EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 5
597-624
Year: 2020

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF BARK OF MANGIFERA INDICA AGAINST INH AND RIF INDUCED HEPATOTOXICITY IN RATS

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Article Received on 10/03/2020 **Article Revised on 30/03/2020** **Article Accepted on 19/04/2020**

1. ABSTRACT

The present study was undertaken to evaluate the hepatoprotective activity of methanol extract of bark of Mangifera indica. It was evaluated in normal and Isoniazid (INH), Rifampicin (RIF) induced hepatotoxicity in rats. Liv.52 was used as a standard drug at a dose of 500mg/kg body weight given in oral route. Albino wistar rats with Isoniazid and Rifampicin induced hepatotoxicity were divided into 4 groups of 6 each. Hepatoprotective effect as two different doses 200 and 400 mg/kg body weight of methanolic extract of Mangifera indica would be investigated for 28 days to evaluate dose dependent activity. Effect of methanolic extract of Mangifera indica on Serum glutamate pyruvate transaminase, Serum glutamate oxaloacetic transaminase Alkaline phosphatase, total cholesterol, total bilirubin were estimated for 0^{th}, 7^{th}, 14^{th}, 21^{st}, 28^{th} day and antioxidant parameters were checked on 28^{th} day of the scarification

KEYWORDS: Mangifera indica, Albino –Wistar rats, Isoniazid, Rifampicin, Hepatoprotective activity.

1. INTRODUCTION

Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury to it or impairment to its function may leads to complications on one's health.[1] Drug-induced liver injury (DILI) is a major health problem that challenges not only health care professionals but also the pharmaceutical industries and drug regulatory agencies. According to the United States, Drug-induced liver injury (DILI) accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of Acetaminophen (APAP-39%) and idiosyncratic liver injury triggered by other drugs (13%).[2] Because of the significant patient morbidity and mortality associated with DILI, the U.S. Food and Drug Administration (FDA) has removed several drugs from the market including Bromfenac, Ebrotidine, and Troglitazone.[3] Other hepatotoxic drugs such as Risperidone, Trovafloxacin, and Nefazodone, have been assigned "black box" warnings.[4] DILI is the most common cause for the withdrawal of drugs from the pharmaceutical market.[5,6]

The development of new diagnostic and therapeutic methods is regularly improving the management and prognosis of most of the diseases but it is also associated with the occurrence of new iatrogenic diseases. Drug-induced liver injuries were one of the major problems. Liver toxicity is developing and remains the first cause of drug-induced death and withdrawl of drugs from the pharmaceutical market. Despite improvement in toxicological studies and in the safety analysis of clinical trials, the frequency of drug hepatotoxicity has not decreased from the last decade. The spectrum of liver damage caused by drugs is very broad. Indeed, all cells present in the liver can be affected by drugs. Almost the entire spectrum of liver injuries can be reproduced by drugs. This explains the concern of physicians, health authorities and pharmaceutical companies about drug hepatotoxicity.[6]

World Health Organization (WHO) estimates that about 80% of populations living in developing countries rely almost exclusively on traditional medicines for their primary health care needs. Since the medicinal plants are the backbone of traditional medicine, that 3300 million people in the under developed countries utilize medicinal plants on a regular basis. This assumption does not include the developed countries where there have been a great fascination for the herbal medicines and dietary food supplements in the last 10 years.

Due to the toxic and adverse effects of synthetic medicines being observed around the globe, herbal medicine have made a comeback to improve the fulfillment of our present and future health needs.[7]

Consumption of medicinal herbs or herbal preparations is tremendously increasing in order to identify alternative approaches to improve the quality of life and maintain a good health.[9] Developing drugs from natural products may reduce the risk of toxicity and maintain its therapeutic effectiveness, when the drug is used clinically.[8]

Tuberculosis (TB) is one of the most common infectious disease which gradually swallows the life span of human beings. The global prevalence of tuberculosis was 32% (1.86 billion people) and mortality rate was 23%.[9] The number of estimated cases of tuberculosis was 7.96 million in 1997 with 80% of all incident tuberculosis cases being found in 22 countries and more than 40% in five south-east Asian countries. In India, pulmonary tuberculosis is one of the major causes for adult deaths. Isoniazid (is nicotinic acid hydrazide-INH) was the first effective bactericidal drug used to treat tuberculosis and till far, an important part of most antitubercular drug regimens. Rifampicin (RIF), which is another effective bactericidal drug, was added to the regimen in 1962 and has remained the most effective antitubercular combination along with Isoniazid. Although both these drugs are very potent against the tuberculosis bacillus, both are well-known hepatotoxic drugs.[10]

Isoniazid can cause mild to moderate elevation of plasma liver enzyme activity in 10-20% of patients and severe hepatotoxicity in approximately 0.5-2%. The incidence of hepatotoxicity is approximately 2.6% with isoniazid-rifampicin co-administration but only 1.6% with isoniazid treatment alone and 1.1% with rifampicin alone; suggesting the higher incidence of severe hepatotoxicity in patient's co-treated with these two drugs.[11,12] The base of evidence for isoniazid hepatotoxicity is a level of risk in the range of 5 to 20 cases of hepatotoxicity expected per 1000 persons receiving treatment with 1% to 10% cases.[13]

As hepatotoxicity is potentially a serious adverse effect of these currently used anti-tubercular chemotherapeutic regimens, a search for an alternative drug useful for the prophylactic treatment of hepatotoxicity induced by anti-tubercular remains important. Efforts to explore hepatoprotective effect of any natural product thus carry a great clinical significance. Therefore, the present study was undertaken for the evaluation of hepatoprotective activity of methanolic extract of bark of Mangifera indica against Isoniazid and Rifampicin in rats.[6]

1.1. LIVER PHYSIOLOGY

The liver is an important and largest organ. Hepato or Hepaticis a medical term from the Greek word hepar which means liver. It has a wide range of functions which are detoxification, protein synthesis, and production of biochemicals necessary for digestion. The liver is necessary for survival, a human can only last up to 24 hours without liver function.

Liver is divided into two principal lobes, which are large right lobe and smaller left lobe. These two lobes separated by the falciform ligament and each lobes made up of many functional units called lobules, each lobule consist of specialized epithelial cells called hepatic cells or hepatocytes.[14]

These hepatocytes are the biosynthetic engines of the liver which allow synthesizing and secreting an array of proteins and metabolic enzymes. Sinusoid cells are lined by endothelial cells and these act as barrier in between blood and hepatocytes. These sinusoid cells are lined by other two types of cells namely.[15]

a. Kupffer cells
b. Stellatic cells

- Kupffer cells are capable of removing and phagocytosing old and defective blood cells, bacteria and other foreign materials.
- Stellatic cells are responsible for storing fat and vitamin-A.

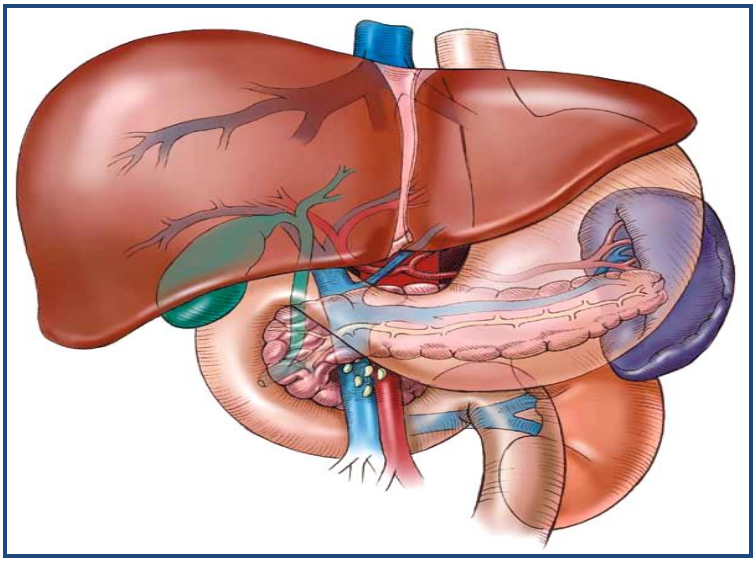


FIG. 1: General Structure of Liver (HUMAN).

One of the liver's most interesting abilities is self-repair and the regeneration of damaged tissues. In clearing the toxins of body, the liver is damaged by exposure to harmful substances. If a failing liver can be supported for a certain period of time, it might regenerate and allows the patient to survive and regain a normal life. The liver's most important task is to filter toxic substances from the body, including alcohol and many other different medications.[16,17]

Table 1: Physiological Functions of Liver.

S.No	Function	Components
1	Hemostatis	Glucose, Proteins, Fat and cholesterol Hormones Vitamins, in particular fat-soluble ones (A, D, E, K)
2	Synthesis	Proteins including the clotting factors (~50g/day) Bile acids (important in fat digestion) Heparin (anti-coagulant) Somatomedins (hormones that promote growth in bone, soft tissues) Estrogen, Angiotensinogen, Cholesterol Acute phase proteins
3	Storage	Vitamins, Glycogen, Cholesterol, Iron, copper, Fats
4	Excretion	Cholesterol, bile acids, phospholipids Bilirubin, Drugs Poisons including heavy metals Hormones
5	Filtration	Poisons Nutrients including amino acids, sugars, and fats, Bilirubin, bile acids IgA, Drugs Dead or damaged cells in circulatory system
6	Immune	Excretes IgA into digestive tract Kupffer cells (macrophages) filter out antigens

1.2. HEPATOTOXICITY

Xenobiotics or toxic chemicals directly or indirectly damage the liver's important cellular functions. So it leads to faster accumulation of toxins in the liver which leads to "Liver damage" called as "Hepatotoxicity".

An obvious sign of hepatic injury is, the liver cell membrane is damaged causing leakage of variety cellular enzymes that are released into the blood stream from cytosol, thereby causing an increased enzyme level in the serum, so it may not able to perform it functions optimally.[18]

Liver damage occurs by either due to direct damage to the liver cells or due to a secondary damage resulting from obstruction of the bile flow. Rarely, it can be due to obstruction to the blood flow either in the portal vein or in the hepatic vein.

Hepatotoxins are the agents which produces the hepatic damage, these are medical agents, chemical agents, natural chemical (e.g.: Microcystins), and herbal remedies.[19,20] Liver damage can be caused by viruses, drugs, systemic diseases and malignancies.[20]

Drug induced liver injury (DILI) is the most common reason for attrition of lead compounds during drug development.[21] Usually, the time of onset of acute injury is within months of initiating a drug. More than 900 drugs, toxins and herbs have been reported to cause liver injury and drugs account for 20-40% of all instances of fulminant hepatic failure.[22,28] Approximately 75% of the idiosyncratic drug reactions result in liver transplantation or death. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures.[2]

Drugs are an important cause of liver injury, several classes of therapeutic drugs shows hepatotoxicity (Table-2), and these affecting metabolism and musculoskeletal systems. Hepatotoxicity associated with drug used accounted for 10.7% in USA, 2.85% in UK, 4.2% in New Zealand and 9.7% in France.[15]

Different class of drugs showing hepatotoxicity are anti psychotic(clozapin)[24], anticancer(Trabectedin)[25], antiepileptic(phenobarbitol, carbamazipine)[26], antifungal (Thiocetamide, Ketoconazole)[27], anti-tubercular drugs (Isoniazid, Rifampicin)[32], NSAIDS(Diclofenac)[33], antipyretics(Paracetmol).[29]

DILI accounts for 7% of reported drug adverse effects, 2% of jaundice in hospitals, and approximately 30% of fulminant liver failure. DILI has replaced viral hepatitis as the most apparent cause of acute liver failure. Food and drug administration (FDA) has withdrawn drugs or mandated relabeling for severe or fatal liver injury exceeding 1 in 50,000 individuals.[34]

1.3. HEPATOTOXICITY AFFECTS ON LIVER ENZYMES

Biochemical markers i.e., serum alanine transferase, serum alkaline phosphatase and bilirubin are used to indicate liver damage.[35] Liver injury is defined as rise in either

a) Alanine transferase (ALT) level more than three times of upper limit of normal (ULN),
b) Alkaline phosphatase (ALP) level more than twice ULN
c) Total bilirubin level more than twice ULN when associated with increased ALT or ALP.

