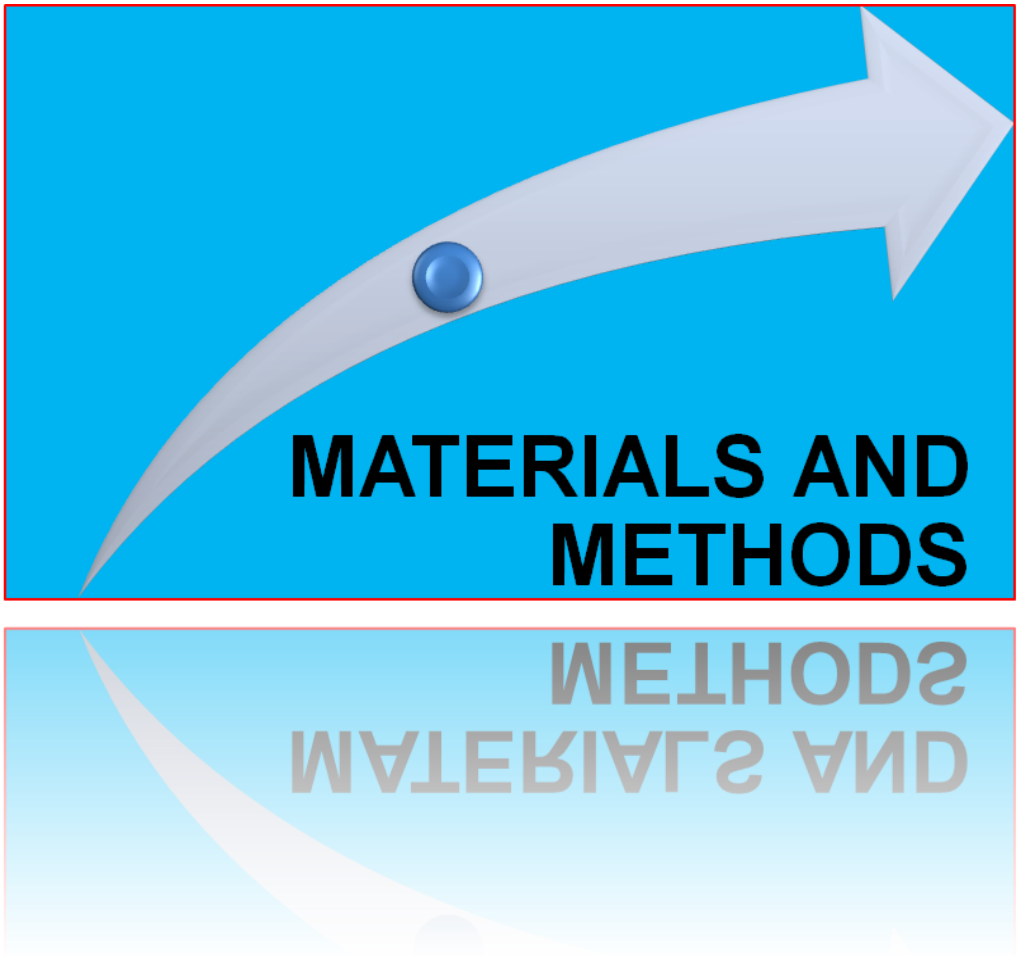
Medicinal uses:

A good number of species *Polygonum* have been used traditionally from a long time for a number of ailments in the indigenous system of medicine. The medicinal properties attributed to the species of *Polygonum* are demulcent and pectoral, astringent and tonic, diuretic, emetic, purgative, febrifuge, vesicant,

vulnerary, insecticide and anthelmintic [117] Besides it also possess antiviral [118] and antibacterial [119] properties. The root stocks are reported to be used in piles, jaundice, debility and consumption. The herb possess antibacterial activity against micrococcus pyrogens and diplococcus pyrogens. Some naturally occurring flavonoids like kaemphferol-3-methyl ester, quercetrin, quercetin shows antifungal activity. But fresh juice of the leaves possesses poisonous properties

Pharmacological activities:

Polygonum glabrum has been mentioned in Ayurveda and is known as *Rasna,* though its clinical use does not appear to include nervous disorders. It has been studied for its anti-inflammatory [120] and antidepressant [121] properties. The findings show that *Polygonum glabrum* extract is clinically effective as anti-inflammatory drug and works by the mechanism of action similar to that of NSAIDs. PG also has been researched for anthelmintic activity [122], which showed activity against *Hymenolepis nana* var. *fraternal.* PG contains several compounds of biological interest, including the sesquiterpenes, a broad spectrum of flavanoids [123] and polyphenols [124].


MATERIALS AND
METHODS

MATERIALS AND METHODS

Materials:

Chemicals and reagents

Serum parameters were estimated using analysis kits purchased Robonik diagnostics Pvt Ltd, Navi Mumbai, India (SGOT, SGPT, TP and Bilirubin) and. Silymarin was a gift from Micro Labs, Hosur, Tamil Nadu, India. Rifampicin and Isoniazide were obtained from Lupin Ltd., Mumbai, India; anaesthetic ether was from TKM Pharma, Hyderabad, India; and ethanol and chloroform were from Rankem Ltd, New Delhi, India.

Diagnostic kits:

Diagnostic kits used for estimation of, SGOT, SGPT, ALP, Total protein, bilirubin were procured from **Robonik Pvt Limited.**

Instruments:

Autoanalyzer (Robonik), Refrigerator centrifuge (MPW-350R),UV-Spectrophotometer (UV-1601, Shimadzu Corporation, Kyoto, Japan), Mini Lyotrap (LTE Scientific Ltd.), Research centrifuge (Remi industries, Mumbai) and homogenizer (Remi Motors, Mumbai). Dhona balance (M/S Dhona instruments Pvt. Ltd., Kolkata, India).

Plant material and extraction

The bark of *Polygonum glabrum* Willd plant was collected during the march 2013 from Sri Venkateshwara University, Tirupati, India. The plant was authenticated by Dr. Madhava Chetty, Department of Botany and voucher specimen of the plant were preserved at institute herbarium library. Bark was separately washed, wiped-dry, and subsequently reduced to a coarse powder. About 100 g of the bark were separately extracted for 24 h with 75% ethanol with intermittent vigorous shaking. The extracts were filtered, concentrated with a rotary evaporator and dried over a water bath at 45°C. The residue from the plant parts were used for experimental analysis[125.]

PRELIMINARY PHYTOCHEMICAL STUDIES[126]

Ethanolic extract of the plant of *Polygonum glabrum* Willd were subjected to chemical tests for the identification of their active constituents.

Tests for carbohydrates and glycosides

A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates.

A. Molisch's Test

Filtrate was treated with 2-3 drops of 1% alcoholic α-naphthol solution and 2ml of con. H2SO4 was added along the sides of the test tube. Appearance of violet coloured ring at the junction of two liquids shows the presence of carbohydrates. Another portion of the extract was hydrolysed with HCl for few hours on a water bath and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides.

B. Legal's Test:

To the hydrolysate, 1ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

C. Borntrager's Test: Hydrolysate was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammoniacal layer acquires pink colour showing the presence of glycosides.

4.2.4 Tests for alkaloids: A small portion of the methanol extract was stirred separately with few drops of dil. HCl and filtered. The filtrate was treated with various reagents as shown for the presence of alkaloids.

Mayer's reagent - Creamy precipitate

Dragandroff's reagent - Orange brown precipitate

Hager's reagent - Yellow precipitate

Wagner's reagent - Reddish brown precipitate

4.2.5 Tests for phytosterol

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol.

Libermann Burchard Test:

The residue was dissolved in few drops of acetic acid, 3 drops of acetic anhydride was added followed by few drops of con. H2SO4. Appearance of bluish green colour shows the presence of phytosterol.

4.2.6 Tests for fixed oils

Spot test:

Small quantity of extract was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

4.2.7 Tests for gums and mucilages:

Small quantity of the extract was added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties for the presence of gums and mucilages.

4.2.8 Tests for Saponins:

The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam shows the presence of saponins.

4.2.9 Tests for proteins and free amino acids:

Small quantity of the extract was dissolved in few ml of water and treated with following reagents.

A. Millon's reage Appearance of red colour shows the presence of protein and free amino acids.

B. Ninhydrin reagent - Appearance of purple color shows the presence of proteins and free amino acids.

C. Biuret test - Equal volumes of 5% NaOH solution and 1% copper sulphate solution were added. Appearance of pink or purple colour shows the presence of proteins and free amino acids.

