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Review Article

# A Review on Hepatoprotective Potential of Some Indigenous Medicinal Plants

**Rekha Khatri, Anju Dhiman***

Department of Pharmaceutical Sciences, Maharshi Dayanand University, Haryana, India

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## ABSTRACT

Every year, around 2 million individuals die as a result of liver disease, 1 million as a result of cirrhosis complications, and 1 million as a result of viral hepatitis and hepatocellular carcinoma. Hepatotoxicity can result in cytotoxicity, cholestasis, steatosis, fibrosis, cirrhosis, hepatitis, liver cancer, and other health problems. Drinking alcohol, using chemicals, using medicines, having an autoimmune disease, and becoming ill are the most common causes of hepatotoxicity. These chemicals have a direct effect on the mitochondrial respiratory chain, cytochromes p-450, gluthione S-acyltransferase, and the mitochondrial permeability transitional pore, among other things. The mechanism of drug-induced hepatotoxicity could be an innate or idiosyncratic hepatotoxin reaction. There are several modern medications that can be used to treat liver disorders. Current pharmacological treatments for liver illnesses are increasingly ineffective, have long-term harmful consequences, and are prohibitively expensive in developing nations. As a result, research into inexpensive, easily accessible medicinal plants that do not need to be processed under strict pharmaceutical standards appears to have sparked a lot of interest as alternative remedies for the sickness. This review covered the many types of liver illness, drug-induced hepatotoxicity processes, and *in vivo* and *in vitro* hepatotoxicity models. This study delves into the phytoconstituents and pharmacological effects of plants found to have hepatoprotective qualities. The purpose of this study is to evaluate the potential for hepatoprotection of numerous indigenous medicinal herbs.

**Keywords:** Hepatotoxicity, Liver disease, Indigenous medicine, *In vivo*, *In vitro*, Hepatoprotection

***Correspondence:** Anju Dhiman, Department of Pharmaceutical Sciences, Maharshi Dayanand University, Haryana, India, E-mail: anju.dhiman@mdurohtak.ac.in

## INTRODUCTION

Around 2 million people die each year from liver disease, one million from cirrhosis complications and one million from viral hepatitis and hepatocellular cancer. Around 2 billion people use alcohol worldwide, with up to 75 million people diagnosed with alcohol use disorders and at risk of developing alcohol-related liver disease. Diabetes affects approximately 400 million individuals, and obesity or overweight affects over 2 billion people and both are risk factors for non-alcoholic fatty liver disease and hepatocellular cancer. While there has been progress in understanding the causes of liver illness and creating therapies, there are still considerable obstacles to overcome. Hepatic dysfunction is a major health problem that researchers are trying to solve. A multitude of factors, including biological, chemical, and pharmacological overdose, have been associated to liver disease. Hepatocytes, vascular cells, and bile ducts may all be involved in the liver injury (Asrani SK, *et al.*, 2019).

## LITERATURE REVIEW

### *Types of liver disease*

**Biliary obstruction:** Biliary obstruction causes jaundice due to a blockage in bile flow. Obstructive jaundice is caused by lesions in the major extra hepatic bile duct, such as cancer, impacted bile stones, or sclerosing cholangitis. Secondary biliary cirrhosis can develop after a bile duct obstruction has been present for a long time.

**Metabolic disease:** Metabolic diseases of the liver can be either hereditary (genetic) or acquired. Hereditary hyperbolic rubinemias and difficulties concerning the intermediate metabolism of lipids, carbohydrates, proteins, and heavy metals are only a few examples of metabolic anomalies of the liver.

**Congenital metabolic disorder:** Gilbert syndrome, Rotor syndrome, and Dubin-Johnson syndrome are the most well-known congenital jaundice disorders. Defects in genetic enzymes, such as alpha-1-antitrypsin deficiency, can cause liver injury, which can progress to cirrhosis (Anand H, *et al.*, 2002).

**Acquired metabolic disorders:** A range of consumed items, such as toxins, medicines, meals and beverages, can cause metabolic disturbance in liver cells. Hepatomegaly, alcoholic hepatitis and cirrhosis are three kinds of liver disease caused by alcohol. Hepatitis can be caused by a variety of drugs, including methyldopa, nitrofurantoin, isoniazid, ketoconazole, and acetaminophen.

**Acute viral hepatitis:** It is a complete illness characterized by a severe hepatocyte assault. There have been five hepatotropic viruses acknowledged. Hepatitis A (HAV) is an orally transmitted, acute, self-limiting disease. Hepatitis B (HBV) and Hepatitis C (HCV) viruses be spread *via* replace of bodily fluids in blood transfusions or association through sexual activity. Hepatitis D virus is a type of virus which causes inflammation during combination with Hepatitis B. The Hepatitis E virus is spread through the intestine and causes self-limiting illnesses. Chronic hepatitis is a rare but serious side effect of HBV and mixed HBV-HDV infection (Acharya SK, *et al.*, 2006).

**Cirrhosis:** Cirrhosis, a chronic liver disease marked by the widespread fibrosis and regenerating lumps that replaces the normal liver parenchyma in a dispersed manner. Cirrhosis is caused mostly by drinking habits and action of hepatitis virus (Schuppan D and Afdhal NH, 2008).

**Liver tumour:** Mostly tumours are formed by bile ducts and liver cells. Hepatic capsule and portal tracts containing Kupffer cells and connective tissue cells are less susceptible for tumor formation. The most frequent primary malignant liver tumor is hepatocellular carcinoma (malignant hepatoma). Cholangiocellular carcinoma is a kind of bile duct cancer.

**Necrosis:** Hepatic necrosis can be localized, widespread, or diffuse. Zonular necrosis can occur in any part of the lobule depending on the agent. Intrinsic (predictable) hepatotoxins cause zonal necrosis, but idiosyncratic pharmacological reaction will be responsible for vast or diffuse necrosis. Central necrosis causes distinctive lesion through chloroform, carbon tetrachloride, acetaminophen and bromobenzene centrizonal necrosis.

**Degeneration:** Hepatocytes show damage to sub-necrotic such as hydropic degeneration as well as eosinophilic degeneration.

**Steatosis:** A fatty liver can be caused by a variety of factors. Hepatocytes are loaded with small fat droplets that do not remove the nucleus in this type of fatty liver. Alcohol and methotrexate, for example, cause macrovascular steatosis (Powell EE, *et al.*, 2005).

**Cholestatic injury:** Viral, chemical, and drug-induced liver injuries are well-known toxicological issues. Hepatotoxicity is thought to be caused by a toxic intermediate that binds covalently to hepatocytes, causing centrilobular hepatic necrosis. The Lipid peroxidation and the Thiol group oxidation are two alternative reasons for necrosis. Lipid peroxidation by its free radical product, $CCl_3$, is one of the principal causes for carbon tetrachloride ($CCl_4$)-induced hepatopathy. Inhibition of free radical generation or scavenging (antioxidant activity) is critical in giving protection against liver injury. Lipid peroxidation membranes are mostly made up of lipids. In addition to their important function of compartmentation, cell membrane lipids are involved in cell responses to a varied range for external stimuli which includes hormones, growth factors, and neurotransmitters. Oxidative breakdown of Polyunsaturated Fatty Acids (PUFA) in the cell membrane is known as Lipid Peroxidation. Many redox activities produce oxygen radicals and active forms of oxygen. To prevent this damaging activity, antioxidant mechanisms are built within the cells. Examples include enzymes such as catalase, superoxide dismutase, glutathione reductase, and others, and the non-enzymatic substances such as glutathione, vitamin E, and carotene. The fundamental cause of oxidative stress appears to be the overproduction of free radicals, which is associated to vitamin A, C, and E deficiency as well as a reduction in the levels of the above-mentioned enzymes. As a result, superoxide dismutase, glutathione reductase, and lipid peroxidation levels are employed as markers of oxidative stress in the liver, and their measurement is to be considered as one of the most important indicators of impaired liver function (Zollner G and Trauner M, 2008).