Toxicants produce parenchymal injury, hepatocytes dies because of apoptosis or necrosis.

To altered membrane permeability or fine cell disruption that accompanies necrotic death, cytosolic enzymes (ALT, AST and SAP), 5-nucleotidase and sorbitol dehydrogenase, and occasionally mitochondrial marker enzymes (e.g.: ornithine carbamyltransferase) are released into the blood. The serum glutamyl pyruvate transaminase andserum glutamyl oxalacetic acid transaminaseare elevated above 1000 IU/l in the liver cell damage and the other three enzymes are elevated in bile flow obstruction.[36]

Abnormalities in the level of fats in the blood stream include elevated LDL cholesterol, reduced HDL cholesterol, and elevated triglycerides. Bilirubin, which is metabolic product of hemoglobin, is elevated in liver damage. If the damage is mainly to liver cells the indirect component of bilirubin is increased. If the liver damage is mainly due to bile flow obstruction then the direct component of bilirubin is increased. If both forms of liver damages are present then both the components of bilirubin are increased.

1.4. TYPES OF HEPATOTOXICITY

Drugs or herbal medicines induce several types of hepatic damage, which includes.[15]

2.4.1 Toxic hepatic damage

This type of hepatic damage is initiated by the interaction of the drug or active components of the herb with hepatic cytochrome P_{450} enzymes. These produce electrophilic molecules or oxygen free radicals that interact with the hepatic cell membranes causing the damage or necrosis of the hepatocytes. The elevation of cytosolic ca^{+2} due to presence of drugs results in the damage of the hepatic mitochondria and the membranes integrity.[37,15]

The classical example of toxic hepatic damage is high dose of paracetmol and phenytoin, isoniazid, starvation or chronic alcohol consumption also produces hepatotoxicity.[10,15,38]

2.4.2 Allergic hepatic damage

This type of Hepatic damage caused by antigen formed through the combination of a hapten of the drug or the active constituent of an herb with a specific liver protein is accompanied with skin rash, fever, gastrointestinal upset, jaundice and hepatomegaly.[15]

Several drugs are shown to induce allergic hepatic damage; these include the combination of amoxicillin-clavulanate, sulphonamides, and phenytoin and anti-HIV drugs.[15]

2.4.3 Idiosyncratic hepatotoxicity

Some established drugs produce unpredictable non-allergic hepatotoxicity. This type of reaction has latent period from weeks up to months following use of those drugs. This type of hepatotoxicity differentiates from allergic (reaction) hepatotoxicity by the absence of fever, rash or eosinophilia.[15]

E.g. Isoniazid, valproic acid, ketoconazole, methyldopa, Diclofenac and troglitazone.[31]

2.4.4 Hepatic cholestasis

Synthesis of bile salt is a one of the function of the liver, which is stored and concentrated in gall bladder and secreted into the duodenum. Some drugs interrupt in the bile flow and secretion from the hepatocytes into the gall bladder via binding of the liver bile salts into the liver or by interaction with bile salts transporting proteins. This interruption results in the accumulation of the bile inside the liver, which leads to cholestasis and appearance of high level of bilirubin in the blood and precipitation of jaundice.[15]

This results in damage of hepatocytes due to excess accumulation of toxic bile acids in the hepatocytes because of failure of camlicular and other intracellular processes. Many synthetic drugs produce hepatic cholestasis.[37]

E.g. Chlorpromazine, hydrochlorothiazide, tenoxicam, estrogens, erythromycin, ibuprofen, captopril, prochlorperazine, amoxicillin clavulanate, co-trimoxazole, metformin, lyproterone, pravastatin, tetrahydrocannabinol and parenteral nutrition.[37]

2.4.5 Granulomatous hepatitis

This type of hepatitis is characterized by infiltration of inflammatory cells and granulomatous changes in the hepatic parenchyma and the portal region. It is accompanied with low grade fever and chronic fatigue. In these type small rounded foci of epitheloid cells and round cells with multi nucleated giant cells surrounded by eosinophil's.[15]

It can result due to chronic use of various drugs like, ticlopidine, allopurinol, carbamazepine, aspirin, dicloxacillin, glibenclamide, rosiglitazone, norfloxacin, procainamide, mebendazole, methimazole, phenytoin, hydralazine, diltiazem, methyldopa, sulfonamides, and amoxicillin, clavulanic acid.[15]

2.4.6 Hepatic veno-occlusive Disease

This type of disease obtained from closure of the small intra hepatic veins by loose connective tissues in absence of thrombi, it leads to massive hepatocellular necrosis. It generally observed as hepatomegaly, ascites, jaundice and weight gain. It is usually induced by high dose of anti-cancer drugs such as azathioprine, cyclophosphamide, thioguanine, and combination of cis-platinum-cyclosphamide and dicarbazine.[15]

2.4.7 Hepatic Phospholipidosis

It is a minor hepatic disorder, it can be induced by some Amphiphilic dugs, and accumulate in the lysosome's and interact with their phospholipids and it leads to suppression of lysosomal function which also induces hepatomegaly.[15]

E.g. Amiodarone.

2.4.8 Non-Alcoholic Steatohepatitis

It resembles as fatty liver with inflammation and necrosis in absence of alcohol consumption due to excessive release of free fatty acids from the accumulated hepatic triglycerides and accompanied by an elevation of hepatic aminotransferases and cause hepatomegaly and also accompanied by a decrease in S-adenosyl methionine.[15]

This type of disease observed in obesity, diabetes mellitus type-2 or hyperlipidemias patients. Various drugs induce this type of disease.

E.g. Amineptine and valproate, olanzapine and risperidone, tamoxifen, corticosteroids, amiodarone, zidovudine, didanosine (2, 3-dideoxy inosine) and high dose of tetracycline and aspirin and paracetmol nutrition.

2.4.9 Liver Fibrosis and Cirrhosis

Some drugs directly produce liver fibrosis and cirrhosis without inducing any elevation of hepatic aminotransferases or bilirubin. This generally induced by high dose of methotrexate, methyldopa and CCl_4, vitamin-A. Chronic administration of CCl_4 may leads to cirrhosis.[39]

2.4.10 Liver Tumors

Some drugs involve in inducing carcinogenicity in the liver. The drugs are methotrexate, cyclosporine and nitrofurantoin.[39]

1.5. DIAGNOSIS OF LIVER DAMAGE

The initial steps in diagnosis of the drugs and medicinal herbs-induced hepatic damages are.[40,15]

- Thorough grasping of the patient's medical history that include the types of drugs and/or herbal medicines used together with their doses frequency and duration of their use.
- The medical health of the patient should be considered.
- Hepatic function tests: Various biochemical parameters should be measured. These include measurement of blood levels of bilirubin, hepatic transaminases.

E.g. aspartate aminotransferase (AST) and alanine aminotransferase (ALT) together with α-glutamyl transpeptidase and alkaline phosphatase enzymes.

- Bilirubin (total) - to diagnose jaundice and assess severity
- Bilirubin (unconjugated) - to assess for hemolysis
- Alkaline phosphatase - to diagnose cholestasis and infiltrative disease
- AST/serum glutamate oxaloacetic transaminase (SGOT) - to diagnose hepatocellular disease and assess progression of disease.
- ALT/serum glutamate pyruvate transaminase (SGPT) - ALT relatively lower than AST in persons with alcoholism
- Albumin - to assess severity of liver injury (HIV infection and malnutrition may confound this).
- Gamma globulin - large elevations suggestive of autoimmune hepatitis, other typical increase observed in persons with cirrhosis
- Prothrombin time after vitamin K - to assess severity of liver disease
- Anti-mitochondrial antibody - to diagnose primary biliary cirrhosis

➢ All diseases known to induce hepatotoxicity similar to drugs and medicinal herbs-induced hepatotoxicity such as sarcoidosis and tuberculosis should be excluded.

➢ Performance of serological tests to check the presence and absence of the following antibodies:

- IgM antihepatitis A virus
- Antihepatitis B/and C viruses
- IgM anti-cytomegalovirus
- IgM anti Epstein-Bar virus capsid antigen
- Herpes simplex virus
- Anti-nuclear
- Anti-mitochondrial

➢ Besides these, the presence or absence of hepatitis-B surface antigen and hepatitis-C RNA should be examined.

➢ Performance of ultra-sound guided percutaneous liver biopsy.

➢ If possible the response to re-challenge with the suspected drug should be performed.

There are many possible causes of liver dysfunction other than liver damage. Thus, if the blood test indicates the liver is not functioning properly, additional tests may be conducted to determine the cause of the problem.

2.5.1 Imaging studies

Imaging studies are used to exclude causes of liver pathology, after which a diagnosis can be made.

- **Ultrasonography:** Ultrasonography is inexpensive compared with CT scanning and MRI is performed in only a few minutes. Ultrasonography is effective to evaluate the gall bladder, bile ducts, and hepatic tumors.

- Computed tomography (CT) scanning: CT scanning can help detect focal hepatic lesions 1 cm or larger and some diffuse conditions. It can also be used to visualize adjacent structures in the abdomen.

Magnetic resonance imaging (MRI) Scanning: MRI provides excellent contrast resolution. It can be used to detect cysts, hemangiomas, and primary and secondary tumors. The portal vein, hepatic veins, and biliary tract can be visualized without contrast injections.

2.5.2 Liver Biopsy

A biopsy is a histopathologic evaluation procedure in which a small sample of liver tissue is taken and examined under a microscope. Looking at the tissue is the best way to determine if the cells are healthy or damaged. A liver biopsy is not essential in every case, but a morphologic pattern consistent with the expected pattern provides supportive evidence.

2.5.3 Endoscopic Retrograde Cholangiopancreatography (ERCP)

ERCP is a procedure that helps to determine whether the liver dysfunction is due to blockage of the common bile duct, the tube which carries bile from the liver to the gall bladder. ERCP uses an endoscope, a long, flexible, lighted tube that allows physician to see inside of the stomach and inject dye into the main bile duct, which make the biliary ducts visible on an x-ray.

1.6. PHARMACOLOGICAL MODELS FOR INDUCING HEPATOTOXICITY

Hepatotoxicity occurs due to:

1. Inorganic compounds
2. Organic agents
3. Synthetic compounds

Inorganic compounds producing hepatotoxicity are arsenic, phosphorous, copper, iron.

Organic agents include certain naturally occurring plant toxins such as pyrrolizidine alkaloids, mycotoxins and bacterial toxins.

The synthetic groups of organic compound are a large number of medicinal agents and the exposure to hepatotoxic compounds may be occupational, environmental or domestic.

2.6.1 Carbon tetrachloride induced hepatotoxicity

Carbon tetrachloride (CCl_4) is often used to induce oxidative stress related hepatitis. The reactive metabolites such as trichloromethyl (CCl_3) and trichloromethyl peroxy (CCl_3OO) radicals emanated from CCl_4 initiate peroxidation of membrane unsaturated fatty acids. This lipid peroxidation of membrane seriously impairs its function and produces liver injury. Liver damage induced by CCl_4 is commonly used model for the screening of hepatoprotective drugs.[41]

CCl_4 induces hepatotoxicity depending upon different doses through different routes of administration.