4.2.10 Tests for phenolic compounds and tannins

Small quantity of the extract was taken separately in water and tested for the presence of phenolic compounds and tannins using following reagents.

A. Dil.FeCl3 solution (5%) -violet colour

B. 1% solution of gelatin containing 10% NaCl - white precipitate

C. 10% lead acetate solution - white precipitate.

4.2.11 Tests for flavonoids

A. With aqueous Sodium hydroxide solution:

Blue to violet colour (anthocyanins), yellow colour (flavones), yellow to orange (flavonones)

B. With Con. H2SO4:

Yellow orange colour (anthocyanins), yellow toorange colour (flavones), orange to crimson (flavonones)

C. Shinoda's test

Small quantity of the extract was dissolved in alcohol and to that a piece of magnesium followed by Con. HCl drop wise was added and heated. Appearance of magenta colour shows the presence of flavonoids.

The results of preliminary phytochemical studies of the plant extract are presented in

Table1. Phytochemical screening of methanolic extract of Polgonum glabrum Willd.

Phytoconstituents	Presence or Absence
Carbohydrates	+
Glycosides	+
Fixed oils and fats	-
Gums & mucilage	-
Potein & amino acids	+
Saponins	+++
Tannins	+
Phytosterols	++
Flavonoids	++
Alkaloids	+++

6 Experimental Animals:

Wistar albino male rats (180–220 g) was obtained from the central animal house of Sigma Institute of Clinical Research and administration Pvt Ltd Hyderabad. The animals were housed at room temperature (22-28 ºC) for 12 hr dark and 12 hr light cycle and given standard laboratory feed and water *ad-libitum*. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee (769/2010/CPCSEA).

Acute oral toxicity study

Procedure:

Acute toxicity studies were performed according to OECD-423 guidelines category IV substance (acute toxic class method). Albino mice (n=3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 hrs with free access to water only. The *Polygonum glabrum* Willd, India were administered orally with a maximum dose of 2000 mg/kgbody weight. The mortality was observed for three days. If mortality was observed in 2/3 or 3/3 of animals, then the dose administered was considered as a toxic dose. However, if the mortality was observed only one mouse out of three animals then the same dose was repeated again

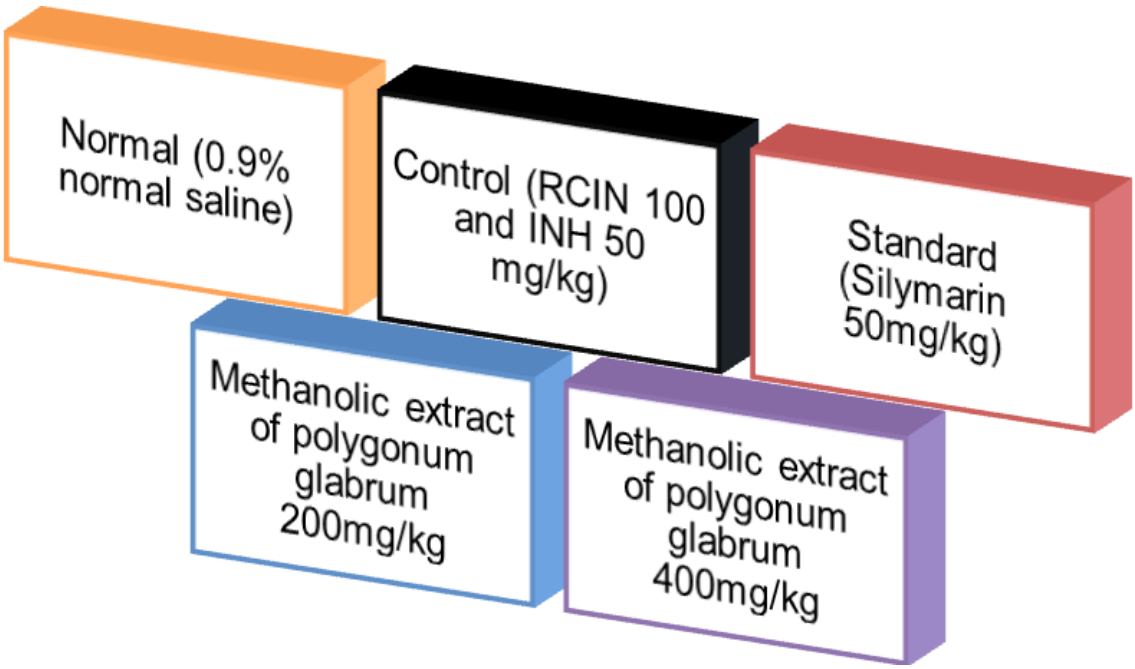
to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher dose (Organization for economic Co-operation and development, 2001)[127]

Observations

Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours) and daily thereafter, for a total of 14 days. All observations were systematically recorded with individual records being maintained for each animal. Observations included changes in skin, mortality and general behavioural pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. No death was observed till the end of study.

HEPATOPROTECTIVE ACTIVITY

Experimental design



Healthy Albino Wistar male rats weighing 180-220 g were used. They were randomized into 5 groups of 6 animals each as follows:

Group I: Normal received the vehicle viz. normal saline (2 ml/kg).

Group II: Controls Received RCIN and INH (100 +50 mg/kg p.o.)**[5]** at every 72 h for 21 days.

Group III: Received silymarin 50 mg/kg p.o. for 21 days and simultaneously administered RCIN and INH (100 +50 mg/kg p.o.) every 72 h.

Group IV: Received MPG 200 mg/kg p.o for 21 days and simultaneously administered a RCIN and INH (100 +50 mg/kg p.o.) every 72 h.

Group V: Received MPG 400 mg/kg p.o. for 21 days and simultaneously administered RCIN and INH (100 +50 mg/kg p.o.) every 72 h.

At the end of all experimental methods, all the animals were sacrificed by cervical decapitation. Blood samples were collected, allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters. The separated serum was used for the estimation of total bilirubin, direct bilirubin, SGOT, SGPT, ALP and total proteins (TP). The animals were sacrificed by administering an excess of ether and their livers were removed.

Assessment of liver function:

Biochemical parameters i.e., aspartate amino transferase (AST)[128] alanine amino transferase(ALT)[128], alkaline phosphatase (ALP)[129], total bilirubin[130] and total protein[131] were analyzed according to the reported methods. The liver was removed, weighed and morphological changes were observed. A 10% of liver homogenate was used for antioxidant studies such as lipid peroxidation (LPO)[132], superoxide dismutase (SOD)[133], catalase[134].

Histopathological studies:

Liver slices fixed for 12 hrs in Bouin's solution were processed for paraffin embedding following standard micro techniques (18). 5µm sections of liver grained with alum haematoxylin and eosin were observed microscopically for histopathological changes.

Biochemical Estimation

SGOT/AST[135]

Principle

Kinetic determination of the aspartate aminotransferase (GOT) activity :

$$\text{L - Aspartate} + \alpha\text{ – Ketoglutarate} \xrightarrow[\text{MDH}]{\text{GOT}} \text{Oxaloacetate} + \text{L-Glutamate}$$

Oxaloacetate + NADH + H ⟶ L-Malate + NAD

Assay procedure 1: Two Reagent procedure

Pipette in to test tubes	**Sample/Control**
R1	800µl
R2	200 µl

Mix and Incubate at 37°C for 2 minutes then add

Pipette in to test tubes	**Sample/Control**
Working reagent	1000µl
Sample/Control	100 µl

Mix and after a 60 seconds incubation at 37°C measure the change of absorbance per minute (ΔA/minute) during 180 seconds.

Calculation :

Activity of Sample (U/L) = Δ A/Min X 1746

SGPT/ ALT[136]

Principle

Kinetic determination of the GPT activity

$$\text{L - Alanine} + \alpha\text{ – Ketoglutarate} \xrightarrow{\text{GPT}} \text{Pyruvate + L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^{+}$$

ASSAY PROCEDURE 1: Two Reagent procedure

Pipette in to test tubes	**Sample/Control**
R1	800µl
R2	200 µl

Mix and Incubate at 37°C for 2 minutes then add

Pipette in to test tubes	**Sample/Control**

Working reagent	1000µl
Sample/Control	100 µl

Mix and after a 60 seconds incubation at 37°C measure the change of absorbance per minute (ΔA/minute) during 180 seconds.