### *Liver injury's biochemical and functional symptoms*

In the subject of toxicology, the impact of drugs or chemicals on liver function is a hot topic. There is nothing specific parameter which can be used to determine "liver function" because liver performs various activities (Shivaraj G, *et al.*, 2009). The advancement in the liver function test has been typically corresponded to the greater understanding of biochemistry of liver. Because the liver may play a role in determining the chemical's biological action, the test becomes a proxy for hepatic function. Paracetamol-induced hepatotoxicity models are being developed for assessing hepatoprotective effectiveness. The levels of numerous physiologically important enzymes were increased in paracetamol-induced liver injury.

**Transaminases:** Transaminases catalyze the transfer of an amino acids group to alfa keto acid, results in the generation of amino acid and keto acid. This is how amino acids like alanine, aspartic acid, and others are made. Serum enzyme activity and liver necrosis have a proportionate relationship. Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) levels in blood plasma rises when they are released from damaged liver tissue. Elevated transaminase levels, which can be 10 to 100 times greater than normal SGOT and SGPT activity, are usually indications of this in clinical settings (Limdi JK and Hyde GM, 2003).

**Bilirubin levels:** One of the liver's natural duties is to eliminate the hemoglobin-bilirubin breakdown product into bile. As a result, a liver function test can be used to assess the liver's ability to extract bilirubin from the blood and excrete it into the bile. This aberrant state can only be diagnosed in this situation by calculating plasma bilirubin levels. Although an increase in blood bilirubin levels is associated with severe parenchymal injury, it is a somewhat insensitive indicator of chemical hepatic injury. Substances like $CCl_4$ produce enough parenchymal injury to generate a significant rise in SGPT activity, although they have little effect on bilirubin levels.

**Cholesterol values:** Fatty liver is defined as a more than 5% raise in the lipid content of liver. One of the reasons for this is the presence of toxic substances. $CCl_4$ inhibits protein synthesis as well as protein-lipid conjugation. As a result, the liver cell's ability to secrete lipoprotein is hampered.

**Hepatotoxicity:** Despite the fact that the majority of pharmaceuticals are digested without causing harm to the liver, numerous deadly and near-fatal drug reactions occur each year. Enzyme all of these variables contribute to the accumulation of hepatocyte toxins, including genetic alterations that lead to the creation of toxic metabolites and the depletion of substrates required for metabolite detoxification. A few medicines produce metabolites that induce liver damage in a consistent and dose-dependent way. Hepatocytes are harmed either directly or indirectly when the activities related to intracellular and membrane integrity are disrupted, or when damages occur to immune-mediated membrane (Bhakuni GS, *et al.*, 2016).

### *Mechanism of hepatotoxicity*

The majority of pharmaceuticals and xenobiotics are lipophilic, allowing for facile absorption across intestinal membranes (Jaeschke H, *et al.*, 2002). Hepatic metabolism converts them to hydrophilic state, allowing them to be easily eliminated. Exogenous compounds are metabolized largely through phase 1 and phase II processes in the liver. The cytochrome P450 is situated in the endoplasmic reticulum of liver, is primarily responsible for oxidative, reductive, hydroxylation, and de-methylation reactions in Phase I. Phase I reactions create hazardous intermediates, which are then transformed into harmless molecules by phase II reactions, which is known as detoxification pathways. Phase II reactions result in the formation of a water-soluble metabolite that is readily eliminated by conjugating molecules with hydrophilic moieties such as glucuronide, sulphate, or amino acids. In phase II reactions, glulathione-S-transferase is involved. This phase, on the other hand, might lead to the formation of unstable reactive species precursors, which can cause hepatotoxicity. The mechanism with respect to liver damage can be classified into pathological and chemical in nature. Pathophysiologic hepatic damages include hepatocyte disruption, transport protein disruption, cytolytic T-cell activation, hepatocyte death, mitochondrial disruption, and bile duct injury. Chemically induced liver damage can be caused by direct or intrinsic or predictable medication responses, as well as indirect or unexpected idiosyncratic drug reactions. The liver's reaction to damage follows a predictable pattern that can be investigated at the tissular, cellular, and molecular levels, regardless of the origin of injury. *In vitro* hepatic systems offer a better technique to screen for hepatotoxic compounds and investigate the mechanisms by which toxins injure the liver.

### *Diagnostic tests for liver*

The dilution of hepatotoxicity is aided *via* amount of several enzyme levels which may be present in the blood (Thapa BR and Walia A, 2007). Determination for serum bilirubin, urobilinogen, and other biomarkers aids in determining the liver's normal capacity to transport organic anion and evaluating the liver's main function, namely drug metabolism. Several enzymes are responsible for critical chemical reactions in the liver. Although, there is a dysregulation of liver enzymes in the diseased state of the liver, these chemical biomarkers are essential for detecting hepatotoxicity.

**Transaminase of Aspartate (AST):** Protein synthesis is aided by the liver enzyme aspartate amino transferases (serum glutamic oxaloacetate transaminase). L-aspartate produces oxaloacetate and glutamate, whereas AST catalyzes the formation of a-ketoglutarate. Oxaloacetate combines with Nicotinamide Adenine Dinucleotide Hydrogen (NADH) to produce Nicotinamide Adenine Dinucleotide (NAD) in the presence of malate dehydrogenase. The AST is proportional to the rate of NADH oxidation with respect to NAD, which is evaluated by the drop in absorbance. Other organs where it can be found is the brain, muscles, kidney and heart. Injury to any one of these tissues might result in rise in blood pressure. Normal

levels range from 7 to 40 U/L.

**Alanine Transaminase (ALT):** ALT also known as alanine aminotransferase or Serum Glutamic Pyruvic Transaminase (SGPT), is one of the indicator used for hepatotoxicity, which is also required for amino acid catabolism and the production of glucose from non-carbohydrate carbon molecules. ALT is also in charge of catalyzing the amino group transfer process within L-alanine and a-ketoglutarate, which results in glutamate and pyruvate. ALT is determined by the rate of oxidation from NADH to NAD, which will be assessed as a decrease within absorbance. Normal levels range from 5 to 50 U/L. This enzyme is released at a greater level when the liver is injured.

**Alkaline phosphatase:** Hydrolase is an enzyme found in bile and in the cells present in biliary ducts of liver. At alkaline pH, alkaline phosphatase hydrolyzes p-nitrophenylphosphate to create phosphate and p-nitrophenol. When the activity of the ALP rises in a sample, so does the absorbance, which is used to quantify the rate of p-nitrophenol production. The normal range is 20 to 120 U/L and if liver illness impairs bile excretion, it may rise.

**Total Bilirubin level (TB):** Bilirubin is produced when hemoglobin is broken down, and liver is responsible for excretion of bile. Diazotized sulphanilic acid interacts with and a colorful diazobilirubin molecule will be formed. Within the presence of a caffeine-benzoate accelerator, unconjugated bilirubin binds to sulphanilic acid. The quantity of bilirubin is determined by intensity of the color produced in the sample. In blood normal range of bilrubin should be 0.2-1.2 mg per dL. At the time of injury of liver, there may be difficulty in removal of bilirubin, causing bilirubin to increase in the blood as well as extracellular fluid.

**Liver histopathology:** Trypan blue exclusion and oxygen absorption experiments show that hepatotoxicants diminish the viability of liver cells. $CCl_4$ is metabolized to $CCl_3$ O- in the liver by cytochrome P450, and the reactive oxidative free radical intermediate produced can causes further damage. Hepatocytes' ability to use oxygen is diminished and their viability suffers as a result. To regain capacity of hepatocytes for protection, there are many allopathic as well as herbal formulation is available for hepatoprotection in market.