- 0.5 ml/kg body weight gives i.p after 28 days of feeding animals.[42]
- 0.5 ml/kg body weight of 8% CCl_4 administered by intragastric gavages for 4-12 weeks.[39]
- 0.7 ml/kg body weight gives for 10 days in 3 intervals by i.p route.[43]
- 0.8 ml/kg body weight gives twice a week for 60 days by s.c route.[44]
- 10 ml/kg body weight of 0.3% of CCl_4 dilution with olive oil administrated 1 h after dealt with drug on the seventh day of the seven-day study period.[45]
- 15 ml/100 g body weight CCl_4 administered by subcutaneously for 3 day/week.[46]

2.6.2 Galactosamine induced hepatotoxicity

Animals: Male Wister rats.
Dose: 100-400mg/kg IP or IV.

Mechanism of action

- Galactosamine disrupts the synthesis of essential uridylate nucleotides resulting in organelle injury and ultimately cell death. Depletion of nucleotides decreases the protein synthesis.
- This toxicity brings about an increase in cell membrane permeability leading to enzyme leakage and cell death.
- It decreases the bile flow, number of viable hepatocytes and also rate of oxygen consumption.

Parameters assessed

The parameters assessed are:

1. Extent of liver necrosis
2. Immuno reactivity for macrophages, lymphocytes and extracellular matrix.
3. Serum enzymes like SGOT, SGPT

2.6.3 Thiocetamide induced toxicity

Animals: Male Wister rats
Dose: 100mg/kg SC

Mechanism of action

- It interferes with the movement of RNA from the nucleus to the cytoplasm which may cause cell membrane injury.
- A metabolite of Thiocetamide also causes hepatic injury.

Parameters assessed

The parameters assessed are:

1. Volume of bile flow
2. Number of viable hepatocytes

2.6.4 Alcohol induced liver injury

Animals: Female Wister rats.
DOSE: 0.4ml/kg of 1.25% alcohol solution

Mechanism of action

- Metabolism of alcohol causes hepatic lipid peroxidation which leads to alteration in membrane phospholipids composition, resulting the loss of membrane structure and integrity.
- Ethanol inhibits antioxidant enzymes like superoxide dismutase due to the damaging effects of free radicals.
- Alcohol pre-treatment also stimulates the toxicity of CCl_4 due to increased production of toxic reactive metabolites.

Parameters assessed

The parameters assessed are:

1. Necrotic index
2. Focal necrosis
3. Diameter of the necrotic areas

2.6.5 Paracetmol induced hepatotoxicity

Animals: Male Wister rats
Dose: 800mg/kg
1000mg/kg
1200mg/kg
1g/kg PO[88]

Mechanism of action

- Paracetmol administration causes necrosis of centrilobular hepatocytes characterised by nuclear pyknosis and eosinophlic cytoplasm followed by large excessive hepatic lesion.
- The covalent binding of N-Acetyl-p-benzoquinoneimine, an oxidative product of paracetmol to sulfhydryl groups of proteins, result in lipid peroxidative degradation of glutathione producing cell necrosis.

Parameters assessed

The parameters assessed are:

1. SGOT
2. SGPT
3. Histology score

2.6.6 Chloroform induced hepatotoxicity

Chloroform produces hepatotoxicity with extensive central necrosis, fatty metamorphosis, hepatic cell degeneration and necrosis either by inhalation (for 1 h in atmosphere) or by subcutaneous administration, 0.5 ml/kg.

2.6.7 Antitubercular drug induced hepatotoxicity

Animals: Male Wistar rats
Dose: Isonicotinic acid hydrazide(INH) 100mg/kg-IP.
Rifampicin 100mg/kg(122µmol/kg)
Isonicotinic hydrazide + Rifampicin + pyrazinamide 7.5mg/kg+10mg/kg+35mg/kg.

Mechanism of action

- Though is nicotinic acid hydrazide, rifampicin and pyrazinamide each in itself are potentially hepatotoxic, when given in combination their effect is enhanced.
- INH is metabolised to monoacetylhydrazine, which is further metabolised to toxic product by cytochrome P_{450} leading to hepatotixicity.
- Rifampicin induced cytochrome P_{450} enzyme induction causes an increased production of toxic metabolites from acetylhydrazine and also increases the metabolism of INH to is nicotinic acid and hydrazine both of which are hepatotoxic.
- Pyrazinamide decreases the blood level of rifampicin by decreasing its bioavailability and increasing its clearance.
- When given in combination with INH and rifampicin appears to be associated with an increase incidence of hepatotoxicity

Parameters assessed

The parameters assessed are:

1. Glutathione S transferase
2. Glutathione peroxidase
3. Superoxide dismutase

2.6.8 Heavy metal induced hepatotoxicity

Fluoride

Excessive fluoride exposure for a prolonged period can induce chronic fluorosis. Involvement of free radicals, increased generations of oxygen free radicals had enhanced lipid peroxidation which has been shown to play an important role in fluorosis. So oxidative stress causes the hepatic damage on drinking high-fluoride containing (150 mg/L of sodium fluoride (NaF) for 3 months) water over a prolonged period.[47]

Nickel

Nickel is a common respirable sized particulate pollutant and it breaks down the immunity by affecting the T-cell system and suppresses the activity natural killer cells in rats and mice at a dose level of 800 mg/L in drinking water administered for 8 weeks in the form of $NiSO_4$. $6H_2O$.

2.6.9 Drug induced hepatotoxicity

Drug-induced hepatic injury is the most frequent cause, it is induced by a large number of clinical drugs.

Table 2: Drug Induced Hepatotoxicity Models.

S.NO	DRUG	KIND OF HEPATOTOXICITY	DOSE
1	Paracetamol	Centrilobular liver necrosis and renal failure	800 mg/kg, p.o[48] 250 mg/kg /day p.o,[96] 600 mg/kg, p.o,[49] 640 mg/kg, p.o,[50] 100 mg/kg, p.o[51] 1 g/kg, p.o[52] 2 g/kg, p.o[53] 3 g/kg, p.o[54]
2	Thioacetamide	Centrilobular necrosis	100 mg/kg, s.c for 7 days[57]
3	Cisplatin	Oxidative stress	3.05 mg /kg, i.p, once a day for 5 days[58]
4	Artesunate	Oxidative stress	5 mg/kg/day, p.o[59]
5	**Antitubercular drugs** Isoniazid Isoniazid and rifampicin Isoniazid, rifampicin and pyrazinamide	 Oxidative stress, Chronic hepatitis or cirrhosis	50 mg/kg, i.p for 11 days 50 mg/kg,p.o + 100 mg/kg, p.o, for 7 days 50 mg/kg,po + 50 mg/kg, p.o for 28 days 100 mg/kg, i.p + 100 mg/kg, p.o for21 days 7.5 mg/kg/day + 10 mg/kg/day + 35 mg/kg/day, i.g, for 35 days

2.7. ANTITUBERCULAR DRUGS

Tuberculosis is dangerous diseases and its death is increasing year by year. Intake of isoniazid and rifampicin, the most common antitubercular drugs, lead to fatal hepatotoxic condition.[63]

Isoniazid and Rifampicin

Isoniazid (is nicotinic acid hydrazide - INH), rifampicin (RIF), pyrazinamide (PZA) have been successfull therapeutic agents for the treatment of TB because of high therapeutic efficacy and good patient acceptance and they produce variety of adverse reactions, from these hepatotoxicity is one of the well-known toxic effects. Peroxidation of endogenous lipids has been shown to be a major factor in the cytotoxic action of isoniazid and Rifampicin.[63]

The incidence of hepatotoxicity is high with Isoniazid and Rifampicin combination than with Isoniazid alone.[11,64] Isoniazid can cause moderate abnormalities in serum transaminases leading to hepatotoxicity.[60]

INH is mainly metabolized by N-acetyltransferase and Amidohydrolase. Hydrazine and acetylhydrazine were known toxic metabolites form isoniazid[12], during metabolism of INH, hydrazine can be produced from directly (from INH) and indirectly (from acetylhydrazine). The direct pathway involves hydrolysis of the amide bond of INHto produce is nicotinic acid and monoacetylhydrazine.[11] The indirect pathwayinvolves acetylation of INH to acetyl-INH by *N*-acetyltransferase, hydrolysis of acetyl-INH to is nicotinic acid and acetylhydrazine, and hydrolysis or deacetylation of acetylhydrazine to hydrazine.[65,69]

Cytochrome P_{450s} (CYPs) are associated with the development of isoniazid induced hepatotoxicity, particularly CYP2E1 and CYP1A1.[66,67] Additionally, CYPs are through to contribute to the additive or synergistic effects of rifampicin or isoniazid- induced hepatotoxicity. CYP2E1 has been considered as an important determinant of human susceptibility to toxicity. CYP2E1 produces free radicals independent of a ligand which can cause cell damage from lipid peroxidation and DNA strand breaks. Our previous study revealed that hepatic CYP2E1 activity correlated with isoniazid hepatotoxicity. CYP1A1 catalyzes the bioactivation of many promutagens and procarcinogens, which also contribute to free radical generation.[12]

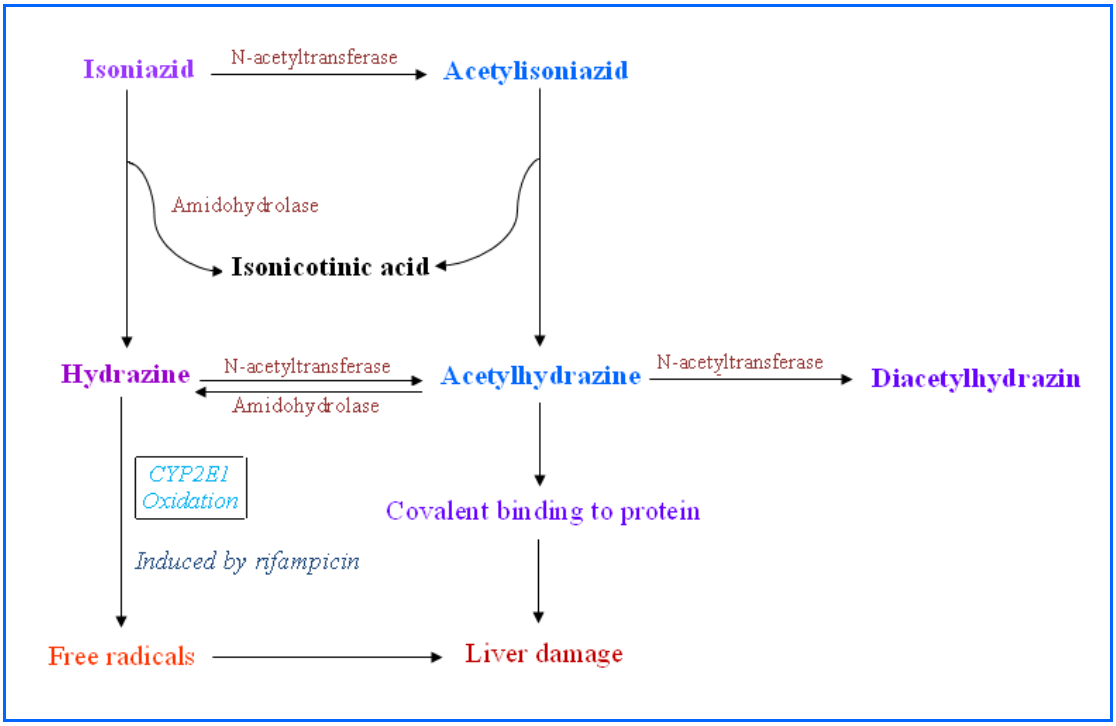


FIG. 2: Pathways of Isoniazid Metabolism.