Calculation:

Activity of Sample (U/L) = Δ A/Min X 1746

ALKALINE PHOSPHATASE[137]

p-Nitrophenyl phosphate is converted to p-nitrophenol and phosphate by alkaline phosphatase. The rate of formation of p-Nitrophenol is measured as an increase in absorbance which is proportional to the ALP activity in the sample.

ALP

p-Nitrophenyl phosphate ⟶ p-Nitrophenol + Phosphate

Assay procedure 1: Two Reagent procedure

Pipette in to test tubes	**Sample/Control**
R1	800µl
R2	200 µl

Mix and Incubate at 37°C for 2 minutes then add

Pipette in to test tubes	**Sample/Control**
Working reagent	1000µl
Sample/Control	100 µl

Mix and after a 60 seconds incubation at 37°C measure the change of absorbance per minute (ΔA/minute) during 180 seconds.

Calculation:

At 405 nm with mono reagent procedure and two reagent procedure for 1 cm path light cuvette

Activity of Sample (U/L) = (Δ A/Min) X 2712

TOTAL PROTEIN[138]

Proteins together with copper ions form a violet blue color complex in alkaline solution.The absorbance of the color is directly proportional to the concentration.

Proteins + Cu ——————> Colored complex

Assay procedure

Pipette in to test tubes	**Blank**	**Standard**	**Sample**
Working reagent 1	1000 µl	1000 µl	1000µl
Distill water	20µl	……	……..
Standard	……	20 µl	……..
Sample	…….	……	20 µl

Mix and read the absorbance (A) after 10 minutes of incubation but within 60 minutes.

Calculation:

$$\text{Conc. of unknown Sample} = \frac{\text{Concentration of Standard}}{\text{Abs. Standard – Abs. of Reagent Blank}} \times \text{Abs. of unknown Sample – Abs. of Reagent Blank}$$

BILIRUBIN TOTAL & DIRECT[139]

PRINCIPLE:

In the determination of total bilirubin, bilirubin is coupled with diazotized sulphanilic acid in the presence of caffeine benzoate solution to produce azobilirubin which has maximum absorbance at 546 nm. Direct Bilirubin in presence of diazotized sulphanilic acid forms a red coloured azo compound in acidic medium which has maximum absorbance at 546 nm.

Assay procedure: 1 - Total Bilirubin End Point (Differential)

	Sample Blank / Control Blank	**Sample/ Control**
Reagent R1	100 µl	100 µl
Reagent R2	—	50 µl
Reagent R3	1000 µl	1000 µl
Sample / Control	100 µl	100 µl

Mix, let stand at room temperature for 5 minutes and read the absorbance of sample against the sample blank.

Calculation:

Concentration in Sample (mg/dl) = (Abs. of Sample – Abs. of Sample Blank) X 14 (mg/dl) Concentration in Sample (µmol/L)= (Abs. of Sample – Abs. of Sample Blank) X 239 (µmol/L)

Assay procedure: 2 - Direct Bilirubin End Point (Differential)

	Sample Blank / Control Blank	**Sample/ Control**
Reagent R1	100 µl	100 µl
Reagent R2	—	50 µl
Reagent R4	1000 µl	1000 µl
Sample / Control	100 µl	100 µl

Mix, let stand at room temperature for EXACTLY 5 minutes and read the absorbance of sample against the sample blank.

Calculation:

Concentration in Sample (mg/dl) = (Abs. of Sample – Abs. of Sample Blank) X 14 (mg/dl) Concentration in Sample (µmol/L)= (Abs. of Sample – Abs. of Sample Blank) X 239 (µmol/L)

ENZYME ASSAY

A portion of liver was taken from all the groups, and a 30% w/v homogenate was prepared in 0.9% buffered KCl (pH 7.4) for the estimation of protein, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and malondialdehyde (MDA).

1. Lipid Peroxidation[140]:

Malondialdehye, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of peroxidation reaction. Malondialdehye reacts with thiobarbituric acid (TBA) to produce red colored species which is measured at 532 nm.

Reagents:

1. TBA-TCA-HCl reagent.

15% w/v TCA, 0.375 %w/v TBA and 0.25 N HCl.

This solution was mildly heated to assist the dissolution of TBA1 ml of liver homogenate was combined with 2 ml of TCA-TBA-HCL reagent and mixed

thoroughly. The solution was heated for 15 min. in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. the absorbance of the supernatant was measured at 532 nm against a blank that contains all the reagents minus the liver homogenate. The malondialdehyde concentration of the sample can be calculated using an extinction coefficient of $1.56x10^5M^{-1}cm^{-1}$

Malondialdehyde concentration (M) = Absorbance/1.56×10^5

2. Estimation of Catalase[141]

In the presence of catalase, H_2O_2 shows a continual decrease in absorbane in UV range. The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240nm (E_{240} = 0.00394 ±0.0002 ltr $mmol^{-1}$ mm^{-1}). The difference in absorbance (Δ A 240) per unit time is a measure of the catalase activity.

Reagents:

1. PBS 50 mM; pH7.0
Dissolve (a) 6.81 of KH2PO4 and (b) in the proportion 1:1.5(v/v).
2. H_2O_2 (0.17 mM): dilute 0.16 ml of (30%w/v) H_2O_2 with Phosphate buffer to 100 ml.

The catalase activity was determined spectrophotometrically according to the protocol of Claiborne (1985).The reaction mixture (2 ml) contained 1.95 ml 10 mM H2O2 in 60 mM phosphate buffer (pH 7.0).The reaction was started by adding 0.05 ml supernatant and the absorbance was followed for 3 min at 240 nm. Phosphate buffer (60 mM, pH 7.0) was used as a reference. The extinction coefficient of 0.04 $mM^{-1}cm^{-1}$ was used to determine the specific activity of catalase. A unit of catalase is defined as the quantity, which decomposes 1.0µmole of H_2O_2 per min at pH 7.0 at 25^0C, while this H_2O_2 concentration falls from 10.3to 9.2mM.The data was expressed as U/mg protein

4. Estimation of glutathione (GSH)[142]

Principle: GSH is a non-protein compound containing sulphydryl group in its structure. DTNB (5,5' di thio bis (2-Nitrobenzoic acid) is a disulfide chromagen that is reduced by sulphydryl compounds to an intensly yellow coloured compound. The absorbance of the reduced chromagen is measured at 412 nm and is directly proportional to the GSH concentration.

Reagents:

1. 10% Trichloroacetic acid (TCA)
2. Phosphate buffer (0.2M) pH 8.0
   0.218 g Sodium dihydrogen phosphate and 2.641 g disodium hydrogen phosphate in 100 ml distilled water
3. DTNB (0.6 mM) (pH 8)
   11.9 mg in 50 ml Phosphate buffer

GSH was measured by the method of Moran et al (1979).The liver homogenate proteins were precipitated by 10% TCA, centrifuged and the supernatant was collected. 1 ml of supernatant was mixed with 6 ml of 0.2 M Phosphate buffer pH 8.0 and 1 ml 0.6 mM DTNB and incubated for 10 min at room temperature. The absorbance of the samples was recorded against the blank at 412 nm and the GSH concentration was calculated from the standard curve by multiplying with the dilution factor (mannervik1985; tetza 1969)

Isolation of liver

Liver was carefully removed and washed with ice cold saline solution and pressed between pads of filter paper and weighed. A portion of the liver was preserved in 10% *v*/*v* neutral formalin for histopathological studies.

Histopathological Examination

A portion of liver tissue was preserved in 10% formaldehyde solution for histopatholigical studies. Haematoxyline and eosin were used for the staining agent and later microscopic slides of the liver photographed at the 100x magnification.

Statistical analysis

The data were expressed as mean±SEM values and tested with one way analysis of variance (ANOVA) followed by the Dunnett's multiple comparison test.$p<0.05$ consider as a significant value.

RESULTS

RESULTS

Phytochemical constituents present in the methanolic extract of *Polygonum glabrum.*

Phytoconstituents	Presence or Absence
Carbohydrates	+

Glycosides	+
Fixed oils and fats	-
Gums & mucilage	-
Potein & amino acids	+
Saponins	+++
Tannins	+
Phytosterols	++
Flavonoids	++
Alkaloids	+++

Percentage yield after extraction with methanol (70%)

Plant name : *Polygonum glabrum Willd*

Part used : Whole plant

Solvent used : Methanol

Yield : 13 grams

% Yield : 7.5%

Acute oral toxicity

Acute oral toxicity was carried out according to OECD guideline. MPG was safe upto 2000mg/kg.