## In vivo *models for hepatoprotection*

Hepatotoxicity is caused by metabolites of chemical substances. Metabolites of these substances cause many clinical and physiologic changes. Glutathione and neutrophils have been discovered to be important in chemically induced hepatotoxicity. Organelles such as mitochondria, cytoskeleton, endoplasmic reticulum, microtubules, and the nucleus may be affected by activation and inhibition of signaling kinases, transcription factors, and gene expression profiles.

**Galactosamine-induced liver necrosis:** Galactosamine generates a discursive type of damage in humans that looks like viral hepatitis and acute self-limiting hepatitis, replete with necrosis, inflammation, and regeneration, comparable to a drug-induced sickness. On first day of the experiment, segregated doses of 100 mg/kg to 400 mg/kg D-galactosamine were administered to induce acute hepatotoxicity. To produce liver cirrhosis, male Westar rats was administered D-galactosamine (500 mg/kg) three times a week for one to three months (Guguen-Guillouzo C, Guillouzo A, 2010).

Rats should be administered D-galactosamine in divided doses ranging from 100 to 400 mg/kg intraperitoneally or intravenously over the course of one day. Male Westar rats (110-180 gm) should be utilized to develop liver cirrhosis, and 500 mg/kg D-galactosamine should be injected intraperitoneally 3 times weekly for 1-3 months to induce liver cirrhosis. Every day, potentially protective chemicals should be administered orally with meals or *via* gavage. The rats should be slaughtered at various intervals, and the liver is obtained by autopsy (Mohamed MA, *et al.*, 2016).

**Paracetamol induced hepatotoxicity:** Non-steroidal anti-inflammatory drugs among the most commonly recommended medications for the treatment of hepatoprotection in recent time. These medications are used to treat a variety of pain and inflammation diseases such as arthritis, musculoskeletal illnesses and other distressing conditions caused by injury. Non-steroidal anti-inflammatory drugs effects on inflammation, pain, and fever can be separated into three categories. Glutathione depletion in the liver leads to liver injury and increases lipid peroxidation (Ahmad F and Tabassum N, 2012).

Wistar rats (150-200 g) of both sexes should be utilized. A single dosage of 2 g/kg of body weight paracetamol should be given orally. The test medicine is administered to the animals for six days before they will be given paracetamol, and then they will be given paracetamol again on the seventh day. After 24 hours, the animal is sacrificed, boold/serum being used for biochemical studies and the liver being utilized for histology examinations (Mahadevan SB, *et al.*, 2006).

**Antitubercular medicines induced hepatotoxicity:** Drugs used to treat Tuberculosis (TB) such as Rifampicin with isoniazid will cause hepatotoxicity. Tuberculosis (TB) is still a major public health issue. For many years, a highly effective tuberculosis treatment regimen has been employed. These medications may create certain issues due to their long-term regimen of 4-8 months. Hepatocyte damage is caused by cytochrome P450 2E1 metabolizing acetyl hydrazine into a reactive acylating species that binds covalently to liver cell macro molecules. Isoniazid raises SGOT, SGPT, ALP and bilirubin levels at the same time as lowering total protein and albumin levels (Lebda MA, *et al.*, 2013).

Rats used in this investigation should be Wistar/Sprague dowley rats, any sex, weighing 150-200 g. Rifampicin (RMP) and Isoniazid (INH) should be given as an intravenous injection once a day for 15 days at a dose of 50 mg/kg body weight. For 15 days, the test drug should be given together with the RMP+INH combination on a daily basis. The rats are beheaded at the end of the experiment and their livers are examined biochemically and histologically (Rezaie A, *et al.*, 2013).

**Carbon tetrachloride induced liver fibrosis:** In laboratory, $CCl_4$ is the most extensively utilised hepatic toxicant for animals for the investigational research of liver toxicity (Rao CV, *et al.*, 2012). Cytochrome P-450 2E1 in hepatocytes produces $CCl_3$, a toxic metabolite of $CCl_4$. The formed free radical give's reaction with lipids and proteins to generate the trichloromethyl peroxyl radical. These radical targets the lipid lying on the endoplasmic reticulum more quickly than the trichloromethyl free radical, resulting in acute centrolobular necrosis. From the histopathology investigations it is clear that different type of fibrosis occurs within different time period such as

- Significant fibrosis: 2-4 weeks
- Severe bridging fibrosis: 5-7 weeks
- Cirrhosis: 8-9 weeks

Each group, twenty female Westar rats (100-150 g) should be used. For eight weeks, the rats will be administered orally twice a week with solution of equal proportion of $CCl_4$ (1 mg/kg) and olive oil. The animals are given chow and have unrestricted access to water. Only olive oil should be given to the control group. Except on Sundays, when only one dose should be administered, test and regular medications should be given twice a day. The animals should be weighed every week, and at the eighth week, they should be killed for histological study under ether anaesthesia.

**Alkyl alcohol induces liver necrosis:** The liver is particularly prone to ethanol's harmful effects. In the liver, alcohol is widely responsible to induce fatty infiltration, hepatitis, and cirrhosis (Xu JY, *et al.*, 2010). When alcohol substitutes fatty acids in mitochondria, fat infiltration develops and changes in the cell membrane's lipid content or fluidity that might affect cellular activity. Alcohol changes the phospholipids in membranes

and causes lipid peroxidation. The generation of oxy free radicals is amplified by the action of ethanol oxidation in the liver. The free radical effect is caused by a reduction in catalase Superoxide dismutase and Gluthione peroxidase.

After fasting overnight, female Westar rats (120-150 g) should be given water and *ad libitum*. The test medicine should be given to 10 rats the next morning, either orally or intravenously and after 1 hour, 0.4 ml/kg of 1.25% allyl solution should be given orally. The test drug should be given again on the second day, and the liver is removed for histological analysis on the third day.

**Ranitidine induced hepatotoxicity:** Ranitidine (RTD) is harmful because of its metabolites, which causes oxidative damage to the liver and can create an immunological allergic reaction (Gulati K, *et al.*, 2018). Fibrosis, bile duct expansion, lymphocyte, plasma cell, polymorph, and eosinophil infiltration are all symptoms of this condition.

A receives simply a vehicle, Group B receives RTD (50 mg $kg^{-1}$ i.m.), and Group C receives Silymarin as a benchmark. The rats should be intoxicated for 21 days after receiving RTD (50 mg $kg^{-1}$ daily, i.m.). After monitoring thiopentone sodium sleeping duration in all groups of animals, they anaesthetize using ether on the 22$^{nd}$ day. Once the blood is collected, the livers should be removed and stored.

**Thioacetamide induced hepatotoxicity:** Thioacetamide (TAA) is a white crystalline solid organosulfur chemical, water soluble compound (Hemieda FA and Elnga MA, 2005). It is used to synthesize organic and inorganic compounds as a source of sulphide ions. It is commonly used to induce fibrosis and damage hepatocytes. Thioacetamide is considered to be safe because it is not showing any harmful effect on liver; on the other hand, thioacetamide intermediate metabolite (thioacetamide-s-oxide), covalently binds with hepatic macromolecules, varying cell permeability and rising intracellular $Ca^{2+}$ concentration, causing cellular damage and necrosis hepatocytes.

**Diethyl Nitrosamine (DEN) induced hepatotoxicity:** The majority of nitrosamines, which have the chemical structure R1 N (-R2)-N=O, are carcinogenic (Akhtar T and Sheikh N, 2013). DEN utilized in the production of cosmetics, insecticides, and the majority of rubber products. The carcinogenic reagent N-Nitrosodiethylamine (DEN) is frequently employed. Diethylnitrosamine is hydroxylated by CYP2E1 in the liver to produce ethyldiazonium ion, which binds with nucleophiles and cause DNA damage, resulting in hepatocyte necrosis within the periportal areas, as well as centro-portal fibrotic septa. Hepatocellular cancer is caused by low doses of DEN given over a long period of time. As a result, this model is particularly useful for researching the progression of liver fibrosis to Hepatocellular Cancer (HCC).