INH is mainly inactivated by N-acetyltransferase 2 (NAT2) mediated acetylation, resulting in acetylisoniazid, which is hydrolyzed to acetylhydrazine. The later is further oxidized by cytochrome P_{450}2E1 (CYP2E1) to form hydroxylamines, which are intermediates in the formation of established hepatotoxic metabolites. The most widely accepted theory is that isoniazid metabolism produces reactive metabolites that bind to and damage cellular macromolecules in the liver.[68]

The hydrazine metabolite of INH and is subsequent effect on CYP2E1 induction is involved in the development of INH induced hepatotoxicity. Isoniazid induced hepatitis is associated with ballooning, focal hepatocyte necrosis, with minimal cholestasis.[11] Recent studies show that polymorphism of the N-acetyltransferase2 (NAT2) genes and glutathione-s transferases (GST) are the two major susceptibility risk factors for anti-tubercular therapy (ATT) induced hepatotoxicity.[70]

Bis-*p*-nitrophenyl phosphate (BNPP), an amidohydrolase (amidase) inhibitor, preventedacetyl-INH-induced hepatocellular damage. Inhibition of acetyl-INH-inducedhepatocellular damage by BNPP could be due to inhibition of productionof hydrazine from acetylhydrazine.[65]

Rifampicin is a powerful inducer of mixed function oxidase, increases the hepatotoxicity of Isoniazid by enhancing the production of toxic metabolites from acetylhydrazine.[11]

These drugs mediated oxidative damage is generally attributed to the formation of the highly reactive oxygen species, which act as stimulator of lipid peroxidation and source for destruction and damage to the cell membrane.[71] Alterations of various cellular defence mechanisms consisting of enzymatic and non-enzymatic components [reduced glutathione (GSH)] have been reported in isoniazid and rifampicin-induced hepatoxicity.[65]

2.8. TREATMENT OF LIVER DISEASES

Generally there is no established treatment for drugs- or herbs-induced hepatotoxicity. The best that can be done is the withdrawal of the offending drug or herb. Beside these, it can be pointed that some benefits have been observed with corticosteroids in allergic hepatotoxicity and with betaine, clofibrate and ursodeoxycholic acid and metformin for non-alcoholic steatohepatitis, of all of drugs-induced hepatotoxicities, the only one that can be effectively treated is that of paracetamol via use of *N*-acetylcysteine.[72,75,40,15]

Hepatitis C virus infection is the leading cause of chronic liver disease, it can treat by using interferon and ribavirin is effective in 30% - 40%.[74] Hepatitis B is another major cause of cirrhosis and hepatocellular carcinoma. It can treated by interferon is only 40 percent, and the response rate to second-line treatment using orally administered lamivudine is only about 30 percent.[74]

Liv.52, is an Ayurvedic formulation containing various herbo mineral principles designed to combat liver injury and to protect liver against damage.

Table 3: Composition of LIV 52.

Composition of Liv.52		
PLANT	**PLANT PART**	**QUANTITY (mg/tablet)**
Capparis spinosa	Bark	65
Cichorium intybus	Seeds	65
Solanum nigrum	Whole plant	32
Cassia occidentalis	Seeds	16
Terminalia arjuna	Bark	32
Achillea millefolium	Seeds	16
Tamarix gallica	Whole plant	16
*It also contains'mandur bhasma'(33 mg/tablet) which is prepared from ferric oxide, triturated in the juices of many hepatic stimulants and cholagogues.		

It has been reported that Liv.52 protects the liver from the hepatotoxicity of paracetamol, anti-cancer drugs, antibiotics, oral contraceptives, alcohol, allyl alcoholand carbon tetrachloride. Administration of Liv.52 in children suffering from malnutrition has improved liver function tests.[75]

2.8.1. Natural medicine

There are numerous plants and traditional formulations available for the treatment of liver diseases.[76] About 600 commercial herbal formulations with claimed hepatoprotective activity are being sold all over the world. Around 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess hepatoprotective activity. In India, more than 93 medicinal plants are used in different combinations in the preparations of 40 patented herbal formulations. However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy. Some herbal preparations exist as standardized extracts with major known ingredients or even pure compounds which are being evaluated.[82]

Table 4: Herbal Formulations for Hepatoprotection.

S.No	NAME OF THE FORMULATION	INDICATION	DOSE
1.	Livergen (Standard Pharmaceuticals, Serampore, West Bengal)	Ayurvedic medicine, gastrointestinal and hepatic regulator	2-4 teaspoon twice daily
2.	Livokin (Herbo-med, Kolkata)	Ayurvedic medicine, for hepatic dysfunction	1-2 teaspoon 2 to 3 times daily
3.	Octogen (Plethico Pharmaceuticals Ltd., Indore)	Ayurvedic medicine, highly potent hepatoprotective	As directed by physician
4.	Stimuliv (Franco-Indian Pharmaceuticals Pvt. Ltd., Mumbai)	Ayurvedic medicine, liver stimulant and tonic	1-2 teaspoon 2 to 3 times daily
5.	Tefroliv (TTK Pharma Pvt. Ltd., Chennai)	Ayurvedic medicine, standardized liver formulation for effective hepatic regeneration	1 teaspoon thrice daily or as directed by physician

3. MATERIALS AND METHODS

3.1 Plant Profile

Mangifera indica is one of the most important tropical plants marketed in the world. It is a large tree that grows in tropical and subtropical regions, whose fruits are widely appreciated by the population. There are many traditional medicinal uses for the bark, leaves and roots of mangifera indica thorough globe.

FIG 3: Mangifera Indica.

3.2 Taxonomy of classification
Division : Mangoliophyta
Class : Mangoliopsida
Subclass : Rosidae
Family : Anacardiaceae
Genus : Mangifera
Species : Indica

3.3 Botany
A large tree with a dense and spreading crown. Leaves are oblong to oblong-lanceolate, 10-30 cm long. The flowers are yellow, small, 3-4 mm long, borne on erect and hairy panicles, as long as the leaves. The fruit is yellow, fleshy, oblong-ovoid, and slightly compressed.

3.4 Cultivation
It is a widely cultivated tree for its fruit. It has several varieties in cultivation; the most popular are the "carabao" and "piko," and the former the preferred export variety. The fruits are of varying shades of yellow, elliptical and somewhat flattened, thin-skinned, with a large flattened seed in the center, surrounded by the edible yellow pulp.

3.5 Distribution
Widely cultivated for its delicious fruit.

3.6 Chemical constituents and properties
Mangiferin; mangin; piuri-yellow dye; benzoic acid; citric acid; tannin, 10%. The leaves contain 43-46 percent euxanthin acid and some euxanthon. Seed contains a fixed oil, oleostearin. The bark exudate yields a resin, gum, ash, and tannin. Mangostine, 29-hydroxymangiferonic acid, mangiferin and flavonoids have been isolated from the stem bark. Leaves and flowers yield an essential oil containing humulene, elemene, ocimene, linalool and nerol.

3.7 Properties
• Root, diuretic; bark, astringent; seeds, astringent and mifuge; leaves, pectoral.
• Considered antiseptic, antibacterial, anti-inflammatory, diaphoretic, stomachic, vermifuge, cardiotonic and laxative.

3.8 Parts used
Leaves, kernel, bark and fruit.

3.9 Uses
- Good source of iron (deficient in calcium); excellent source of vitamins A, B, and C. Fruit contains citric, tartaric and malic acids.
- Decoction of root is considered diuretic.
- Bark and seeds are astringent.
- Resin is used for aphthous stomatitis.
- Cough: Drink infusion of young leaves as needed.
- Diarrhoea: Take decoction of bark or kernel as tea.
- Hot lotion from bark used for rheumatism.
- Gum resin from bark, mixed with coconut oil, used for scabies and other parasitic skin diseases.
- Juice of leaves used for dysentery.
- Tea of leaves with a little honey used for hoarseness and aphonia, 4 glasses daily.
- Powdered dried leaves, 1 table spoon to a cup of warm water, 4 times daily, used for diabetes.
- Ashes of burned leaves used for scalds and burns.
- Infusion of young leaves used in asthma and cough.
- Tea of powdered dried flowers, 4 times daily for diarrhoea, urethritis.
- Juice of peel of unripe mangoes used for skin diseases.
- Seed is vermifuge and astringent.
- Infusion of powdered dried seeds used for asthma, diarrhoea, dysentery, menorrhagia, bleeding piles, round worms.
- In Indian traditional medicine, seeds used for diarrhoea.

3.10 Proven studies
• Antibacterial / Photochemical: Study showed that leaf extracts of M. indica possess some antibacterial activity against Staphylococcus aureus, E. coli, and Pseudomonas aeruginosa provides a basis for its medical use in Uganda. Phytochemical study showed saponins, steroids and triterpenoids, alkaloids, coumarins, anthracenocides, flavonones, tannins and reducing sugars.[93]
• Anti-asthmatic: Mangifera indica stem bark effect on the rat trachea contracted by acetylcholine and histamine: Study showed MI blockage of histaminic and muscarinic receptors, supporting the traditional use of MI stem back in the treatment of asthma.[87]
• Immunostimulant: Immunomodulatory activity of alcoholic extract of Mangifera indica L in mice: Study showed increased humoral antibody titer and delayed type hypersensitivity in mice suggesting a potential for a drug with immunostimulant properties.[88]
• Antihyperglycemic: Antihyperglycaemic effect of Mangifera indica in rat: Study showed leaf extract of MI possess hypoglycemic activity, possibly due to reduction in intestinal absorption of glucose.+[92]
• Flavonoids / Antihyperlipidemic Effect: Flavonoids from M indica eefectively reduce lipid levels in serum and tissues of rats with induced-hyperlipidemia. Degradation and elimination of cholesterol were enhanced.[89]
• Antioxidant: Oral administration of flavonoids showed significant antioxidant action in cholesterol-fed experimental rats. The activities of free radical-scavenging enzymes were significantly elevated and lipid peroxide content was significantly reduced in flavonoid-treated hypercholesterolemic rats.[90]
• Anti-diarrheal Activity: Study of the methanolic and aqueous extracts of seeds of M indica showed significant anti-diarrhea activity, the effect partly attributed to the effect on intestinal transit.[86]
• Anti-Diabetic Activity: Study showed all extracts had significant antihyperglycemic effect in type 2 model rats. The ethanol extracts of stem-barks reduced glucose absorption gradually during the whole perfusion period in type 2 rats.[92]

• Polyphenols / Antiulcerogenic Activity: Study showed oral pretreatment with mangifera leaf decoction decreased the severity of gastric damage in induced-gastric lesions. Two main phenolic compounds isolated were mangiferin and C-glucosyl-benzophenone. The findings show the potential gastroprotective properties of the aqueous decoction from M indica leaves.[94]
• Antiinflammatory, Analgesic and Hypoglycemic: Anti-inflammatory, analgesic and hypoglycemic effects of Mangifera indica Linn. (Anacardiaceae) stem-bark aqueous extract: Results of the study support the folkloric use of the plant for painful arthritic and other inflammatory conditions, as well as T2DM.[95]
• Anti-Cancer / Polyphenols: A Texas Agrilife Research study by food scientiists Dr. Susanne Talcott and Dr Steve Talcott found that polyphenol extracts from mango promote anticancer activity in certain colon and breast cancer cells in lab.[96]

3.11 Plant collection

Mangifera indica was collected from surrounding areas of Nellore authentified by Prof. P.JAYARAMAN, PLANT ANATOMY RESEARCH CENTRE, WEST TAMBARAM CHENNAI. Bark was separated, washed in water, chopped into pieces and then shade dried.

3.12 Preparation of extract

50 g of powder of *Mangifera indica* bark was weighed and extracted with 300ml of various solvents like pet ether, benzene, chloroform, ethyl acetate, methanol and aqueous in a soxhlet apparatus for 72 hrs. Then the solvent is subjected to distillation and concentrated the extracts. The extract is concentrated with rotary evaporator under reduced pressure and the dried extract was weighed and the percentage yield of the extracts was calculated.