Acute Toxicity Record Sheet of *Polygonum glabrum Willd*

Title: Evaluation of LD50 ***Polygonum glabrum Willd***

Drug*: Polygonum glabrum Willd* Dose: 2000 mg/kg BW Species: Albino mice Sex: Male & Female

Duration: 24 hours

S.no.	**Code**	**Toxicity**		**Time**	**Observation**										
		Onset	**Stop**	**Of Death**	**Skin colour**	**Eyes**	**Resp**	**CNS**	**Tre**	**Con**	**Sali**	**Diah**	**Sleep**	**Leth**	**Com**
1.	**MPG**	x	x	x	x	x	x	x	X	x	x	X	x	x	X

(*TRE-Tremor, CON-Convulsions, SALI- Salivation, Diah - Diarrhea, LET-Lethargy) **x** = Negative, ♋ = Positive

MPG: Methanolic extract of *Polygonum glabrum Willd*

Effect of extract of methanolic extract of *Polygonum glabrum willd* on liver weight.

Antitubercular drugs (Rifampicin+Isoniazied) treatment in rats resulted in enlargement of liver which was evident by increase in the wet liver. The groups treated with standard silymarin 50mg/kg ($p<0.05$) and MPG 200mg/kg (non-significant), 400mg/kg ($p<0.05$) extract showed significant restoration of wet liver weight.

Effect of methanolic extract of *Polygonum glabrum willd* on serum biochemical markers in RIF/INH induced hepatoxicity in rats.

The damage to the structural integrity of liver is commonly assessed by the determination of serum amnino-transferas activities. The liver weight and SGPT, SGOT, ALP, bilirubin and total protein.

SGOT

Rifampicin+Isoniazied administration resulted in significant ($p<0.001$) elevation of SGOT levels as compared to normal group. Treatment with standard drug silymarin 50mg/kg showed significantly ($p<0.01$) reduction of SGOT level as compared to control group. Administration of extract MPG 200mg/kg and 400mg/kg had showed significant reduction of SGOT ($p<0.05$, $p<0.01$) as compared to control group.

SGPT

Rifampicin+Isoniazied administration resulted in significant ($p<0.001$) elevation of SGPT levels as compared to normal group. Treatment with standard drug silymarin 50mg/kg showed significantly ($p<0.001$) reduction of SGPT level as compared to control group. Administration of extract MPG 200mg/kg and 400mg/kg had showed significant reduction of SGPT level ($p<0.01$, $p<0.001$) as compared to control group.

ALP

Rifampicin+Isoniazied administration resulted in significant ($p<0.001$) elevation of ALP levels as compared to normal group. Treatment with standard drug silymarin 50mg/kg showed significantly ($p<0.001$) reduction of ALP level as compared to control group. Administration of extract MPG 200mg/kg and 400mg/kg had

showed significant reduction of ALP level ($p<0.01$, $p<0.05$) as compared to control group.

Total Protein

Rifampicin+Isoniazied administration resulted in significant ($p<0.001$) elevation of total protein levels as compared to normal group. Treatment with standard drug silymarin 50mg/kg showed significantly ($p<0.001$) reduction of total protein level as compared to control group. Administration of extract MPG 200mg/kg and 400mg/kg had showed significant reduction of total bilirubin level ($p<0.001$, $p<0.01$) and direct bilirubin level ($p<0.0$)as compared to control group.

Bilirubin

Rifampicin+Isoniazied administration resulted in significant ($p<0.001$) elevation of total and direct bilirubin levels as compared to normal group. Treatment with standard drug silymarin 50mg/kg showed significantly ($p<0.001$) reduction of total and direct bilirubin level as compared to control group. Administration of extract MPG 200mg/kg and 400mg/kg had showed significant reduction of total ($p<0.001$, $p<0.05$) and direct bilirubin level ($p<0.01$, $p<0.05$) as compared to control group

Effect of methanolic extract of *Polygonum glabrum Willd* on *in-vivo* Antioxidant parameters.

Catalase RCIN and INH treatment caused a significant ($P<0.001$) decrease in the level of catalase, in liver tissue when compared with control group (table 3). The liver of silymarin 50mg/kg treated animals showed a significant increase ($p<0.001$) in antioxidant enzymes levels compared to INH and RCIN treated rats.The treatment of MPG at the doses of 200 and 400 mg/kg resulted in a significant ($p<0.01$, $p<0.001$) increase of enzymic antioxidants when compared to RCIN and INH treated rats.

GSH

RCIN and INH treatment caused a significant ($P<0.001$) decrease in the level of GSH in liver tissue when compared with control group (table 3). The liver of silymarin 50mg/kg treated animals showed a significant increase ($p<0.001$) in antioxidant enzymes levels of GSH as compared to INH and RCIN treated rats. The treatment of MPG at the doses of 200 and 400 mg/kg resulted in a significant

(p<0.001, p<0.001) increase of enzymic antioxidants of GSH when compared to RCIN and INH treated rats.

LPO

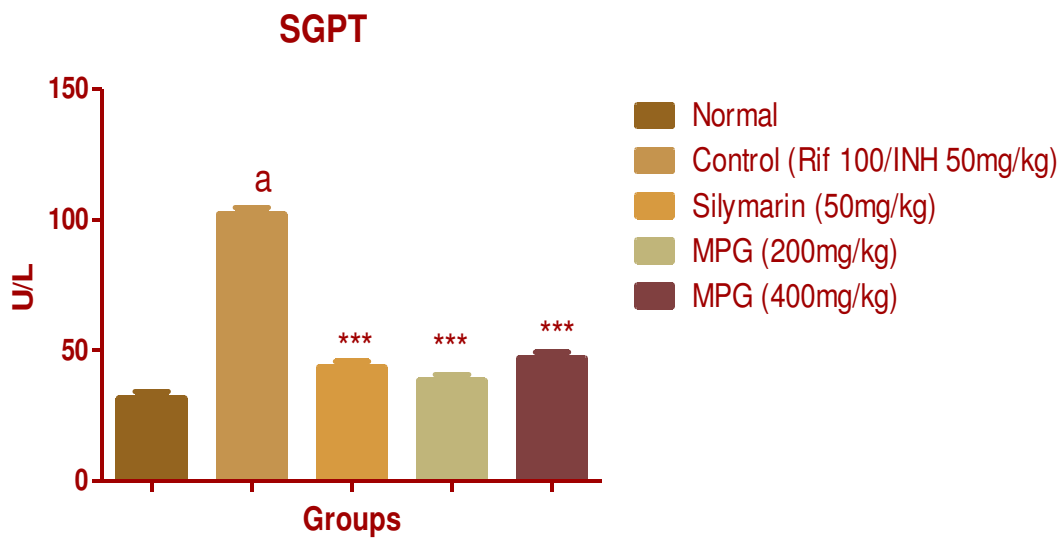
RCIN and INH treatment caused a significant (P<0.001) decrease in the level of LPO in liver tissue when compared with control group (table 3). The liver of silymarin 50mg/kg treated animals showed a significant increase (p<0.001) in antioxidant enzymes levels of LPO as compared to INH and RCIN treated rats. The treatment of MPG at the doses of 200 and 400 mg/kg resulted in a significant (p<0.05, p<0.001) increase of enzymic antioxidants of LPO when compared to RCIN and INH treated rats.

Table 1: Effect of of methanolic extract of *Polygonum glabrum Willd* on serum biochemical parameters.