Animals must be put on a heating pad during the nitrosamine administration process to maintain a constant temperature of 37°C and avoid hypothermia. Animals with age of 14-days are given a single DEN dosage of 10 to 25 mg/kg body weight. DEN doses of 50 to 90 mg/kg body weight are given in animals between the ages of five and six weeks. Inject the solution into mice with disposable sterile syringes. When the injections are finished, hazardous waste container is used to dispose of used syringe all used syringes and needles. Animals in the control group should be given a comparable volume of 0.9 percent NaCl one hour after injection, and mice should be checked for abnormalities at regular intervals. Mice should be slaughtered at the end of the experiment by cervical dislocation, and blood, liver (and other organs) should be taken for further research. Animal corpses and excess biological waste should be disposed of in a hazardous waste receptacle.

**Aflatoxin B1 induced hepatotoxicity:** Molds like *Aspergillus flavus* and *Aspergillus parasiticus* create aflatoxin, which is a naturally occurring mycotoxin (Behairy A, *et al.*, 2021). There are at least 13 different forms of Aflatoxin found in nature, in which Aflatoxin B1 found to be the most poisonous. In animals consuming low dietary amounts, Aflatoxin B1 (AFB), a hepatotoxin, is produced by certain Aspergillus fungus, such as *Aspergillus flavus*. In the liver, cytochrome Alfatoxin B1 is converting into an intermediate (exo-8, 9-epoxide) with the help of P450. Epoxide converts into quickly into AFB1's dialdehyde and attaches to protein, contributing to acute toxicity. This may result in compensatory liver hyperplasia, which may enhance the integration of mutations into the DNA of cellular DNA that target guanine residues. AFB1 raises SGOT, SGPT, alkaline phosphatase, and bilirubin levels in the blood while lowering cholesterol levels.

Male Sprague-Dawley rats (200-250 g) should be used to induce hepatotoxicity. Rats should put in steel cages with a temperature-controlled (250°C) environment with free access to food and water. They'll be split into four groups, each with five animals. Extract solution (36 mg/4 ml) in distilled water should be administer to two groups (dose 36 mg/kg/day) for 2 days. Extract-treated rat groups are given a 0.3 percent aflatoxin B1 solution in Dimethylsulfoxide (DMSO). Aflatoxin B1 is produced in a similar amount in rats that are not given the extract. The vehicle is distributed to the other two control groups. Liver damage should be significant *via* significant changes during the activity of microsomal and serum enzymes, all animals should be decapitated 72 hours after receiving the AFB treatment (Číž M, *et al.*, 2010). Serum samples should be collected from blood samples at the moment of rat sacrifice will be used to determine liver injury indicators such as Sorbitol Dehydrogenase (SDH), Serum Glutamic Pyruvic Transaminase (SGPT) and Serum Glutamic Oxalacetic Transaminase (SGOT). To determine specific enzyme actions, kits should be preferred. After collection of blood samples, the animals' livers should be quickly removed and extract the microsomes with described procedure.

## In vitro *models for hepatoprotection*

**Fresh hepatocyte culture:** To isolate liver cells, a modified approach is applied. Anesthesia is done with the treatment of pentobarbital sodium (Ramboer E, *et al.*, 2015). At 37°C, femoral vein will be injected with 1000 IU of heparin, followed by a perfusion for 20-minute at flow rate 30 ml per minute with the calcium-free 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (1% bovine serum albumin fraction V). Liver will be expanding during this phase lead to change in color of liver progressively from dark red to greyish white. Enlarged liver will be perfused by a 50 ml Trypsin phosphate versene glucose solution, follow by a 20-minute perfusion with calcium-free HEPES buffer (collagenase solution (0.075%)+calcium chloride). Transfer this cell suspension into a conical flask (sterile) followed by agitation for 5 minutes with a magnetic stirrer to release hepatocytes into the solution. Filter the cell suspension through a nylon mesh (250 mesh) and centrifuge at 1000 rpm for 15 minutes. Remove the supernatant and carefully resuspend the loosely packed cell pellet in calcium-free HEPES buffer. This washing process should be repeated by three times. The Trypan blue dye exclusion method is used to determine cell viability. Ham's F 12 medium (10% newborn calf serum+antibiotics+10-6M dexamethasone+10-8 bovine insulin) is used to grow hepatocytes. Incubate the hepatocytes in humidified incubator at 37°C for 30 minutes

**Parameter to be measured:** Aspartate Amino Transferase (ASAT), Alanine Amino Transferase (ALAT), Alkaline Phosphatase (ALP), Triglycerides (TGL), Total protein, Albumin, Total Bilirubin (TB), Lactate Dehydrogense (LDH)

**Primary human hepatocytes culture (Galisteo M, *et al.*, 2006):** All buffers are created in a sterile atmosphere

- Perfusion buffer I-Hank's Balanced Salt Solution (HBSS, without $Ca^{2+}$ and $Mg^{2+}$)
- Perfusion buffer II-Hank's Balanced Salt Solution HBSS (with $Ca^{2+}$ and $Mg^{2+}$)

- Perfusion buffer II plus collagenase II: Collagenase II (1000 U)+300 ml Perfusion buffer II

The solution should be used within 30 minutes of being prepared. At the moment of perfusion, the solution should be warm.

***Prepare William's complete medium:*** William medium+L-Glutamine (2 mM)+Fetal bovine serum (5%)+Insulin (100 mM)+Dexamethasone (100 mg)+Penicillin (100 IU/ML)+Streptomycin (100 mg/ml). Warm these buffers in a water bath for 30 minutes at 42°C, giving in a 37-degree Celsius output temperature at the cannula.

***Isolation of liver by rat perfusion method:***

- Perfusion system made up of a peristaltic perfusion pump, silicone tubing and water bath. The peristaltic perfusion pump's flow rate should be set at 10 ml per min.
- Rat (300 g) is anaesthetized with ketamine+xylazine from the route intraperitoneal (ip). To assess anesthetic depth, press your toes together, abdominal hair will be shaved and treat abdomen with betadine+ethanol when the rat no longer reacts to the unpleasant stimuli. A midline incision is needed to get access.
- Viscera should be shift right outside of the abdominal cavity and in exposed hepatic portal vein introduce 18-gauge angiocath in exposed hepatic portal vein.
- Connect the perfusion tube to the needle at the low flow rate (10 ml per minute) and begin the infusion *in situ* with Perfusion Buffer I that has been pre-warmed (37°C).
- The liver should pale almost instantly if done correctly. When successful cannulation is confirmed, efflux will be done by incision in the Inferior Vena Cava (IVC). Another test for good cannulation is applying mild pressure to the IVC with a sterile swab; liver begin to expand from all lobes quickly. It is recommended that the flow rate be raised to 25 mL/min. The color of the liver should lighten.
- Keeps the flow flowing for another 6 minutes by switching to Perfusion buffer II+collagenase II.
- At the interval of 5 sec apply pressure to the IVC on a regular basis through the help of cotton swab. As liver swells, hepatic cell dissociation improves, shortening total digesting time and boosting final output.
- After collagenase perfusion, liver should be look mushy. Under tissue cell culture hood, Liver will be dissected and liver will be placed in a sterile beaker which is pre-chilled having William's complete medium.