FIG. 4: Preparation of Extract.

The percentage yield of extracts was tabulated in the Table no.5.

Table 5: Percentage Yield of Mangifera Indica In Different Solvents.

S.NO	EXTRACT	PERCENTAGE YIELD
1	Petroleum ether	5.92
2	Benzene	4.13
3	Chloroform	4.05
4	Ethyl acetate	2.65
5	Methanol	6.89
6	Water	3.98

3.13 Phyto chemical tests

3.13.1 Test for carbohydrates

- **Molisch's test:** To the 2 ml of test solution, alcoholic naphthol was added and then few drops of Conc. Sulphuric acid was added through sides of the test tube. Purple to violet colour ring appeared at the junction.
- **Barfoed's test:** To the 1 ml of test solution, 1ml of Barfoed's reagent was added and heated on a water bath, the formation of cupric acid confirmed the presence of the monosaccharide.
- **Fehling's test:** Fehling's A&B of equal volumes was taken and heated on a water bath. To this 2ml of test solution was added and again heated. Brick red precipitate was formed.
- **Benedict's test:** To the 2ml of sample solution, 2 ml of Benedict's reagent was added and heated on a water bath. Reddish brown precipitate was formed.

3.13.2 Test for proteins and amino acids

- **Biuret test:** To the 2 ml test solution, 2 ml of Biuret reagent was added. The formation of violet colour confirmed the presence of proteins.
- **Millon's test:** To the 2 ml of test solution, 2 ml of Millon's reagent was added. The formation of white precipitate confirmed the presence of amino acids.
- **Ninhydrin test:** To the 2ml test solution, 2ml of Ninhydrine solution was added and boiled on a water bath. The formation of violet colour confirmed the presence of amino acids.

3.13.3 Test for steroids

- **Salkowski test:** The extract was treated with few drops of concentrated sulphuric acid, the formation of red colour at lower layer indicated the presence of steroids or the formation of yellow coloured lower layer indicated presence of triterpenoids.
- **Libermann-Burchard test:** The extract was treated with few drops of acetic anhydride, boiled and cooled. After adding the conc. Sulphuric acid from sides of test tube, a brown colour ring was formed at the junction between two layers and upper layer turned into green which confirmed the presence of steroids and formation of deep red colour indicated presence of triterpenoids.

3.13.4 Test for alkaloids

- **Dragendroff's reagent:** To the 2 ml of sample solution, Dragendroff's reagent (potassium bismuth

iodide solution) was added. Reddish brown precipitate was formed.

- **Mayer's reagent:** To the 2 ml of sample solution, Mayer's reagent (potassium mercuric iodide solution) was added. Cream colour precipitate was formed.
- **Hager's reagent:** To the 2 ml of sample solution, Hager's reagent (saturated solution of picric acid) was added. Yellow colour precipitate was formed.
- **Wagner's reagent:** To the 2 ml of sample solution, Wagner's reagent (Iodine-potassium iodide solution) was added. Reddish brown precipitate was produced.

3.13.5 Test for glycosides

Cardiac glycosides

- **Baljet's test:** 2 ml of test solUtion was treated with picric acid or sodium picrate. Orange color was formed.
- **Keller-killani test (test for deoxy sugars):** The drug was extracted with chloroform and evaporated to dryness. 0.4 ml of glacial acetic acid was added containing trace amount of $FeCl_3$. Transfer the solution to a small test tube-acetic acid layer shows blue colour.

Saponin glycosides

- **Foam test:** Place 2 ml solution of drug in water in test tube, shake well, stable froth was formed.

Anthraquinone glycosides

- **Borntrager's test:** Boil the test material with 1 ml of H_2SO_4 in a test tube for 5 min. Filter while hot. Cool the filtrate and shake with equal volume of dichloromethane or CHCl3. Separate the lower layer of dichloromethane or CHCl3 and shake it with half of its volume of dil.NH3. A rose pink to red colour was produced in ammonical layer.

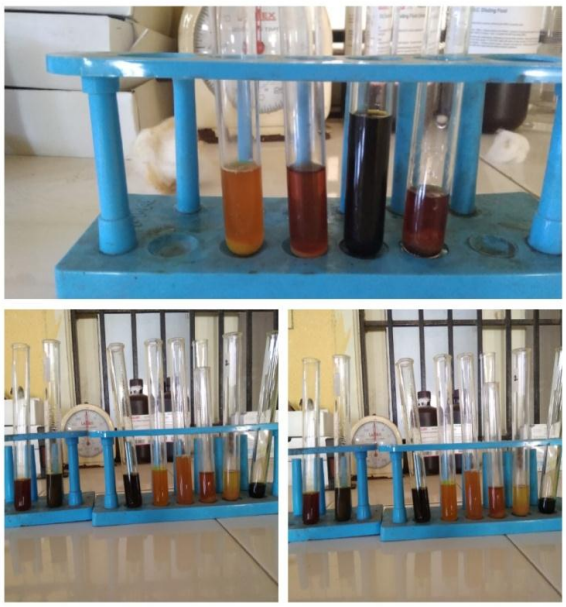


FIG 5: Phytochemical Tests.

3.13.6 Test for tannins and phenolic compounds

- **$FeCl_3$ test:** The extract was treated with $FeCl_3$ solution, formation of blue color confirmed the presence of hydrolysable tannins and formation of green colour confirmed the presence condensed tannins.

3.13.7 Test for flavonoids

- **Schinoda test:** To the 2 ml of test solution, few magnesium trunings was added and conc. HCl drop wise. Pink, crimsion red, green to blue colour appeared after few minutes.

For *Mangifera indica*

Various chemical tests were performed for the pet. ether, benzene, chloroform, ethyl acetate, methanol and aqueous extracts of Mangifera indica the results were mention in below in table no 6 as follows.

Table 6: Showing Results for Different Phytochemical Tests for Different Extracts.

S.NO	Name of the Test	P .E	B.E	C.E	E.E	M.E	W.E
	Test for Carbohydrates						
1.	Molisch's test	-	-	-	-	+	-
2.	Fehling's test	-	-	+	-	+	-
3.	Barfoed's test	-	-	-	-	+	-
4.	Benedict's test	-	-	+	-	+	-
	Test for Proteins and amino acids						
5.	Biuret test	-	-	-	-	+	-
6.	Millon's test	-	-	-	-	+	-
7.	Ninhydrin test	-	-	-	-	+	-
	Test for steroids						-
8.	Salkowski test	-	+	-	+	+	-
9.	Libermann Burchard	-	+	-	+	+	
	Test for Alkaloids						
10.	Dragendorff's test	+	+	-	+	+	+
11.	Wagner's test	+	-	+	+	+	+
12.	Mayer's test	+	+	+	+	+	+
13.	Hager's test	+	+	-	+	+	+
	Test for Cardiac glycosides						
15.	Baljet test	-	-	+	-	-	-
16.	Keller killiani test	-	-	-	-	-	+
	Test for Saponin glycosides						
17.	Foam test	-	-	-	-	-	-
	Test for Anthra quinone glycosides						
18	Borntragger's test	-	-	-	-	+	-
19.	**Test for Tannins and phenolic compounds**	-	-	-	-	+	-
	Test for Flavanoids						
20.	Shinoda test	-	-	-	-	+	-

3.14 Animals

Female wistar rats (150-200 g) body weight were obtained from Raghavendra enterprises, Bangalore, and they were housed under standard husbandry conditions, 25±5^{0}C temperature, 12 h light/dark cycle with standard rat feed (Pravan agro Ltd. India) with water *adlibitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee of.

3.15 Drugs and chemicals

DTNB (5, 5-di thio bis 2-nitro benzoic acid) was procured from Sigma-Aldrich Pvt. Ltd. India; liv.52 was procured from Himalaya herbals Banglore, isoniazid and rifampicin procured from Lupin Ltd. India and all other chemicals and reagents used were of analytical grade, procured from SD fine chemicals Ltd. India.

SGPT, SGOT, alkaline phosphatase, total cholesterol and HDL, total bilirubin and Total protein estimation kits were procured from Kamineni Life Sciences Pvt. Ltd. India.

3.16 Instruments

Analytical UV-Visible spectrophotometer (Analytical technologies Ltd. Model no: AUV 2060).

3.17 Acute toxicity study

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method). Wistar albino rats of either sex were selected randomly and divided into five groups (n = 6). The animals were fasted overnight and methonolic extract in doses of 500, 1000, 2000, 4000 mg/kg body weight, were administered orally to II – V groups. Group I which received vehicle (water) served as control. The animals were observed continuously for 2 hr, and then intermittently for 6 hr and at the end of 24 hours, the number of deaths was noted to determine LD_{50} of the extract.

Animals were also observed for behavioral, neurological and autonomic profiles simultaneously.

3.18 Hepatoprotective activity

Hepatotoxicity was induced in the present study, using isoniazid (200 mg/kg, p.o) and rifampicin (200 mg/kg, p.o) and liv.52 (500 mg/kg, p.o) was used as standard drug.[63]

Total animals were divided into five groups, each group containing 6 animals and treated for 28 days, as given in treatment schedule (Table 7).

Table 7: Treatment schedule- Hepatoprotective activity.

Groups	Treatment (28 days)	Purpose
I	Vehicle (distilled water)	To serve as normal animal
II	INH (200 mg/kg, p.o) and rifampicin (200 mg/kg, p.o)	To serve as control
III	INH (200 mg/kg, p.o) and rifampicin (200 mg/kg, p.o) and liv 52 (500 mg/kg, p.o)	To serve as standard
IV	INH (200 mg/kg, p.o) and rifampicin (200 mg/kg, p.o) and *Mangifera indica* bark extract (200 mg/kg, p.o)	To assess the hepatoprotective activity (at low dose)
V	INH (200 mg/kg, p.o) and rifampicin (200 mg/kg, p.o) and *Mangifera indica* bark extract (400 mg/kg, p.o)	To assess the hepatoprotective activity (at high dose)

3.19 Collection of blood sample

The blood samples were with drawn on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day from the retro orbital venous plexus of rats without any coagulant for the separation of serum. After collecting the blood in eppendorf tubes kept aside for 1 hour at room temperature and then serum was isolated by centrifugation at 2000 rpm for 15 min and stored until analyzed for various biochemical parameters.

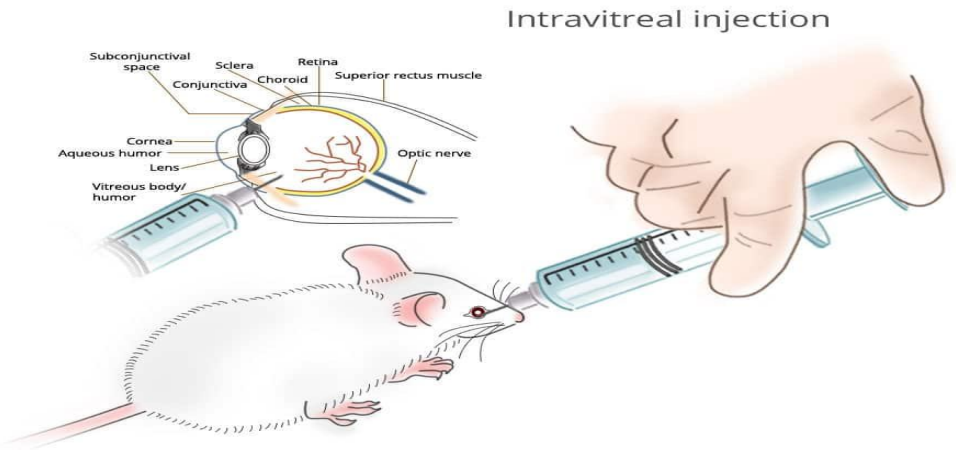


FIG 6: Collection of Blood Sample from Retro Orbital Venous Plexus of Rats.