GROUPS	Liver weigt g/100 g BW	SGPT U/L	SGOT U/L	ALP U/L
Normal control	2.84±0.12	31.86±2.34	51.94±1.64	76.87±1.80
Positive control (CCl4 1.5 ml/kg)	3.14±0.09a	102.3±2.34a	165.6±1.82a	212.3±3.05a
Standard (Silymarin, 100 mg/kg)	2.77±0.48 *	43.64±2.12***	89.89±1.13**	123.3±2.05***
EME (200 mg/kg)	3.12±0.15ns	38.59±2.20**	68.02±1.62*	142.7±1.83**
EME (400 mg/kg)	2.98±0.09*	47.05±2.22***	87.70±1.51**	159.0±2.33*

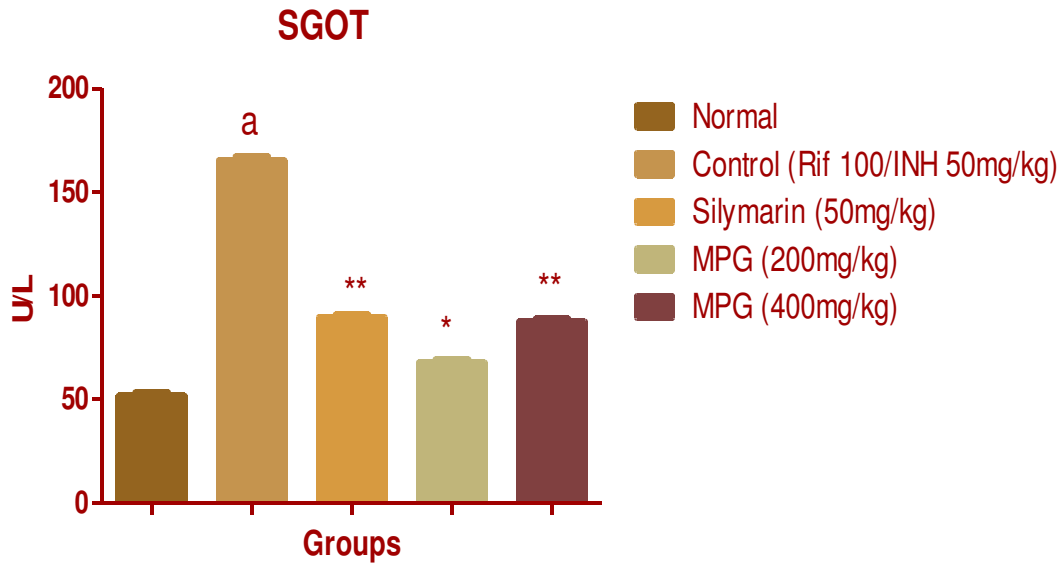
All the values are expressed as mean±SEM, ns= not significant, N=6, One way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test, *p<0.05, **p<0.01 and ***p<0.001 as compared to Normal control group. and ^{a}p<0.001 as compared to Normal group.

Fig.no.1: Effect of of methanolic extract of *Polygonum glabrum Willd* on serum SGPT.



All the values are expressed as mean±SEM, ns= not significant, N=6, One way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test ***p<0.001 as compared to Normal control group. and [a]p<0.001 as compared to Normal group.

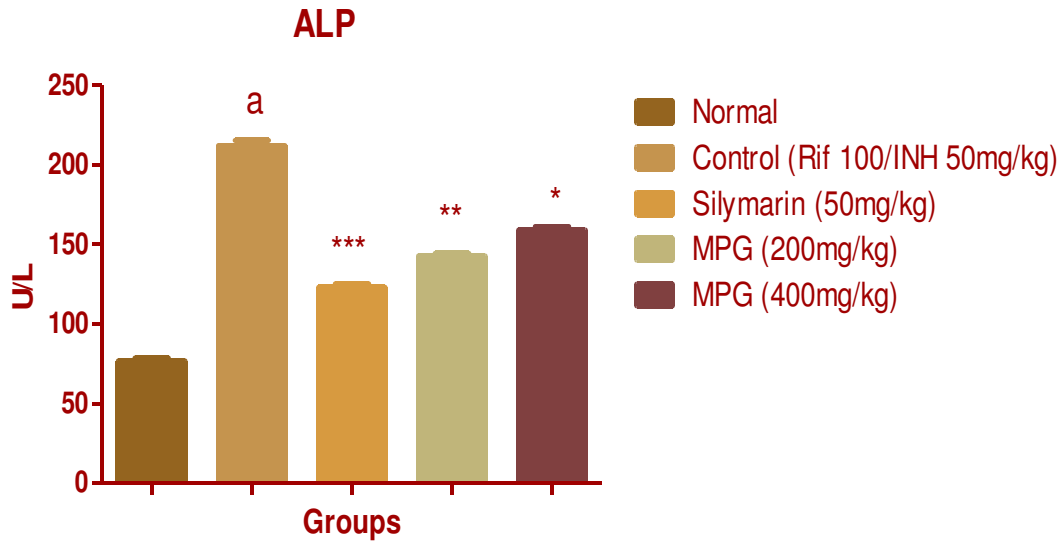
Fig.no.2: Effect of of methanolic extract of *Polygonum glabrum Willd* on serum SGOT.



GROUPS	Total protein (g/dl)	Bilirubin (mg/dl)	
		Total	Direct
Normal control	10.54±0.73	0.18±0.00	0.18±0.02
Control (RIF+INH)	3.29±0.35a	2.10±0.33a	0.54±0.03a
Standard (Silymarin, 50 mg/kg)	7.56±0.34***	0.29±0.02***	0.33±0.01***
MPG (200 mg/kg)	6.92±0.33**	0.33±0.02***	0.30±0.01**
MPG (400 mg/kg)	8.56±0.46***	0.41±0.01*	0.40±0.01*

All the values are expressed as mean±SEM, N=6, One way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test, *p<0.05 and **p<0.01 as compared to Normal control group. and [a]p<0.001 as compared to Normal group.

Fig.no.3: Effect of of methanolic extract of *Polygonum glabrum Willd* on serum ALP.

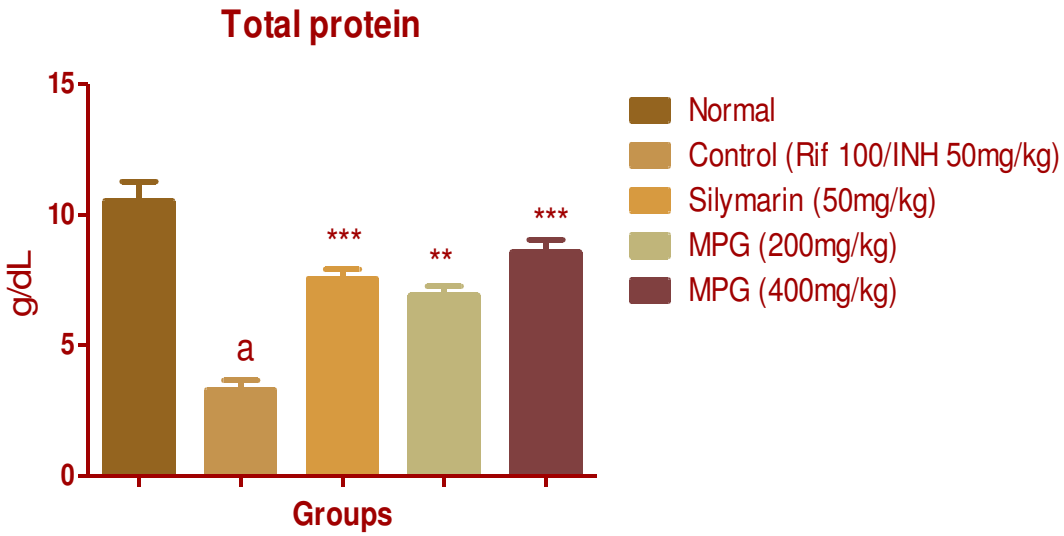


All the values are expressed as mean±SEM, N=6, One way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test, *p<0.05, **p<0.01 and ***p<0.001 as compared to Normal control group. and [a]p<0.001 as compared to Normal group.

Table 2: Effect of of methanolic extract of *Polygonum glabrum Willd* on serum total protein and bilirubin.

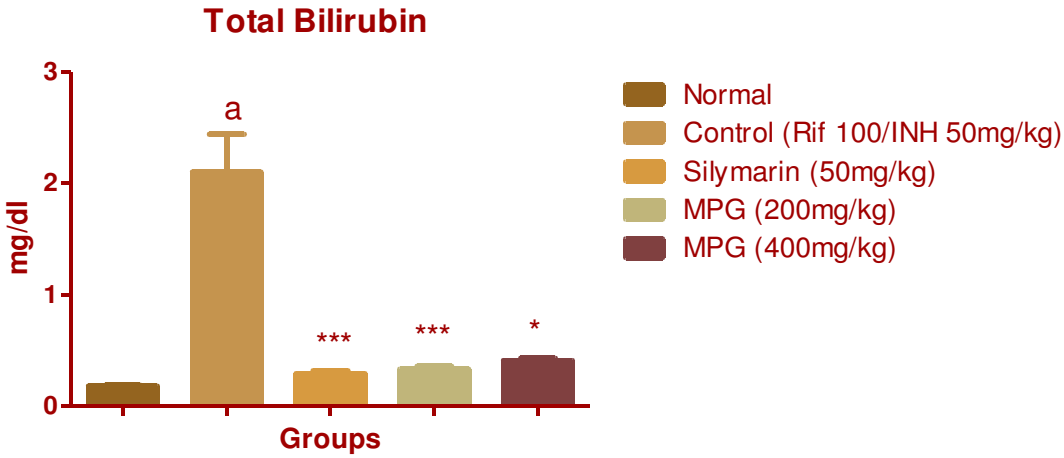
All the values are expressed as mean±SEM, N=6, One way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test, *p<0.05, **p<0.01 and ***p<0.001 as compared to Normal control group and [a]p<0.001 as compared to Normal group.