***Isolation of hepatocyte cell:***

- Within the cell culture hood gently scatter the cells with in a sterile Petri plate into William's complete medium with a cell scraper.
- To eliminate connective tissues and undigested tissue bits, 100 mm pore size cell strainer will be used to strain the cell dispersion into a 50 ml conical tube.
- A 40-millilitre cell suspension will be centrifuged for 3 minutes at 50 g × at 4°C in William's complete medium.
- Supernatant should be removed and cell should be gently suspended in 40 mL. Wash the cells in cold William's complete medium and then again centrifuge the suspension.
- Supernatant should be removed from the cells, then carefully suspend them in 25 mL of William's complete medium. Fill the tube halfway with PBS and gently mix in 25 mL of 90 percent Percoll solution.
- At 4°C, centrifuge 200 g for 10 minutes. The living cells are on the bottom of the Percoll gradient have two layers-bottom layer contain living cells and top layer contain dead cells.
- Centrifuge the 20 mL warm medium, which is used to re-suspend the cell pellet. Washing of cell should be done with 30 mL William's complete warm medium.
- Hemocytometer is used for counting of cells in the cell suspension and evaluate trypan blue staining for cell viability.

**Human hepatocyte culture:**

- Preferred concentration of dilute cells should be put in warm William's complete medium. Cell culture plates are used for plating cell in the preferred volume. Hepatic cell cultures can be grown on uncoated plates.
- Cell density should reach 60-70 percent confluence in a normal prep with >85 percent viable cells, allowing for interaction of cells whereas hepatocytes develop upto maximum cell size.
- Put plates for 30 minutes in the cell culture hood without disturbances and then put them in the incubator to ensure an even monolayer of hepatocytes or to prevent cells from congregating in the well's center.
- Cells culture at 37°C within environment having 5% carbon dioxide and 95% air. After 4 hours of growth, there is continues growth in cells during the same serum-containing medium otherwise be moved to a serum-free medium. When cells are cultured in a serum-free media, they keep their morphology while avoiding the deleterious effects of hormones.
- Allow cells to recuperate and develop for at least one night before attempting an experiment. We recommend cells intended meant for testing in 24 hours, since they preserve enzyme activity.
- Allow at least one night for cells to recover and develop before performing an experiment. We recommend testing the cells within 24 hours of receiving them, since this may help preserve enzyme performance.

***Parameter measured:*** Lactate Dehydrogense (LDH), Growth Stimulation Hormone (GSH)

**Immoralised cell lines:** On a regular basis, HepG2 cells will be grown and subculture as monolayers into DMEM (10% newborn calf serum) (Al-Naqeeb MA, *et al.*, 2003). Before being used into the experiments, the cells in this study were batch cultivated for 10 days. The cells are extracted and plated in 96 well Nunclon microtitre plates at 30,000 cells per well. After that, let it rest for 24 hours at 37°C within $CO_2$ (5%) humidified environment. After that, cells are treated with a toxicant. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction test is used to detect cytotoxicity by measuring the vitality of HepG2 cells at the end of the study. After 1 hour of incubation, the test solution from each well is sucked out and replaced with 50 ml of MTT produced in Minimum Essential Medium (MEM) without phenol red. The plates are gently shaking before being incubated at 37°C for 3 hours in a humidified 5 percent $CO_2$ environment. After removing the supernatant, the plates were gently agitated with 50 mL propanol to dissolve the formazan that had formed. The absorbance at 540 nm is measured using a microplate reader.

***Parameters:*** Lipid Peroxidation (LPO), Aspartate Amino Transferase (ASAT), Alanine Amino Transferase (ALAT), Growth stimulating hormone.

## DISCUSSION

### *Potential herbal drugs for hepatoprotection*

Some of the potential medicinal plants having hepatoprotective properties that have been reported are mentioned in *Table 1*.