3.20 Liver parameters

At the end of the treatment schedule weight of the animals was noted and then followed by sacrificed by cervical dislocation, excision of liver.

- One lobe of the liver was immediately processed for antioxidant parameters.
- Other lobe of the liver was stored in 10% formalin solution for histological preparations.

3.21 Bio Chemical Studies

3.21.1 Estimation of SGPT (Serum glutamyl pyruvate transaminase)

(Modified IFCC UV- kinetic method)

Principle: SGPT catalysis the transfer of amino groups from L-alanine to 2-oxoglutarate. The rate of reaction is monitored using a coupling enzyme lactate dehydrogenase (LDH), where by the pyruvate formed is converted to lactate in the presence of NADH. The oxidation of NADH is measured by monitoring the decrease in absorbance at 340 nm.

$$\text{L-Alanine} + \text{2-Oxoglutarate} \xrightleftharpoons{\text{SGPT}} \text{Pyruvate} + \text{L- Glutamate}$$

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

Procedure

Pipette into test tube as follows.

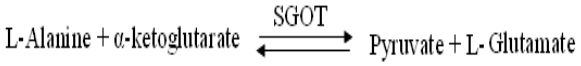
Table 8: Procedure for Estimation of Sgpt.

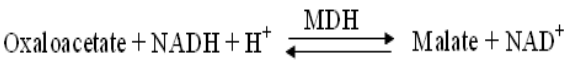
Reagent	Test
Enzyme reagent	1.0 ml
Serum/plasma	0.1 ml

Assay temperature: 37^0 C
Mix well and read absorbance against distilled water at 340 nm as follows
A_0 - exactly after 1 min.
A_1, A_2, A_3 - exactly after every 30 seconds for 1 min 30 seconds.
Calculate the average change in absorbance per minute (ΔA/min)
At 340 nm in **IU/L = ΔA/min × 1746 × tf**
Temperature conversion factor (tf) 1:0 at 37^0 C

3.21.2 Estimation of SGOT (Serum glutamyl oxaloacetic acid transaminase) (Modified IFCC UV- Kinetic method)

Principle: SGOT catalysis the transfer of an amino group from L-alanine to α-ketoglutarate. The rate of reaction is monitored using a coupling enzyme malate dehydrogenase (MDH), where by the oxaloacetate formed is converted to malate in the presence of NADH. The oxidation of NADH is measured by monitoring the decrease in absorbance at 340 nm.





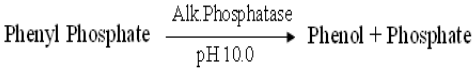
Procedure
Pipette into test tube as follows.
Table 9: Procedure for Estimation of Sgot.

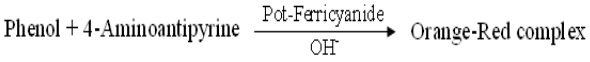
Reagent	Test
Enzyme reagent	1.0 ml
Serum/plasma	0.1 ml

Assay temperature: 37^0 C
Mix well and read absorbance against distilled water at 340 nm as follows
A_0 - exactly after 1 minute
A_1, A_2, A_3 - exactly after every 30 seconds for 1 min 30 seconds
Calculate the average change in absorbance per minute
At 340 nm in **IU/L = ΔA/min × 1746 × tf**
Temperature conversion factors (tf) 1:0 at 37^0 C

3.21.3 Estimation of Alkaline phosphatase (Kind and King's Method)

Principle: Alkaline phosphatase from serum converts phenyl phosphate to inorganic phosphate and phenol at pH 10.0. Phenol so formed reacts in alkaline medium with 4-aminoantipyrine in presence of the oxidizing agent potassium ferricyanide and forms an orange-red colored complex, which can be measured colorimetrically. The colour intensity is proportional to the enzyme activity.





Procedure
Table 10: Procedure for Estimation of ALP.

Reagent	Blank (B)	Standard (S)	Control (C)	Test (T)
Working buffer	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Substrate purified water	1.5 ml	1.5 ml	1.5 ml	1.5 ml
Mix well and incubate at 37^o C for 3 min				
Serum	-	-	-	0.05 ml
Reagent 3	-	0.05 ml	-	-
Mix well and incubate at 37^o C for 15 min				
Reagent 2	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Serum	-	-	0.05 ml	-

Mix well after addition of each reagent and measure the absorbance at 510 nm of blank (B), standard (S), control (C) and test (T) against purified water.

Calculations

$$\frac{\text{O.D. Test} - \text{O.D. Control}}{\text{O.D. Standard} - \text{O.D. Blank}} \times 10$$

3.21.4 Estimation of Total cholesterol (Chod-Pod/ Phosphotungstate Method)

Principle
BCholesterol+$H_2O \xrightarrow{CHE}$Cholesterol + Free fatty acid
Cholesterol+$O_2 \xrightarrow{CHO}$Cholest - 4ene 3-one + H_2O_2
H_2O_2 + Phenol + 4-amino antipyrine$\xrightarrow{POD}$Red Quinoneimine complex + H_2O

Procedure

Pipette into 3 test tubes labelled blank (B), standard (S), and Total cholesterol (T_c) as shown below.

Table 11: Proceduure for Estimation of Total Cholesterol.

Reagent	1.0 ml procedure			3.0 ml procedure		
	B	S	T_c	B	S	T_c
Cholesterol reagent I	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Cholesterol standard II (conc. 200 mg/dl)	--	10 µl	--	-	20 µl	--
Specimen	--	--	10 µl	--	--	20 µl
Distilled water	--	--	--	2.0 ml	2.0 ml	2.0 ml

Mix well and incubate for 5 min at 37°C or 10 min at room temperature. Read the absorbance of standard (S), total cholesterol (T_c) against blank at 505 nm.

Calculations

Total cholesterol (in mg/dl) = **(Abs. of T_c / Abs. of S) X 200**

3.21.5 Estimation of HDL

Principle

Cholesterol + $H_2O \xrightarrow{CHE}$ Cholesterol + Free fatty acid

Cholesterol+$O_2 \xrightarrow{CHOD}$ Cholest-4ene 3-one + H_2O_2

H_2O_2 + Phenol + 4-amino antipyrine $\xrightarrow{POD}$ Red Quinoneimine complex + H_2O

On addition of precipitating reagent to the serum, followed by centrifugation, HDL fraction remains in the supernatant while the lipoproteins precipitate out.

Procedure

Step 1: **Pipette into the centrifuge tube.**

Serum / plasma	0.2 ml
Precipitating reagent (3)	0.3 ml

Mix well and allow standing at room temperature for 5 min. Centrifuge at 3000 rpm for 10 min to get a clear supernatant. If supernatant is not clear (high TGL level) dilute the sample 1:1 normal saline and multiply the result with 2.

Step 2: **Pipette into 3 test tubes labelled blank (B), standard (S), HDL cholesterol (T_H) as shown below.**

Table 12: Procedure for Estimation of HDL.

Reagent	1.0ml procedure			3.0 ml procedure		
	B	S	T_H	B	S	T_H
Cholesterol reagent(1)	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
HDL Cholesterol standard (4) (conc. 50 mg/dl)	--	100 µl	--	-	200 µl	--
Supernatant (from step1)	--	--	100 µl	--	--	200 µl
Distilled water	100 µl	--	--	2.2 ml	2.0 ml	2.0 ml

Mix well and incubate for 5 min at 37°C or 10 min at room temperature. Read the absorbance of standard (S), HDL cholesterol (T_H) against blank at 505 nm.

Calculations

$$\text{HDL cholesterol (in mg/dl)} = \frac{(\text{Abs. of } T_H)}{\text{Abs. of S}} \times 50$$

3.21.6 Estimation of Bilirubin (Modified Jendrassik & Grof's Method)

Principle

Conjugate (Direct) bilirubin present in serum reacts with diazotized sulphanilic acid to yield azobilirubin which absorbs at 546 nm. Total bilirubin present in serum reacts with diazotized sulphanilic acid in presence of activator to yield azobilirubin, which absorbs at 546 nm.

Bilirubin+ Diazotized sulfanilic acid $\longrightarrow$ Azobilirubin compound

Procedure

Pipette into test tubes labelled blank (B) and test (T) as follows:

Table 13: Procedure for Estimation of Bilirubin.

Reagent	Sample Blank (B_T)	Test (T_T)
Total Bilirubin reagent	1.0 ml	1.0 ml
Total Diazo reagent	---	25 µl
Mix well and proceed		
Serum sample	50 µl	50 µl

Mix well and incubate at room temperature for 10 min and read absorbance of total sample blank (B_T) and total test (T_T) at 546 nm.

Calculations

Total Bilirubin in mg/dl= **(Abs of T_T – Abs of B_T) X 26.31** (Factor)

3.22 Estimation of antioxidant parameters

Preparation of homogenate

The animals were sacrificed and excised liver was weighed, the homogenate was prepared as follows.

Reagent

1. 0.25 M sucrose solution 85.87 g of sucrose was dissolved in 1000 ml of distilled water.
2. 10 mm tris buffer solution: 1.2 g of tris was dissolved in 900 ml of distilled water. pH was adjusted to 7.4 with 1M HCl and diluted up to 1000 ml.

Procedure

Excised livers were cross chopped with surgical scalps into fine slices and were chilled in the cold 0.25 M sucrose, quickly blotted with filter paper. The tissue was minced and homogenized in ice cold 10 mm tris HCl buffer (to pH 7.4) at a concentration of 10% (w/v) with 25 stokes of tight teflon pestle of glass homogenizer at a speed of 2500 rpm. The prolonged homogenization under hypotonic condition was designed to disrupt as far as possible the ventricular structure of cells so as to release soluble protein and leave only membrane and non-vascular matter in sedimentation form. It was then centrifuged at 5000 rpm at 20^o C temperature and clear supernatant was separated and used to estimate superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT) and lipidperoxidation (LPO).

3.22.1 *In-vivo* antioxidant parameters

3.22.1.1 Super oxide dismutase (SOD)

Principle

Rate of auto oxidation of epinephrine and the sensitivity of this auto oxidation to inhibition by SOD were augmented as pH was raised from 7.8 -10.2, O_2 generated by xanthine oxidase reaction, caused by the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per O_2 introduced. The auto oxidation of epinephrine proceeds by at least two distinct pathways only one of which is free radical chain reaction involving O_2 and hence inhabitable by SOD.

Reagents

1. Carbonate buffer (0.05 M, pH 10.2): 16.8 g. of sodium bicarbonate and 22 g of sodium carbonate were dissolved in 500 ml of distilled water and final volume was made up to with distilled water.
2. Ethylene diamine tetra acetic acid (EDTA) (0.49 M): 1.82 g of EDTA was dissolved in 1000 ml of distilled water.
3. Epinephrine (3 mm): 9.9 mg of epinephrine bitartrate was dissolved in 10 ml of 1M HCl solution.
4. SOD standard: dissolve 1mg (1000 units/mg) of SOD from bovine liver in 100 ml of carbonate buffer.