Fig.4: Effect of of methanolic extract of *Polygonum glabrum Willd* on serum total protein.



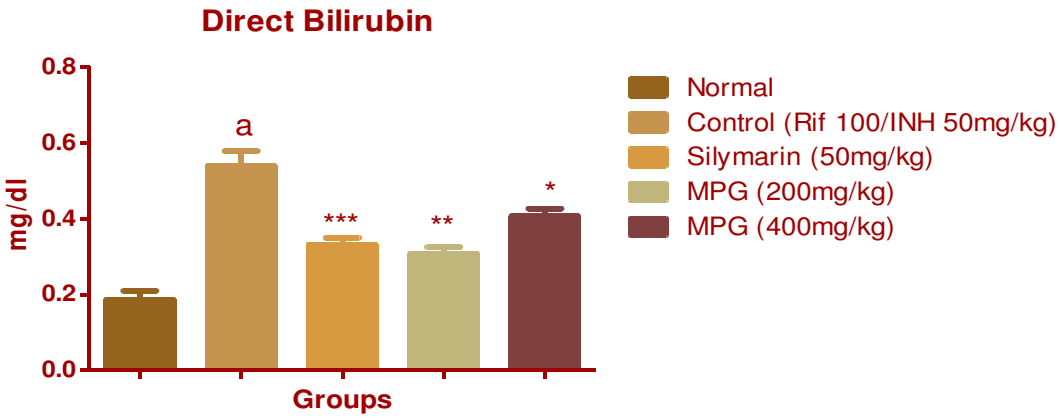
All the values are expressed as mean±SEM, N=6, One way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test **p<0.01 and ***p<0.001 as compared to Normal control group and [a]p<0.001 as compared to Normal group

Fig.5: Effect of of methanolic extract of *Polygonum glabrum Willd* on serum total bilirubin.



All the values are expressed as mean±SEM, N=6, One way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test, *p<0.05 and ***p<0.001 as compared to Normal control group. and [a]p<0.001 as compared to Normal group

Fig.6: Effect of of methanolic extract of *Polygonum glabrum Willd* on serum direct bilirubin.



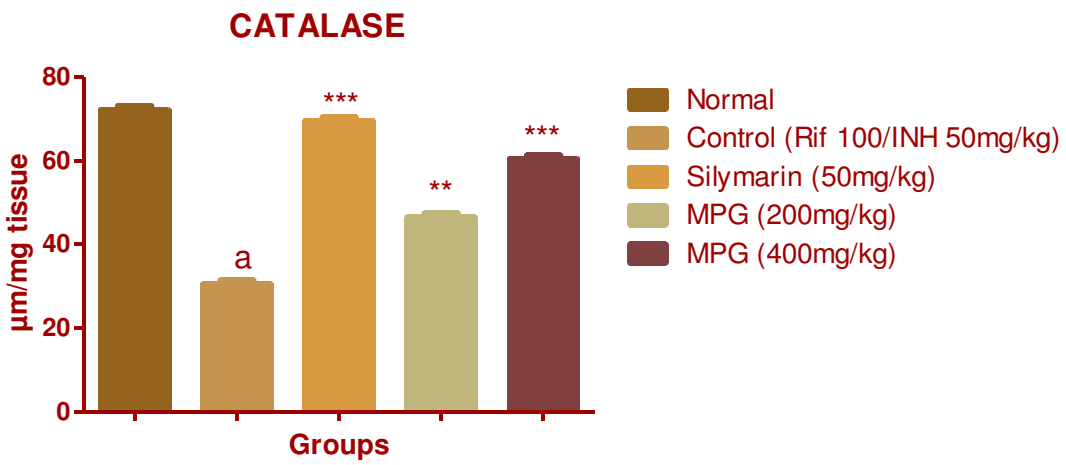
All the values are expressed as mean±SEM, N=6, One way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test, *p<0.05, **p<0.01 and ***p<0.001 as compared to Normal control group. and [a]p<0.001 as compared to Normal group.

Table.4: Effect of of methanolic extract of *Polygonum glabrum Willd* on Invivo Antioxidant parameters.

Treatment group	*In-vivo* Antioxidant parameters		
	CATALASA µm/mg tissue	**GSH µm/mg tissue**	**LPO µm of /H_2O_2/mg tissue**
Normal	72.33±0.81	3.17±0.05	9.97±0.27
Control (RIF+INH)	30.63±0.84a	1.13±0.06a	24.39±0.59a
Standard Silymarin (50m/kg)	69.70±0.74***	2.42±0.07***	15.64±0.37***
MPG 200mg/kg	46.59±0.91**	1.93±0.01***	15.90±0.65*
MPG 400mg/kg	60.57±0.82***	1.80±0.02***	17.53±0.58***

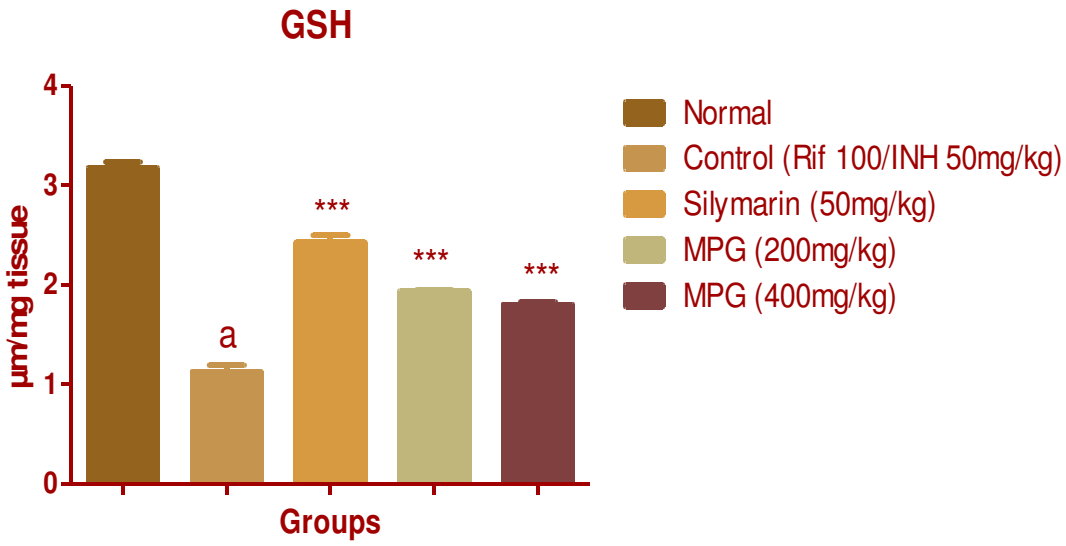
All the values are expressed as mean±SEM, N=6, One way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test, *p<0.05, **p<0.01 and ***p<0.001 as compared to Normal control group. and [a]p<0.001 as compared to Normal group.

Fig.no.7: Effect of *Polygonum glabrum Willd* on catalase level in RCIN and INH -induced hepatotoxicity rats.