**Table 1: List of reported hepatoprotective activity of some medicinal plants**

| S. No. | Name | Synonym | Biological source | Part used | Chemica constituents | Reported action | References |
|---|---|---|---|---|---|---|---|
| 1 | Antamool | *Asclepias asthmatica* L. | *Tylophora indica* (Burm. f.) Merr. (Apocynaceae) | Dried leaves | Quercetin, α- and β-amyrins, cetyl-alcohol, phytosterol, resin, pigments, glucose, wax | Anticancer, Antioxidant, Antiasthmatics, Antiallergic, Hepatoprotective, | Sharma MM, *et al.*, 2013 |
| 2 | Amla | *Diasperus nanus, P. niruoides* | *Phyllanthus amarus* Schum. Et. Thonn. | Dried leave | Vitamin C, Phyllanthin, Hypophyllanthin, Corilagin | Liver disorder, Kidney stones | Pramyothin P, *et al.*, 2007 |
| 3 | Amaltas | *Bactyrilobium fistula, C. bonlandiana, C. excelsa, C. fistula* | *Cassia fistula* L. (Fabaceae) | Fresh fruit | Oxalic Acids, Tannins, Oxyanthraquinones, Anthraquinone derivatives | Anti-inflammatory Antioxidant, Antidiabetic, Hepatoprotective, Antimicrobial | Das S, *et al.*, 2008 |
| 4 | Arjuna | *Terminalia cuneata, Chuncoa glabra, Pentaptera angustifolia* | *Terminalia arjuna* Roxb. (Combretaceae) | Bark | Tannin, Triterpenoids, Phytosterol, Gallic acid | Cardio protective action, used in cirrhosis of liver | Doorika P and Ananthi T, 2012 |
| 5 | Asian Spiderflower | *Arivela viscosa, C. acutifolia, C. icosandra, P. viscosa, Sinapistrum visosum* | *Cleome viscosa* Linn. (Capparaceae) | Whole plant | Terpenes, Flavonoids, Phenol carboxylic acid, Polyphenols | Antimicrobial activity, Antitumor, Analgesic, Antiemetic, Antitumor, Anticonvulsant, Hepatoprotective activity | Meera R, *et al.*, 2009 |
| 6 | Basil | *Ocimum album, O. anisatum, O. nigrum* | *Ocimum basilicum* L. (Lamiaceae) | Dried leaves | Essential oils, Linalool, Methyl chavicol | Headache, Cough, diarrhoea, constipation, Hepatoprotective action | Gilani AU and Janbaz KH, 1995 |
| 7 | Berberry | *Berberis afghanica, B. heteracantha, B. angustifolia, B. vulgari* | *Berberis lyceum* Royle. (berberidaceae) | Whole plant | Alkaloids, Tartaric acid, Citric acid | Anti-inflammatory action, Immunomodulatory action, Dental plaque, Hepatoprotective action | Singh B, *et al.*, 2001 |
| 8 | Bhringaraj | *Verbesina prostrate, E. undulata, C. oederi* | *Eclipta alba* L. (Asteraceae) | Leaves | Tridecanol, c-sitosterol, Oleic acid, Eicosyl ester, Cyclocholestan, Octadecenoic acid, Dodecanoic acid | Anti-inflammatory, antioxidant, Antidiabetic, Hepatoprotective | Gupta NK and Dixit VK, 2009 |
| 9 | Bitter leaf | *Verninia randii, V. giogii, V. vogeliana, V. weisseana* | *Verninia amygdalina* Del (Asteraceae) | Dried leaves | Flavonoids, Alkaloids, Steroids, Serpenoids, Glycosides, Tannins, Phenols, Saponins, and the absence of Anthraquinones | Antioxidant, Antidiabetic, Anti-allergic, Anti-inflammatory, Anticancer, Antimicrobial, Antimalarial, Antifertility, Antifungal, Antibacterial, Hepatoprotective action | Adesanoye OA and Farombi EO, 2010 |
| 10 | Black creeper | *Apocynum frutescens, Echites frutescens, Quirivelia frutescens* | *Ichnocarpus frutesecens* L. (Apocynaceae) | Whole plant | Polyphenols, Terpenoids, Alkaloids, Phytosterols, Carbohydrates, Coumarins, Glycosides, Flavonoids, Saponins, Anthroquinones and Steroids | Antidiabetic, Antimicrobial, Wound healing activity, Hepatoprotective activity, Anti-inflammatory, Analgesic Antipyretic, Antiurolithiatic, Antitumor activity | Dash DK, *et al.*, 2007 |
| 11 | Black night shade | *Solanum americanum* | *Solanum nigrum* L. (Solaneaceae) | Dried leaves | Polyphenols, anthocyanidin, Gentisic acid, Luteolin, Apigenin, Kaempferol, and m-coumaric acid | Anticancer, Immunomodulatory, Antimicrobial, Anti-convulsant, Hepatoprotective | Liu FP, *et al.*, 2016 |
| 12 | Chicory | *Cichorium caeruleum, C. hirsutum, C. glabratum, C. officinale* | *Cochorium intybus* L. (Asteraceae) | Seeds | Inulin, Coumarins, Flavonoids, Sesquiterpenoids, Triterpenoids, Steroids, Organic acid | Antimicrobial, Anthelmintic activity, Antimalarial, Hepatoprotective, Antidiabetic | Pandey A, *et al.*, 2011 |
| 13 | Chirata | *Swertia angustifolia, S. chinensis, S. japonica, S. cordata* | *Swertia chirata* L. (Gentianaceae) | Dried leaves | Glycoside-Amarogentin, Gentio-pectin | Liver disorder, Eyes, Heart remedy, Stomach bloating | Mukherjee S, *et al.*, 1997 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 14 | Corrinder | *Corriandrum sativum* | *Corriandrum sativum* L. (Apiaceae) | Dried leaves | Decenoic acid, Capric acid, Linalool, Terpenoids | Antioxidant, Sedative-Hypnotic activity, Anticonvulsant, Diuretic activity, Hepatoprotective activity | Roy CK, *et al.*, 2006 |
| 15 | Green cress | *Arabis chinensis, Cardamum sativum, Crucifera nasturtium, Lepia sativa, Nasturtium crispum* | *Lepidium sativum* L. (Brassicaceae) | Seeds | Fatty acid, Beta Sitosterol, Alpha Sitosterol, Tocopherol | Asthma bronchitis, Cough, Liver aliments | Sultana S, *et al.*, 2005 |
| 16 | Guava | *Guajava pyriferra* L. | *Psidium guava* L. (Myrtaceae) | Fruit dried leaves | Terpenoid, Gujanoic acid, Oleanolic acid, Quercetin | Antioxidant, hepatoprotection, Antiallergy, Antimicrobial, Antigenotoxic, Antispasmodial, Cytotoxin, Cardioactive, Anticough, Antidiabetic, Anti-inflammatory | Abuelgasim AI, *et al.*, 2008 |
| 17 | Jatamansi | *Fedia grandiflora, Nardostachys chinensis, P. jatamansi, Valeriana jatamansi* | *Nordostochys jatamansi* DC (Caprifoliaceae) | Rhizomes | Jatamansone, Jatamanshic acid | Epilepsy, Hysteria, Convulsions, Antiarrhythmic flitter | George M, *et al.*, 2016 |
| 18 | Kanak champa | *Cavanilla acerifolia, Dombey acerifolia, Pentapetes acerifolia, Pterospermadendron acerifolium* | *Pitrospermum acerifolium* L. (Sterculiaceae) | Dried leaves | Methyl protocatechuate, Vanillic acid, Protocatechuic acid, β-sitosterol-3-O-β–D-glucoside | Anti-inflammatory and Analgesic, Antiulcer, Wound healing, Anthelmintic activity, Hepatoprotective | Verma PC, *et al.*, 2009 |
| 19 | Kapur-kachri | *Gandasulium spicatum, H. acuminatum, H. album, G. sieboldii* | *Hedychium spicatum* Buch. ham (Zingiberaceae) | Rhizomes | Alpha pinene, Beta pinene, Limonene,1,8 cineole,2-Alkanones, Linalool, Camphore | Nausea, Bronchial asthma, Halitosis, Vomiting, Liver complaints | Chattopadhyay RR, *et al.*, 1992 |
| 20 | Kutki | *Gandasulium spicatum, H. acuminatum, H. album, G. sieboldii* | *Picchoriza kurrao* Royle (Serophulariaceae) | Rhizome | Glycoside-Kutkin, Picroside, Leithoside, Glucose, Wax, Cathritic acid | Therapy for liver and lung disease, Antioxidant, Immunomodulating agent | Al-Razzuqi R, *et al.*, 2012 |
| 21 | Licorice | *Glycyrrhiza glandulifera* | *Glycyrrhiza glabra* L. (Fabeceae) | Root | Glycyrrhizin, Glycyrrhizic acid, Liquiritin, isoliquiritin, hispaglabridin A and B | Antitussive, Expectorant, Antimicrobial, Anticoagulant, Memory enhancing activity, Antiulcer Antioxidant, Anti-inflammatory Anticarcinogenic, Antimutagenic, Hepatoprotective, | Vargas-Mendoza N, *et al.*, 2014 |
| 22 | Milk thistle | *Carduus marianus L.* | *Silybum marianum* L. (Asteraceae) | Root rhizome | Silybin. Isosilybin, Silydianin, Quercetin, Betain | Antidiabetic, Hepatoprotection, Hypocholesterolaemic, Antihypertensive, Anti-inflammatory, Anticancer, Anti-viral, Cardio protective activity | Choudhary GK and Singh SP, 2018 |
| 23 | Neem | *Melia azadirachta, Antelaea azadirachta, M fraxinifolia, M. japonica* | *Azadirachta indica* (Meliaceae) | Dried leaves | Azadirachtin Quercetin, Catechins, Carotenes and Vitamin C | Antimalarial activity Antifungal activity Antibacterial activity, Antioxidant, Hepatoprotective | Chen RR, *et al.*, 2020 |
| 24 | Noni | *Morinda angustifolia, M. aspera, P. chrysorhiza, S. citrifolia* | *Morinda citrifolia* L. (Rubiaceae) | Fruit juice | Aspartic acid, Glutamic acid and Isoleucine | Antibacterial, Antiviral, Antifungal, Antitumor, Hepatoprotective, Analgesic | Al-Razzuqi R, *et al.*, 2012 |
| 25 | Turmeric | *Curcuma domenstica* | *Curcuma longa* L. (Zingiberaceae) | Rhizome | Curcumin, Demethoxycurcumin and Curcuminoids | Anti-inflammatory, Antioxidant, Anti-cancer, Antidiabetic, Antifungal, Antiprotozoal, Wound healing | Sadashiva CT, *et al.*, 2019 |

### *Allopathic drugs for hepatoprotection*

Drugs available in market for hepatoprotection are Viboliv, Metadoxil, Alcoliv, Hepatoz, Prohep Forte, Hepsi and Osilma etc. Hepatotoxicity is most typically manifested as liver malfunction or damage caused by an excessive amount of medicines or xenobiotics. Hepatotoxicants are exogenous substances that cause liver harm, such as medicine overdose and industrial chemicals. Although the specific mechanism of drug-induced liver injury induced by drug is uncertain, it appears to entail two paths: Direct hepatotoxicity and indirect hepatotoxicity. Hepatotoxin showing direct hepatotoxicity is carbon tetrachloride, thioacetamide, acetaminophen, galactosamine, fulvine, phalloidin, ethyl alcohol, flatoxins. Hepatotoxins showing indirect hepatotoxicity include methyl testosterone, chlorpropamide, tetracycline, halothane, phenytoin, methyldopa and sulphonamide. For the treatment of liver problems, a limited number of contemporary drugs are available (Gupta K, *et al.*, 2019). In the early stages of acetaminophen intoxication, N-acetylcysteine is used. During valproate toxicity, L-carnitine may be beneficial. Pruritus can be relieved with cholestyramine. Ursodeoxycholic acid reduces absorption of intestine and inhibits synthesis of cholesterol and storage. Ursodeoxycholic acid is primarily utilized into the treatment related with chronic disorder of hepatic system. Chelates of penicillamine include various metals that eliminates through the kidneys. Other medications include: Antiviral drugs such as steroids, antibiotics and many more are used to treat liver disorders. Many of today's drugs have severe side effects. Synthetic medicine has a wide range of interactions, contra-interactions, side effects, and toxicity including vomiting, fatigue, dry mouth, insomnia diarrhoea, constipation, dizziness, anemia depression, seizures, swelling, high blood sugar, impotency, fainting confusion, and finally lead to death. Many antibiotics used for hepatoprotection are frequently associated with stomach distress or allergic responses. Interferon causes flu-like symptoms with fever and body aches as a side effect. Due to severe side effects of synthetic drugs, herbal treatment has been widely accepted for liver hepatoprotection.