Procedure

0.5 ml of sample was diluted with 0.5 ml distilled water, to this 0.25 ml ethanol, 0.5 ml of chloroform (all chilled reagents) were added. The mixture was shaken for 1 min and centrifuged at 200 rpm for 20 min. The enzymatic activity of supernatant was determined, to it 0.05 ml of carbonate buffer (0.05 M pH 10.2) and 0.5 ml EDTA (0.49 M) was added. The reaction was initiated by addition of 0.4 ml epinephrine and the change in optical density/mm was measured at 480 nm. SOD was expressed as units/mg. protein change in optical density/mm 50% inhibition of epinephrine to adrenochrome transition by enzyme is taken the enzyme unit. Calibration curve was prepared using 10-125 units SOD.

$$SOD = \frac{(0.025\text{-}Y)}{Y \text{ X } 50} \text{ X } 100$$

Y- Final reading – initial reading

3.22.1.2 Catalase (CAT)

(Hugo E. Aebi method)

Principal

$H_2O_2 \longrightarrow H_2 + O_2$

$RDOH \longrightarrow H_2O + ROH + A$

Decomposition of H_2O_2 = Decrease in absorbance at 240 nm

Reagents

1. Phosphate buffer (50 mm, pH7.0):

A. Dissolve 6.81 g KH_2PO_4 in distilled water and make up to 1000 ml.

B. Dissolve 8.9 g NaH_2PO_4. $2H_2O$ in distilled water and make up to 1000 ml.

Mix the solution **A** and **B** in proportion 1:15 (v/v)

2. Hydrogen peroxide (30 mm/I): Dilute 0.34 ml of 30% Hydrogen peroxide with phosphate buffer up to 100 ml.

Procedure

Dilute homogenate 20 times with Phosphate buffer pH 7.0

Blank	Test
4 ml of homogenate diluted with 2 ml of phosphate buffer P^H 7, and take absorbance at 254 nm for 3 min. with 30 sec. interval	2 ml of homogenate diluted with 1 ml of H_2O_2 (8.5 micro lit. in 2.5 ml phosphate buffer (50mM/l. pH 7.0) and take the absorbance at 240 nm for 3 min. with 30 sec. interval. (Add H_2O_2 just before taking O.D)

Calculation

Log (A / B) × 2297.3

Where,

A: Initial absorbance

B: final absorbance (after 30 second)

Units = µ moles of H_2O_2 consumed/min/mg

3.22.1.3 Reduced glutathione (GSH)

Reagents

1. TCA (10% w/v) solution: Accurately weighed 10 g of TCA was dissolved in 100 ml of distilled water.
2. Phosphate buffer (0.2 M, pH 8)
3. DTNB reagent (0.6 M): 60 mg of 5, 5- dithio bis (2-nitro benzoic acid) was dissolved in 100 ml of 0.2 M sodium phosphate (pH8).
4. Standard glutathione: Prepared by dissolving 10 mg of reduced glutathione in 100 ml of distilled water.

Procedure

To 1 ml of sample, 1 ml of 10% TCA was added. The precipitated fraction was centrifuged and to 0.5 ml supernatant, 2 ml DTNB was added. The final volume was made up to 3 ml with phosphate buffer. The colour developed was read at 412 nm. The amount of glutathione was expressed as µg of GSH/mg protein reduced glutathione was used as standard (100 µg/ml).

$$X = \frac{(Y - 0.0046)}{0.0034}$$

Y – Absorbance of test sample

3.22.1.4 Lipid peroxidation (LPO)

Reagents

1. Thiobarbituric acid: 0.67% w/v in 1M tris hydrochloride pH -7, 0.67 g of thiobarbituric acid was dissolved in 100 ml of distilled water.
2. Trichloroacetic acid (20% w/v): 20 g of TCA was dissolved in 100 ml of distilled water.
3. Standard malondialdehyde (0-25 n.mol)

A stock solution containing 50 mm/ml of 1,1,3,3-tetra ethoxy propane in tris hydrochloride buffer in pH -7, 10 ml of stock solution was diluted to 100 ml to get a working standard 50 nm malondialdehyde/ml. This was used for preparation of calibration curves.

Procedure

2 ml of sample was mixed with 2 ml of 20% TCA and kept in ice for 15 min. The precipitate was separated by centrifugation and 2 ml of samples of clear supernatant solution were mixed with 2 ml aq. 0.67% TBA solution. This mixture was heated on a boiling water bath for 10 min. It was cooled in ice for 5 min and absorbance read at 535 nm. The values were expressed as nm of MDA formed/mg of protein values are normalized to protein content of tissues (Slater and Sawyer, 1971).

$$X = \frac{(Y + 0.002)}{0.0026086}$$

Y – Absorbance differences of final (after 3 min) and initial reading of test sample.

4. RESULTS

The results obtained for the effect of methanolic stem bark extract of Mangifera indica on SGPT, SGOT, ALP, effect on total cholesterol, HDL, bilirubin(serum analytical methods) and the effect on SOD, Catalase, reduced GSH, lipid peroxidation (tissue bio chemical methods) and histology are as follows.

Table 14: Effect of Methanolic Extract of Mangifera Indica on SGPT.

Group	Treatment	SGPT (IU/L)				
		0 day	7th day	14th day	21st day	28th day
I	Normal	34.5±3.5	34.7±3.5	34.9±4.1	35.4±5.7	35.1±2.1
II	Control INH+RIF (200 mg/kg)	30.2±5.5	112.8±17.2	143.4±2.3	165.5±6.1	188.7±4.1
III	Standard INH+RIF (200 mg/kg) + liv52 (100 mg.kg)	37.3±2.3	68.3±5.7	92.3±3.9	101.2±7.2	113.1±5.4
IV	INH+RIF (200 mg/kg) + *Mangifera indica*(200 mg/kg)	32.4±3.9	88.2±4.6	102.5±4.8	122.4±8.9	133.3±6.2
V	INH+RIF (200 mg/kg) + *Mangifera indica* (400 mg/kg)	38.9±6.2	74.7±9.2	97.9±5.1	113.8±3.3	135.7±3

a = $p< 0.001$, when compared to normal animals
b = $p< 0.001$, when compared to control animals

5. DISCUSSION

5.1. Pharmacological studies

5.1.1. Acute toxicity studies

The Methanolic bark extract of *Mangifera indica* was found to be safe since no animal died even at the maximum single dose of 4000 mg/kg when administered orally, and the animals did not show any gross behavioral changes. Hence, 1/20 and 1/10 of maximum therapeutic dose (4000 mg/kg) was selected for the present study.

5.2. Hepatoprotective activity

5.2.1. Effect on SGPT

Rats treated with INH+RIF (G-II) showed a significant increase in SGPT levels on 7th, 14th, 21st and 28th day, when compared to normal group (G-I). The group (III) rats treated with standard drug liv.52 (500 mg/kg) showed a significant decrease in SGPT levels on 7th, 14th, 21st and 28th day, when compared to control (G-II).

The groups (IV and V) receiving methanolic bark extract of *mangifera indica*(200 mg/kg and 400 mg/kg) shows a dose dependent decrease on SGPT levels on 7th, 14th, 21st and 28th day when compared to control group (G-II) (Table-6.1).

5.2.2. Effect on SGOT

A significant increase in SGOT levels was observed in rats treated with INH+RIF (G-II) on 7th, 14th, 21st and 28th day, when compared to normal group (G-I). The group (G-III) rats treated with standard drug liv.52 (500 mg/kg) showed a significant decrease in SGOT levels on 7th, 14th, 21st and 28th day, when compared to control (G-II).

The groups (IV and V) receiving methanolic bark extract of *Mangifera indica*(200 mg/kg and 400 mg/kg) showed a dose dependent decrease in SGOT levels on 7th, 14th, 21st and 28th day when compared to control group (G-II) (Table-6.2).

5.2.3. Effect on ALP

Administration of INH + RIF induced a significant increase in ALP levels on 7th, 14th, 21st and 28th day in control group (G-II) when compared to normal group (G-I).

On treatment with liv 52 induced a significant decreases in ALP levels on 7th, 14th, 21st and 28th day in standard group (G-III) when compared to control group (G-II).

Groups (IV and V) treated with methanolic bark extract of *Mangifera indica*induces a significant decreases in ALP levels on 7th, 14th, 21st and 28th day both the doses has shown dose dependent when compared to control group (G-II) (Table-6.3).

5.2.4. Effect on total cholesterol

A significant increases in total cholesterol levels in INH + RIF treated group (G-II) on 7th, 14th, 21st and 28th day when compared to normal group (G-I).

Liv.52 treated group (G-III) shows significant decreases in total cholesterol levels on 7th, 14th, 21st and 28th day when compared to control group (G-II).

Methanolic bark extract of *Mangifera indica*treated groups (IV and V) shows a significant decreases in total cholesterol levels on 7th, 14th, 21st and 28th day in both

doses has shown dose dependent when compared to control group (G-II) (Table-6.4).

5.2.5. Effect on serum HDL-cholesterol

INH + RIF receiving groups (G-II) shows a significant increases in HDL- cholesterol levels on 7th, 14th, 21st and 28th day when compared to normal group (G-I).

Liv.52eceiving group (G-III) shows significant decreases in HDL- cholesterol levels on 7th, 14th, 21st and 28th day when compared to control group (G-II).

methanolic bark extract of *mangifera indica*treated groups (IV and V) shows a significant decreases in HDL-cholesterol levels on 7th, 14th, 21st and 28th day in both doses has shown dose dependent when compared to control group (G-II).

5.2.6. Effect on total bilirubin

Administration of INH + RIF induced a significant increase in total bilirubin levels on 7th, 14th, 21st and 28th day in control group (G-II) when compared to normal group (G-I) .

On treatment with liv 52 induced a significant decrease in total bilirubin levels on 7th, 14th, 21st and 28th day in standard group (G-III) when compared to control group (G-II).

Groups (IV and V) treated with methanolic bark extract of *Mangifera indica*induces a significant decreases in total bilirubin levels on 7th, 14th, 21st and 28th day both the doses has shown dose dependent when compared to control group (G-II) (Table-6.6).

5.3. *In vivo* antioxidant parameters

In the present study, various antioxidant parameters were assessed in the pancreas at the end of the study on 29th day.

Administration of INH + RIF diminished the antioxidant status in liver by decreases the catalase, GSH, SOD levels and increases the LPO levels in control group (G-II) when compared to normal group (G-I).

Standard group (G-II) treated with liv.52 increases the antioxidant status in liver by increasing the SOD, catalase, GSH levels and decreases the LPO levels when compared to control group (G-II).

Both doses of methanolic bark extract of *Mangifera indica*shows the significant increases in antioxidant status in liver by increasing the SOD, catalase, GSH levels and decreasing the levels of LPO has shown dose dependent when compared to control group (G-II) (table6.7) (Graphs-6.7,6.8,6.9,6.10).

Histological examination of liver slices

Histological examination of the normal liver slices showed normal hepatic parenchyma. There was no sign of inflammation or necrosis in these animals (Fig- 6.11). In INH–RIF treated group of animals showed moderate to heavy lobular inflammation and moderate portal triaditis with piecemeal necrosis or focal lobular inflammation (Fig- 6.12).

Pre-treatment with liv.52, 500 mg/kg dose showed almost normal liver lobule with no sign of necrosis and portal triads. Only a few inflammatory cell are observed in the centrizonal area (Figure- 6.13).

In the liver cells of rats treated with hydro-alcoholic root extract of *Mangifera indica* at 200 mg/kg dose showed reduction of necrosed area and inflammatory infiltrates in centrizonal area with disappearance of inflammatory infiltrate around portal triad (Figure- 6.14). *Mangifera indica* at 400 mg/kg dose showed greater reduction of the necrosed area and sparse inflammatory cell infiltration (Figure-6.15) as compared to 200 mg/kg dose. Hence, *Mangifera indica* showed dose dependent reduction in the necrosis and inflammatory infiltration.