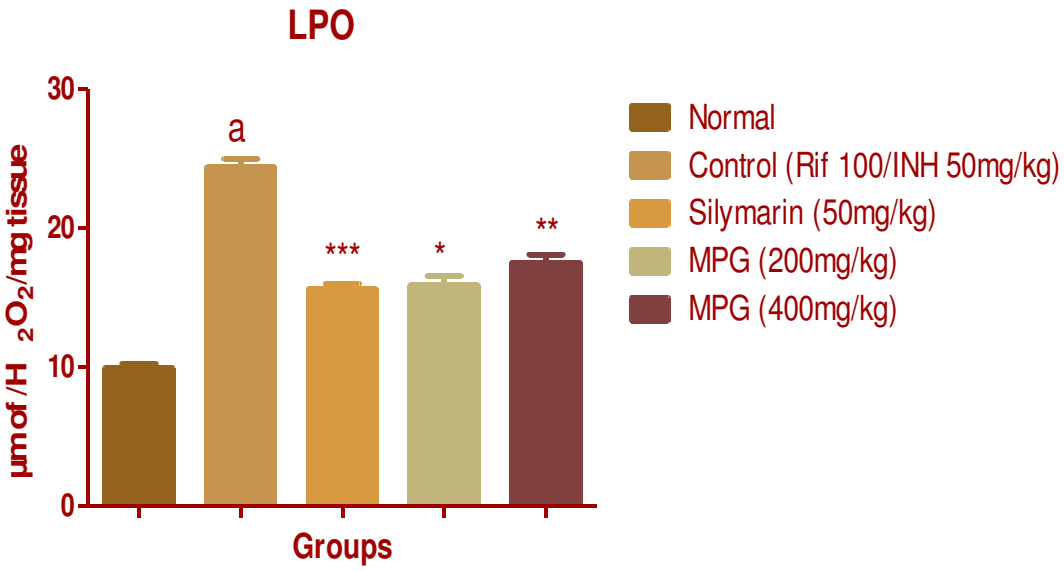
All the values are expressed as mean±SEM, N=6, One way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test, **p<0.01 and ***p<0.001 as compared to Normal control group. and [a]p<0.001 as compared to Normal group.

Fig.no.8: Effect of *Polygonum glabrum Willd* on GSH level in RCIN and INH -induced hepatotoxicity rats.



All the values are expressed as mean±SEM, N=6, One way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test ***p<0.001 as compared to Normal control group. and [a]p<0.001 as compared to Normal group.

Fig.no.9: Effect of *Polygonum glabrum Willd* on LPO level in RCIN and INH -induced hepatotoxicity rats.



All the values are expressed as mean±SEM, N=6, One way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test *p<0.05, **p<0.01and ***p<0.001 as compared to Normal control group. and [a]p<0.001 as compared to Normal group.

Histopathological studies on liver in antitubercular drug induced (RIF+INH) hepatotoxicity.

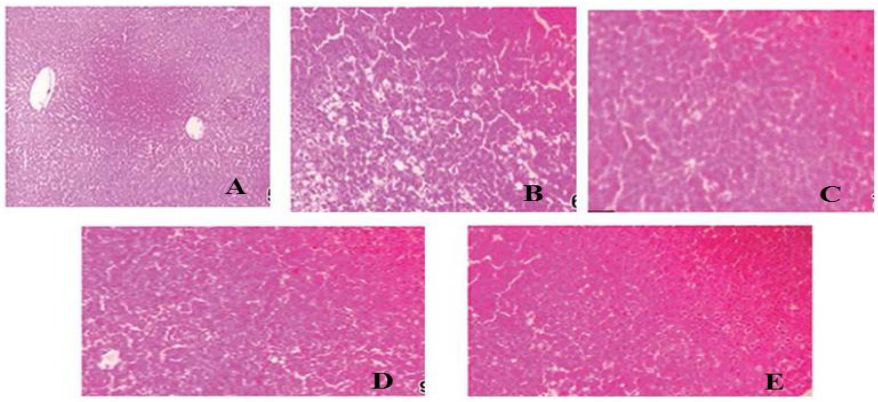
Normal group: The architecture of liver parenchyma appeared intact. There were seen scattered mononuclear inflammatory cells and epithelioid granulomas with in the parenchyma. Periportal and perivenular infiltration by aggregates of mononuclear inflammatory cells were seen. Some of the central veins show thrombosis.

Control group: The architecture of liver parenchyma appeared partly effaced. There are seen degenerating hepatocytes amidst normal hepatocytes. The parenchyma showed areas of necrosis with mixed inflammatory infiltration. Periportal infiltration by mixed inflammatory cells comprising of lymphocytes, neutrophils and histocytes were seen.

Standrad Silymarin group: The architecture of liver parenchyma appeared intact. Focal areas showed degenerating and regenerating hepatocytes.The parenchyma showed aggregates of lymphocytes, macrophages and histocytes. Periportal and perivenular infiltration by mononuclear inflammatory cells comprising of lymphocytes and histocytes were seen.

MPG 200mg/kg group: The architecture of liver parenchyma appeared intact. There were seen some regenerating hepatocytes amidst normal hepatocytes. The parenchyma showed aggregates of mononuclear inflammatory cells. Periportal and perivenular infiltration by scattered mononuclear inflammatory cells comprising of lymphocytes and histocytes were seen. bile duct proliferation was seen at some areas.

MPG 400mg/kg group: The architecture of liver parenchyma appeared intact. There were seen dilated and congested sinusoids between the hepatocytes. The parenchyma shows few epitheloid granulomas. Periportal and perivenular infiltration by scattered mononuclear inflammatory cells were seen. Some of the central veins showed congestion.



Histopathology of Liver

A: Normal group

B: Control (RIF+INH)

C:Standard (Silymarin 50mg/kg)

D: MPG 200mg/kg

E: MPG 400mg/kg

DISCUSSION

Drug-induced hepatotoxicity is a potentially serious adverse effect of the currently used anti-tubercular chemotherapeutic regimens containing RCIN and INH. The conversion of

monoacetyl hydrazine, a metabolite of INH, to a toxic metabolite via cytochrome P450 leads to hepatotoxicity. RCIN induces cytochrome P450 enzyme resulting an increased production of toxic metabolites from acetyl hydrazine (AcHz). RCIN can also increase the metabolism of INH to isonicotinic acid and hydrazine, both of which are hepatotoxic. The plasma half-life of AcHz (metabolite of INH) is shortened by RCIN and AcHz is quickly converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which is related to the higher incidence of liver necrosis caused by RCIN and INH combination [143]. In addition to these mechanisms; oxidative stress induced hepatic injury is one of the important mechanisms in hepatotoxicity produced by anti-tubercular drugs [144,145].Earlier it has been well documented that both ALT and AST are considered among the most sensitive markers to assess hepatocellular damage leading to liver cell necrosis [146,147].

ALP, which is secreted from the lysosomes, is also a marker enzyme for assessing liver damage. Slight to moderate increases in ALP (1-2 times normal) occurred in liver disorders[148]. Estimating the activities of serum marker enzymes, like AST, ALT, ALP, total bilirubin, can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released in to the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage.

The enhanced activities of these serum marker enzymes observed in RCIN and INH treated rats in our study correspond to the extensive liver damage induced by RCIN and INH. Results indicate that MPG administration could blunt RCIN and INH -induced increase in activities of different marker enzymes of heptocellular injury, viz. AST, ALT, ALP, total bilirubin and total protein, (Table 1-2) suggesting that MPG possibly has a protective influence against RCIN and INH induced hepatocellular injury and degenerative changes. Antitubercular drugs mediated oxidative damage is generally attributed to the formation of free radicals, which act as stimulator of lipid peroxidation and source for destruction and damage to the cell membrane[149].

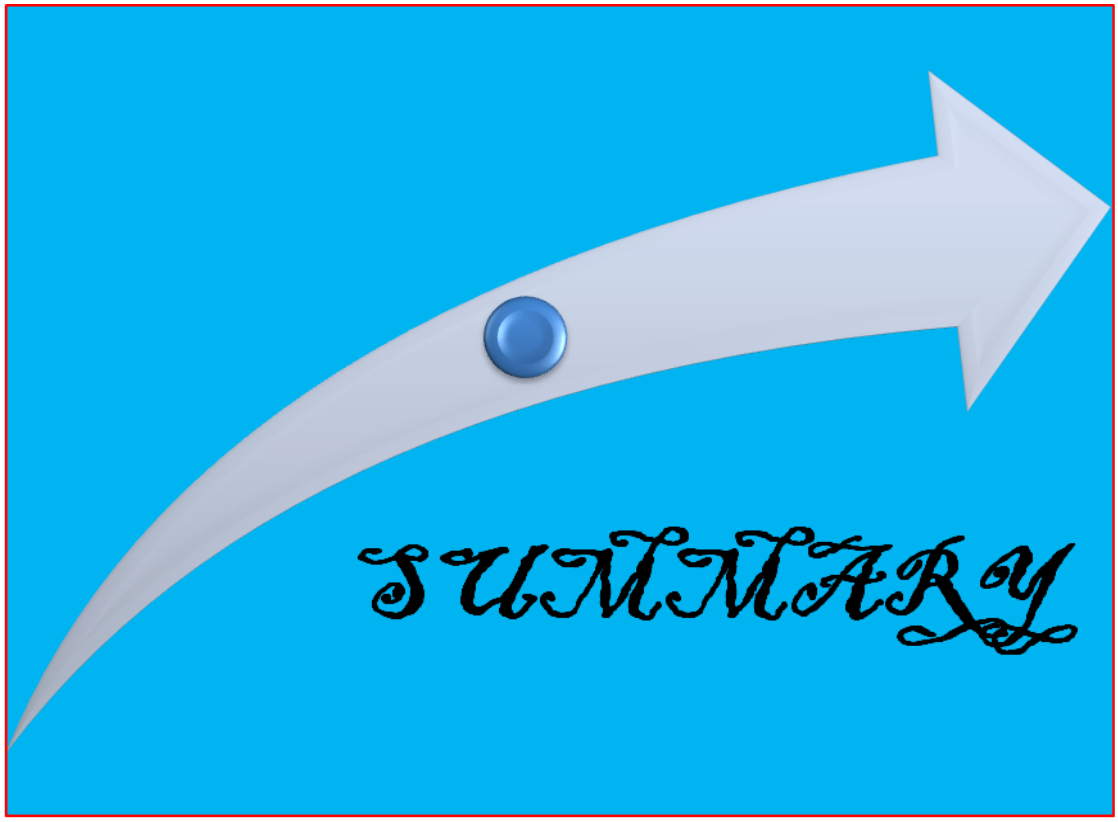
In previous report suggested that, there did not seem to be clear evidence that INH proves much more injuries than RIF and, in this connection, they consider that it is the combination of these two drugs that confer the additive, or even synergistic, potential of liver toxicity than either agent alone, as conjectured[150,151]. In our study, RCIN and INH treatment produced the elevation in the levels of LPO. Treatment of the rats with MPG significantly reduced the elevated levels of LPO.