### *Advantages of marketed herbal drugs for hepatoprotection*

Herbal medications are more extensively employed as hepatoprotective agents than allopathic pharmaceuticals because they are less expensive, have a higher cultural acceptance, are more compatible with the human body, and have fewer negative effects. Liv-52, Livergen, Octogon, Stimuliv, Tefrolivare few examples of herbal formulation available in market for hepatoprotection with minor side effects like dizziness, nausea, constipation, headache. Liver injury therapies have recently received a lot of attention due to the toxic effects of several allopathic drugs that cause liver damage. As a result of their low toxicity and high healing effectiveness, researchers have focused their attention on herbs showing traditional use in hepatoprotective activity in liver showing toxicity. Investigation of several folk medicine for their hepatoprotective activity are done on animal experiments. Traditional treatments have been used to benefit from herbal medicine's positive effects on human health. Researchers are working hard to uncover new sources of hepatoprotective substances because of the hazards involved with manufactured drugs. The bulk of hepatoprotective drugs on the market for treating various forms of liver disorders have been derived from plants in recent years, in the form of single plant preparations or poly herbal mixtures. Rural population in developing countries considered Folkloric herbs essential for health care for quality of life. The pharmacological ingredients of many herbal remedies have yet to be fully discovered. The majority of commercially available formulations contain sulphur-containing amino acids, which are thought to prevent lipid peroxidation. Treatment with allopathic medications is still haphazard, and there is no systematic treatment available. Sulphur-containing amino acids, which are expected to prevent lipid peroxidation, are found in the majority of commercially available formulations. Allopathic pharmaceutical treatment is still random, and no systematic treatment is available (Ahmad AS and Sharma R, 2020). Herbal medications are in significant demand for basic healthcare in both developed and developing nations because to their broad biological and therapeutic activity, better safety margins, and lower cost (Kshirsagar A, *et al.*, 2008).

## CONCLUSION

Hepatotoxicity is a major public health problem. Because herbal treatments are less costly, have a greater cultural acceptance, are more compatible with the human body, and have less side effects, they are more often preferred as hepatoprotective agents than allopathic pharmaceuticals. New sources of hepatoprotective chemicals are being sought by researchers. Many herbal treatments are yet to be scientifically explored in terms of their pharmacological components. This study delves into the phytoconstituents and pharmacological effects of plants found to have hepatoprotective qualities. The purpose of this study is to evaluate the potential for hepatoprotection of numerous indigenous medicinal herbs.

## REFERENCES


1. Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol. 2019; 70(1): 151-171.
2. Anand H, Nulty J, Dhawan A. Cell therapy in congenital inherited hepatic disorders. Best Pract Res Clin Gastroenterol. 2022; 56: 101772.
3. Acharya SK, Madan K, Dattagupta S, Panda SK. Viral hepatitis in India. Natl Med J India. 2006; 19(4): 203-217.
4. Schuppan D, Afdhal NH. Liver cirrhosis. Lancet. 2008; 371(9615): 838-851.
5. Powell EE, Jonsson JR, Clouston AD. Steatosis: Co-factor in other liver diseases. Hepatology. 2005; 42(1): 5-13.
6. Zollner G, Trauner M. Mechanisms of cholestasis. Clin Liver Dis. 2008; 12(1): 1-26.
7. Shivaraj G, Prakash D, Vinayak H, Avinash M, Sonal V, Shruthi K. A review on laboratory liver function tests. Pan Afr Med J. 2009; 12(6):17-23.
8. Limdi JK, Hyde GM. Evaluation of abnormal liver function tests. Postgrad Med J. 2003; 79(932): 307-312.
9. Bhakuni GS, Bedi O, Bariwal J, Deshmukh R, Kumar P. Animal models of hepatotoxicity. Inflamm Res. 2016; 65: 13-24.
10. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of hepatotoxicity. Toxicol Sci. 2002; 65(2): 166-176.
11. Thapa BR, Walia A. Liver function tests and their interpretation. Indian J Pediatr. 2007; 74: 663-671.
12. Guguen-Guillouzo C, Guillouzo A. General review on *in vitro* hepatocyte models and their applications. Methods Mol Biol. 2010; 640(2); 1-40.
13. Mohamed MA, Eldin IM, Mohammed AE, Hassan HM. Effects of *Lawsonia inermis* L.(Henna) leaves' methanolic extract on carbon tetrachloride-induced hepatotoxicity in rats. J Intercult Ethnopharmacol. 2016; 5(1): 22.
14. Ahmad F, Tabassum N. Experimental models used for the study of antihepatotoxic agents. J Acute Dis. 2012; 1(2): 85-89.
15. Mahadevan SB, McKiernan PJ, Davies P, Kelly DA. Paracetamol induced hepatotoxicity. Arch Dis Child. 2006; 91(7): 598-603.
16. Lebda MA, Taha NM, Korshom MA, Mandour AE-WA, Goda RL. Ginger (*Zingiber officinalis*) potentiate paracetamol induced achronic hepatotoxicity in rats. J Med Plants Res. 2013; 7(42): 3164-3170.
17. Rezaie A, Fazlara A, Karamolah MH, Shahriari A, Zadeh HN,

Pashmforosh M. Effects of *Echinacea purpurea* on hepatic and renal toxicity induced by diethylnitrosamine in rats. Jundishapur J Nat Pharm Prod. 2013; 8(2): 60.