Hepatotoxicity of anti-TB drugs is a serious problem because it causes significant morbidity and mortality that requires modification of the drug regimen.[98] The incidence of anti-TB drug induced hepatotoxicity has been reported to be higher in developing countries and some factors such as liver disease, incorrect use of drugs, malnutrition and more advanced tuberculosis have been implicated for the increase in hepatotoxicity.[98] It is estimated that the incidence of clinically relevant hepatotoxicity is 3% in the US, 4% in UK and 11.5% in India.[98]

DIH due to isoniazid prophylaxis has been more commonly observed with advanced age. In published reports, the relative risk of DIH due to isoniazid (INH) prophylaxis ranged from 0/1000 in persons under the age of 20 years; 2.8/1000 in persons aged <35 years, compared to 7.7–19.2/1000 observed in those aged above 50 years. When isoniazid was used to treat TB in combination with other drugs, DIH was found to occur more often in older patients.[98,100]

The increased risk of hepatotoxicity with INH and rifampicin (RIF) combination has been attributed to the interaction between the metabolism of isoniazid and rifampicin. INH is metabolized in the liver primarily by acetylation and hydrolysis, and it is these acetylated metabolites that are thought to be hepatotoxins.[101] Acetyl-isoniazid, the principal metabolite is converted to monoacetyl hydrazine. The microsomal p450 enzymes convert monoacetyl hydrazine to other compounds resulting in hepatotoxicity. RIF is thought to enhance this effect by enzyme induction.[98] RIF induces cytochrome P450 enzyme causing an increased production of toxic metabolites from acetyl hydrazine (AcHz). RIF 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

RIF 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 INH and RMP in combination.

Serum transaminases, serum alkaline phosphatase and serum bilirubin have been reported to be sensitive indicators of liver injury. Serum transaminases are important class of enzymes linking carbohydrate and amino acid metabolism. Aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT) are present majority in the liver and these are also found in the cardiac muscles, skeletal muscles, pancreas, lungs, kidney, brain, etc., SGPT concentration is highest in the liver and therefore, it appears to a more sensitive test to hepatocellular damage than SGOT.[102]

There is no suitable drug for treating hepatotoxicity, in regard of these herbs are implicated as potential hepatoprotective agents. Therefore, the present study attempts to study the hepatoprotective and antioxidant property of *Mangifera indica* against anti-TB drug induced hepatotoxicity, in rats of wistar strain. Biochemical parameters of hepatotoxicity and oxidative stress were analyzed from serum and liver homogenates to assess the hepatoprotective activity. Further histopathological study was also carried out to confirm the pathological changes.

The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due INH and RIF induced peroxidative damage or altered permeability of membrane.[35] Increased protein catabolism and urea formation that are seen in antitubercular drugs-induced hepatocellular damage and necrotic lesions in the hepatocytes may also be responsible for the increase of these amino tranferases activities in liver. Similar increase in levels of SGPT and SGOT in the hepatic cells were also observed in the present study, when treated with combination of INH+RIF.

In the present study, co-administration of methanolic bark extract of *Mangifera indica* with INH+RIF significantly decreased the levels of these diagnostic marker enzymes (SGPT and SGOT) and the effect was observed to be dose dependent.

Alakaline phosphatase was found to increase in the group- II animals treated with INH+RIF. ALP activity on endothelial cell surfaces is responsible for the conversion of adenosine nucleotides to adenosine, a potent vasodilator and anti-inflammatory mediator that results from injury. So, following injury, accumulation of interleukin-6 can lead to production of adenosine by alkaline phosphatase and subsequent protection from ischemic injury. This may be the reason for the increment in ALP in intoxicated rats due to liver cell necrosis.[63]

In the present study, co-administration of methanolic bark extract of *Mangifera indica*with INH+RIF decreased the levels of these ALP marker enzymes in the serum.

Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures.[104] The toxic metabolite hydrazine is produced, which further binds covalently to the macromolecule and causes peroxidative degradation of lipid membrane of the adipose tissue. In view of this, the reduction in levels of SGOT, SGPT and ALP caused by the *Mangifera indica* is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by INH+RIF. Similar mechanism for hepatoprotective action were proposed for *Ginkgo biloba*, *Strychno spotatoru* and *Momordica dioica* Roxb.[105,104]

The major disorder encountered in antitubercular drugs-induced hepatitis is fatty accumulation in the liver, which develops either due to excessive supply of lipids to the liver or interference with lipid deposition. The pathogenesis is multifactorial, reflecting complex biosynthetic, enzymatic and catabolic dearrangement in lipoprotein metabolism.[106] In the present study, the levels of total cholesterol were higher in INH and RIF administered rats, indicating that the antitubercular drugs-induces hypercholesterolemic condition. The increase in cholesterol levels in the liver might be due to increased uptake of LDL from the blood by the tissues.[107] The abnormal cholesterol deposition is favoured by the dangerous tendency of cholesterol to undergo passive exchange between the plasma lipoproteins and the cell membranes.[108] Hence, protective HDL-cholesterol levels were reduced in the animals treated with INH+RIF.

In the present study, co-administration with methanolic bark extract of *Mangifera indica*reduced the elevation in the levels of total cholesterol induced by anti-TB drugs. The levels of protective HDL-cholesterol were also prominently increased when animals treated with *Mangifera indica*.

The probable mechanism responsible may be due to the decrease in the biosynthesis of cholesterol in the liver or by inhibiting enzymes responsible for the synthesis of cholesterol, by the chemical constituents of M*angifera indica.* This effect may also be responsible for an improvement in the serum HDL-levels. *Tridax procumbent* is also reported to have beneficial effect on lipid profile due to similar mechanism.[109]

Determination of serum bilirubin represents an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary disease and severe disturbance of hepatocellular function.[109] The disaggregation of polyribosomal profiles induced by toxins is also

associated with the inhibition of protein synthesis, which may be partially responsible for the fatty liver, probably not necrosis although it contributes to disabling of the cell.[63] Hence, decrease in protein levels and increase in total bilirubin was observed in animals when treated with INH+RIF.

In the present study, co-administration of methanolic bark extract of *Mangifera indica*with INH+RIF increased the levels of these total proteins and decreased the total bilirubin levels in the serum.

Cytochrome P_{450} is one of the liver enzymes, considered responsible for damage of hepatic cells.[32] *Mangifera indica* ***may*** inhibit these enzymes, thus enhance in the level of total proteins and decrease in the levels of total bilirubin. Thus was observed the hepatoprotective action may be mediated through the inhibition of UDP-sugar derivatives, enhancement of glycoprotein biosynthesis and stabilisation of cell membrane and inhibition of lipid accumulation by its hypolipidemic property, which are the few common mechanisms attributed for hepatoprotective activity for natural drugs.[110]

The results of histopathological parameters also support the results of biochemical parameters and explain the hepatoprotective activity of*Mangifera indica.*

Free radicals alter the structural and functional integrity of cells by a variety of mechanisms, including lipid peroxidation, sulfhydryl oxidation and proteolysis and shearing of the nuclear material. Healthy cells can scavenge free radicals effectively by their defensive system (antioxidant effects). In short, there is a dynamic relationship between reactive oxygen species and antioxidants in the human body. In some pathological conditions, such as cells suffering ischaemic insult, the sudden generation of reactive oxygen species can dramatically upset this balance with an increased demand on the antioxidant defence system. Natural antioxidants are depleted accompanied by accumulation of reactive oxygen species. In such a situation, natural products can play an important role in two aspects: enhance the activity of original natural antioxidants and neutralize reactive oxygen species by nonenzymatic mechanisms.[111] It is reported that oxidative stress is also involved in liver damage.[112] Hence, effect of *Mangifera indica*on oxidative stress induced by INH+RIF was studied.

Isoniazid and rifampicin induced hepatitis is due to their biotransformation to reactive metabolites that are capable of binding to cellular macromolecules.[71] As an alternative to inducing cellular damage by covalent binding, there is evidence that these antitubercular drugs cause cellular damage through the induction of oxidative stress, a consequence of dysfunction of hepatic antioxidant defense system. The role of oxidative stress in the mechanism of isoniazid and rifampicin-induced hepatitis has been reported by Attri et al., (2000).

Lipid peroxidation is a common event in toxic phenomenon, is regulated by the availability of substrate in the form of polyunsaturated fatty acids (PUFA). Although it occurs to a limited extent under normal physiological conditions, but external factors can augment this process so that it escapes cell control leading to degradation of lipids in the cell membrane and eventually causing membrane damage and death of cell.

In the present study, free radicals formed either by the reaction of metabolites of INH+RIF with oxygen or by the interaction of superoxide radicals with H_2O_2, seem to initiate peroxidative degradation of membrane lipids and endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to formation of lipid peroxides which in turn give products like MDA that cause loss of integrity of cell membrane and damage to hepatic tissue.[114]

Reduced glutathione is one of the most abundant non-enzymatic biological antioxidant present in the liver[114]; it efficiently scavenges reactive toxic metabolites of antitubercular drugs. As a substrate for antioxidant enzymes glutathione peroxidase (GPX), glutathione reductase (GR), it protects cellular constituents from the damaging effects of peroxides formed during metabolism and other ROS. Liver injury has been observed when GSH stores are markedly depleted. In our study, similar decrease in GSH was observed on administration of INH+RIF.

It is known that SOD, CAT constitutes a mutually supportive team of antioxidant enzymes which provides a defense system against ROS. Catalase is an enzymatic antioxidant, a heamoprotein which catalyses' the reduction of hydrogen peroxide to water and oxygen and protects the tissue from highly reactive hydroxyl free radicals.

In the present study, SOD and catalase decreased in INH+RIF treated animals may be due to an excessive formation of superoxide anions.

Concomitant administration of methanolic bark extract of *Mangifera indica*and INH+RIF effectively increased the GSH, SOD and CAT activities and also decreased the MDA levels which may be attributed to the scavenging of radicals by methanolic bark extract of *Mangifera indica* resulting in protection of these enzymes.

Flavonoids, present in the *Mangifera indica* is known to quench the free radical by maintaining antioxidant levels.[115] Thus, it can be suggested that significant antioxidant activity shown by *Mangifera indica*may be due to presence of mangostin, 29-hydroxymangiferoic acid.

The present study indicates that the methanolic bark extract of *Mangifera indica*may be used as an effective hepatoprotective agent. Further studies on isolation and

structural determination of active principles might be worthy.

6. CONCLUSION

Hepatotoxicity has a considerable impact on health because many of the hepatic reactions induced by pharmaceutical preparations can be very severe. Drug-induced hepatotoxicity (DIH) is a leading cause of liver injury. DIH is an important and commonly encountered adverse effect with antituberculosis treatment (ATT). Isoniazid and rifampicin are first line drugs used for tuberculosis chemotherapy and are associated with hepatotoxicity.

Therefore, the hepatoprotective activity of methanolic bark extract of *Mangifera indica* was evaluated against antitubercular drug induced hepatotoxicity in rats. The parameters monitored in the present study were SGPT, SGOT, ALP, total cholesterol, HDL, total protein and total bilirubin. The *in vivo* antioxidant parameters LPO, GSH, SOD and catalase were also assessed in the liver homogenates.

The methanolic bark extract of *Mangifera indica* has shown hepatoprotective effect with a significant decrease in SGOT, ALP, total cholesterol and total bilirubin levels and significant increase in HDL. It is also observed from the present study that *Mangifera indica*offers significant protection against antitubercular drug induced oxidative stress, noted by decrease in MDA levels and increase in SOD, CAT and GSH levels.

Hence, the results obtained in this study proved the efficacy of methanolic bark extract of *Mangifera indica*as hepatoprotective agent and the effect was observed to be dose dependent. Further studies for better understanding of the mechanism of action and hepatoprotective activity might be note worthy.

7. ACKNOWLEDGEMENT

I would like to express my sincere regards to our guide A.Vidhyadhari mam, (M.pharm), in Dept. of pharmacology, and our Principal DR. P.Venkatesh (M.pharm, Ph.D) and our academic incharge DR. Hepcy kalarani(m.pharm, Ph.D), our R&D Director DR. R.prema mam (M.pharm,Ph.D) and thanks to all our project members and all our classmates who helped us for this throughout research process.

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