Alterations of various cellular defense mechanisms consisting of enzymatic antioxidant components [superoxide dismutase (SOD), catalase, GSH have been reported in RCIN and INH -induced hepatoxicity (Table 3). The RCIN and INH administered animals exhibited significantly ($p<0.001$) low levels of hepatic enzymatic antioxidant components, and significantly increased with co-administration of MPG (200 and 400 mg/kg) at the all the doses and in silymarin treatment group after 21 days. On the day of 21st enzymatic antioxidant components levels significantly increased with co-administration of MPG at the lower and higher doses (200 and 400 mg/kg) and in silymarin treated group.

Histopathological studies, treatment with different doses of MPG produced mild portal triaditis and absence of necrosis when compared with control. Treatment with silymarin also shows mild portal triaditis and absence of necrosis and vacuoles. All these results indicate a hepatoprotective potential of the extract. The results of the present study suggested that MPG possess hepatoprotective activity against the hepatotoxicity induced by the combination of two antitubercular drugs.

CONCLUSION

In conclusion, the results of this experiment demonstrate a potent hepatoprotective action of methanolic extract of *Polygonm glabrum Willd* in Rifampicin and INH-induced oxidative stress and liver toxicity in rats. Such effects can be correlated directly with its ability to reduce lipid peroxidation and enhance the antioxidant defence status. Thus methanolic extract of *Polygonm glabrum Willd* may be used as a safe and effective alternative chemo preventive and protective agent in the management of liver diseases.


SUMMARY

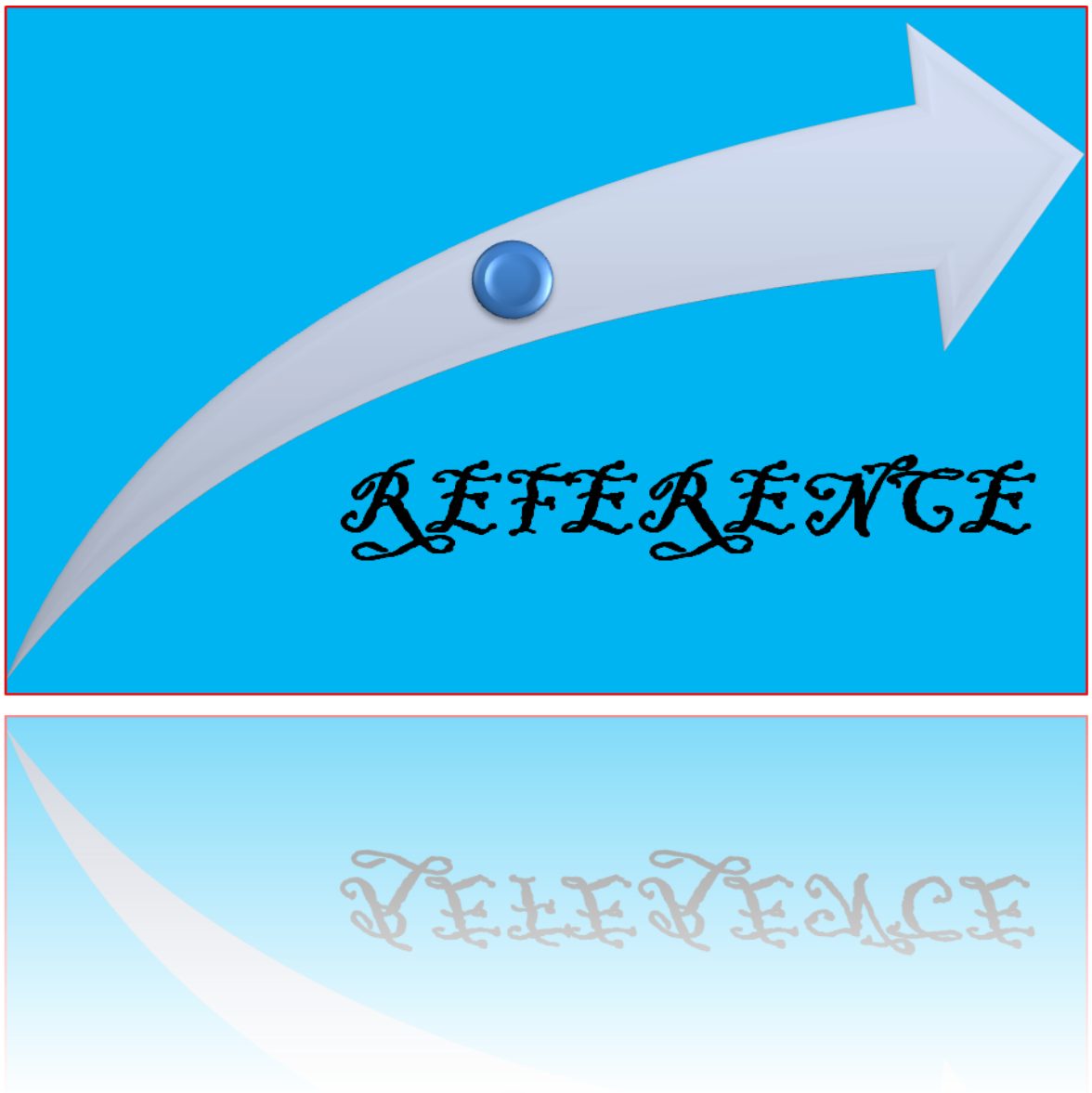
SUMMARY

The present study was aimed to assess the hepatoprotective activity of methanolic extract of *Polygonum glabrum Willd.*

LD50 studies were conducted in albino rats with methanolic extract of polygonum glabrum according to OECD guidelines 423 and was found safe upto the dose level of 2000 mg/kg confirming its non-toxic nature. The hepatoprotective activity was studied in antitubercular drugs induced hepatoxic animal models. The physical parameters such as liver weight and biochemical parameters like SGOT, SGPT, ALP, Total protein and Bilirubin. Antioxidant parameters like catalase, GSH and LPO and histopathology reports of livers were also considered to confirm hepatoprotection.

Antitubercular drugs induced hepatotoxicity was significantly prevented by pretreatment with methanolic extract of Polygonum glabrum Willd. Decrease in wet liver weight and reduction in biochemical parameters levels like serum SGOT, SGPT, ALP, Total protein bilirubin and increase in ROS scavenging enzyme activities such as catalase GSH and LPO after treatment with methanolic extract of Polygonum glabrum Willd confirmed the hepatoprotective effect of ectract under study. In liver injury models in rats restoration of hepatic cells with minor fatty changes and absence of necrosis after treatment with extract was observed indicating satisfactory hepatoprotection.

Based on improvement in serum marker enzyme levels, physical parameter and antioxidant parameters and histopathological studies it was concluded that methanolic extract of Polygonum glabrum possesses significant hepatoprotective activity in the doses used.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