18. Rao CV, Rawat AK, Singh AP, Singh A, Verma N. Hepatoprotective potential of ethanolic extract of *Ziziphus oenoplia* (L.) Mill roots against antitubercular drugs induced hepatotoxicity in experimental models. Asian Pac J Trop Med. 2012; 5(4): 283-288.
19. Xu JY, Su YY, Cheng JS, Li SX, Liu R, Li WX, *et al.* Protective effects of fullerenol on carbon tetrachloride-induced acute hepatotoxicity and nephrotoxicity in rats. Carbon. 2010; 48(5): 1388-1396.
20. Gulati K, Reshi MR, Rai N, Ray A. Hepatotoxicity: Its mechanisms, experimental evaluation and protective strategies. Am J Pharmacol. 2018; 1(1): 1004.
21. Hemieda FA, Elnga MA. Biochemical and histological studies on H2-receptor antagonist ranitidine-induced hepatotoxicity in rats. Indian J Exp Biol. 2005; 43(9): 782-785.
22. Akhtar T, Sheikh N. An overview of thioacetamide-induced hepatotoxicity. Toxin Rev. 2013; 32(3): 43-46.
23. Behairy A, Elkomy A, Elsayed F. Liquorice (*Glycyrrhiza glabra*) hydroethanolic extracts effectively alleviate thioacetamide-induced hepatotoxicity and hematotoxicity in experimental rats through their antioxidant nature. Int J Pharmacol Toxicol. 2021; 9(1): 10-16.
24. Číž M, Čížová H, Denev P, Kratchanova M, Slavov A, Lojek A. Different methods for control and comparison of the antioxidant properties of vegetables. Food Control. 2010; 21(4): 518-523.
25. Ramboer E, de Craene B, de Kock J, Berx G, Rogiers V, Vanhaecke T, *et al.* Development and characterization of a new human hepatic cell line. EXCLI J. 2015; 14: 875-889.
26. Galisteo M, Suárez A, Montilla MP, Fernandez MI, Gil A, Navarro MC. Protective effects of *Rosmarinus tomentosus* ethanol extract on thioacetamide-induced liver cirrhosis in rats. Phytomedicine. 2006; 13(1-2): 101-108.
27. Al-Naqeeb MA, Thomson MA, Al-Qattan K, Kamel FA, Mustafa TA, Ali MU. Biochemical and histopathological toxicity of an aqueous extract of ginger in female rats. Kuwait J Sci Eng. 2003; 30(2): 35-48.
28. Sharma MM, Verma RN, Jamal SM, Batra A, Rao DV. An anti-asthmatic plant: *Tylophora indica* burm. F. merrill-A review. Agric Rev. 2012; 33(3): 192-201.
29. Pramyothin P, Ngamtin C, Poungshompoo S, Chaichantipyuth C. Hepatoprotective activity of *Phyllanthus amarus* Schum. et. Thonn. extract in ethanol treated rats: *In vitro* and *in vivo* studies. J Ethnopharmacol. 2007; 114(2): 169-173.
30. Das S, Sarma G, Barman S. Hepatoprotective activity of aqueous extract of fruit pulp of *Cassia fistula* (AFCF) against carbon tetrachloride ($CCL_4$) induced liver damage in albino rats. J Clin Diagn Res. 2008; 2(5): 1133-1138.
31. Doorika P, Ananthi T. Antioxidant and hepatoprotective properties of *Terminalia arjuna* bark on isoniazid induced toxicity in albino rats. Asian J Pharm Tech. 2012; 2(1): 15-18.
32. Meera R, Devi P, Kameswari B, Madhumitha B, Merlin NJ. Antioxidant and hepatoprotective activities of *Ocimum basilicum* Linn. and *Trigonella foenum-graecum* Linn. against $H_2O_2$ and $CCl_4$ induced hepatotoxicity in goat liver. Indian J Exp Biol. 2009; 47(7): 584-590.
33. Gilani AU, Janbaz KH. Preventive and curative effects of *Berberis aristata* fruit extract on paracetamol-and $CCl_4$-induced hepatotoxicity. Phytother Res. 1995; 9(7): 489-494.
34. Singh B, Saxena AK, Chandan BK, Agarwal SG, Anand KK. *In vivo* hepatoprotective activity of active fraction from ethanolic extract of *Eclipta alba* leaves. Indian J Physiol Pharmacol. 2001; 45(4): 435-441.
35. Gupta NK, Dixit VK. Evaluation of hepatoprotective activity of *Cleome viscosa* Linn. extract. Indian J Pharmacol. 2009; 41(1): 36.
36. Adesanoye OA, Farombi EO. Hepatoprotective effects of *Vernonia amygdalina* (astereaceae) in rats treated with carbon tetrachloride. Exp Toxicol Pathol. 2010; 62(2): 197-206.
37. Dash DK, Yeligar VC, Nayak SS, Ghosh T, Rajalingam R, Sengupta P, *et al.* Evaluation of hepatoprotective and antioxidant activity of *Ichnocarpus frutescens* (Linn.) R. Br. on paracetamol-induced hepatotoxicity in rats. Trop J Pharm Res. 2007; 6(3): 755-765.
38. Liu FP, Ma X, Li MM, Li Z, Han Q, Li R, *et al.* Hepatoprotective effects of *Solanum nigrum* against ethanol-induced injury in primary hepatocytes and mice with analysis of glutathione S-transferase A1. J Chin Med Assoc. 2016; 79(2): 65-71.
39. Pandey A, Bigoniya P, Raj V, Patel KK. Pharmacological screening of *Coriandrum sativum* Linn. for hepatoprotective activity. J Pharm Bioallied Sci. 2011; 3(3): 435.
40. Mukherjee S, Sur A, Maiti BR. Hepatoprotective effect of *Swertia chirata* on rat. Indian J Exp Biol. 1997; 35(4): 384-388.
41. Roy CK, Kamath JV, Asad M. Hepatoprotective activity of *Psidium guajava* Linn. leaf extract. Indian J Exp Biol. 2006; 44(4): 305-311.
42. Sultana S, Perwaiz S, Iqbal M, Athar M. Crude extracts of hepatoprotective plants, *Solanum nigrum* and *Cichorium intybus* inhibit free radical-mediated DNA damage. J Ethnopharmacol. 1995; 45(3): 189-192.
43. Abuelgasim AI, Nuha HS, Mohammed AH. Hepatoprotective effect of *Lepidium sativum* against carbon tetrachloride induced damage in rats. J Anim Vet Adv. 2008; 1(3): 20-23.
44. George M, Joseph L, Deshwal N, Joseph J. Hepatoprotective activity of different extracts of *Pterospermum acerifolium* against paracetamol induced hepatotoxicity in albino rats. Pharm Innov J. 2016; 5(3): 32-36.
45. Verma PC, Basu V, Gupta V, Saxena G, Ur Rahman L. Pharmacology and chemistry of a potent hepatoprotective compound Picroliv isolated from the roots and rhizomes of *Picrorhiza kurroa* royle ex benth.(kutki). Curr Pharm Biotechnol. 2009; 10(6): 641-649.
46. Chattopadhyay RR, Sarkar SK, Ganguly S, Banerjee RN, Basu TK, Mukherjee A. Hepatoprotective activity of *Azadirachta indica* leaves on paracetamol induced hepatic damage in rats. Indian J Exp Biol. 1992; 30(8): 738-740.
47. Al-Razzuqi R, Al-Jawad FH, Al-Hussaini JA, Al-Jeboori A. Hepatoprotective effect of *Glycyrrhiza glabra* in carbon tetrachloride-induced model of acute liver injury. J Phys Pharm Adv. 2012; 2(7): 259-263.
48. Vargas-Mendoza N, Madrigal-Santillán E, Morales-González Á, Esquivel-Soto J, Esquivel-Chirino C, y González-Rubio MG, *et al.* Hepatoprotective effect of silymarin. World J Hepatol. 2014; 6(3): 144-149.
49. Choudhary GK, Singh SP. *In vitro* hepatoprotective efficacy of extract of *Hedychium spicatum* rhizome in paracetamol induced toxicity in HepG2 cell line. Indian J Anim Sci. 2018; 88: 546-549.
50. Chen RR, Liu J, Chen Z, Cai WJ, Li XF, Lu CL. Anthraquinones extract from *Morinda angustifolia* roxb. root alleviates hepatic injury induced by carbon tetrachloride through inhibition of hepatic oxidative stress. Evid Based Complement Alternat Med. 2020.

51. Sadashiva CT, Hussain HF, Nanjundaiah S. Evaluation of hepatoprotective, antioxidant and cytotoxic properties of aqueous extract of turmeric rhizome (Turmesac). J Med Plants Res. 2019; 13(17): 423-430.
52. Gupta K, Taj T, Thansiya B, Kamath JV. Pre-clinical evaluation of hepatoprotective activity of phytol in wistar albino rats. IP Int J Compr Adv Pharmacol. 2019; 4(1): 17-20.
53. Ahmad AS, Sharma R. Comparative analysis of herbal and allopathic treatment systems. Eur J Mol Clin Med. 2020; 7(7): 2869-2876.
54. Kshirsagar A, Vetal Y, Ashok P, Bhosle P, Ingawale D. Drug induced hepatotoxicity: A comprehensive review. Internet J Pharmacol. 2008; 7: 1.